



**THEME [ICT-2011.9.1]
[Challenging current Thinking]**

Grant agreement for: Collaborative project

Annex I - "Description of Work"

Project acronym: SI-CODE

Project full title: " Towards new Brain-Machine Interfaces: state-dependent information coding "

Grant agreement no: 284553

Version date: 2012-05-02

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A1: Project summary

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per project

General information

Project title ³	Towards new Brain-Machine Interfaces: state-dependent information coding		
Starting date ⁴	01/03/2012		
Duration in months ⁵	36		
Call (part) identifier ⁶	FP7-ICT-2011-C		
Activity code(s) most relevant to your topic ⁷	ICT-2011.9.1: Challenging current Thinking		

Abstract ⁹

Brain Machine Interfaces (BMIs) are devices mediating communication between a brain and the external world, and hold the potential for a) restoring motor or sensory functions to people who lost them due to illness or injury, and b) understanding neural information processing through controlled interactions between neurons and external devices. However, the success of BMIs is hampered by the problem that neural responses to external correlates are highly variable because they depend on the internal state of the neural network. We propose to remove this obstacle by developing a radically new generation of "bidirectional BMIs" (which decode information from the recorded neural activity and provide information to the brain by stimulation) employing neural computational strategies and neuromorphic VLSI devices that i) understand how network states influence neural responses to stimuli; ii) use this know-how to discount variability induced by state changes in real time and thus operate with increased bandwidth and performance. We gather a highly interdisciplinary team composed of both mathematical and experimental neuroscientists and of VLSI engineers. We will study the interplay between ongoing network states and stimulus-evoked responses in various nervous systems of different complexity. We will develop advanced algorithms and models of network dynamics to determine the network state variables best predicting and discounting neural variability, and to construct optimal state-dependent rules to decode neural activity. We will implement these algorithms in a new "state-dependent bidirectional BMI" prototype using low-power neuromorphic VLSI circuits that extract in real time network state information and use it to produce outputs optimally suited for both decoding of recorded signals and delivering electrical stimulation to a neural tissue in a given state. This BMI will be tested in a benchmark experiment in rats to guide an external device with closed loop control.

A2: List of Beneficiaries

Project Number ¹	284553	Project Acronym ²	SI-CODE
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List of Beneficiaries

No	Name	Short name	Country	Project entry month ¹⁰	Project exit month
1	FONDAZIONE ISTITUTO ITALIANO DI TECNOLOGIA	IIT	Italy	1	36
2	SCUOLA INTERNAZIONALE SUPERIORE DI STUDI AVANZATI	SISSA	Italy	1	36
3	MAX PLANCK GESELLSCHAFT ZUR FOERDERUNG DER WISSENSCHAFTEN E.V.	MPI-BC	Germany	1	36
4	UNIVERSITAET ZUERICH	UZH	Switzerland	1	36
5	CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE	CNRS	France	1	1

A3: Budget Breakdown

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One Form per Project

Participant number in this project ¹¹	Participant short name	Fund. % ¹²	Ind. costs ¹³	Estimated eligible costs (whole duration of the project)					Requested EU contribution
				RTD / Innovation (A)	Demonstration (B)	Management (C)	Other (D)	Total A+B+C+D	
1	IIT	75.0	T	1,390,099.00	0.00	59,000.00	0.00	1,449,099.00	1,101,574.00
2	SISSA	75.0	T	558,720.00	0.00	5,000.00	0.00	563,720.00	424,040.00
3	MPI-BC	75.0	S	670,456.00	0.00	2,200.00	0.00	672,656.00	505,042.00
4	UZH	75.0	T	579,966.00	0.00	5,600.00	0.00	585,566.00	440,574.00
5 (DEL)	CNRS	75.0	T	0.00	0.00	0.00	0.00	0.00	0.00
Total				3,199,241.00	0.00	71,800.00	0.00	3,271,041.00	2,471,230.00

Note that the budget mentioned in this table is the total budget requested by the Beneficiary and associated Third Parties.

*** The following funding schemes are distinguished**

Collaborative Project (if a distinction is made in the call please state which type of Collaborative project is referred to: (i) Small of medium-scale focused research project, (ii) Large-scale integrating project, (iii) Project targeted to special groups such as SMEs and other smaller actors), Network of Excellence, Coordination Action, Support Action.

1. Project number

The project number has been assigned by the Commission as the unique identifier for your project, and it cannot be changed. The project number **should appear on each page of the grant agreement preparation documents** to prevent errors during its handling.

2. Project acronym

Use the project acronym as indicated in the submitted proposal. It cannot be changed, unless agreed during the negotiations. The same acronym **should appear on each page of the grant agreement preparation documents** to prevent errors during its handling.

3. Project title

Use the title (preferably no longer than 200 characters) as indicated in the submitted proposal. Minor corrections are possible if agreed during the preparation of the grant agreement.

4. Starting date

Unless a specific (fixed) starting date is duly justified and agreed upon during the preparation of the Grant Agreement, the project will start on the first day of the month following the entry into force of the Grant Agreement (NB : entry into force = signature by the Commission). Please note that if a fixed starting date is used, you will be required to provide a detailed justification on a separate note.

5. Duration

Insert the duration of the project in full months.

6. Call (part) identifier

The Call (part) identifier is the reference number given in the call or part of the call you were addressing, as indicated in the publication of the call in the Official Journal of the European Union. You have to use the identifier given by the Commission in the letter inviting to prepare the grant agreement.

7. Activity code

Select the activity code from the drop-down menu.

8. Free keywords

Use the free keywords from your original proposal; changes and additions are possible.

9. Abstract

10. The month at which the participant joined the consortium, month 1 marking the start date of the project, and all other start dates being relative to this start date.

11. The number allocated by the Consortium to the participant for this project.

12. Include the funding % for RTD/Innovation – either 50% or 75%

13. Indirect cost model

A: Actual Costs

S: Actual Costs Simplified Method

T: Transitional Flat rate

F :Flat Rate

Workplan Tables

Project number

284553

Project title

SI-CODE—Towards new Brain-Machine Interfaces: state-dependent information coding

Call (part) identifier

FP7-ICT-2011-C

Funding scheme

Collaborative project

WT1

List of work packages

Project Number ¹	284553	Project Acronym ²	SI-CODE
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LIST OF WORK PACKAGES (WP)

WP Number ⁵³	WP Title	Type of activity ⁵⁴	Lead beneficiary number ⁵⁵	Person-months ⁵⁶	Start month ⁵⁷	End month ⁵⁸
WP 1	Management	MGT	1	5.00	1	36
WP 2	Interplay of ongoing activity and responses to stimuli in mammalian neural	RTD	1	39.00	1	36
WP 3	The role of ongoing activity in the leech nervous system	RTD	2	49.00	1	36
WP 4	Understanding how spontaneous network state influences information transmission across brain regions	RTD	3	41.00	1	36
WP 5	Methodologies for the extraction of state-dependent information from neural activity	RTD	1	47.00	1	36
WP 6	Theoretical analysis of state dependency of stimulus-driven activity in networks of spiking neurons	RTD	1	43.00	1	36
WP 7	Neuromorphic circuits for state-dependent processing in Brain-Machine Interfaces	RTD	4	42.00	1	36
WP 8	State dependent bidirectional BMI (bBMI)	RTD	1	35.00	1	36
WP 9	Dissemination of results	MGT	1	19.00	1	36
				Total	320.00	

WT2: List of Deliverables

Project Number ¹	284553	Project Acronym ²	SI-CODE
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List of Deliverables - to be submitted for review to EC

Deliverable Number ⁶¹	Deliverable Title	WP number ⁵³	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D1.1	Scientific and management periodic report	1	1	2.50	R	PU	18
D1.2	Scientific and management periodic report	1	1	1.50	R	PU	36
D1.3	Final scientific and management report	1	1	1.00	R	PU	36
D2.1	High resolution experimental platform with multisite optical/electrical stimulation	2	1	21.00	R	CO	18
D2.2	Report on state-dependent sensory processing in neuronal assemblies in vitro	2	1	18.00	R	CO	36
D3.1	Report on ongoing activity in the leech nervous system	3	2	25.00	R	CO	18
D3.2	Report on the effectiveness of state dependent decoding in leeches	3	2	24.00	R	CO	36
D4.1	Report on state dependence of neural responses in the vibrissal system	4	1	4.00	R	CO	18
D4.2	Report on how ongoing activity influences transmission of optogenetic stimulation of the LGN	4	3	25.00	R	CO	36

WT2: List of Deliverables

Deliverable Number ⁶¹	Deliverable Title	WP number ⁵³	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D4.3	Report relationship between ongoing activity, neuromodulatory stimulation and sensory stimulation	4	3	12.00	R	CO	36
D5.1	Algorithms identifying the most informative network state parameters	5	1	24.00	R	CO	18
D5.2	Algorithms in open source format and under an open source license on a dedicated website	5	1	23.00	R	CO	36
D6.1	Report on state dependence of stimulus-driven dynamics in large networks of spiking neurons	6	1	22.00	R	CO	18
D6.2	Report on models that best explain experimental ongoing dynamics	6	1	21.00	R	CO	36
D7.1	Tape-out of custom VLSI multi-neuron chip	7	4	20.00	R	CO	18
D7.2	Multi-neuron chip application to measurement of recorded network state	7	4	22.00	R	CO	36
D8.1	Report on algorithms improving the performances of bBMs	8	1	15.00	R	CO	18
D8.2	Report on the integration of a	8	1	10.00	R	CO	36

WT2: List of Deliverables

Deliverable Number ⁶¹	Deliverable Title	WP number ⁵³	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
	neuromorphic VLSI and a state-dependent bBMI						
D8.3	Report on the benchmark test of the VLSI-based state-dependent bBMI	8	1	10.00	R	CO	36
D9.1	Dissemination plan	9	1	1.00	R	PU	3
D9.2	Website and intranet	9	1	2.00	R	PU	3
D9.3	Dataset repository	9	3	8.00	R	PU	36
D9.4	Update of data analysis SW portals	9	1	8.00	R	PU	18
Total				320.00			

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP1	Type of activity ⁵⁴	MGT
Work package title	Management		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	1		

Objectives

- Project administrative and scientific coordination
- Ensuring relationships with EC
- Web-site management and external cooperation arrangement

Description of work and role of partners

The management of SI-CODE will ensure that the project is completed within the terms of the contract with the European Commission. This includes ensuring that:

- i- Appropriate agreements and management framework are in place between the partners;
- ii- All the project's activities are properly coordinated with appropriate levels of legal, contractual, ethical, financial and administrative management of the consortium;
- iii- Proper operational project management is provided throughout the project;
- iv-)The project completes its work to the expected timescales, resources and quality levels; v- Appropriate reporting to the European Commission is undertaken.

Task 1.1 (M1-M36) Administrative coordination.

The financial (costs, budgets) and continuous follow-up of the project. This will contain the contractual reporting activities with the EC, including the reports on the work progress, the deliverables, the preparation and attendance of the Review meetings and of a project meeting with the Project Officer at Month 9. This will also include the preparation of the project meetings (follow-up meetings, kick-off meeting, etc.), with the preparation of the related documentation (agenda, action points, etc.). The management activities, like controlling and transmitting the information between the partners are also targeted here.

Task 1.2 (M1-M36) Project scientific coordination

Follow-up of the research advancement considering the scientific objectives targeted by the project. This will include several aspects like the reviewing of the reports and deliverables, the publishing of scientific papers with the results of the Consortium, etc. The Consortium will also make sure that the periodic report to be submitted to the EC covers aspects of research findings with the potential of dual use, if applicable.

Task 1.3 (M1-M6) Intranet implementation

Implementation of the project intranet: it will constitute a vital management and communication tool amongst the project participants.

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	4.00
4	UZH	1.00
Total		5.00

WT3: Work package description

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D1.1	Scientific and management periodic report	1	2.50	R	PU	18
D1.2	Scientific and management periodic report	1	1.50	R	PU	36
D1.3	Final scientific and management report	1	1.00	R	PU	36
Total			5.00			

Description of deliverables

D1.1) Scientific and management periodic report: Overall administrative and scientific project management report. This report will be a coherent set composed of an extended Executive Summary followed by chapters reporting on the other deliverables due at this date and closing with an annex listing the project publications in the period [month 18]

D1.2) Scientific and management periodic report: Overall administrative and scientific project management report. This report will be a coherent set composed of an extended Executive Summary followed by chapters reporting on the other deliverables due at this date and closing with an annex listing the project publications in the period [month 36]

D1.3) Final scientific and management report: Final scientific and management report: [month 36]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS5	Review meeting #1	1	18	
MS11	Review meeting #2	1	36	
MS12	Project meeting with the Project Officer	1	9	

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP2	Type of activity ⁵⁴	RTD
Work package title	Interplay of ongoing activity and responses to stimuli in mammalian neural		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	1		

Objectives

- Development of a new platform for multisite optical/electrical stimulation and high-density recordings
- Characterization of the properties of ongoing activity and of its influence upon neural responses to stimuli in networks at high spatiotemporal resolution

Description of work and role of partners

Task 2.1 (M1-M12) Development of an experimental set-up for multisite optical stimulation and high spatiotemporal electrophysiological recordings. High-resolution APS-MEAs (4096 microelectrodes, sampling rate of 8kHz/channel at full frame) will be combined and adapted with an optical and electrical stimulation system enabling spatially and temporally defined photo-stimulation patterns and local electrical stimuli to be delivered. The existing hardware/software of the APS-MEA platform will be upgraded to manage both optical/electrical stimulations. In particular, a projection approach based on a Digital Micromirror Device (DMD) will be adopted to enable scaling of laser excitation of ChannelRhodopsins2 (488nm) and halorhodopsin (594nm) from hundreds of nano-meters to hundreds of micro-meters to selectively stimulate or inhibit neuronal targets. Involved Partners: IIT, SISSA

Task 2.2 (M1-M18) Experiments aimed at characterizing the ongoing activity during different states of the cortical network. To study input-output function with respect to the state of the network we will make use of neuronal networks (random or topologically defined) interfaced with MEA-based systems (T1.1). Analyses of recorded signals will include: (i) first- and second-order statistics (mean firing rate, ISI, CV, etc.); (ii) cross-correlation and functional connectivity estimation; (iii) power spectrum density. The intrinsic activity will be manipulated by optogenetic photo-modulation and pharmacological manipulations. Expected output: Experimental characterization and control of the internal state of neuronal assemblies. Involved Partners: IIT, SISSA

Task 2.3 (M6-M24) Experiments aimed at characterizing the activity under stimulation. The stimulation patterns will be defined on the basis of their spatio-temporal features starting from simple artificial stimuli to more complex time-varying spatio-temporal patterns to unravel how the network makes use of the ongoing activity to account for previously received stimulation. Temporally uncorrelated stimuli from spatially close/far away sites and temporally correlated (time-varying inputs) stimuli from single site or from spatially distributed sites will be used, and robustness of information coding will be made by adding external noise to the stimuli. Expected output: Characterization of state-dependent input-output functions. Involved Partners: IIT, SISSA

Task 2.4 (M12-M30) Decoding and extraction of the state-dependent input-output function. By applying the innovative analysis methods from WP4 we will attempt to develop decoding procedures, based on the description of the ongoing activity obtained in T1.2-1.3, with the aim to properly decode form state-dependent neural activity the original sensory stimulation reaching performances close to 100 %. Involved Partners: IIT, SISSA

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	34.00

WT3: Work package description

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
2	SISSA	5.00
	Total	39.00

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D2.1	High resolution experimental platform with multisite optical/electrical stimulation	1	21.00	R	CO	18
D2.2	Report on stet-dependent sensory processing in neuronal assemblies in vitro	1	18.00	R	CO	36
		Total	39.00			

Description of deliverables

D2.1) High resolution experimental platform with multisite optical/electrical stimulation: [month 18]
 D2.2) Report on stet-dependent sensory processing in neuronal assemblies in vitro: [month 36]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS1	Experimental data-set on ongoing activity	3	18	
MS8	Understanding the interplay between ongoing activity, incoming stimuli and neural responses	1	36	

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP3	Type of activity ⁵⁴	RTD
Work package title	The role of ongoing activity in the leech nervous system		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	2		

Objectives

- To obtain a complete characterization of the ongoing activity in the leech nervous system
- To understand how this ongoing activity is affecting the input-output relationships in the presence of controlled sensory inputs

Description of work and role of partners

Task 3.1 (M1-M12) Statistical analysis of the ongoing activity in the leech nervous system. We will characterize the spatio-temporal scales of ongoing state changes by measuring how the cross-correlation of pairs of single neurons depends on the bin-width and the degree of correlation between the global electrical activity obtained from single roots and from different ganglia, and we will relate single neuron firing during spontaneous activity to massed measures of neural activation such as LFPs. We will compare properties of the spontaneous activity in isolated ganglia, in chains of two connected ganglia and in semi-intact leeches, where the entire leech nervous system is present. Involved Partners: SISSA

Task 3.2 (M1-M12) Relation between the ongoing activity and spontaneous behaviour. We will compare the ongoing activity recorded from interneurons and other neurons in leech ganglia from semi-intact preparations with the observed behaviour. We will also use a preparation composed by a ganglion and a piece of skin connected to it to understand the behavioural outcome of ongoing activity and the behavioural responses of stimuli presented at different states of ongoing dynamics. This is possible because when a burst of ongoing activity occurs in the ganglion the skin contracts spontaneously. By collecting data for several hours we will establish on a solid statistical basis the relation between behaviour and the ongoing electrical activity of the neuronal assembly under investigation. Involved Partners: SISSA, IIT

Task 3.3 (M6-M24) Ongoing activity and sensory processing. We will investigate: how ongoing activity affects sensory processing. We will use a variety of different sensory stimulations of different complexity. In the simplest case we will evoke a controlled number of spikes in a single mechanosensory neurons; we will touch the skin with an appropriate stimulator; we will combine visual and mechanical stimulations. In these experiments we will collect a large number of different trials, possibly around 1000, in order to have a vast repertoire of processing also in the presence of different states of the intrinsic dynamics. Involved Partners: SISSA, IIT

Task 3.4 (M6-M24) Pharmacological modulation of the ongoing activity and sensory processing. We will modulate the intrinsic dynamics by blocking inhibitory synapses – with bicuculline or picrotoxin - and therefore by increasing the global level of the ongoing activity or by blocking excitatory synapses – with blockers of NMDA receptors such as APV – and therefore silencing the ongoing activity. In this way we will be able to sample in a better and possibly exhaustive way all the possible internal states and to determine their influence on information processing. Involved Partners: SISSA, IIT

Task 3.5 (M12-M36) Decoding and extraction of state-dependent information. We will use methodology of WP4 to decode sensory information in the presence of the ongoing activity. We will attempt to develop decoding procedures, based on the description of the ongoing activity obtained in T1.1-1.4, able to properly identify the original sensory stimulation close to 100 %. Involved Partners: SISSA, IIT

WT3: Work package description

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	3.00
2	SISSA	46.00
	Total	49.00

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D3.1	Report on ongoing activity in the leech nervous system	2	25.00	R	CO	18
D3.2	Report on the effectiveness of state dependent decoding in leeches	2	24.00	R	CO	36
		Total	49.00			

Description of deliverables

D3.1) Report on ongoing activity in the leech nervous system: [month 18]

D3.2) Report on the effectiveness of state dependent decoding in leeches: [month 36]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS1	Experimental data-set on ongoing activity	3	18	
MS8	Understanding the interplay between ongoing activity, incoming stimuli and neural responses	1	36	

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP4	Type of activity ⁵⁴	RTD
Work package title	Understanding how spontaneous network state influences information transmission across brain regions		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	3		

Objectives

- To determine how ongoing activity interacts with optogenetic, sensory and electrical stimulation in vivo
- To determine how this interaction affects transmission of network responses to a stimulus across brain areas

Description of work and role of partners

Task 4.1 (M1-M24) Relation between optogenetic stimulation of the geniculostriate pathway and spontaneous cortical state on V1 responses.

We will stimulate optogenetically the Lateral Geniculate Nucleus while recording simultaneously neural activity (i.e. LFPs and multiunit spiking activity) both in the optogenetically stimulated LGN region and in the in the early areas of visual cortex (e.g. V1, V2) of anaesthetized rats and monkeys using MRI compatible electrodes. The neural and fMRI recordings of LGN and V1/V2 will be used not only for examining the effects of stimulation but also for the study of inter-area communication in period of resting activity and stimulation respectively. A number of analysis and modelling methodologies (described in WP4-5) will be used to achieve the latter goal. Involved Partners : MPI-BC, IIT

Task 4.2 (M12-M36) Relationship between electrical stimulation of noradrenergic neuromodulatory pathways and spontaneous cortical state on cortical sensory responses.

We will examine the effects of noradrenergic neuromodulation on both cortical state and the sensory induced activity in prefrontal and somatosensory cortex, and to examine the relationship between cortical responses to sensory stimulation, activity of LC-noradrenergic system and ongoing cortical activity. We will apply different patterns of electrical stimulation to the LC and monitor cortical activity using multielectrode arrays. The whole-brain BOLD signal will be used to investigate how the ongoing cortico-coerulear network state may determine the transmission of the response to the stimulus across brain areas and what brain regions are affected by the LC stimulation. Involved Partners : MPI-BC, IIT

Task 4.3 (M6-M18) Relationship between electrical stimulation and spontaneous cortical state on cortical responses between two brain regions by using a high-density array of electrodes.

We will use two high-density stimulation and recording electrodes in two different brain areas to understand how the state of the on-going activity influences the response in the same region and the postsynaptic activity of a connected region. We will place a dense array of stimulating electrodes into the barrel cortex (region A) and a similar recording array in the vibrissae representation of motor cortex (region B). After placing the electrodes, we will stimulate region A with different spatiotemporal patterns of electrical micro-stimulation and we will simultaneously record the response of region A and B. We are going to use methods described in WP4 to characterize the network-state parameters that influence the neural response in both regions and the information that can be extracted by the knowledge of both state and neural response. Involved Partners : IIT, MPI-BC

Task 4.4 (M12-M36) Relationship between sensory stimulation and spontaneous cortical state on cortical responses between two brain regions by using a high-density array of electrodes

We will use the same setup as in T3.3 but we will replace electrical micro-stimulation with sensory stimulation to explore the state dependence of sensory induced activity Sensory stimulation will be provided by controlled movements of the whisker principal to the location of the recording electrode. Involved Partners : IIT, : MPI-BC

WT3: Work package description

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	4.00
3	MPI-BC	37.00
Total		41.00

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D4.1	Report on state dependance of neural responses in the vibrissal system	1	4.00	R	CO	18
D4.2	Report on how ongoing activity influences transmission of optogenetic stimulation of the LGN	3	25.00	R	CO	36
D4.3	Report relationship between ongoing activity, neuromodulatory stimulation and sensory stimulation	3	12.00	R	CO	36
Total			41.00			

Description of deliverables

D4.1) Report on state dependance of neural responses in the vibrissal system: [month 18]

D4.2) Report on how ongoing activity influences transmission of optogenetic stimulation of the LGN: [month 36]

D4.3) Report relationship between ongoing activity, neuromodulatory stimulation and sensory stimulation: [month 36]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS1	Experimental data-set on ongoing activity	3	18	
MS8	Understanding the interplay between ongoing activity, incoming stimuli and neural responses	1	36	

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP5	Type of activity ⁵⁴	RTD
Work package title	Methodologies for the extraction of state-dependent information from neural activity		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	1		

Objectives

- To provide the novel set of analysis algorithms to determine how neural networks encode state dependent information;
- To use these algorithms to determine what neural response parameters define the network state and influence state dependent information transmission

Description of work and role of partners

Task 5.1 (M1-M12) Develop the main computational methods for robust calculation of the information quantities needed for the state-dependent information analysis.

We will develop a new set of algorithms that can be applied to experimental and simulated datasets and provide quantitative answers about which network state parameters and neural codes carry most information. Involved partners: IIT

Task 5.2 (M6-M24) Use models and analysis to understand the rules and mechanisms of state dependent information coding.

We will apply these algorithms systematically to models to derive a set of rules between state parameters and enhanced encoding of stimuli given the knowledge of the state parameters. The expected output is a set of putative network state parameters that in models consistently increase the information that can be extracted from the population. Involved Partners: IIT

Task 5.3 (M18-M36) Use methods to analyze data provided by experimental collaborators.

We will collaborate with each experimental partner to first fine-tune the algorithms to the statistics and nature of each dataset, and then to use them to determine the set of actual network state parameters that increase the information that can be extracted from the population in each experimental condition. Involved Partners: IIT, SISSA, MPI-BC

Task 5.4 (M18-M36) Open source delivery of state-dependent information extraction algorithms.

We will create a website containing the final set of open source algorithms and some example (simulated and real) datasets for analysis. Involved Partners: IIT, SISSA, MPI-BC

Task 5.5 (M18-M36) Fine tuning of optimal state parameters for VLSI-based real time detection of state dependence.

The information theoretic methods will be used to find the optimal parameters values to measure both "activity" and "dynamic" state parameters for online state detection by VLSI circuits. This will be first done by analysing VLSI state detection in offline neural data and will be finally performed to optimize the parameters to run the closed loop bBMI. Involved Partners: IIT, UZH

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	33.00
2	SISSA	5.00

WT3: Work package description

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
3	MPI-BC	6.00
4	UZH	3.00
Total		47.00

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D5.1	Algorithms identifying the most informative network state parameters	1	24.00	R	CO	18
D5.2	Algorithms in open source format and under an open source license on a dedicated website	1	23.00	R	CO	36
Total			47.00			

Description of deliverables

D5.1) Algorithms identifying the most informative network state parameters: [month 18]

D5.2) Algorithms in open source format and under an open source license on a dedicated website: [month 36]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS4	Release of software tools for extracting state-dependent stimulus information	1	18	
MS7	Multi-neuron chip for state-dependent parameters evaluation	4	36	

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP6	Type of activity ⁵⁴	RTD
Work package title	Theoretical analysis of state dependency of stimulus-driven activity in networks of spiking neurons		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	1		

Objectives

- Perform a systematic numerical and analytical investigation of the dynamics of models of networks of excitatory and inhibitory spiking neurons with realistic synaptic kinetics
- Characterize asynchronous and synchronous states in network models
- Understand the mechanisms controlling transitions between different types of states in network models
- Obtain a LFP model which is compatible with experimental data (LFP spike triggered average, membrane potential-LFP correlations) in different states
- Analyse systematically stimulus-induced dynamics in network models, as a function of the network state immediately preceding stimulus presentation
- Provide a dynamical model to be implemented in silico for real time state detection

Description of work and role of partners

Task 6.1 (M1-M12) Analytical and numerical investigation of ongoing activity in networks of spiking neurons
We will extend analytical tools developed previously by CNRS to the investigation of the dynamics of networks of excitatory and inhibitory neurons with realistic kinetics. This will allow us to determine parameter regions in which various types of states (asynchronous, or synchronous) are observed. In all network states, various types of observables (in particular those related to LFP) will be compared to experimental data. Involved Partners: IIT, SISSA, MPI-BC

Task 6.2 (M6-M24) Analytical and numerical investigation of the interplay between ongoing activity and stimulus-driven activity in networks of spiking neurons
We will use methods from statistical physics and theory of dynamical systems to investigate systematically stimulus-driven dynamics in networks of spiking neurons, as a function of the state of the network immediately preceding stimulation. Involved Partners: IIT, SISSA, MPI-BC

Task 6.3 (M18-M36) Determination of models that best explain experimental ongoing dynamics.
We will compare model results with the experimental datasets and WP1-3 and characterize which network parameters and architectures best explain the observed data (LFPs, spike rate dynamics, dependence of response upon ongoing activity) and allow the highest amount of extraction of state dependent information. Involved Partners: IIT, UZH

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	40.00
4	UZH	3.00
	Total	43.00

WT3: Work package description

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D6.1	Report on state dependence of stimulus-driven dynamics in large networks of spiking neurons	1	22.00	R	CO	18
D6.2	Report on models that best explain experimental ongoing dynamics	1	21.00	R	CO	36
Total			43.00			

Description of deliverables

D6.1) Report on state dependence of stimulus-driven dynamics in large networks of spiking neurons: [month 18]

D6.2) Report on models that best explain experimental ongoing dynamics: [month 36]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS2	Neuromorphic circuit specification completed	4	18	
MS3	Multi-neuron chip for state-dependent parameters evaluation	1	36	
MS7	Multi-neuron chip for state-dependent parameters evaluation	4	36	

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP7	Type of activity ⁵⁴	RTD
Work package title	Neuromorphic circuits for state-dependent processing in Brain-Machine Interfaces		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	4		

Objectives

- Development of neural models of state-dependent computation using biophysically realistic networks of neurons and synapses, compatible with the constraints of neuromorphic VLSI implementations.
- Design and fabrication of neuromorphic chip that implements the networks defined above.
- Implementation of the models using the chip developed within a re-configurable (AER) multi-chip set-up.
- Evaluation of the system's state-dependent computational capabilities: decoding neural trajectories and learning to decode time-varying signals, with controlled stimuli (computer generated spike sequences).

Description of work and role of partners

Task 7.1 (M1-M6) Constrain the models explored by WP5 to feasible VLSI implementations. We will collaborate with WP4 and WP5 to define the specifications for neural models of state-dependent computation using biophysically realistic networks of neurons, synapses, and learning mechanisms, compatible with the constraints imposed by the neuromorphic VLSI implementations. Expected output: specifications for constraining computational models. Involved partners: UZH, IIT

Task 7.2 (M6-M24) Design and implement adaptive and plastic neuromorphic circuits for estimating the state of ongoing neural activity in real time.

In this task we will design analog circuits that implement the computational operators of the state variable detections derived in WP4-5. We will implement spiking neuron models and dynamic synapse circuits that exhibit the properties specified in task 1. In a first prototype we will integrate a set of neural population models with discretized sets of synaptic weights. In a second prototype we will integrate large numbers of plastic synapses (of the order of tens of thousands) and large numbers of neurons (for the order of tens of hundreds) onto a single chip layout. Both chips will be sent out to fabrication using a standard CMOS process. Expected output: Fabrication of two prototypes of new neural model using biophysically realistic network of neurons implemented in a neuromorphic VLSI device. Involved Partners: UZH, IIT

Task 7.3 (M9 - M30) Device characterization and testing.

In this task we will prepare the infrastructure for testing, evaluating, and applying the neuromorphic chip to real-time experiments. This requires the design of custom printed-circuit-boards (PCBs) to host the full-custom chips, the development of custom interfacing boards and systems to interface it to standard PCs. Once both chip and PCBs will be available, we will integrate everything into an evaluation system and characterize its basic response properties. Expected output: Setup for interfacing custom chip to PCs, and basic characterization of the system. Involved Partners: UZH, IIT

Task 7.4 (M18 – M36) Evaluate and test models and relative neuromorphic implementation for state-dependent decoding of neural activity and encoding of stimulation parameters, as defined in WP5.

In this task we will stimulate VLSI neural networks with recorded spiking activity and modulate their activity with the estimated network state. Specifically, we will implement the algorithms developed in WP5 for state-dependent information extraction. Expected output: feasibility study of decoding and encoding of neural activity and stimulation using multi-neuron VLSI chips. Involved partners: UZH, IIT

WT3: Work package description

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	15.00
4	UZH	27.00
Total		42.00

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D7.1	Tape-out of custom VLSI multi-neuron chip	4	20.00	R	CO	18
D7.2	Multi-neuron chip application to measurement of recorded network state	4	22.00	R	CO	36
Total			42.00			

Description of deliverables

D7.1) Tape-out of custom VLSI multi-neuron chip: [month 18]

D7.2) Multi-neuron chip application to measurement of recorded network state: [month 36]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS2	Neuromorphic circuit specification completed	4	18	
MS7	Multi-neuron chip for state-dependent parameters evaluation	4	36	

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP8	Type of activity ⁵⁴	RTD
Work package title	State dependent bidirectional BMI (bBMI)		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	1		

Objectives

- To design and develop a framework that establish a bidirectional communication between the brain and an external device able to include the state-information improving the communication bandwidth
- To evaluate the improvements obtained by introducing a neuromorphic VLSI device in a closed loop state-dependent bBMI

Description of work and role of partners

Task 8.1 (M1-M12) Development of mathematics of bBMI.

In this task we will develop a novel mathematical framework based on the algorithms already used in the preliminary experiments. These algorithms will establish updated decoding and encoding interfaces both to improve the performances of the bidirectional communication system and to be integrated with an additional input that takes into account the state of the network. Expected output: specifications of decoding and encoding algorithms. Involved partners: IIT

Task 8.2 (M12-M24) Realization and testing of non-state dependent bBMI.

In this task we will realize a non state-dependent bBMI both to test the consistency of the new mathematical framework and to define a set of parameters for performance evaluation and assessment of the bBMI paradigm, as common base for the following benchmark tests. Anesthetized rats will be used as subjects to run and validate the bBMI. Expected output: specifications of parameters defining the performances of the bBMI. Involved partners: IIT

Task 8.3 (M24 – M36) Realization and testing of a VLSI-based state-dependent bBMI

In this task we will realize a state-dependent bBMI introducing the neuromorphic VLSI chip developed in WP6. To realize such integration, we will develop a dedicated hardware and software framework to correctly interface the neuromorphic VLSI device in the closed-loop real-time configuration. We will design and run a benchmark test to evaluate and compare the performances of the integrated state-dependent neuromorphic closed-loop bBMI system with the non state-dependent implementation. Awake rats chronically implanted with microwire arrays will be also used as subjects to test the ability of the brain of modulating the neural activity to control the bBMI. Involved partners: IIT,UZH

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	32.00
4	UZH	3.00
Total		35.00

WT3: Work package description

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D8.1	Report on algorithms improving the performances of bBMIs	1	15.00	R	CO	18
D8.2	Report on the integration of a neuromorphic VLSI and a state-dependent bBMI	1	10.00	R	CO	36
D8.3	Report on the benchmark test of the VLSI-based state-dependent bBMI	1	10.00	R	CO	36
Total			35.00			

Description of deliverables

D8.1) Report on algorithms improving the performances of bBMIs: [month 18]

D8.2) Report on the integration of a neuromorphic VLSI and a state-dependent bBMI: [month 36]

D8.3) Report on the benchmark test of the VLSI-based state-dependent bBMI: [month 36]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS6	Realization and testing of non-state dependent bBMI	1	18	
MS9	Realization and testing of state dependent bBMI	1	36	

WT3: Work package description

Project Number ¹	284553	Project Acronym ²	SI-CODE
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One form per Work Package

Work package number ⁵³	WP9	Type of activity ⁵⁴	MGT
Work package title	Dissemination of results		
Start month	1		
End month	36		
Lead beneficiary number ⁵⁵	1		

Objectives

- Ensure proper dissemination of results to a large variety of potentially interested actors (media and general audiences, defined target groups and opinion leaders, scientific community, etc.)
- Guarantee the projects long-term impact through a planning for take-up activities
- Ensure that the results of the project are properly prepared for exploitation

Description of work and role of partners

Task 9.1 Dissemination plan (M1-M3)

The consortium shall survey existing conferences, workshop and forums in order to select those that will be more beneficial to the project. Once the survey is finished, a dissemination plan will be elaborated with the objective of optimising disseminating efforts. In this way, the consortium will try to synchronise new discoveries with the deadlines and schedules of selected dissemination events. Special attention will be provided to topics which could raise issues of dual use/misuse.

Task 9.2 Project website (M1-M3)

A project website will be immediately set up. On this website the public project results will be published, together with links to our open source neural data analysis software portals (see also task 4.4), where the new algorithms resulting from the project's activity will be included.

Task 9.3 Project image (M1-M3)

The Consortium plans to define a clear project image, with a pleasant look and feel and a strong graphical characterization. It will represent an important tool for strengthening the project's identity amongst participants, as well as towards external stakeholders.

Task 9.4 Clustering actions (M12-M36)

We will ensure potential exchange of information with other complementary EU or FP7 projects by deliberately adopting an 'open systems' approach to knowledge sharing, in which all new knowledge from the Project is freely available to all in order that it should have maximum impact in terms of re-use and exploitation by other research projects and initiatives. The Consortium will moreover develop and deliver training programmes, aimed at spreading knowledge and competence in the findings of the Project concerning the new experimental platform and the unified framework for in vitro and in vivo experiments, as well as the planned new artificial devices resulting from the project.

Task 9.5 Experimental dataset (M3-M36)

The main experimental datasets resulting from the project will be archived with the metadata format of the repositories CARMEN (www.carmen.org.uk) and STA toolkit (<http://neuroanalysis.org/>) to promote their exploitation and maximal impact in neuroscience.

Task 9.6 Data analysis software (M12-M36)

The IIT open source neural data analysis software portals (www.ibtb.org and <http://code.google.com/p/pyentropy/>) will be upgraded to include the new state-dependent information extraction algorithms developed in this proposal. In this way also the new set of algorithm resulting from the SI-CODE project will be given open access and could be exploited and reuse by other researchers.

WT3: Work package description

Task 9.7 Contribution to portfolio and concertation activities at FET-Open level in order to support scientific cooperation at the FET-Open level and broad public awareness of the project achievements.

Person-Months per Participant

Participant number ¹⁰	Participant short name ¹¹	Person-months per participant
1	IIT	11.00
2	SISSA	4.00
3	MPI-BC	2.00
4	UZH	2.00
Total		19.00

List of deliverables

Deliverable Number ⁶¹	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature ⁶²	Dissemination level ⁶³	Delivery date ⁶⁴
D9.1	Dissemination plan	1	1.00	R	PU	3
D9.2	Website and intranet	1	2.00	R	PU	3
D9.3	Dataset repository	3	8.00	R	PU	36
D9.4	Update of data analysis SW portals	1	8.00	R	PU	18
Total			19.00			

Description of deliverables

D9.1) Dissemination plan: [month 3]
 D9.2) Website and intranet: [month 3]
 D9.3) Dataset repository: [month 36]
 D9.4) Update of data analysis SW portals: [month 18]

Schedule of relevant Milestones

Milestone number ⁵⁹	Milestone name	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS10	Dataset and code repository	1	36	

WT4: List of Milestones

Project Number ¹	284553	Project Acronym ²	SI-CODE
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List and Schedule of Milestones

Milestone number ⁵⁹	Milestone name	WP number ⁵³	Lead beneficiary number	Delivery date from Annex I ⁶⁰	Comments
MS1	Experimental data-set on ongoing activity	WP2, WP3, WP4	3	18	
MS2	Neuromorphic circuit specification completed	WP6, WP7	4	18	
MS3	Multi-neuron chip for state-dependent parameters evaluation	WP6	1	36	
MS4	Release of software tools for extracting state-dependent stimulus information	WP5	1	18	
MS5	Review meeting #1	WP1	1	18	
MS6	Realization and testing of non-state dependent bBMI	WP8	1	18	
MS7	Multi-neuron chip for state-dependent parameters evaluation	WP5, WP6, WP7	4	36	
MS8	Understanding the interplay between ongoing activity, incoming stimuli and neural responses	WP2, WP3, WP4	1	36	
MS9	Realization and testing of state dependent bBMI	WP8	1	36	
MS10	Dataset and code repository	WP9	1	36	
MS11	Review meeting #2	WP1	1	36	
MS12	Project meeting with the Project Officer	WP1	1	9	

WT5: Tentative schedule of Project Reviews

Project Number ¹	284553	Project Acronym ²	SI-CODE
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Tentative schedule of Project Reviews

Review number ⁶⁵	Tentative timing	Planned venue of review	Comments, if any
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Project Effort by Beneficiary and Work Package

Project Number ¹	284553	Project Acronym ²	SI-CODE
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Indicative efforts (man-months) per Beneficiary per Work Package

Beneficiary number and short-name	WP 1	WP 2	WP 3	WP 4	WP 5	WP 6	WP 7	WP 8	WP 9	Total per Beneficiary
1 - IIT	4.00	34.00	3.00	4.00	33.00	40.00	15.00	32.00	11.00	176.00
2 - SISSA	0.00	5.00	46.00	0.00	5.00	0.00	0.00	0.00	4.00	60.00
3 - MPI-BC	0.00	0.00	0.00	37.00	6.00	0.00	0.00	0.00	2.00	45.00
4 - UZH	1.00	0.00	0.00	0.00	3.00	3.00	27.00	3.00	2.00	39.00
5 - CNRS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	5.00	39.00	49.00	41.00	47.00	43.00	42.00	35.00	19.00	320.00

Project Effort by Activity type per Beneficiary

Project Number ¹	284553	Project Acronym ²	SI-CODE
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Indicative efforts per Activity Type per Beneficiary

Activity type	Part. 1 IIT	Part. 2 SISSA	Part. 3 MPI-BC	Part. 4 UZH	Part. 5 CNRS	Total
1. RTD/Innovation activities						
WP 2	34.00	5.00	0.00	0.00	0.00	39.00
WP 3	3.00	46.00	0.00	0.00	0.00	49.00
WP 4	4.00	0.00	37.00	0.00	0.00	41.00
WP 5	33.00	5.00	6.00	3.00	0.00	47.00
WP 6	40.00	0.00	0.00	3.00	0.00	43.00
WP 7	15.00	0.00	0.00	27.00	0.00	42.00
WP 8	32.00	0.00	0.00	3.00	0.00	35.00
Total Research	161.00	56.00	43.00	36.00	0.00	296.00
2. Demonstration activities						
Total Demo	0.00	0.00	0.00	0.00	0.00	0.00
3. Consortium Management activities						
WP 1	4.00	0.00	0.00	1.00	0.00	5.00
WP 9	11.00	4.00	2.00	2.00	0.00	19.00
Total Management	15.00	4.00	2.00	3.00	0.00	24.00
4. Other activities						
Total other	0.00	0.00	0.00	0.00	0.00	0.00
Total	176.00	60.00	45.00	39.00	0.00	320.00

WT8: Project Effort and costs

Project Number ¹	284553	Project Acronym ²	SI-CODE
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Project efforts and costs

Beneficiary number	Beneficiary short name	Estimated eligible costs (whole duration of the project)						Requested EU contribution (€)
		Effort (PM)	Personnel costs (€)	Subcontracting (€)	Other Direct costs (€)	Indirect costs OR lump sum, flat-rate or scale-of-unit (€)	Total costs	
1	IIT	176.00	685,785.00	11,000.00	213,027.00	539,287.00	1,449,099.00	1,101,574.00
2	SISSA	60.00	250,400.00	5,000.00	98,800.00	209,520.00	563,720.00	424,040.00
3	MPI-BC	45.00	243,315.00	2,200.00	86,500.00	340,641.00	672,656.00	505,042.00
4	UZH	39.00	273,079.00	0.00	92,900.00	219,587.00	585,566.00	440,574.00
5	CNRS	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total		320.00	1,452,579.00	18,200.00	491,227.00	1,309,035.00	3,271,041.00	2,471,230.00

1. Project number

The project number has been assigned by the Commission as the unique identifier for your project. It cannot be changed. The project number **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

2. Project acronym

Use the project acronym as given in the submitted proposal. It cannot be changed unless agreed so during the negotiations. The same acronym **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

53. Work Package number

Work package number: WP1, WP2, WP3, ..., WPn

54. Type of activity

For all FP7 projects each work package must relate to one (and only one) of the following possible types of activity (only if applicable for the chosen funding scheme – must correspond to the GPF Form Ax.v):

- **RTD/INNO** = Research and technological development including scientific coordination - applicable for Collaborative Projects and Networks of Excellence
- **DEM** = Demonstration - applicable for collaborative projects and Research for the Benefit of Specific Groups
- **MGT** = Management of the consortium - applicable for all funding schemes
- **OTHER** = Other specific activities, applicable for all funding schemes
- **COORD** = Coordination activities – applicable only for CAs
- **SUPP** = Support activities – applicable only for SAs

55. Lead beneficiary number

Number of the beneficiary leading the work in this work package.

56. Person-months per work package

The total number of person-months allocated to each work package.

57. Start month

Relative start date for the work in the specific work packages, month 1 marking the start date of the project, and all other start dates being relative to this start date.

58. End month

Relative end date, month 1 marking the start date of the project, and all end dates being relative to this start date.

59. Milestone number

Milestone number: MS1, MS2, ..., MSn

60. Delivery date for Milestone

Month in which the milestone will be achieved. Month 1 marking the start date of the project, and all delivery dates being relative to this start date.

61. Deliverable number

Deliverable numbers in order of delivery dates: D1 – Dn

62. Nature

Please indicate the nature of the deliverable using one of the following codes

R = Report, **P** = Prototype, **D** = Demonstrator, **O** = Other

63. Dissemination level

Please indicate the dissemination level using one of the following codes:

- **PU** = Public
- **PP** = Restricted to other programme participants (including the Commission Services)
- **RE** = Restricted to a group specified by the consortium (including the Commission Services)
- **CO** = Confidential, only for members of the consortium (including the Commission Services)

- **Restreint UE** = Classified with the classification level "Restreint UE" according to Commission Decision 2001/844 and amendments
- **Confidentiel UE** = Classified with the mention of the classification level "Confidentiel UE" according to Commission Decision 2001/844 and amendments
- **Secret UE** = Classified with the mention of the classification level "Secret UE" according to Commission Decision 2001/844 and amendments

64. Delivery date for Deliverable

Month in which the deliverables will be available. Month 1 marking the start date of the project, and all delivery dates being relative to this start date

65. Review number

Review number: RV1, RV2, ..., RVn

66. Tentative timing of reviews

Month after which the review will take place. Month 1 marking the start date of the project, and all delivery dates being relative to this start date.

67. Person-months per Deliverable

The total number of person-month allocated to each deliverable.

PART B

COLLABORATIVE PROJECT

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B1. Concept and objectives, progress beyond state-of-the-art, S/T methodology and work plan

B1.1 Concept and project objectives

1.1.1 SI-CODE concepts

Brain Machine Interfaces (BMIs) are devices mediating communication between the brain and the external world, and hold the potential for reaching two closely related objectives: a) restoring motor or sensory functions to people who lost them due to illness or injury, and b) gaining a deeper understanding of neural information processing through the controlled interaction between neural populations and a virtually unlimited variety of external devices. Yet, there is a most severe obstacle to improve BMI performance and bring it to the level needed for a major impact on both healthcare and basic science: neural responses to incoming stimuli suffer from a very large variability originating mostly from the dependence of neural responses upon the internal state of the network. We propose a radically new strategy to remove this obstacle, consisting in three closely related steps: i) characterizing comprehensively the origin and the rules of state dependency of neural responses; ii) understanding how to use these rules to discount in real time the neural variability introduced by state changes; and finally iii) developing a new concept of bi-directional Brain-Machine Interfaces which exploits our characterization of state dependency of neural responses to increase the bandwidth of communication with the brain.

Our ideas stem from the following three Concepts.

Concept 1: Responses of real neurons are state dependent

Our understanding of brain processes such as sensory or motor function is mostly based on mapping changes in external correlates to changes in neuronal activity. Yet, this view suffers from a fundamental limitation. In stark contrast with most artificial devices designed for sensing or control, neural responses to stimuli or external correlates are highly variable: repeated presentation of the same external correlates elicit each time a very different neural response. It is believed that the main reason of this variability is that neural responses, and brain processes in general, are “state dependent”: neural responses do not depend only on the current stimulus or external correlate relevant to the area under consideration, but also on a number of variables expressing the internal ongoing dynamics and the internal state of the neural microcircuit network (Buonomano and Maass, 2009). This picture applies to essentially all levels of organization of nervous systems, from the neural cultures employed in in-vitro studies to invertebrates to both anaesthetized and awake behaving mammals.

Recent years have witnessed a significant effort to characterize the statistical properties of internally generated changes in network states and the mechanisms behind their generation. These studies have concentrated on studying the dynamics of the spontaneous neural responses in absence of stimulation. Overwhelming evidence indicates that almost all biological brains – and the great majority of networks and assemblies of biological neurons – are characterized by a large spontaneous ongoing electrical activity: neurons fire action potentials (“spikes”), synapses release transmitters in the absence of any sensory input or stimulation. This spontaneous electrical activity is often correlated over large spatial scales, often larger than the spatial correlations of stimulus evoked activity (Arieli et al., 1996). In

cortex of higher mammals, ongoing activity is characterized by transitions between different states of network excitability or of synchronization between neurons (Schroeder and Lakatos, 2009; Steriade et al., 1993). Transitions between these states likely occur because of fluctuations in internal network variables, such as the balance between excitation and inhibition, which can in principle profoundly control the cell's integrative and electrophysiological properties, the amplitude and time course of synaptic inputs and the timing and probability of spiking (Logothetis, 2008). Changes in the internal "state" variables are to some extent measurable from integrative massed measures of extracellularly recorded neural activity: for example the dynamics of the functional balance between excitation and inhibition (determining the circuit's excitability) is reflected e.g. in the phase of low-frequency Local Field Potentials (LFPs)), whereas the level of network synchronization is detectable e.g. by examining the relative power of high vs. low-frequency LFPs.

While the mechanisms generating these ongoing state changes are beginning to be unravelled, their impact on network information processing is still poorly understood, largely because the interaction between ongoing activity and the response to an applied stimulus has been incompletely characterized so far. A first goal of SI-CODE is to fill this gap in knowledge by characterizing comprehensively:

- the rules of interaction between spontaneous activity, network state and incoming stimulus;
- how these interactions determine the local and global response in the brain.

We will use experimental models over a wide range of complexity, from simple nervous systems of invertebrates (leech) to the cortex of mammals (rats and macaque monkeys), in vivo and in vitro. We will gather empirical evidence about state dependence of neural responses by applying electrical and/or optogenetic stimulation to nervous systems while recording, prior and during stimulation, different measures of neural activity (such as spiking activity or LFPs) from which we will estimate both neural responses to stimuli and neural state variables. Neural activity will be measured over a wide range of spatiotemporal scales, from advanced high-density CMOS-MEA (Multi Electrode Arrays) to multielectrode systems for stimulation (both electrical and optogenetic) and recording, to fMRI measures to study the effect of electrical stimulation of a single site over several areas (see MS1, to be achieved by month 18). Recording activity over a wide range of spatial scales is of paramount relevance to the goal of understanding the principles of interactions between networks states and incoming stimuli. Indeed, bursts of electrical activity in hippocampal slices originate from hub neurons and propagate with a specific spatio-temporal pattern (Bonifazi et al., 2009) and similarly, in the rat somatosensory cortex, sensory inputs and the intrinsic dynamics propagate with different spatial patterns (Sakata and Harris, 2009). Therefore, an important part of our project will consist in applying stimulations to nervous systems at a very fine spatial scale and in measuring in details the electrical activity prior and post stimulation. To do this, it is necessary to record the electrical activity of a very large number of neurons. This will be accomplished in-vitro by developing and using advanced high-density CMOS-MEA (i.e., APS-MEA) coupled with precise optogenetic stimulation (WP2); by using in-vivo high density multielectrode arrays for the electrical stimulation and recording from connected neural populations; and by using systems for measuring (by BOLD fMRI) the effect of electrical stimulation of a single site in the whole brain (WP4). Moreover, we will consider

both electrical and optogenetic stimulation of the nervous tissue to understand if they interact in a different or similar way with ongoing activity.

Concept 2: The neural response variability due to its state-dependency can be discounted once we understand it

An important implication of state dependency of neural responses is that it makes them highly variable. From the point of view of a single neuron, the dependence of its response on the network state looks like “noise” or variability, limiting its reliability. Therefore, many authors have described the effect of ongoing activity as a nuisance severely limiting their ability to carry information. Understanding how brains can reach such high performance in the face of this variability is one of the most crucial, and yet unaddressed, challenges in neuroscience. The classic approach of neurophysiology has been to simply remove it by averaging neural responses over several stimulus repetitions (“trials”). However, this strategy is clearly not the one used by our brains, which perceive complex stimuli or perform complex actions on a single trial basis, without resorting to averaging over many trials. However, the analysis of experimental data shows that the noise introduced by the intrinsic dynamics is very - possibly too - large at the level of a single neuron. Yet, this ongoing dynamics does not appear to be an insurmountable obstacle to reach high performances. Our brains are able to perceive complex stimuli on a single trial basis even in the presence of environmental distracters. Because of this, scientists are now beginning to reassess the role of ongoing activity and to regard it as a crucial (if yet not understood) aspect of neural computation rather than an obstacle to it. Here we hypothesize that the nervous system copes with the high trial-to-trial variability due to state dependency because other neurons can use knowledge of their network’s state to predict this variability and then “discount” it. Thus, decoding a neural response must consider the brain state (“context”) at which it was generated. We believe this will be possible, based on both the evidence that simple dynamical model can predict trial-to-trial response variability on the basis of network states (Curto et al., 2009) and because the intrinsic dynamics has spatial and temporal correlations different from those of responses evoked by sensory inputs or appropriate stimulations. The differences in scale of the intrinsic and the stimulus induced component are therefore a further tool to separate out the two components and discount the variability induced by the spontaneous intrinsic dynamics. Building on these findings, we will develop advanced algorithms (MS4, to be achieved by month 18) and mathematical models of network dynamics (MS3, to be achieved by month 36) which will determine the network state variables best predicting neural variability. These mathematical tools will enable us to characterize quantitatively the details of state dependence of neural responses to stimuli, and this in turn will enable us to construct explicit state dependent decoding rules which discount this variability and thus extract more information from neural activity (MS8, to be reached by month 36).

The experimental data:

- will be used to determine which network variables (e.g. LFPs, firing rate envelopes, etc) best predict the trial-to-trial variability;
- will provide the basis to develop mathematical models of the dynamical interaction between network variables and the stimuli;
- will be used to construct explicit algorithms for a novel and effective state-dependent decoding of neural responses.

To ensure that these data and algorithms make the largest possible impact on neuroscience, we will release in the public domain both the key experimental datasets and the algorithms (MS 10, to be achieved by month 36).

Concept 3: Knowing state-dependency of responses enlarges the bandwidth for two-way communication with brains

The characterization of the origin of neural response variability from state changes has fundamental implications for improving BMIs, as explained next. The first realizations of BMIs simply decoded the recorded neural activity (e.g. from motor cortex) and translated it into commands for controlling an external device (e.g. a robotic arm). These decoding-based BMIs have the limitation that they require users to keep a constant focus of attention on the execution of a detailed motor command, and that they do not directly sense non-visual information relevant to the task (i.e. non-kinematic information, such as weight or rigidity of a manipulated object). These problems are propelling research towards bidirectional BMIs (bBMIs), which, in addition to decoding neural activity and translating it into a desired action, also provide the brain with external information relevant to the progress of the action in the form of electrical stimulation of the neural tissue. The problem of trial-to-trial variability of neural responses resulting from their state-dependent nature is the most serious obstacle in communicating effectively with the nervous systems through both types of BMIs. The ability to measure and discount variability due to state changes holds the promise to increase the bandwidth for communication with the brain both in the decoding direction (since variability limits the amount of information available to control the device) and in the information-injecting direction (since it limits the reliability by which a desired neural activity can be evoked by a given stimulation). In order to explore the feasibility of using state dependence to improve the bandwidth for communication in bBMIs, it is important not only to understand the principles by which network state variables affect neural responses, but also to be able (in real time) to continuously measure these state variables and compute how to use them to discount variability. This can only be done by developing hardware which can do these operations continuously and in real time on the recorded neural data. In the long term, to be clinically relevant, this hardware will have to be chronically implantable. Full custom Very Large Scale Integration (VLSI) technology is essential for implementing efficient BMIs that have the characteristics mentioned above (Lebedev and Nicolelis, 2006). The size and power-budgets imposed by these types of applications leave no alternative, and require the design of state-of-the-art analog and mixed signal analog/digital VLSI circuits. This is something that has been widely recognized by both scientific and commercial communities, and as a result, there are now substantial resources being allocated to the design and development of such devices. As this is an emerging technology, the main focus is currently on signal acquisition, signal conditioning and (wireless) signal transmission. Example of state-of-the-art research includes the work done by a wide range of US groups, including our colleagues R. Harrison and R. Sarpeshkar (Harrison, 2008; Sarpeshkar et al., 2008), as well as a set of EU groups (IMEC, etc.). Neurophysiologists are still investigating the properties of neural recordings and are still carrying out basic research on the processing methods that are best suited for extracting information from the neural recordings. As the algorithms are often computationally intensive, all explorations are currently being done off-line. So the state-of-the-art in BMI design is currently focusing all efforts on faithfully reproducing neural signals

and reliably transmitting them for further off-line processing. Future generations of BMI devices however will have to include neural signal processing directly at the device level, especially if the BMI device will be used for both sensing and stimulating neural tissue. In this project we focus on these additional and complementary circuit and signal processing strategies that will be needed in future generations of BMI technologies. Specifically, we explore on-chip neural processing strategies and circuits based on implementations of biophysically realistic networks of spiking neurons. We investigate the use of neuromorphic circuits and systems to exploit bio-inspired design for building compact, reliable and low-power devices (Sarpeshkar06), using technologies that are fully compatible with the BMI devices being currently built by our colleagues (Harrison et al., 2007).

SI-CODE's technological benchmark will be the realization, in anaesthetized rats, of a new prototype of "state dependent bBMIs" which guides efficiently movement of an artificial system (see MS9, to be achieved by month 36). The BMI will use a Force Field as a control policy for a simplified "reaching" movement by exploiting the knowledge of the state of the brain networks communicating with the BMI. This BMI prototype will detect network state variables in real time using low-power neuromorphic Very Large Scale Integration (VLSI) circuits (to be first designed by month 18 -see MS2- and to be completed by month 36 - see MS7), and use state information to both decode better neural activity and to evoke more reliably the intended responses to electrical stimuli, thereby injecting more accurate information into the brain.

1.1.2 Relation to the call FET Open, Objective ICT-2011.9.1

SI-CODE proposes to lay the scientific foundations necessary for a new generation of BMIs with a radically improved bandwidth of communication with the brain, therefore paving the way for a very significant breakthrough in the use of information technologies in healthcare. SI-CODE first identifies the main problem for successful BMI development to be in the variability of neural responses, which is in turn largely caused by the state dependence of neural responses. SI-CODE proposes a radically new concept to uproot this problem: if the state dependence of neural responses is understood, then the variability induced by this dependence can be tamed and discounted.

The foundational nature of the idea proposed here demands that SI-CODE puts a very significant effort in laying the scientific foundations for the new paradigm proposed, which is based on understanding the state dependence of neural responses. This work will provide a completely novel set of principles and explicit algorithm which decode neural activity by using state information to discount state-induced variability. These algorithms and knowledge will elucidate the role of ongoing activity on neural information coding, thereby leading to a paradigm shift in studies of neural coding (which previously considered state dependence mostly as noise or nuisance rather than an integral part of the neural messages) and will have profound implications on the way neuroscientists will measure and interpret their data and will think of brain function.

We recognize that to make the fundamental progress in understanding state dependence of neural responses, we must rely on deep synergies between different scientific disciplines and on bringing together the information collected at different levels of investigation. For this reason, SI-CODE founds its planned breakthroughs on bringing together: experimental

neuroscience at very different levels and scales of organization of the nervous system; new advanced technology for highly parallel and simultaneous multiple scale recordings of neural activity; formal approaches to neural network dynamics developed in theoretical physics; information theory concepts and electronic circuit designs developed in advanced theoretical engineering.

To ensure that this research will have the largest impact and will contribute to shift current thinking, we will archive in public repositories the main experimental datasets and all the new state-dependent information extraction algorithms developed in this proposal.

SI-CODE introduces into the project an important collaboration with a leading US scientist (Prof. F.A. Mussa-Ivaldi, Northwestern University), who is a pioneer of the development of bidirectional BMIs and will participate in SI-CODE as visiting Professor to partner 1 (IIT). This collaboration introduces skills which are currently not present at the EU level, thereby strengthening Europe's scientific and technology base, contributing to its global leadership in ICT, as well as contributing to raise global interest in the foundational component of our scientific and technological progress.

1.1.3 Targeted breakthrough and its relevance towards a long-term vision

In ageing societies, nervous system diseases are having increasingly high social costs. To cure these diseases, it is imperative to improve our understanding of brain function. In this endeavor, Information Technology can play an important role by providing BMIs which improve the quality of life and the autonomy of patients who lost motor or sensory function due e.g. to multiple sclerosis, amyotrophic lateral sclerosis, spinal cord injuries, stroke. In particular, the development of efficient decoding interfaces is crucial to interpret and execute actions expressed by activity of motor or pre-motor areas, whereas artificial sensory channels are crucial both to restore sensory function and to provide a real-time feedback which can replace the tactile or proprioceptive signals necessary for motor tasks. The greater goal of restoring motor functions after paralysis calls for the establishment of a genuine two-way communication between brain and devices which implements both of these components that are necessary for essentially all tasks in everyday life.

B1.2 Progress beyond the state of the art

1.2.1 State-of-the-art

a. State-of-the-art in neuro-electronic interfaces - Baseline

In the early 80's, advancements in micro-fabrication technologies enabled the introduction of Micro-Electrode Arrays (MEAs), which first allowed in-vivo and in-vitro (Gross et al., 1982) multi-site, long-term recordings of the electrical activity of neuronal populations and extracellular stimulation from one or more electrodes, thus enabling experimental investigations of their collective dynamics and computational properties. Now commercially available (e.g. Multi Channel Systems, Reutlingen, Germany; Panasonic, Osaka, Japan; Ayanda Biosystems, Lausanne, Switzerland), MEAs are the subject of continuous microtechnology developments (Pearce and Williams, 2007). Recently, fully-integrated monolithic Complementary Metal-Oxide Semiconductor MEAs (CMOS-MEA) with on-chip amplification, multiplexing and analogue-to-digital conversion (Eversmann et al., 2003; Frey et al., 2009; Gandolfo et al., 2011; Imfeld et al., 2008) were validated for in-vitro applications

on cultured neuronal networks and ex-vivo brain tissue. In particular, a new MEA platform was presented (Berdondini et al., 2009) based on the Active Pixel Sensor concept (APS) implemented in CMOS and aimed at overcoming current spatial-temporal resolution limitations by managing acquisitions from high density arrays of 4096 microelectrodes at a full frame rate of 7.8 kHz/channel and with a 21 μm electrode separation. This platform enables to bring MEA technology in the imaging field for the localization and tracking of electrophysiological signals (Gandolfo et al., 2011), it was shown to improve the significance of statistical network activity parameters (Maccione et al., 2010) and, with adequate surface chemistry and cell culture protocols, it can record at single cell resolution from large networks of thousands of neurons.

State-of-the-art in neuro-electronic interfaces – Progress to be made

The performance and success of our research will be demonstrated by: development of a novel high-density Micro-Electrode Array with 4096 electrodes featuring high-resolution spatio-temporal recording combined with optical, electrical and pharmacological stimulation; and validation of such a system by using it for the recording of responses of in vitro neural networks.

b. State-of-the-art on intrinsic dynamics of neuronal assemblies and on the state dependency of neural responses- baseline

Neuronal networks often display irregular spontaneous bursting activity: these bursts can occur in simple nervous system as in the leech and in cultures of hippocampal (Bonifazi et al., 2005) and cortical (Chiappalone et al., 2007) neurons with remarkably similar properties. From a computational point of view, arrhythmic spontaneous bursts represent the noise of the system under investigation and it is important to determine their statistical properties. Evoked activity in stimuli driven experiments is always superimposed to this ongoing background activity, which contributes to the trial-to-trial (Arieli et al., 1996; Azouz and Gray, 1999). Importantly, the spontaneous background activity displays spatial and temporal correlation (Chiu and Weliky, 2001; Fiser et al., 2004; Segev et al., 2002). Recently, theoretical (Abbott and Rohrkemper, 2007; De Arcangelis et al., 2006) and in vitro experimental (Beggs and Plenz, 2003, 2004; Pasquale et al., 2008) studies suggested that the brain might operate as a self-organized critical system. Recently, Plenz and colleagues detected neuronal avalanches in vivo, confirming the space-time scale-invariant organization of these neural patterns (Gireesh and Plenz, 2008; Petermann et al., 2009; Plenz and Thiagarajan, 2007) as for in vitro preparations. A recent work demonstrated that the presence of neuronal avalanches maximize information capacity and transmission (Shew et al., 2011).

A few studies have begun to suggest that the structure of ongoing activity prior to stimulation is one of the main determinants of the large trial-to-trial variability of responses observed across repeated presentation of the same stimulus. Arieli and colleagues (Arieli et al., 1996) using optical imaging of Voltage Sensitive Dyes dynamics found that the averaged change in depolarization level of a visual cortical patch could be predicted as a linear function of the stimulus and the depolarization level preceding the stimulus. Similar results were obtained when considering the membrane potential value at the time of the presentation of a visual stimulus and the spiking response to the stimulus (Azouz and Gray, 1999). Recently, Curto and colleagues (Curto et al., 2009) extended these results showing that the massed/averaged spiking response of an auditory cortical patch can be modelled by a simple dynamical system.

This system predicts correctly that the interaction between input and cortical responses switches from a linear to a non-linear regime depending whether cortex is in a desynchronized or in a synchronized state, which could be determined by the power spectrum of recorded Local Field Potential (LFP).

State-of-the-art on intrinsic dynamics of neuronal assemblies and on the state dependency of neural responses- progress to be made

The above results suggest that knowledge of ongoing activity prior to the stimulus can be used to discount this variability and thus greatly increase the information that we can obtain from the neural response. However, this has not been demonstrated or exploited yet, and the investigation of this issue is the purpose of SI-CODE. In order to achieve this goal, SI-CODE will: collect a number of experimental datasets suited to document the state dependence of neural responses; it will use the experimental observations to develop quantitative relationships (i.e. equations) relating network state, incoming stimulus, and neural response; and it will use these equations to discount a significant fraction of trial to trial variability.

SI-CODE will provide the most comprehensive knowledge of how network state variables influence neural responses to stimuli, going beyond current knowledge in many ways: i) it will show how ongoing activity interacts with stimulation (most previous studies considered ongoing spontaneous dynamics without stimulation); ii) it will study multiple levels of organization, increasing the chance of discovering “general” rules; iii) the unprecedentedly high scale and resolution of recording/stimulation in vitro will extend the observation to the cellular level and enable the exploration of the scale requirements needed to define effective neural state variables; iv) it will provide information about the effect of network state not only on local response but also on transmission across areas. The latter is crucial for developing bBMIs, which require decoding information from motor areas and injecting information into sensory areas respectively.

c. State-of-the-art in neural coding - baseline

The search for the neural code began many decades ago, with the discovery that the number of spikes elicited by peripheral neurons depends on simple stimulus features (Adrian, 1928). It continues today with the exploration of different putative codes, and with systematic comparisons between the information in individual codes and behavioural performance (Panzeri et al., 2010). An important of neural codes is defined by time: neuronal responses evolve over time, and the temporal structure of neural activity can only be neglected at the cost of losing considerable information (Panzeri et al., 2010). Over the past few years, it has become clear that the neural code is also distributed along time: sensory information is multiplexed in neural responses at least in two timescales, a slow one related to slow network fluctuations, and a fast one related to precise spike times (Kayser et al., 2009; Lisman, 2005). Multiplexing information over different time scales increases the encoding capacity of neural responses, enables disambiguation of stimuli that cannot be discriminated at a single response timescale, and makes sensory representations stable to the presence of variability in the sensory world. Thus, temporal multiplexing could be a key strategy used by the brain to form an information-rich and stable representation of the environment. One key property of the low frequency (< 10 Hz) components of the temporal code (Belitski et al., 2008; Kayser et al., 2009; Lisman, 2005; Montemurro et al., 2008) is that they are expressed by parameters (such as the phase of slow LFPs) which were traditionally associated with the state of ongoing

network state rather than with responses to stimuli (Saleem et al 2010). The above described multiplexing based on such parameters of neural activity raises the possibility that the nervous system encodes state dependent information, and that the observation of neural activity at different temporal scales may be key to the separation of the state and the stimulus-driven component of neural activity. This coding hypothesis has not been tested systematically yet, and this testing is one of the goals of SI-CODE.

State-of-the-art in neural coding – progress to be made

The consortium will individuate the state dependent and the stimulus driven components and neural activity, document their interplay, and find explicit algorithms that allow the extraction of higher amounts of stimulus information from neural activity when taking into account their state dependence. SI-CODE will achieve this by:

- *Developing a novel set of algorithms for “state-dependent” decoding of neural responses to stimuli.* These algorithms will provide quantitative answers about which network state parameters and neural codes carry most information, and will explain how to discount the response variability due to network state changes;
- *Determining the “neural state variables”, i.e. the neural responses features that best measure the network state and best predict state-dependent neural responses to stimuli.* This will be achieved by applying systematically the above algorithms to all datasets to derive a set of measurable network state parameters which consistently increase the information extracted with state dependent decoding. These will provide empirical bases for the modelling, VLSI, and BMI work described below.

d. State-of-the-art in neuronal modeling of ongoing activity - baseline

Starting from the mid-1990s, several theoretical studies (Amit and Brunel, 1997; Brunel, 2000; Tsodyks and Sejnowski, 1995; van Vreeswijk and Sompolinsky, 1996, 1998) investigated the conditions for observing low-rate ongoing activity in randomly connected networks of excitatory and inhibitory neurons. These studies reproduce some of the major features of background activity as observed in cortex: highly irregular firing (approximately Poisson); and wide distribution of average firing rates. In a systematic study of the parameter space in such networks, (Brunel, 2000) found that different network states (an asynchronous irregular state; and two types of synchronous states, characterized by slow and fast oscillations, respectively) could be obtained depending on the level of external inputs, and the balance between excitation and inhibition. Later studies by Brunel and collaborators have focused on the fast oscillatory state. Brunel and Wang (Brunel and Wang, 2003) have shown how the frequency of the global network oscillation depends on the properties (in particular time constants) of excitatory and inhibitory synapses. Finally, Mazzoni et al (Mazzoni et al., 2008) were able to extend these models to go beyond spike train dynamics and include a tractable but realistic quantification of the LFP, based on a weighted sum of the averaged excitatory and synaptic inputs on pyramidal cells of the network. They were able to reproduce well the LFP dynamics observed experimentally in anesthetized monkeys (Belitski et al., 2008). Another line of work considered conductance-based synapses rather than current-based synapses. It was found in particular that the asynchronous irregular state can be observed in such networks even in absence of external inputs, provided synapses are conductance-based (Kumar et al., 2008; Vogels et al., 2005). In all these models, the network connectivity was

not structured, and background activity was represented by a single network state. Other investigators have built more structured models of primary visual cortex that have a multiplicity of attractors (so-called 'orientation states', see e.g. (Blumenfeld et al., 2006)). In the presence of external noise, such networks can potentially exhibit a much more complex ongoing activity, which corresponds to wandering among multiple attractor states (see e.g. (Goldberg et al., 2004)).

State-of-the-art in neuronal modeling of ongoing activity – progress to be made

The main progress that SI-CODE will make in neural modeling will be to obtain models of the ongoing activity and to evaluate its influence on coding and decoding from a theoretical perspective. In particular, we will develop mathematical models of neural networks that will reveal the mechanisms of the dynamical interaction between neural network state variables and the stimuli. These models will reveal the principles of state-dependent neural communication and will lead to formulating new hypotheses for more effective state-dependent decoding of neural responses and to experimental refinements.

e. State-of-the-art on neuromorphic devices - baseline

Neuromorphic circuits are a class of hybrid analog/digital circuits that implement hardware models of biological systems. It has been argued that these types of circuits can be used to develop a new generation of computing technologies based on the organizing principles of the biological nervous system (Boahen, 2005; Sarpeshkar, 2006). The styles of computation used in neuromorphic circuits are fundamentally different from those used by conventional computers. As the biological systems they model, they process information using energy-efficient asynchronous, event-driven, methods and most importantly are adaptive and fault-tolerant. These characteristics offer an attractive alternative to conventional computing strategies, especially if one considers the advantages and potential problems of future advanced VLSI fabrication processes. By using massively parallel arrays of computing elements, exploiting redundancy to achieve fault tolerance, and emulating the neural style of computation, neuromorphic VLSI architectures can exploit to the fullest potential the features of advanced scaled VLSI processes and future emerging technologies, naturally coping with the problems that characterize them, such as device inhomogeneities, and imperfections (Indiveri, 2007). A library of neural primitives such as leaky Integrate and Fire neurons (Indiveri, 2007), dynamic synapses with learning and adaptation (Bartolozzi and Indiveri, 2007) is now available and constantly updated to include additional features that more closely resemble the properties of neural computation on VLSI systems. These primitives will be used to implement systems which have to interact with the real nervous system in real-time. Therefore they have biologically plausible time constants (i.e., of the order of milliseconds), so that they are inherently synchronized with the real world events. In recent years reconfigurable networks of spiking neurons with dynamic synapses, showing types of competitive-cooperative style of computation, have been developed (Chicca et al., 2006; Choi, 2005; Mallik et al., 2005; Serrano-Gotarredona et al., 2005). Typically they have been used in multi-chip configuration for processing data from neuromorphic sensors and for building reconfigurable networks of neurons populations, thanks to the AER spike-based asynchronous communication protocol. This approach gives the opportunity to explore diverse connectivity profiles and computing architectures. Nevertheless, once the needed network architecture has been established, it can be integrated on single chips where it

directly receives inputs from low-power VLSI neuro-engineering circuits that are currently being developed for signal conditioning and acquisition from implanted electrodes.

State-of-the-art on neuromorphic devices – progress to be made

SI-CODE will develop a new class of “state dependent” low power neuromorphic VLSI circuits that extract in real time network state information and use it to produce outputs optimally suited for both decoding of recorded signals and for delivering electrical stimulation to the brain. These devices will greatly increase the bandwidth for two-way communication with the brain, paving the way for future chronic BMI implants. This will be done partly in collaboration with a US scientist (F.A. Mussa-Ivaldi) who is a worldwide leader in bBMIs, thereby contributing to implementing highly strategic worldwide alliances in this rapidly growing field.

f. State-of-the-art on Brain Machine Interfaces (BMIs)-baseline

So far, the development of BMIs has proceeded along two separated tracks. There are sensory interfaces, such as the cochlear implants (Loeb, 1990) that transform external physical events into neural stimuli for the brain. And there are motor interfaces that decode activities from cortical regions to generate commands for external devices (Velliste et al., 2008) such as a robotic arm. A limitation of current BMI approaches based on decoding neural activity is that they require users to keep a constant focus of attention on the execution of detailed motor commands. In these set-ups, feedback is limited to vision, which involves long delays and requires gaze to be constantly on the moving device. Furthermore, non-kinematic information, such as the weight, rigidity and temperature of a manipulated object, are not directly sensed.

State-of-the-art on Brain Machine Interfaces (BMIs)-progress to be made

What is now needed in order to overcome these limitations is an interface capable to establish a two-way communication between the brain and the external world (Mussa-Ivaldi et al., 2010). Laying down the foundations to achieve this goal is one of the aims of SI-CODE. It will construct the proof of concept for a new class of bidirectional state-dependent BMIs which employ neural computational strategies and neuromorphic VLSI devices able to remove in real-time the main current limit to BMI performance: neural response variability.

B1.3 S/T Methodology and associated work plan

In addition to the standard Work Packages on Management (WP1) and Dissemination (WP9), the project contains experimental Neurophysiological Work Packages (WPs 2-4) and theoretical/ analytical/technological Work Packages (WPs 5-8). Theoretical models (WP6) will work together with progress in development of analysis algorithms (WP5) of experimental findings to refine the understanding of state-dependent neural coding. This will in turn result to the development of VLSI circuits (WP7) which can detect network state online and lead to our final benchmark, the realization of state-dependent bBMI emulating the function of the spinal cord (WP8).

All procedures for animal experiments (WP2-4 and WP8) will be carried out to the highest ethical standards (see Section B5 for full details on Ethical Issues). The basic design for all experiments is the same: to measure stimulus-response relationships and to characterize how they depend on network state variable. A given number of different spatiotemporal stimulation patterns shall be presented to the network and the neural activity (spikes or LFPs) will be recorded prior and during stimulation. Each of the employed stimulation patterns will

be repeated several times (≥ 50 trials per stimulation pattern) so that we can compute probabilities of response given the network “state”. We will then analyse them first with standard techniques to characterize the statistics of variables describing ongoing activity (cross-correlations, spatial coherence, spectral analysis, avalanche and bursting analysis) in order to obtain empirical characterizations of relationship between neural response, stimulus and state variables. This will provide a basis for further analyses on the same data using the novel algorithms developed in SI-CODE (WP5).

1.3.1 WP2: Interplay of ongoing activity and responses to stimuli in mammalian neural assemblies in vitro

Cortical networks in vitro usually generate self-sustained synchronized bursts of activity, alternated with periods of low and irregular firing. This phenomenon is well-known, and it has been extensively described in the literature (Corner et al., 2002; Marom and Shahaf, 2002), but still the mechanisms which give rise to this spontaneous massive activation remain unclear. The oscillatory nature of such intrinsic dynamics suggests a strong resemblance with slow-wave oscillations occurring during non-rapid-eye-movement sleep and at the earlier stages of embryonic CNS development (Corner, 2008).

Investigation of the mechanisms at the basis of the emergence of ongoing activity

From an experimental viewpoint, in-vitro preparations coupled with the recently validated high-density microelectrode array devices (4096 electrodes, 8 kHz full-array sampling rate) offer a unique possibility to investigate in large neuronal networks and under long-term experimentation the mechanisms at the basis of the emergence of ongoing activity. With this platform we will characterize at high spatiotemporal resolution ongoing activity states and response modulations expressed by networks of cortical neurons. Indeed, we will exploit the peculiar features provided by the high-electrode density of 580 electrodes/mm² for the detailed characterization of the network dynamics for the precise localization of activity propagation initiation sites and for the classification of spatiotemporal activity patterns at multiple scales (e.g., center of activity trajectories, neuronal avalanches). To investigate the role of the network functional organization at the cellular level, we will also study networks of different size and topology by varying cellular densities and by constraining network architectures with bio-chemical and physical cues. Finally, to sample responses by modulating a wide range of network states, we will perform a pharmacological dissection of the different synaptic currents present in the neuronal networks under investigation and possibly responsible for the generation of spontaneous oscillations. As an example, previous studies were aimed at exploring the effects of blocking the excitatory/inhibitory synaptic transmission, mediated by either NMDA or GABA_A receptors, by APV or picrotoxin/bicuculline (BIC), respectively. These experiments were carried out in both networks of leech neurons and networks of dissociated rat hippocampal neurons plated onto standard MEAs (Mazzoni et al., 2007). Moreover, the same antagonist of inhibitory synapses, BIC, and acetylcholine (ACh), an agonist of the cholinergic pathways, were delivered to rat cortical neurons, probing induced changes in the bursting dynamics and on the degree of synchronization level (Chiappalone et al., 2007)).

Therefore, we will investigate the effects of different compounds known to block synaptic transmission and to affect the ongoing activity. In particular, we plan to use:

- *standard agonists/antagonists of the excitatory NMDA/non-NMDA synaptic receptors and of the inhibitory GABA_A/GABA_B pathways;*
- *standard agonists of the cholinergic pathways, such as ACh or carbachol.*

Recent studies have shown that the stimulation of cortical neurons *in vitro* with carbachol mainly induces a loss of oscillatory synchronization, leading up the network to a desynchronized state more similar to the *in vivo* activity of awake animals (Tateno et al., 2005). We believe that the implications of such desynchronization for coding would be of great interest.

Investigating the input-output relationships in presence of ongoing activity

To study the input-output functions and interplay of these relationships with the on-going activity, after a preliminary characterization of network states and spontaneous activity we will employ cortical or hippocampal cultures (from rat embryos) plated on standard (i.e. commercially available) MEAs (e.g. Multichannel Systems, Reutlingen, Germany). This will allow us to exploit the capability of standard MEAs to both record and electrically stimulate from each electrode. Then, cortical cultures will be plated also on high-density CMOS MEAs (HD-MEAs – cf. Fig. 1) and the stimulation patterns will be provided by optical stimulation, either coupled with standard extracellular stimulation from one or more locations (i.e. by means of tungsten electrodes) or alone.

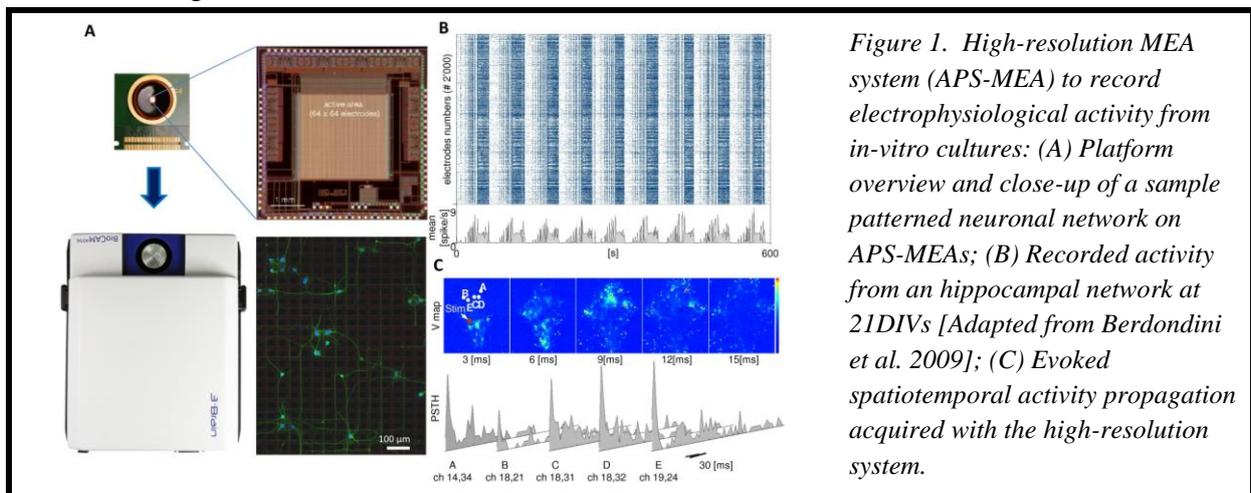


Figure 1. High-resolution MEA system (APS-MEA) to record electrophysiological activity from in-vitro cultures: (A) Platform overview and close-up of a sample patterned neuronal network on APS-MEAs; (B) Recorded activity from an hippocampal network at 21DIVs [Adapted from Berdondini et al. 2009]; (C) Evoked spatiotemporal activity propagation acquired with the high-resolution system.

With optical stimulation we will investigate cellular-/population-type specific modulation effects on the overall network activity delivering input stimuli from one or more locations. To do so, the high-resolution MEA platform will be integrated with an electrical and optical stimulation system projecting photo-stimulation patterns with Digital Micromirror Devices (DMDs) up to 2000fps and shaping laser beams down to diffraction limits. To selectively excite/inhibit cellular neuronal targets, adapted optogenetic probes and laser wavelengths will be used (e.g. ChannelRhodopsins2 and halorhodopsin). In order to explore whether state-dependent activity encodes also the time between stimuli, we will design a class of experiments with stimuli having one or two different stereotyped spatial structures, but with different inter-stimulation timing or time varying inputs with relatively short inter-stimulation-pattern times to get some effect of the previous pattern on the current stimulus.

The possibility of constraining the network architecture with chemical cues will be exploited by using micro-contact printing techniques demonstrated to preserve network confinement for long-term experimentation. Specific patterns will be defined by stamping appropriate adhesion molecules to obtain interconnected populations or to identify a specific neuronal population to which stimulation is delivered. We will develop specific experimental protocols in which the stimulation patterns can be of electrical (standard MEA, APS-MEA) or optical (APS-MEA) nature. The stimulation patterns will be defined on the basis of their spatial-temporal features starting from simple artificial stimuli to more complex time-varying spatial-temporal patterns mimicking natural sensory inputs. We will first investigate basic parameters for both electrical and optical stimulation such as:

- *amplitude, frequency or duration of the stimulus;*
- *position in the array: single site/multiple site (i.e. focal vs. distributed stimulation).*

We will apply simple patterns of stimuli, with the following features:

- *temporally uncorrelated stimuli from spatially close/far away sites;*
- *temporally correlated (time varying inputs) stimuli from single site or from spatially distributed sites.*

We will make use of these stimulation patterns in biological preparations characterized by a different condition of the ongoing activity (i.e. either spontaneous or chemically manipulated) and in presence or not of external noise. The internal dynamics of our networks can be modulated in different ways, thus providing a different activity substrate during which we can deliver our spatio-temporal stimuli. For example, we can think of:

- *Stimulating our networks in the presence of a ‘dominant’ bursting activity (e.g. by blocking inhibitory synaptic pathways by the addition of Bicuculline);*
- *Stimulating our networks during a reduced bursting activity – quieter state (e.g. by blocking fast/slow excitatory synaptic pathways by means of CNQX/APV);*
- *Stimulating our network in absence of synchronized network bursts (i.e. a state in which the bursting activity is still present but such activity is uncorrelated) (Tateno et al., 2005);*
- *Stimulating our networks during a background stimulation which reduce or abolish bursting behavior (Wagenaar et al., 2005).*

More specifically, our experimental protocols will be based on the following procedures:

- *A given number of different spatio-temporal stimulation patterns (i.e. see above) are presented to the network and the neural activity, in terms of spikes or LFPs, is recorded continuously prior, during and after stimulation. Each of the stimulation patterns employed will need to be repeated several times (i.e. 50 trials or more per stimulation pattern) so that we can compute information, variability and probability of response given the “state” of the network (see below).*
- *The effect of spatial scale of stimulation and the importance of diversities of spatial scales of spontaneous vs. induced activity will be investigated by systematically play with stimulation patterns of different spatial scale or location.*

The work in WP2 will be carried out by IIT (L Berdondini as leading investigator, plus personnel budgeted in SI-CODE) with contributions from SISSA.

1.3.2 WP3: The role of ongoing activity in the leech nervous system

The in-vivo experimental analysis will first take advantage of the peculiarity of the *leech nervous system*.

In leeches a sensory input can be applied either by delivering the physical stimulation, such as a mechanical input to the skin or a visual or a chemical stimulation to the animal, or by impaling a sensory neurons and evoking a controlled number of spikes.

We will analyse the spontaneous firing of single neurons and inter-neurons and their role in the generation of spontaneous bursts of ongoing activity. In order to characterize the time and space scales of ongoing dynamics, we will measure how the cross-correlation of pairs of single neurons during spontaneous activity depends on the bin-width and the degree of correlation between the global electrical activity obtained from single roots and from different ganglia, and how ongoing single neuron activity relates to more massed activation measures such as LFPs. Subsequently, we will compare properties of the spontaneous activity in isolated ganglia, in chains of two connected ganglia and in semi-intact leeches, where the entire leech nervous system is present. We will investigate how the ongoing activity affects responses to the various types of sensory stimuli outlined above in different conditions of internal states.

In the leech nervous system, it is possible to modify and control the ongoing activity by blocking inhibitory or excitatory synapses. By manipulating activity in this way, we will sample in an exhaustive way all the possible internal states and determine their influence on information processing.

We will also use a preparation composed by a ganglion and a piece of skin connected to it to understand the behavioural outcome of ongoing activity and the behavioural responses of stimuli presented at different states of ongoing dynamics. This is possible because when a burst of ongoing activity occurs in the ganglion, the skin contracts spontaneously.

In these experiments we will collect a large number of different trials, possibly almost thousand, in order to have a vast repertoire of information processing also in the presence of different states of the ongoing activity. Once these experimental data have been obtained, we will use the methodologies described in section 1.3.4 in order to extract information on the stimulus in a state-dependent way.

The work in WP3 will be carried out by SISSA (V Torre as leading investigator, plus personnel budgeted in SI-CODE) with contributions from IIT.

1.3.3 WP4: Understanding how spontaneous network state influences information transmission across brain regions

This WP will use *mammalian in-vivo system* to investigate the interaction between ongoing network states and neural response in sensory thalamus and cortex, and monitor the state-dependent propagation of neural signals across brain areas by means techniques such as combined multisite recording, microstimulation and whole-brain fMRI. We will carry out three series of experiments summarized in the following paragraphs. In the first series we will use optogenetic stimulation of the sensory thalamic nucleus to target cell-type specific rather

than axonal stimulation. The experiments will use the geniculostriate visual pathway that we have been studying by means of electrical stimulation (Logothetis et al., 2010). In the second series of experiments we will use high-density stimulation and recording microelectrode arrays in two different cortical areas of anaesthetized rats to study neural activity evoked by electrical and sensory stimulation of the somatosensory-motor pathways. In the third sets of experiments we shall stimulate electrically neuromodulatory nucleus, Locus Coeruleus (LC), and study sensory-evoked responses in cortical targets of the LC. The multisite silicone probes will permit both stimulation of the LC and recording neuronal activity in the close proximity (~100-500µm) to the stimulation site. Thus, the variability of sensory responses will be evaluated depending on cortical state and the activation of a neuromodulatory system in the brainstem. Given that LC activity may modulate the cortical state itself, this latter experiment will be useful in the long term to understand how to control brain states in addition to discounting the response variability that they introduce.

Relation between optogenetic stimulation of the geniculostriate pathway and ongoing cortical state on visual cortex responses

Our recent studies showed that electrical stimulation (ES) of LGN abolishes the activity of microcircuits in the striate cortex (Logothetis et al., 2010). In specific, each electrical pulse in LGN induces a sequence of a very brief excitation in V1 (by means of the input, usually stellate cells), followed by a long lasting (close to half a second) profound inhibition of the supragranular neurons of the area. The profound inhibition of an area's output during the stimulation of its afferent has important implications for our understanding of signal propagation in general and function of neural prosthetics specifically. Any ES-induced neural activity will propagate to some extent through cortico-subcortico-cortical pathways, rather than cortico-cortical connectivity. These effects are strikingly observable with fMRI: stimulation of V1 afferents disrupts the propagation of signals from V1 to extrastriate areas and induces a strong Negative Bold Response observed in these areas during electrical stimulation; disruption of propagation is due to the strong synaptic inhibition that follows the over-synchronized spatiotemporal profile of ES-elicited thalamic-input, rather than being due to reduced excitability; for the very same state of suppressed V1 spiking, BOLD responses in the area can be negative or positive, reflecting the overall thalamocortical synaptic activity, here corresponding to the product of pulse efficiency and the frequency of stimulation. All in all, BOLD maps made during electrical stimulation reveal areas that have lost afferent signals rather than projection fields and regions involved in certain type of cognitive behaviour. As discussed in detail in our recent publication (Logothetis et al., 2010), shunting of the V1 output by ES stimulation of the LGN is entirely due to a spatiotemporal blurring of the afferent activation. In brief, electrical stimulation excites axons in an undifferentiated manner and therefore does not elicit the spatio-temporal activation patterns (including correct synaptic delays) which are necessary to respect the operational principles of the recurrent cortical microcircuits.

A potentially better spatially and temporally controlled stimulation may be achieved by using optogenetic stimulation. This would permit selection of neuronal type (reduce spatial blurring) and stimulate somata rather axons, thereby potentially reducing also the temporal synchronicity of the afferents spikes in V1.

In SI-CODE we are therefore setting out to repeat the aforementioned experiments by using

optogenetic stimulation. We will stimulate optogenetically the LGN while recording simultaneously neural activity (i.e. LFPs and multiunit spiking activity) both in the optogenetically stimulated LGN region and in the in the early areas of visual cortex (e.g. V1, V2) of anaesthetized rats and monkeys using MRI compatible electrodes. The neural and fMRI recordings of LGN and V1/V2 will be used not only for examining the effects of stimulation but also for the study of inter-area communication in period of resting activity and stimulation respectively. All experiments will be conducted in rats and in the non-human primate (macaque monkeys) under anaesthesia. During optogenetic stimulation we shall combine activation of an analog of the microbial opsins channelrhodopsin-2 (ChR2), a light sensitive non-selective cation channel, with high-field functional imaging. For details of the methodology of fMRI recordings, we refer to (Logothetis et al., 2010).

Relationship between electrical stimulation and spontaneous cortical state on cortical responses between two brain regions by using a high-density array of electrodes.

An alternative and complementary approach will be constituted by the use of two high-density stimulation and recording electrodes in two different brain areas to understand how network states influence the response of a connected region. We will stimulate the vibrissal primary somatosensory cortex S1 of anaesthetized rats with various spatiotemporal patterns using an array of stimulating multielectrode arrays while simultaneously recording (with a similar array) responses in the vibrissae representation of motor cortex M1. Rats will be anaesthetized with a mixture of Zoletil (30 mg/kg) and Xylazine (5 mg/kg). We will also study how the sensory-stimulation response of region A and its connected region B depends on the state of the network prior to the whisker stimulation. The state of the network is derived by applying the algorithms developed in WP5 by analyzing the activity recorded during the pre-stimulus periods.

Neuromodulatory pathways and spontaneous cortical state on cortical sensory responses.

We will study the effects of ongoing cortical state in prefrontal and somatosensory cortex of mammals upon the stimulation of a neuromodulatory (noradrenergic) input. We will apply patterns of electrical stimulation to the Locus Coeruleus (LC; a major noradrenergic site). Initially the experiments will be carried out in urethane-anaesthetized rats. Specifically, in this series of experiments, we will stimulate electrically the Locus Coeruleus while recording sensory responses in the somatosensory and prefrontal cortices. LC neurons project to most brain regions, including the paleo- and neocortex (Jones et al., 1977), and LC terminals have both nonsynaptic release sites that may provide paracrine-type neuro-transmission (Sara, 2009), as well as conventional synapse-like appositions with postsynaptic specializations pointing to the co-existence of wiring transmission (Aston-Jones and Cohen, 2005). NE may increase neural responsiveness by reducing afterhyperpolarization (e.g. by blocking Ca^{2+} -dependent K^+ currents) or decrease it by enhancing GABA-induced inhibition (Sara, 2009). Thus, the activity of LC-noradrenergic system may strongly contribute to the modulation of the cortical state (Constantinople and Bruno, 2011). Standard surgical procedures will be applied for the placement of recording and stimulating electrodes. A custom-developed MRI-compatible electrode drive will be used for positioning and fixation of the electrodes. Details of MRI compatible electrodes were reported previously (Logothetis et al., 2001). We will also use a new generation of MRI-compatible multichannel silicon probes (NeuroNexus Technologies) allowing simultaneous recording and stimulation. Rats will be first implanted

with microelectrodes and then placed in the 7T MR scanner. The minimal stimulation parameters will be adjusted to obtain a reliable BOLD signal. The cortical recordings will be used to determine both the network state prior to sensory stimulation and the neurophysiological responses to sensory stimuli with or without LC stimulation. The fMRI combined with electrophysiological recordings and microstimulation in rats will be conducted with the approaches that have been optimized and are successfully used by MPI-BC (Canals et al., 2008).

The work in WP4 will be carried out by MPI-BC (N Logothetis as leading investigator, plus personnel budgeted in SI-CODE) with contributions from IIT.

1.3.4 WP5: Methodologies for the extraction of state-dependent information from neural activity

Having characterized the intrinsic dynamics, we will try to develop decoding procedures able to extract state-dependent information, i.e. to determine the input also in the presence of bursts of the intrinsic dynamics. Therefore:

- *We will first identify a set of potential internal state variables that may influence the neural response to stimuli, and we will then construct a set of algorithms to compute these putative state variables;*
- *We will then determine the best state variable candidates as the ones that add most information.*

Candidates for parameters measuring state variable and algorithms to compute state variables

In the neocortex, the word “state” is used with two different meanings, corresponding to two different time scales at which the behaviour of the network is considered. Following (Curto et al., 2009) we shall classify brain state variables into two types (“dynamic state variables” and “activity state variables”) depending on whether they describe the ongoing dynamics of the network on a long scale (seconds) or short scale (tens to hundreds of milliseconds) respectively. We shall refer to the state of the cortex, in the sense of the dynamics of network activity on a time scale of seconds or more and as reflected in the LFP power spectrum, as its “dynamic state”. The cortical state variations during the sleep cycles are an example of states whose changes may be well described by changes in dynamic state variables: during waking or rapid eye movement sleep, the cortex operates in a desynchronized state, characterized by high-frequency low-amplitude LFPs; during slow-wave sleep, the cortex operates in a synchronized state, characterized by higher amplitude, lower-frequency LFPs characterized by an alternation of “upstates” of higher generalized activity and “downstates” of network silence (Steriade et al., 1993). Cortical activity during behaviours such as quiet resting shows an intermediate pattern in which downstates of reduced length and depth are observed (Luczak et al., 2009; Petersen et al., 2003; Poulet and Petersen, 2008). Under anaesthesia, the cortex usually operates in the synchronized state. However, under some anaesthetics (such as urethane), desynchronized periods may occur spontaneously (Curto et al., 2009). In the following, we will refer to “dynamics state variables” to describe the variables that describe cortical states which persist on long scale.

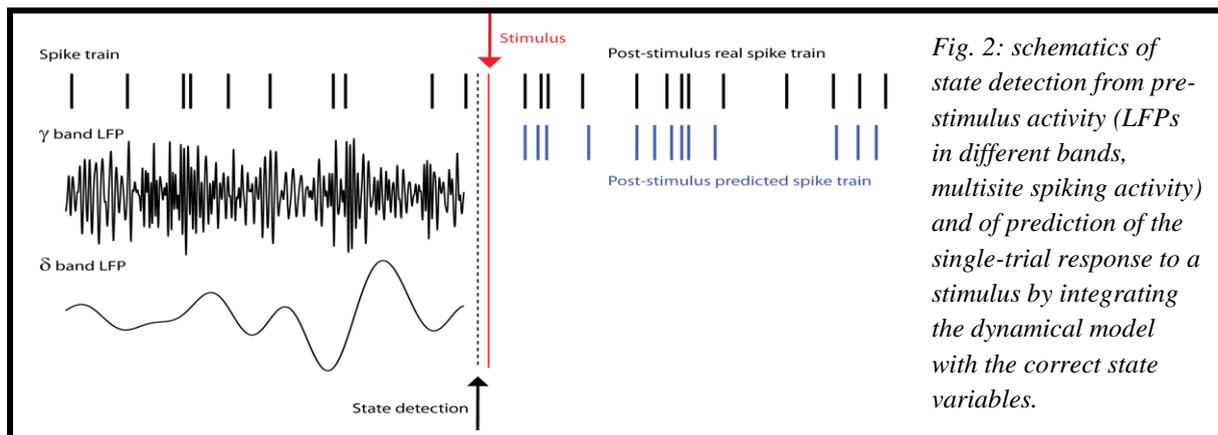
The word “state” is also used to refer to fluctuations in instantaneous network activity at time scales of the order hundreds of milliseconds, as in the case of changes between Up and Down states. We will refer to “activity state variables” to describe the variables as those describing cortical states that persist on these shorter time scales.

The basic approach that we will pursue is to measure the network state variables by best fit of the neural recordings prior to the stimulus to a dynamical system model. We will call this the “*dynamical system fit*” method to measure state variables.

The first dynamical system method which we will use is that proposed by Curto, Harris and colleagues. They used the FitzHugh-Nagumo (FHN) model, a simple dynamical system of self-sustained excitation, to model ongoing activity in the auditory cortex (Curto et al., 2009). They could successfully predict the neural response to a stimulus by fitting a FHN model to the 3s of neural ongoing activity preceding the stimulus time. According to this model, we shall estimate two activity state variables (v and w) directly from neural activity (as the mean instantaneous rate and its value integrated over a time scale τ of few tens of ms respectively). We will then estimate five dynamic state variables (a_1, a_2, a_3, b, I), which specify the FHN model, by best fit of the ongoing data. Best fit is done by minimizing $\epsilon^2(t)$ of this equation when the activity variables are computed over the last 3s of spontaneous activity before stimulus application:

$$\frac{dv}{dt} = a_3 v^3 + a_2 v^2 + a_1 v + b w + I + \epsilon(t); \frac{dw}{dt} = (v - w) / \tau \quad (1)$$

As we will see in Sections 1.3.5-6, for designing algorithms and circuits for real time detection of the state variables θ and of their effect upon neural responses r to stimuli, it is useful to describe the dependence between network state, response and stimulus as an equation $r=f(s,\theta)$. The correspondence between the FHN dynamical fit approach and the “ $r=f(s,\theta)$ ” conceptualization is made explicit in the following. Suppose we want to select the stimulus current value s which gives a response r given θ . The set of parameters θ represents all the activity and dynamical parameters of the FHN as explained above, which are obtained from a direct measure of pre-stimulus neural activity and from model fitting respectively (Fig. 2, left part). The response r predicted by our equations (e.g. the time-dependent firing rate in a post-stimulus window) is the outcome of integrating the above equation when using the states parameters arising from best fit of the pre-stimulation data and adding a stimulation transient of amplitude s (the chosen current value). The r that best matches the desired one can be approximated by trying out different values for stimulus amplitude s (Fig. 2, right part).



The second dynamical system method to determine state variables will stem from the modeling work of in WP6. In brief, we will fit the model of WP6 (a network of excitatory and inhibitory spiking neurons) to periods of spontaneous activity and extract (in a way similar to what detailed above) by best fit the dynamical state parameters (i.e. the parameters describing the synaptic couplings in the network). Again, as in the FHN model, the activity state parameters will be taken directly from the measured pre-stimulus activity. The information theoretic metric developed in this WP (Eq. 3) will be the criterion chosen to compare the performance of the FHN and of the recurrent network model.

The optimal way to measure and define the neural parameters (corresponding to both “activity states” and “dynamics state” variables) entering the dynamical fit state estimation procedure are not known a priori and must be determined empirically. Estimating activity variables require extracting components of neural responses at different time scales. The variables were extracted from spikes rates only in (Curto et al., 2009). However, it might be better to extract them from the LFPs as well. Based on a large bulk of literature, our empirical approach to study the optimal definition of state variables would be to consider a frequency-based decomposition of massed neural activity. In this approach, a main candidate to feed for example to recurrent models for estimation of network state would be some ratio between high and low frequency power of the LFP (which is well known to reliably indicating synchronization/desynchronization). The frequency boundaries for defining high vs low frequency regions and compute this ration will be optimized in terms of the state dependent information they transmit (see next subsection). A likely candidate for activity variables, in addition to the instantaneous spike rate and its low-frequency envelope, is the phase of LFPs in the 1-10 Hz frequency range. In fact, the phase of such fluctuations: correlates well with Up/Down state transitions of membrane potentials in anaesthetized preparations (Salem et al. 2010); correlates with the transition in the reduced Up/Down states observed during quiet behaviour (Petersen et al., 2003; Poulet and Petersen, 2008); correlates with fluctuations in network excitability observed in awake behaving animals during sensory tasks (Kayser et al., 2009; Lakatos et al., 2008; Panzeri et al., 2010; Schroeder and Lakatos, 2009); and reflects fluctuations in the input to recurrent networks (Mazzoni et al., 2008; Mazzoni et al., 2010). All the suggestions above were based on results proposed from data taken from the cortical literature, which is where the state dependence of neural responses has begun to be addressed so far. An important question is how these parameters may generalize to cultures or invertebrate preparation. The first consideration is that fluctuations in excitability (such as

Up/Down state transitions) and the synchronization and oscillations described above for cortex are an ubiquitous observation in neural networks, and are indeed also observed networks of cortical neurons cultured in vitro (see Section 1.3.1), and that in general there are similarities between spontaneous activity in both cortex and cultures. Therefore we will first explore systematically the effectiveness of all the above dynamical system methods in providing information rich state variables also with data from WP2-4. Moreover, and as discussed in Section 1.3.1, recent work has also shown that the ongoing dynamics of neural networks in vivo and vitro by power-law distributed event sizes and durations (“neuronal avalanches”). We will quantify ongoing activity as the temporal succession of neuronal avalanches, something which has not been investigated yet. We will evaluate their effectiveness as network state variables by quantifying whether it is possible to predict the response to the stimulus in a given trial in terms of the relative recent history of occurrence of an avalanche at a specific time after a preceding one. Similarly, in densely recorded cortical cultures, we will also aim at detecting repeating spatio-temporal patterns of ongoing activity at different levels, either sets of spikes or bursts appearing with specific latencies on different electrodes. If this approach is empirically successful in predicting variance of stimulus responses, the generation of such sequences will be also investigated by models (WP6) to become part of the dynamical fir procedure.

In general, all the above described initial guesses for network state parameters will be thoroughly applied both to the data of WP2-4 and to the models of WP6 to understand how they should be computed to extract the optimal amount of state dependent information. The models of WP6 are also expected to provide additional theoretically motivated candidates from both dynamic and activity state variables, which we will in turn apply to the data to further refine our measures in a “closed loop” between theory and experiment.

Algorithms to compute information in state variables

This part of the work aims at developing a data analysis method to quantify the performance of candidate neural variables expressing state dependent coding of information about the stimuli. The proposed methods are based on mutual information (abbreviated “information”), which provides a metric to evaluate the performance of candidate neural codes in representing a set of stimuli. Previous studies (recently reviewed in (Panzeri et al., 2010; Quian Quiroga and Panzeri, 2009; Victor, 2006)) of the information carried by neural responses measured the information between stimuli and responses, which is computed using Shannon’s equations from the empirically determined probabilities of response r immediately after the stimulus s , ignoring the state of the network prior the stimulus, as follows:

$$I(S;R) = \sum_{s,r} P(s,r) \log_2 \frac{P(s,r)}{P(r)P(s)} \quad (2)$$

where $P(s,r)$ denotes the joint probability of observing response r together with stimulus s , and $P(s)$, $P(r)$ denote the marginal probabilities of stimuli and responses. The responses r are obtained either from a single neuron or a population of neurons.

In the present proposal we will overcome the limitation arising from ignoring the knowledge of state variables. We will do so by quantifying the effect upon information of observing a set of parameters θ defining the state of the network prior to the stimulus, as well as the neural response to the stimulus. These state parameters will be evaluated according to what

described in the above subsection. The information between stimuli and responses with knowledge of network state can be defined as:

$$I(S;R,\Theta) = \sum_{s,r} P(s,r\theta) \log_2 \frac{P(s,r)}{P(r\theta)P(s)} \quad (3)$$

State-dependent coding is present when $I(S,R,\Theta)$ is much larger than $I(S,R)$. Since state parameters θ cannot carry information by themselves, they will increase the information $I(S,R,\Theta)$ only when they allow a prediction and so the discounting, of the trial-to-trial variability. The traditional use of information in neural coding is to choose the response parameters r that maximize the response information $I(S;R)$ in Eq (2). Here we take a novel approach: we will use the state dependent information $I(S;R,\Theta)$ to determine the best neural parameters. In other words, we will choose, among all candidate neural codes r and state variables θ , those maximizing $I(S;R,\Theta)$. We will perform this analysis on all experimental datasets provided in this proposal.

Information measures suffer from upward biases when they are computed from experimental datasets comprising only a finite number of stimulus-response trials (Panzeri et al., 2007). To eliminate this problem, we will use (and extend to the case of $I(S,R,\Theta)$) appropriate statistical procedures (Montemurro et al., 2007; Panzeri et al., 2007) that we previously developed for $I(S,R)$. When considering low-dimensional representations of r and θ (so that their combined dimension does not exceed 3-4) we will first discretize the parameter range into a finite number of bins to facilitate probability sampling and then we will use bias correcting procedures. When considering high-dimensional representations of r and θ (so that their combined dimension exceeds 3-4) then the bias corrections mentioned above are likely to become ineffective. In such cases we will then use an intermediate stimulus reconstruction step to approximate in a data robust way (Quiñero Quiroga and Panzeri, 2009) both $I(S,R)$ and $I(S,R,\Theta)$.

The work in WP5 will be carried out by IIT (S Panzeri as leading investigator, plus personnel budgeted in SI-CODE) with contributions from SISSA, MPI-BC and UZH.

1.3.5 WP6: Theoretical analysis of state dependency of stimulus-driven activity in networks of spiking neurons

The main goal of this part of the project is *to obtain models of the ongoing activity and to evaluate its influence on coding and decoding from a theoretical perspective*. In particular, we will perform a systematic analysis, using and perfecting analytical tools introduced by (Brunel and Hakim, 1999) and (Brunel, 2000), of the dynamics of randomly connected networks with realistic synaptic kinetics. We will characterize the different types of states (e.g., asynchronous, slow/ fast oscillatory states) in network models. We will determine mechanisms controlling transitions between different types of states: And finally we will compute an LFP in the network model and compare it to experimental data, by quantifying in details patterns of spike rates and FLP spectra, as well as quantities capturing spike-LFP relationships, such as the relationship between LFPs and spike rates; the importance of computing LFPs as well as spike rates originates from the ability of the former to capture network state variations.

We will analyse systematically stimulus-induced dynamics, as a function of the network state immediately preceding stimulus presentation. Three types of scenarios will be considered:

- *a slowly varying stochastic external input that modulates the excitability of the whole network – in this case, there are no well-defined states, rather a continuum of excitability levels of the network;*
- *an external input which leads to transitions between UP/DOWN states of the network – in this case, the distribution of excitability levels is bimodal.;*
- *Up/Down states triggered by the network dynamics – the distribution of excitability levels is again bimodal in this case, but the origin is now intrinsic rather than extrinsic.*

In all cases, we will derive the equations describing the dynamics of the instantaneous firing rates of a local network of excitatory and inhibitory neurons, as a function of stimulus-dependent external inputs, and the stochastic 'activity state variable(s)' leading to variability of the response. Using linear response theory, the Fourier transforms of the instantaneous firing rates of excitatory and inhibitory neurons are given by

$$r_E(\omega) = \frac{(1 + A_{II})R_E\mu_E - A_{EI}R_I\mu_I}{(1 - A_{EE})(1 + A_{II}) + A_{EI}A_{IE}}; \quad r_I(\omega) = \frac{A_{IE}R_E\mu_E + (1 - A_{EE})R_I\mu_I}{(1 - A_{EE})(1 + A_{II}) + A_{EI}A_{IE}} \quad (4)$$

where $\mu(a=E,I)$ are the Fourier transform of the time-dependent inputs to both populations, A_{ab} is given by

$$A_{ab}(\omega) = J_{ab}R_a(\omega)S_{ab}(\omega) \quad (5)$$

where J_{ab} ($a,b=E,I$) are the coupling strengths from population b to population a , R_a is the single neuron ($a=E,I$) transfer function, and S_{ab} are synaptic transfer functions (synapses from b to a). From these equations one can compute in a similar way the Fourier transform of the LFP, and thus its spectrum. In the above equations, the coupling strengths J_{ab} and the parameters specifying the functions R_a and S_{ab} are the 'dynamical state parameters' that will be obtained through a direct fit of the experimental data during spontaneous activity. On the other hand, parameters specifying the time-dependent inputs will be obtained from measured data immediately preceding the stimulation (see Section 1.3.4).

This analysis will help us to interpret experimental data on the variability of stimulus-induced activity and how it can be predicted by ongoing activity preceding the stimulus. Information-theoretic tools developed in the framework of the project (section 1.3.4) will be applied to network simulations, in the same way as they are applied to experimental data with the purpose of determining which exact definitions of measurable state parameters lead to the highest amount of state-dependent information (Eq 3).

We will first fit the data recorded in WP2-4 using the model, to check that the model can indeed reproduce the experimentally observed LFP dynamics. The excitability state will be discretized in a number of states. Then, for each 'state', we will compute the mean LFP spectrum $MFLFP(f,\theta)$ where θ is the parameter used for the excitability state. We will then fit the mean-field equations described above to the data, in order to obtain the parameters of the network model (the "dynamic state variables") that best fit the data. If we get a good fit, we

will proceed to the next step. Otherwise, the model will be modified (by incorporating more details of the biophysics of single neurons and synapses, which will lead to modified transfer functions R and S) until a good fit is obtained.

The networks of spiking neurons that obey the mean-field equations will then be implemented in a neuromorphic VLSI device in WP7. Particular attention will be devoted to a close collaboration between data analysis, modelling and VLSI engineering to calibrate the design and parameter of the neuromorphic devices (WP7).

The modeling work in WP6 will be carried out entirely by partner IIT (with S. Panzeri as the leading investigator, and with a postdoc supported by SICODE and with visiting Prof. N. Brunel), with contributions from UZH regarding the implications of the modeling work for in silico implementations.

1.3.6 WP7: Neuromorphic circuits for state-dependent processing in Brain-Machine Interfaces

We will develop the technology to support the models and algorithms developed in the theoretical and experimental activities. This technology will provide constraints in the development of computational models that enable their neuromorphic implementation and that are compatible with those present in biological neural networks. The neuromorphic VLSI device will comprise a reconfigurable network of Integrate-and-Fire neurons with dynamic synapses capable of implementing a Spike-Timing Dependent Plasticity learning rule. By interfacing the network activity to these VLSI circuits we can find an appropriate set of parameters θ that best describe the state of the network. We will also focus on the development of local adaptation, plasticity, and homeostatic mechanisms. Indeed, in this way we expect to obtain artificial systems able to cope with long-term changes in the input stimuli and in changes of recorded signals (for example due to electrode encapsulation). We will design circuits that implement these mechanisms and fabricate them in VLSI technology using the well tested AMS 0.35 μ m process provided by the Europractice service.

A central part of this activity will be devoted to the design of spike-based neural networks that can implement state-dependent computations. The VLSI networks of spiking neurons developed will be based on recently proposed dynamic synapse circuits and exponential Integrate-and-Fire circuits. In particular, biophysically realistic synaptic dynamics will be implemented using the DPI circuit proposed in (Bartolozzi and Indiveri, 2007). Similarly, the I&F neuron functionality will be implemented circuits comprising an input diff-pair integrator (DPI), which models the neuron's leak conductance, an integrating capacitor which models the neuron's membrane capacitance, and a set of non-linear circuits which are responsible for implementing the neuron's spiking mechanism. The inverting amplifier with positive feedback implements sodium activation and inactivation dynamics. The reset transistor models the Potassium conductance functionality and, together with the constant sub-threshold leak transistor, implements the refractory-period behaviour. A second instance of a DPI models the neuron's Calcium conductance, and produces an after-hyperpolarizing current (I_{ahp}) proportional to the neuron's mean firing rate, and responsible for the spike frequency adaptation mechanism. Multiple instances of these circuits can be integrated onto VLSI chips (e.g. 128 neurons and 4096 synapses on the smallest possible die size -2mmx2mm- offered by the AMS 0.35 μ m).

By using leaky integrate and fire neuron models described above, we can implement a set of recurrent self-sustained oscillators that can capture the dynamics expressed by both dynamical models that we use (WP5) to estimate state variables (the FHN model of (Curto et al., 2009) and the recurrent network model of WP6). The so defined state-dependent parameters can be mapped on the biophysical parameters of the VLSI network such as synaptic time constants and relative recurrent excitatory and inhibitory weights.

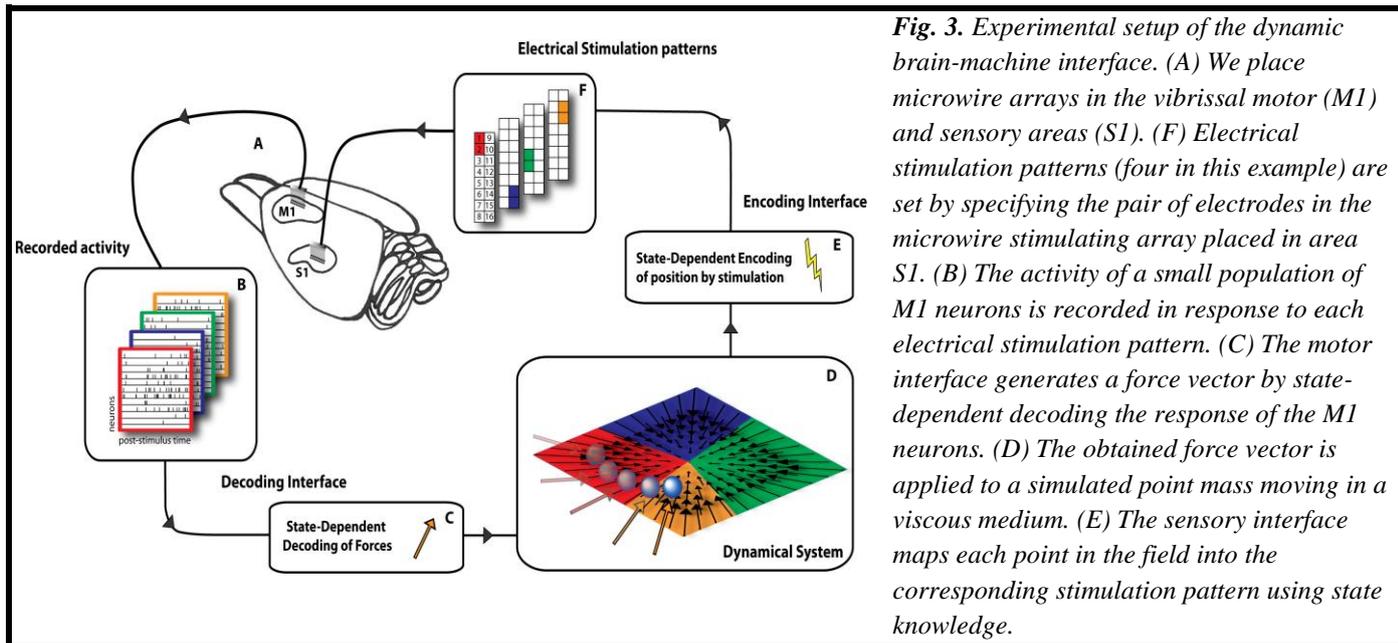
One possible way in which these circuits can detect and discount state dependence can be conceptualized in the following for both types of dynamical models. Suppose that the problem is that of selecting the right stimulus current value to elicit a given response r which carries the specific information that we wish to give to the brain about the current position of the point driven by the bBMI (see WP8). The bBMI has to measure the state θ and then decide which value of current s (out of say $M=10$ different possible values) to apply in order to elicit the desired response r , according to the equation $r=f(s,\theta)$ that is obtained from the analytical work. The “activity state parameters” varying on short scales will be estimated directly from the recorded massed neural activity (instantaneous spike rates, slow spike rate envelopes, LFPs). For this task we will evaluate the use of on-line software spike detection algorithms (of which there are several versions currently available both on the open source and on the commercial market) and of current state-of-the-art analog hardware systems such as those developed by collaborators in the United States (<http://www.intantech.com/products.html> and (Harrison et al, 2009)) and by IIT collaborators (Bonfanti et al., 2011). Then, the VLSI circuit can evaluate the dynamical state parameters by comparing responses of its units to the injection of the “activity state variables” and choosing as dynamic state variables the parameters of those silicon units best matching the observed neural dynamics (some major parameters of the neural network that are not likely to change over time of the BMI experiment will be fixed during an initial calibration period by fitting the recorded LFP to the analytical equations, rather than being continuously evaluated over time). Once the current state variables have been evaluated, then the circuit can predict the responses of the silicon neurons with best matching state parameters to various strength of input. The value of stimulation amplitude s which most closely matches r is then selected and applied to cortex. The problem of decoding motor cortex activity in a state dependent way will be tackled following the same principles.

The work in WP7 will be carried out by UZH (G. Indiveri as leading investigator, plus personnel budgeted in SI-CODE) and IIT (C. Bartolozzi as leading investigator, plus personnel budgeted in SI-CODE).

1.3.7 WP8: State dependent bidirectional BMI (bBMI)

In vertebrates, the communications between brain and limbs are mediated by the spinal cord, a biological interface that combines brain instructions with sensory information and organizes coordinated patterns of muscle forces driving the limbs along dynamically stable trajectories. We will implement a bBMI that establishes bi-directional communication between the sensory and motor areas of the brain of anaesthetized rats and a simulated dynamical system, which will be either a point-mass moving in a force field (FF) or a multi-articulated arm with muscle-like actuators, whose behaviour will also be modelled as viscoelastic FFs. The FF chosen by the experimenter can be seen as a control policy executing a pre-programmed

motor behaviour, in analogy to how the vertebrate spinal cord drives muscles to goals using position-dependent forces.



The bBMI is schematized in Fig. 3. After implanting a multielectrode array of stimulating electrodes (see WP4) in the vibrissal representation of primary somatosensory cortex (S1) and a similar array of recording electrodes in vibrissal motor cortex (M1), we will empirically select a set of electrical stimulation patterns of S1 that modulate responses in M1 reliably. Then we will establish an “encoding interface” (which provides the brain with “sensory” - position and velocity- information about the task progress) which associates a portion of the spatial domain of the force field to each S1 stimulation pattern. This will be done computing distances in terms of spike train metrics between stimulus-evoked activities of M1 neurons, and projecting them onto the FF domain by Multi Dimensional Scaling. Then we will build a “decoding interface” which decodes M1 evoked activity (computing the most likely stimulus eliciting it) and, using force field equations, translates this activity into a force as an input of the dynamical system. After this calibration phase, we will run the bBMI in a real-time closed-loop configuration to probe the “reaching” ability of such system to drive the simulated point mass or the multi-articulated arm from a starting position toward the goal location (an equilibrium point of the FF).

Once the interface, decoding algorithms and methodologies have been established, we shall include in the setup the full-custom VLSI device developed in WP7. The VLSI device will measure the state of S1 cortex in real time and use this measure to fine tune the choice of the electrical stimulus which elicits the desired motor response and thus provides more faithful spatial information. Instances of the same VLSI device will also be used to compute the state of M1 cortex and discount the state-induced variability of M1 neural responses and improve their decoding. The overall effect of the introduction of state dependency is expected to be a much tighter control of the object by the brain, resulting in a much smaller variability of forces at fixed position than that in the non-state dependent bBMI.

In the last phase of this development, we will test the ability of the awake rat’s brain to control the bBMI by modulating the neural activities recorded by the decoder so as to control

a robotic feeder. We will test movement performance both in free space and in the presence of obstacles and unexpected perturbations. We expect that the interface will effectively provide the rat's brain with a closed-loop system that –like the spinal cord- provides access to sensory information and to the motor output, while enforcing a stable behaviour via the pre-defined force field. The state dependent bidirectional BMI will be our final benchmark.

The work in WP8 will be carried out by IIT (A Vato as leading investigator, plus personnel budgeted in SI-CODE, plus contribution of Prof. Mussa-Ivaldi as visiting Professor) with contributions from UZH.

1.3.8 WP9: Dissemination and open access

To maximize the impact of our results, we will disseminate them by 'Open Access' archive of some of the main experimental datasets and of Open Source state-dependent information algorithms on public repositories (either our own websites, such as www.ibtb.org, or public neuroscience repositories of data and software, such as e.g. carmen.org.uk), as well as by standard procedures (international meetings and peer-reviewed publications in high profile journals in general biology, both experimental and theoretical neuroscience and in engineering). Further information on the dissemination of results is provided in section 3.2.

1.3.9 Overall strategy of the work plan

The overall strategy of SI-CODE is reflected in 9 WPs which are tightly interconnected.

- **WP2-4** will provide the multiscale analysis of the role of the ongoing activity in different preparations. WP2 is a transversal scientific-technological activity in which high-density MEA, advanced optogenetic tools and network patterning are developed and integrated. WP3 focuses on a simple invertebrate system that has the advantage to be studied in semi-intact preparations allowing both natural and artificial sensory stimulation. WP4 will investigate network dynamics and information coding in in-vivo mammalian neuronal networks.
- **WP5-6** will be dedicated to building models of the ongoing activity and to develop algorithms for decoding information in state-dependent way. The activities of WP5-6 will be based on the experimental analysis carried out in WP2-4 and will provide the input to WP7-8. WP5 will develop algorithms to be used to decode in a state-dependent way experimental data collected in WP2-4, while WP6 will focus on the development of analytical and numerical models.
- **WP7** will design and construct a new neuromorphic chip for online detection of the cortical network state. Particular care will be devoted to the continuous integration between WP5, WP6 and WP7 to ensure that the design and implementation VLSI real-time state detection circuit fully benefits from knowledge gained by the analysis of state dependent information coding in the various neurophysiological datasets (collected by the analysis of WP5) and by the closely related progress obtained with the neural network models of WP6. Due to the crucial nature of this integration, one position at IIT will be specifically devoted to this purpose.

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- **WP8** will design and develop a VLSI-based state-dependent bidirectional brain machine interface. The knowledge gained by WPs 2-6 and the devices developed by WP7 will be used in developing a state-dependent bBMI as a possible realization of a bidirectional interface between the brain and the external world to obtain a desired behaviour by a controlled external object.

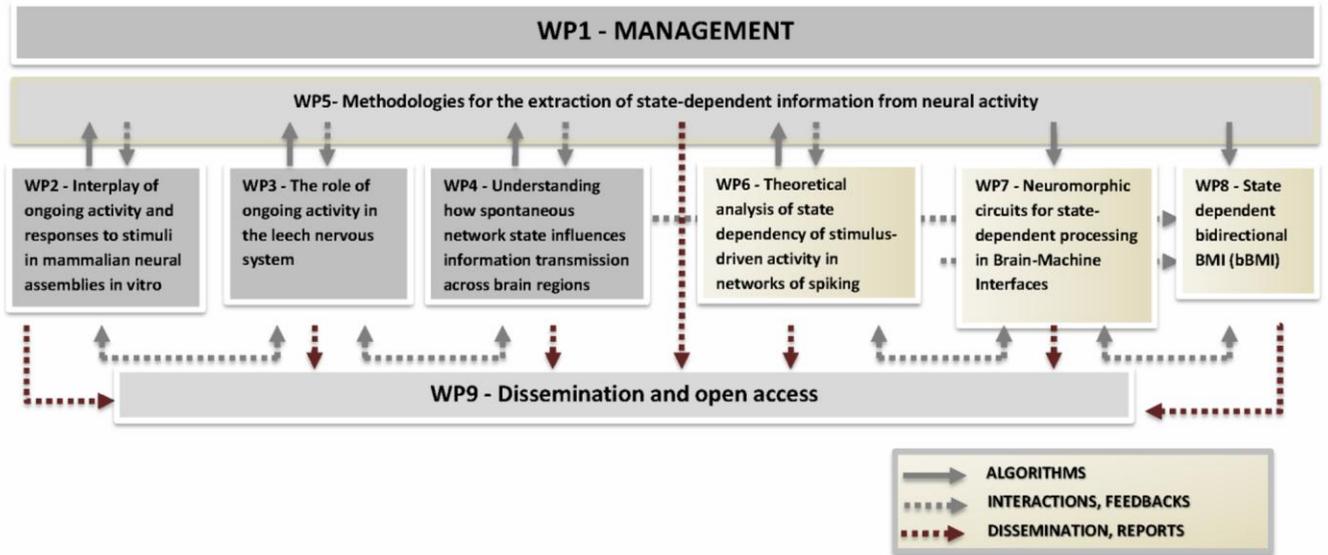
Finally,

- **WPI** will deal with all management and planning issues and ensure SI-CODE remains tightly focused and the interactions between groups remains properly timed.
- **WP9** will coordinate a proper dissemination of the results of SI-CODE, aimed at ensuring that they have the largest possible impact on society, science and healthcare.

As a result of this structure, the overall strategy of SI-CODE will be highly focussed to the solution of a major scientific problem; based on an extensive set of collaborations between partners with a complementary expertise; and finalized to the development of a new class of state dependent bBMIs algorithms based on major advances of the understanding of brain function with allows solving the major current drawbacks of BMIs.

1.3.10 Dependencies between WPs

The following Pert diagram shows the organization of the project in terms of work packages and their components, highlighting the interdependencies (see WP descriptions for details on interdependencies).



1.3.11 Time diagram



1.3.12 Contingency plans

The main risk in the project is that critical results, which other results depend on, will be delayed or not sufficiently accurate. Further risks are related to the specific research activities. To minimize the risks, a contingency plan has been identified in the following table.

	Risk description	Effect	Control strategy	Countermeasure	Impact	Prob.
1	The integration of high-density MEA chips and optical stimulation presents problems	Delays in time scale	Progress monitored and reported according to the Sections above. Initially experiments will be performed on commercially available systems with standard MEA.	Activities are initiated at an early stage and devices are already available within the consortium. SI-CODE includes a partner with great experience in the field of high-density devices.	High	Very-Low
2	Integration and synthesis on the performed multi-scale experimental characterization and analysis present problems	Delays in time scale. Failure to use of the different experimental models for a synthetic computational model	Progress monitored and reported according to the Sections above.	Integration should be guarantee by WP4 and more specifically by the use of the same analysis tools. In case of failure of one experimental models we will concentrate on some of them	Medium	Low
3	The proposed algorithms are not efficient in extracting information about the stimulus in a state-dependent context	Delays in time scale. Partial failure in exploiting the state-dependent information coding	Progress monitored and reported according to the Sections above.	Different approaches are proposed and additional ones could be devised during the project based on the strong experience by the partners. Computational models could be used to test new decoding strategies.	High	Very-Low

	Risk description	Effect	Control strategy	Countermeasure	Impact	Prob.
4	Computational model systems are not able to reproduce state-dependent information coding	Partial failure to reproduce the natural computation in simulated systems	Progress monitored and reported according to the Sections above.	The use of in-vitro cultures will serve also as intermediate modelling system to translate and test understanding from in-vivo experiments to synthetic models	High	Low
5	Over risk 3 Synthesis in a neuromorphic chip not supported by simulated models	Delays in time scale. Difficulties to reproduce the natural computation in HW	Implementation of the neuromorphic chip based on the in-vitro model system and the decoding strategies developed	Activities are initiated at an early stage to allow for initial test and comparison with the experimental models	High	Low
6	Given the constraints of neuromorphic VLSI technology, implementation of the network presents problems	Partial failure in extracting online state parameters and in implementing a true bidirectional closed loop state dependent bBMI	Use of re-configurable multi-chip set-up	Implementation of the neuromorphic chip restricted to only a simpler subclass of state extraction procedures	High	Low
7	Difficulties in testing and using the neuromorphic chip in closed loop BMI	Failure in demonstrating the capability state dependent coding of bidirectional BMIs	Use of simulated model systems Thorough testing and problem solving in open loop BMI	Demonstration of advantages of state dependency simulated data and in an open loop context (decoding and encoding studied separately)	Low	Low

B2. Implementation

B 2.1 Management structure and procedures

The management activities in the project will ensure that the project is completed within the terms of the contract with the European Commission. This includes ensuring that:

- Appropriate agreements and management framework are in place between the partners;
- All the project's activities are properly coordinated with appropriate levels of legal, contractual, ethical, financial and administrative management of the consortium;
- Proper operational project management is provided throughout the project;
- The project completes its work within the expected timescales, resources and quality levels;
- Appropriate reporting to the European Commission is undertaken.

The Consortium has decided to entrust IIT for the management of the project, as it can make available highly qualified staff with experience in management of EU funds both from a scientific and an administrative perspective. In order to ensure clear responsibilities, the following management and decision making structures will be in place. The consortium will be managed by the following components:

- A **Project Coordinator (PC)**, Stefano Panzeri (supported by a **Management Support Team - MST**) who will be responsible for the administration and the management of the project and its resources;
- A **General Assembly (GA)** representing all Partners, as the ultimate decision-making body of the Consortium;
- The **Project Steering Committee (PSC)**: responsible for the coordination of the project activities, the review of the scientific strategy, the management of changes and conflict resolution, providing external high level advice and representation in an international context.

Project Coordinator

The major effort of the management will focus on obtaining an efficient and productive collaboration between all partners. The PC has already verified with the different partners their willingness towards the collaborative effort required by the project. The Steering Committee will make sure that all collaborations take place in due time and according to the planned criteria.

The PC will be the intermediary between the Partners and the European Commission and will:

- be in charge of the overall coordination and management of the Project, dealing with all the contractual, ethical, financial and administrative issues;
- ensure a smooth communication flow between the partners;
- oversee the scientific coordination of the project, including the supervision of the work package leaders and the maintenance of a timetable for keeping track of events, milestones and deliverables;
- monitor that the partners comply with their obligations;
- collect and review information relevant to the submission of reports (progress reports, mid-term and final reports) and other deliverables (including financial statements and

- related certification) to the European Commission, as well as the preparation of annual summaries of the scientific achievements which can be used also for internal purposes;
- prepare meetings, propose discussion points and prepare the agenda of the General Assembly meetings, chair the meetings, prepare the minutes of the meetings and monitor the implementation of the decisions taken at those meetings;
 - Chair the Steering Committee, defining its meeting agenda and the PSC decisions;
 - Transmit promptly to the EC or to whoever submit a request documents and information related to the project;
 - Administer the Community financial contribution and fulfil the financial tasks providing, upon request, the Parties with official copies or originals of documents which are in the sole possession of the Coordinator when such copies or originals are necessary for the Parties to present claims.

General Assembly

The GA will meet annually for reviewing and monitoring the progress of the project as well as identifying appropriate actions for the successful performance of the project. It will be in charge of making decisions or proposals for decisions to be taken by the PSC, particularly decisions of major strategic and scientific relevance. Given the already existing good relationship between the partners, most decisions are expected to be taken by consensus. In cases in which this cannot be reached, the GA will have the last word and decisions will be taken with a majority vote (2/3) by the GA.

Project Steering Committee

The PSC is formed by the Project Coordinator and all the principle investigators involved as Work Package (WP) leaders, namely:

- Luca Berdondini IIT (P1), responsible for WP2
- Vincent Torre SISSA (P2), responsible for WP3
- Nikos Logothetis MPI-BC (P3), responsible for WP4
- Stefano Panzeri IIT (P1), responsible for WP5, WP6 and Project Coordinator
- Giacomo Indiveri UZH (P4), responsible for WP7
- Alessandro Vato IIT (P1), responsible for WP8 and WP9

The Project Coordinator is also responsible for WP1.

The PSC, in close cooperation with the Project Coordinator, will:

- Be responsible for all the scientific aspects of the project, including making sure that deliverables and milestones are timely reached;
- Develop a quality control plan to be applied to all deliverables and services provided by the project;
- Make sure that all the collaborations planned in the proposal occur as scheduled;
- Evaluate the progress of all the work packages and propose corrective actions whenever necessary (e.g. when deliverables are behind schedule or when problems occur which impede a smooth progress of the research)
- Identify potential commercial exploitation of the project's findings and results and take the appropriate measures to profit from them.
- Identify potential risks of dual use/misuse of the project's research results and devise a strategy for minimizing and dealing with them.

Management Support Team

A MST will assist the PCS, the General Assembly and the PC providing the day-to-day management as well as administrative, advisory and infrastructural services to the project. Proposed by the PC, the MST will comprise a project manager and a financial manager assistant, with consolidated experience in the management of regional, national and EU grants, who will be appointed specifically to this project.

Consortium agreement

Before the beginning of the project, all partners will agree upon the terms of a Consortium Agreement, whose specifications must be in line with the interests of the Community as well as with applicable competition rules.

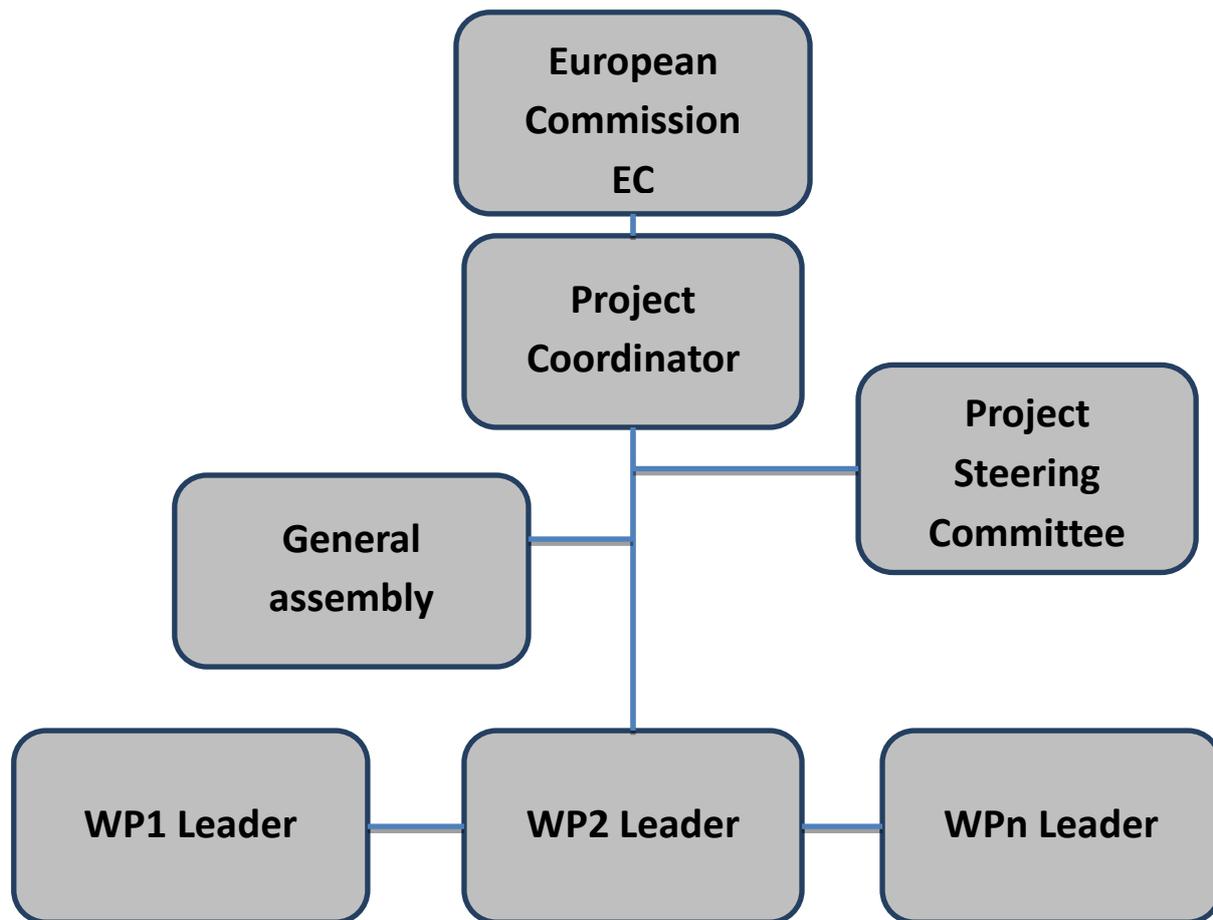
The SI-CODE Consortium has been examining a draft Consortium Agreements using aspects of the developed DESCAs model agreement for FP7: the DESCAs Simplified Model Consortium Agreement, Version 3.0, March 2011, for Small Collaborative Project.

The DESCAs project (<http://www.desca-fp7.eu>) has tried to bring together all of the key groups involved in producing FP6 model consortium agreements, with the aim of producing one consistent modular agreement for FP7 which balances the interests of all key player categories, in the spirit of a responsible partnering which aims at gaining the full benefits of Open Science and Open Innovation.

It will detail issues such as Intellectual Property Rights, prior knowledge, rights to exploitation, and the responsibilities of all of the parties involved in the project. A draft will be circulated to all consortium members for examination and amendment during the negotiation period.

2.1.1 Decision making

The management structure is sketched below. Decisions of the General Assembly are reached on the basis of the votes of all members (one member=one vote), consisting of the representatives of each participating institution and the Project Coordinator. The Project Coordinator has one additional vote.



Project meetings, which serve as platform for discussions, knowledge transfer and decision making, will take place at least twice a year at one of the participating institutions' location. Optionally, meetings may be replaced by telephone conferences.

The PSC will meet whenever needed, either by face-to-face or telephone conference meetings, as a minimum in correspondence with the key milestones of the project such as the completion of each Work Package.

The PC can decide if a telephone conference or a meeting has to be hold and if additional meetings are required. The members of the PSC will stay in close contact, and, if required, additional meetings or telephone conferences will be held on request of the PC or other members of the consortium.

The project is divided into eight work packages (WPs). Leaders as defined in previous sections (see section 2.1) are appointed for each WP.

WP leaders will be responsible for the **planning, monitoring on a daily basis** and **reporting** of the performed WPs and the tasks therein.

2.1.2 Progress monitoring and corrective actions

The project has clearly defined goals and carefully scheduled milestones and deliverables, as described in paragraph “**1.3 S/T methodology and associated work plan**” and in WT3 “**Work Packages description**” table.

Progress monitoring will be done on the basis of detailed Gantt charts available for each WP and task (where reasonable also for sub-tasks). The WP leaders, and subsequently all partners, will report at regular intervals (quarterly) to the PCR. Addressed issues include the realised progress versus scheduled, and the foreseen progress versus scheduled. In agreement with the PSC, the PC may undertake corrective actions if the progress deviates from the project planning. In case that a partner does not fulfill the expectations, budget and task reallocations are possible.

The project's progress assessment will be done also with reference to scientific and management **reports**: a report, describing the overall and individual technical progress and the financial consumptions during the report period including future planning, will be submitted every 18 months to the project's partners and to the EC. Additional internal reports may be added if deemed necessary by the consortium.

2.1.3 Internal reporting and communication & sharing of knowledge

Due to the nature of the project, an efficient and effective communication and knowledge-flow within the partners is very important.

A six-month internal reporting procedure will ensure detailed information on ongoing activities.

The following arrangements will be made to ensure optimum dissemination of knowledge within the consortium:

Project telephone conferences or meetings will be held at least every 6 months. They will serve as the main forum for interactions between all groups and for reviews. The partners of the project will take turns in hosting project meetings to further increase interactions between the participating institutions. In addition, further meetings in smaller groups may be organised in order to directly exchange specific knowledge and results. Minutes of the meetings are prepared by one of the partners appointed by the Project Coordinator.

Internal project reports of each WP have to be delivered to the PC by the WP leaders every six months at deadlines defined by the PC. The partners responsible for sub-WP tasks have to report to the WP leaders according to the deadlines defined by the WP leaders. These reports will be compiled by the coordinator and distributed to all partners..

Intranet: an intranet will be implemented to assist in the management of documents, to allow fast and efficient communication within the Consortium and to optimise external communication and information flow. Partners will be therefore able to share materials and knowledge in an effective way, to update and check the progress remotely and spread information on the project to all external interested parties. The confidential parts of the material will be protected by secure access and control procedures.

Personal exchange: For training in specific techniques as well as for joint experiments and direct exchange of information, members of the different groups may work in the laboratory of the partners as required by the project.

Scientific and management reports: A report, describing the overall and individual technical progress and the financial consumptions during the report period including future planning, will be submitted every 18 months to the project's partners and to the EC.

2.1.4 Risk management strategy

General strategy

The overall management of risks related to the SI-CODE Project will be responsibility of the Project Coordinator who will be responsible for tracking efforts to reduce high risk, combine risk briefings, reports, and documents as delivered by the Work Package (WP) leaders and required for project reviews by the Commission.

The WP Leaders are nevertheless responsible for the Risk Assessment within their work packages, which includes identification, analysis, handling, information (in case of moderate or high risks), monitoring, and tracking efforts to reduce low and moderate risks.

The risk management strategy that the SI-CODE consortium will use includes the accomplishment of the following activities:

- Risk Identification;
 - Risk Estimation;
 - Risk Mitigation activities;
- Risk Ownership, Monitoring and Reporting.

1. *Risk Identification*: The risk identification activity is not bounded at the beginning of the phase. Each time a new risk is detected it shall be managed (identified, assessed...).

Nevertheless, the biggest effort has to be put at the beginning in order to anticipate, as far as possible, the monitoring of possible risk and plan, if the case, mitigation actions. For each risk, a risk form will be completed and kept in a so-called risk forms folder.

2. *Risk estimation*: This risk estimation is carried out on the basis of the likelihood of concerned events and the relevant impact on the project in terms of costs. Risk likelihood will be estimated considering three possible values, namely: low, medium and high. In the same manner SI-CODE will estimate the impact evaluation related to each risk. Each risk will be referenced within a table having as rows the risk likelihood and as columns the possible impact on the project (see figure below).

Impact	high	1	2	3	
	medium	0	1	2	
	low	0	0	1	
		low	medium	high	Likelihood

Level 0: for these risks, no action is required. They are just included in the risk form folder and reviewed by the Project Coordinator to check possible variation of its estimations.

Level 1: an owner is appointed which is in charge of monitoring the risk evolution and reporting to the Project Coordinator. The owner can be a WP leader.

Level 2: like level 1 plus definition of specific mitigation actions. These actions are defined by the Project Coordinator who identifies also possible trigger events to start them. The owner monitors the risks and these trigger events.

Level 3: planned mitigation actions are timely started. The risk is in charge to the Project Coordinator, which closely follows-up the effectiveness of the in-progress mitigation actions.

3. Risk Mitigation activities: Mitigation activities are meant actions undertaken at management level to smooth the impacts of events identified as risks. They will be planned for level 2 and level 3 risks and their description will be provided within the risk forms.

Unmanageable risks, that is to say risks for which the Project Coordinator is not able to deal with in a significant way, shall be highlighted and a proper justification on the lack of mitigation actions should be provided. Mitigation activity shall be followed-up by the Project Coordinator who supervises the accomplishment and verifies the effectiveness of the performed actions.

The Project Coordinator will manage a Risk Mitigation Action List tracing the evolution of the status of each action.

4. Risk Ownership, Monitoring and Reporting

Each identified risk shall have an owner who is responsible for its monitoring and reporting. The Project Coordinator will identify the proper owner (itself included) for all the risks that have been identified with level 1 and 2. Level 0 risks do not have owner, level 3 ones are managed directly by the Project Coordinator.

Each owner reports periodically to the Project Coordinator (during the project meetings) about the risks it is in charge of. It timely reports to the Project Coordinator each event related to level 2 risks (e.g. trigger events).

Specific strategy regarding the risk of needing new expertise not included in the team.

Among the potential risks that may arise during the course of the project, a particularly relevant one is the arising of an unexpected scientific question or scientific problem that seems to require scientific skills not covered by the original consortium.

We minimized this risk at the start by carefully planning what we feel to be a very strong team covering a very wide range of multidisciplinary expertise that includes what is needed to make a significant breakthrough in the investigations of the questions we set in our proposal. Should any new need not covered by the present skills arise, we will exploit the fact that each of the partners works in a high-profile, large and genuinely interdisciplinary research center. Therefore it is likely that any additional expertise that may become necessary during the project could be covered by one of our colleagues at one of the institutions of the beneficiaries. We will first identify such scientists and then invite them to attend our formal or informal meetings, as appropriate. Finally, in the unlikely event that we need something that we cannot find within our consortium or at our own institutions, we will identify scientists outside of our consortium or institutions and involve them in the project. The wide network of collaborators that each of the partner has already in place will be a key resource in this process.

B 2.2 Beneficiaries

Italian Institute of Technology

The Italian Institute of Technology (IIT) is a Foundation created to promote excellence in research in Italy. It is open to the active participation of private organizations to encourage technological development and training in high technology. IIT aims at becoming an international centre of excellence for scientific research in advanced technology, attracting contributions from the world of research. Focusing on synergy and interdisciplinarity, four technological platforms – Neurosciences, Nanobiotechnologies, Drug Discovery Development and Robotics - will develop research activities with the common aim of studying humanoid technologies. The research will result in multiple industrial and social outcomes and impacts regarding various sectors such as manufacturing, medical/surgical, security, and space exploration. Currently, there are over 400 researchers at IIT. IIT participates in SI-CODE with three research units: CNCS, RBCS and NBT.

The CNCS (Center for Neuroscience and Cognitive Systems) at IIT is directed by Prof J. Assad and focuses on a multidisciplinary approach to understanding the computational abilities of the brain. Currently the department focuses on studies with human subjects, including psychophysical techniques, functional imaging, and trans-cranial stimulation, along with computational approaches. The CNCS laboratory contributed to SI-CODE is the Lab of Neural Computation (led by Prof. Stefano Panzeri). This lab is interested in understanding how populations of neurons in sensory structures (visual, auditory and tactile) encode and exchange information. The laboratory investigates these issues by means of computational tools. In particular, the laboratory develops methods (mostly but not only based upon Shannon's Information Theory of communication) for the analysis of time series of neural recordings from multiple locations. The algorithms are designed to determine which neural pathways or network nodes provide information relevant for perception or behavior, what information they carry, and when and how these pathways or nodes exchange information. The laboratory also constructs and uses models of recurrent networks of spiking neurons with the aim of understanding the dynamics of sensory representations under naturalistic stimulation conditions, and to derive simple coding rules describing the transformation between the dynamics of natural stimuli and that of neural network responses.

Role of CNCS in the project. Coordination of the project; Development of all the analysis algorithms for state dependent information encoding and decoding (WP5); Analysis, by means of the aforementioned algorithms, of all (simulated and real) datasets produced in this proposal (WP2-4); Online open source delivering of the code for aforementioned algorithms (WP5); Development of network models of state dependence of neural responses (WP6).

Key investigators:

Stefano Panzeri (Senior Scientist with 15 years of postdoctoral experience, of which 10 years at Faculty level) is the coordinator of Si-CODE and the responsible for the IIT part of the project. He has been at the forefront of studies on neural codes for a decade, by providing some of the most cited algorithms to extract single-trial information from brain recordings, some of the most compelling demonstrations of how the brain uses the time domain to encode information, and the first characterizations of how the low frequency fluctuations of LFPs complement the information carried by precise spike times. He is also an expert in developing network models of cortical dynamics and in using them to understand how networks of

neurons encode information. He has published 100 peer-reviewed articles on neural information processing which were cited more than 2400 times, with four first author articles with more than 100 citations. He has been awarded numerous Personal Research Fellowships (MRC, EU Marie Curie, HFSP) and has served as Vice Chairman of the UK Medical Research Council Panel for Biomedical Informatics. He has received awards of more than 20 grants for more than 8M€ Stefano Panzeri also holds a research Chair at the Institute of Neuroscience and Psychology of the University of Glasgow (UK).

Within this project IIT-CNCS will host (as Visiting Professor) **Nicolas Brunel** from University of Chicago. Brunel (a long term collaborator of both Prof Panzeri and of Prof Logothetis, leading investigator in MPZ) is the world leader in the analytical and numerical analysis of models of cortical dynamics and has provided a crucial input to the project at the submission stage.

The RBCS (Department of Robotics, Brain and Cognitive Sciences) at IIT is directed by Prof. G. Sandini and comprises over 70 scientists and focuses on a multidisciplinary approach to “humanoid technologies” leading to technologies and systems which have human-like features and performance and can directly communicate with the human nervous system. The unit of RBCS contributing the most to this proposal is the Brain Machine Interfaces (BMIs) unit, which is directed by Prof. L. Fadiga (a leading neurophysiologist with 20 years of experience), comprises more than 15 scientists. The department laboratories are equipped with state-of-the-art computational facilities, acute and chronic neurophysiological laboratories, animal facility, humanoid robotic platforms, human behaviour and neurophysiological measurement devices (EMG, TMS, EEG, NIRS), electronic laboratory, and mechanical workshops.

Role of RBCS in the project. Electrical Microstimulation, sensory stimulation and recording with high density multielectrode arrays in somatosensory and motor cortex (WP4), realization of bidirectional state dependent BMIs (WP8), interfacing of neuromorphic chips and neural activity for online state detection (WP7).

Key investigators and Relevant Expertise.

Alessandro Vato (Team Leader) has considerable experience in large scale recordings from somatosensory and motor cortex of both anesthetized and behaving rats (WP4), and in bidirectional BMIs (WP8). **Chiara Bartolozzi** (Team Leader) is a VLSI engineer with track record in designing and producing and testing neuromorphic computational devices and is the coordinator of the EMORPH FET Proactive project. She will be the key scientist in the interaction between modelling (WP6) experimental data analysis (WP5) and VLSI fabrication and test (WP7).

Within this project RBCS will host (as Visiting Professor) **Ferdinando A. Mussa Ivaldi** from Northwestern University in Chicago, a world leader in the development of Brain Machine Interfaces who has pioneered the first proofs of concepts and the conceptual developments of bidirectional BMIs.

The NBT (Department of Neuroscience and Brain Technologies) is directed by Profs. J. Assad and F. Benfenati and has the objective to apply new technologies to the study of the central nervous system. The Department consists of an electrophysiology unit (including neuroelectronic interface laboratories), a molecular and cellular neurobiology unit and a clean room fully equipped for fabrication of neural interfaces.

Role of NBT in the project. Technology development for high-density MEAs and optogenetics; in-vitro experiments on neuronal cultures; in-vitro investigations of state dependency of neural activity. IIT-NBT will coordinate WP2.

Dr. Luca Berdondini, PhD, is responsible for the contribution of the NBT department to this grant. Berdondini is a team-leader focusing on the development of innovative chip-based high-information content neuroelectronic interfacing platforms for fundamental and applied neurophysiology. With a strong background in microengineering and micro-/nano-technology, he pioneered the development of APS-MEAs and of the current high-resolution MEA platform.

SISSA

SISSA (www.sissa.it) is the acronym of Scuola Internazionale Superiore per Studi Avanzati and it is one of the very few Italian institutions dedicated exclusively to graduate and post-graduate training. It is considered one of best scientific institutions in Italy (Nature, vol 438, 16 December 2005, pp 1046-47) according to several recent rankings. Initially concentrated around Theoretical Physics and Mathematics, SISSA has expanded its range of activities to comprise new, highly promising and dynamic fields such as Neuroscience, Genomics and Nanoscience.

Role and expertise of SISSA in the proposal. The SISSA group will be responsible for the analysis of ongoing dynamics in the leech nervous system and all the related statistical analysis. Vincent Torre has been studying from several years the electrical activity in the leech nervous system and its relation to behaviour. In particular, Torre laboratory has already studied several aspects of sensory coding and of the ongoing electrical activity. Therefore his laboratory is highly suited to carry out the activities described in WP2.

Vincent Torre 's scientific career is essentially focused on seeking an interaction between Biology and Physics and, for this reason, it can be described as genuinely interdisciplinary. Vincent Torre has always concentrated his efforts on three major areas of research (Structure and Function of Ionic Channels, Neuronal Networks and Computational Neuroscience) complementary among them, and, over the years, he has given for each one of them major contributions. Vincent Torre has expertise in a variety of electrophysiological recordings with suction pipettes, patch pipettes and intracellular recordings with fine pipettes. He is a physicist by training and he is able to apply sophisticated mathematical tools to analyse and model collected experimental data. Vincent Torre is also expert in image processing and in also in imaging the electrical activity both with Calcium and Voltage sensitive Dyes. At SISSA there are facilities for culturing neurons, stem cells and neuronal networks from rat and mouse brains. There are also excellent facilities for microscopy (confocal, AFM, fluorescence,...) tissue culture, molecular biology, gene expression profiling and bionanotechnology.

Max Planck Institute for Biological Cybernetics

The Max Planck Institute for Biological Cybernetics is one of the 79 independently organized research facilities of the Max Planck Society (MPG) that carry out basic research in the interests of the general public. Research in the Department "Physiology of Cognitive Processes" concentrates on the neural mechanisms of visual perception and object recognition

in primates and uses combined psychophysical and electrophysiological experiments utilizing nuclear magnetic resonance imaging. The department comprises 7 senior scientists, 22 postdocs, 24 PhD students and 22 staff persons (including NMR physicists and veterinarian). The laboratory is equipped with custom-made high field (4.7/40cm) and very high field (7T/60cm) NMR Spectrometers and has direct access to a comprehensive mechanic and electronic workshop that will be extensively used for all the hardware required in this project. The laboratory is equipped with setups for experiments in both alert and anesthetised monkeys and rats.

Main tasks and role in the project. Establish relation between optogenetic stimulation of the geniculostriate pathway and spontaneous cortical state on V1 responses (Logothetis). Establish relationship between electrical stimulation of neuromodulatory pathways and spontaneous cortical state on cortical responses (Eschenko).

Previous experience relevant to these tasks. The laboratory has a very extensive track record on pioneering research and using a variety of techniques to tackle scientific questions. They include human and animal psychophysics, intracortical recordings, EEG studies, HPLC-MS-MS capillary sampling for the detection and quantification of neurotransmitters in the behaving animal, invasive or non-invasive fMRI, MRI-based in vivo connectivity studies, development of MR-detectable smart agents), combined electrical and microstimulation. The Logothetis laboratory is the first worldwide to implement simultaneous invasive neuroscientific techniques (e.g. electrophysiology, injections, stimulation, etc.) with functional MRI. All hardware and software that permitted such measurements were developed in house (several patents). The first study using this multimodal approach, published in Nature 2001, provided first insights into the neural basis of the BOLD imaging mechanism and remains one of the most cited among the top ten papers of the entire Biology field.

Researchers

Professor Nikos K. Logothetis is director at the Max Planck Institute for Biological Cybernetics. He published about 360 articles mainly in high-ranked journals. His recent work includes the application of functional imaging techniques to monkeys and measurement of how the fMRI signal relates to neural activity. He is in the Advisory Board of i) McGovern Institute, M.I.T., ii) Brain and Cognitive Sciences, M.I.T. and iii) POSIT Science Corporation, San Francisco; receiving Editor in the European Journal of Neuroscience (EJN), and associate editor in Trends in Cognitive Sciences (TICS), Neuron, Current Biology, Current Opinion Neurobiology, and regular reviewer for Nature, Nature Neuroscience, J Neuroscience, PNAS, Cerebral Cortex, Cerebral Blood Flow and Metabolism, Journal of Neurophysiology, Experimental Brain Research, and Vision Research. Awards: DeBakey Award for Excellence in Science (1996), the Golden Brain Award of the Minerva Foundation (1999), the Louis-Jeantet Prize of Medicine (2003), and the Zülch-Price for Neuroscience (2004).

Dr Oxana Eschenko is team leader in the Department “Physiology of Cognitive Processes” and has 12 years of postdoc experience. She has an extensive track record in the neurobiology of learning, sleep and memory, and in the neurophysiology of neuromodulatory systems. She has broad expertise in combined fMRI and neurophysiology in the rat.

University of Zurich

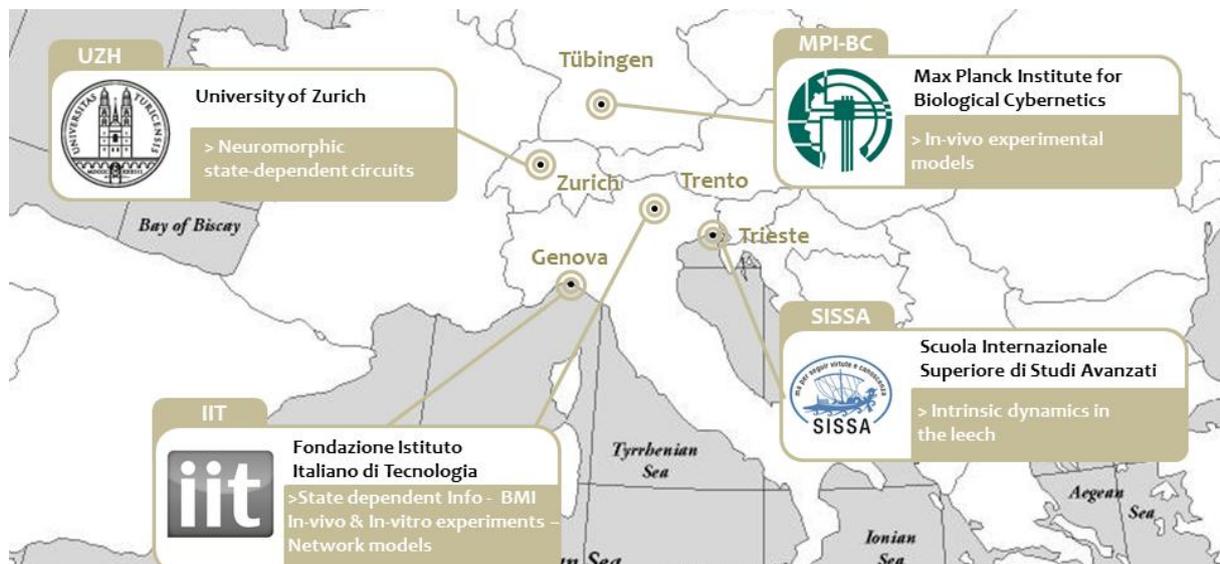
The Institute of Neuroinformatics (INI) is a joint institute of the Swiss Federal Institute (UZH) and University of Zurich (UZH). It was founded in 1995 under the directorship of Profs. Douglas and Martin to carry out experimental, theoretical and applied research with the aim of discovering key principles by which the brain is built and works, and to use this knowledge in practical applications where possible. The Institute has a staff of over 70 persons. It is located in purpose-built accommodation on the University of Zurich Irchel campus. In addition to experimental neurophysiology and neuroanatomy facilities it includes extensive electronic design, development and test equipment, and computer support. The INI environment is highly interactive and interdisciplinary. Personnel range from neuroscientists, computer scientists, engineers, physicists, to psychologists whose expertise we can also draw on when necessary.

Main tasks and Role in the project. UZH will be responsible for designing neuromorphic circuits which implement the neural operators developed by the other consortium partners, and fabricating a VLSI device that will be used to evaluate the state of the neural system we record from and produce state-dependent output signals that can be used to drive conventional BMI circuits for producing appropriate neural stimulations.

Expertise. UZH has extensive experience in the design of neuromorphic VLSI devices and in the implementation of real-time behaving neuromorphic systems, that is well matched to the requirements of this project, and complementary to the competences of all other consortium members.

Prof Giacomo Indiveri graduated in Electronics Engineering from the University of Genova, Italy in 1992 and obtained a post-doctoral fellowship at Caltech from 1994 to 1996. He has since gained more than 10 years experience in the design and fabrication of neuromorphic electronic circuits that implement cortical processing. He is an expert in the construction of spike-based learning, winner-take-all architectures, and hardware attentional systems. Indiveri is member of numerous scientific committees of international conferences on advanced circuits and systems design, and has been co-organizing the annual Neuromorphic Engineering Workshop since 1999. He was appointed associate editor for the 2003 IEEE Transactions on Neural Networks, Special Issue on Neural Networks Hardware Implementations, and recently published the book Analog VLSI: Circuits and Principles, with S-C Liu, J Kramer, T Delbruck, and R Douglas. In addition to his personal involvement, Indiveri will be supported by 1 Post-Doctoral researcher, working on the analog VLSI design and layout activities.

B 2.3 Consortium as a whole



The consortium (see above) is constituted by 4 research groups from 3 different countries. The project partners have not only complementary expertise and facilities but also cross-boundary competences, allowing them to address all the required development tasks. Although the consortium has not collaborated before as a whole, it builds on some existing bilateral collaborations between pairs partners, thereby guaranteeing the implementation of a very effective collaboration. Stefano Panzeri (senior scientist at IIT-CNCS and project coordinator) and the group of Nikos Logothetis at MPI-BC have been collaborating for the last five years on information analysis of neurophysiological and fMRI experimental data and on neural network models and information coding; Chiara Bartolozzi, Team Leader at IIT, has collaborated fruitfully for several years with Giacomo Indiveri (UZH). Despite this common research already in place among some of the partners, the SI-CODE consortium has never worked all together. It is now seeking to join forces to go beyond the single current research towards new innovative research which could benefit not only the single institutions but also the European Research Area at large. Without this grant, this cooperation would not be possible.

The Partners for this proposal have come together in recognition of the potential for very effective collaboration as a result of their complementary expertise and facilities. The collaboration is designed to provide a balanced input of basic and applied research effort. The coordinating group is based at IIT-CNCS and is a worldwide leading group in the field of computational neuroscience and neural information processing; the MPI-BC group is the undisputed world leader in the development of techniques for the simultaneous recordings of neural activity at different spatial and temporal scales; the IIT-NBT and the IIT-RBCS have top class expertise in neuroscience and neuroengineering to microtechnology;; and the UZH group is a world leading group in the field of neuromorphic engineering; the presence of a strong interdisciplinary, neuroscientific oriented group at SISSA with long-lasting experience in neurophysiology and a strong background in biophysics will guarantee excellent interchanges among the different disciplines present in the consortium.

Additionally, the researchers working on the project will have an extensive input and technical help from other faculty members not funded by the project. Within each workpackage, the workpackage leader will have the responsibility of day-to-day scientific and technical management. As described in the Management structure the project steering committee will collectively take the responsibility for monitoring progress against defined deliverables and milestones.

The Project Steering Committee will be responsible for the correct utilization of funds awarded to their respective institutions.

B 2.4 Resources to be committed

Considering the very good level of equipment and facilities of the involved laboratories, no major costs for instruments are foreseen. The laboratories that will carry out experimental works on electrophysiology will upgrade their existing set-up in accordance with the needs to accommodate the new developed systems. Therefore, a small budget for equipment is foreseen for the interested-involved partners. The main allocated budget is for personnel and will include mainly young postdoctoral researchers. More senior positions were requested by SISSA due to the high quality of the senior expertise available there for the experiments to be carried out for this project. The total effort requested (see tables in WorkPackage descriptions) for the entire project consists of 176 man months, of which 151 are requested to the EU and 25 will be contributed by the IIT. The other significant costs are related to the fabrication for the realization of VLSI circuits. Additionally, in order to test and provide a ready-to-use platform to the partners, a budget for the mechanical and electrical workshops is planned. The consortium seeks for a substantial equilibrium of resources allocated among the partners. IIT seeks for more support than other partners because of coordination costs and because IIT effectively makes three independent contributions to SI-CODE (one from the CNCS department, one from the RBCS department, one from the NBT department), each of which is comparable to that of one of the other individual institutions. The research activities of the project include extensive experimental activity, data-analysis (with new methods to be developed as part of the project) on multi-site spike trains, and neural network modeling. Such activities are heavily time consuming and this would justify the relatively large amount of person/months needed for development of some WPs such as WP1-4, and WP6-7. There are two visiting Professors in the project. Prof. Mussa Ivaldi (Northwestern University) will visit IIT-RBCS to contribute to the experimental setting up and the algorithmic implementation of the non-state dependent BMI (leading to Deliverable D8.1) and to contribute to the algorithmic implementation of the state-dependent real time bBMI algorithms in software and hardware (leading to Deliverable D8.3). Prof. Brunel (Chicago University) will visit IIT-CNCS to contribute to the development of the approximated analytical methods for determining the stimulus-state-response transfer functions (leading to Deliverable D6.1) and to contribute to the best fitting of models to experimental data (leading to Deliverable D6.2).

2.4.1 IIT

Personnel Costs				
Role	Number	Salary/month	Man months	Cost

Senior scientist(MGT)	1	11.928	1.5	17.893
Admin. officer (MGT)	1	3.000	2.5	7.500
Senior scientist/Team Leader	3	8.810	9	79.295
Visiting Professor	2	8.000	6	48.000
Post-doc	3	3.901	132	533.097
TOTAL PERSONNEL COSTS				685.785
Other direct Costs				
Description				Cost
Consumables				107.000
Travels (RTD + MGT)				73.126
Equipment				32.900
TOTAL OTHER DIRECT COSTS				213.026

IIT budget for Consumables and Equipment will be used as following:

Consumables		
Amount	Type	Purpose
2,000 €	Purchase and maintenance of experimental rodents	D4.1, D4.3, D8.2, D8.3
3,000 €	Switching headstage for acute recording and electrical stimulation	D4.1, D4.3
2,000 €	Surgery tool for acute experiments and chronic implants of microwires arrays	D4.1, D4.3, D8.2, D8.3
13,000 €	Stimulation and recording high-density microelectrode arrays for acute and chronic experiments.	D4.1, D4.3, D8.2, D8.3
8,000 €	Optical components for light stimulation (objectives, LEDs, waveguides) and electronic components for managing light stimulation combined with high-resolution MEAs recordings (BioCam 4096)	D2.1
15,000 €	Neuroelectronic microelectrode array devices (APS-MEAs chips and MEAs, ~ 250€/device)	D2.1, D2.2, D8.2, D8.3
15,000 €	Animals for dissociated neuronal preparations (rats, mice), ~ 250€/animal	D2.1, D2.2, D8.2, D8.3
12,000 €	Biochemicals for optogenetic probes (for infection, electroporation) and Biopatterning tools (masters for μ -CP, cellular adhesion/inhibiiton molecules)	D2.1, D2.2
6,000 €	Lab consumables for cell cultures preparation, culture and experiments(e.g. Plasticware, Tubing, Small Material)	D2.1, D2.2
9,000 €	Electronics & Mechanical workshop	D2.1
15,000 €	Chemicals for cell culturing, Drugs for chemical stimulation/modulation, Compounds for Immunofluorescence Imaging)	D2.1, D2.2
Equipment		

6,000 €	Multicore workstations dedicated for hard calculations to develop computational methods for the state-dependent information analysis and also to back-up and exchange data creating a website with the developed algorithm and a dataset repository for simulated and real data.	D5.1, D.5.2
17,000 €	High-resolution MEA platform Hardware and Photostimulation Hardware (FPGA board, A/D converters, frame-grabber, 1 PC for data acquisition)	D2.1, D2.2
9,900 €	Multicore workstations for numerical simulations of networks	D6.1, D6.2

2.4.2 SISSA

Personnel Costs				
Role	Number	Salary/month	Man months	Cost
Full Professor	1	13.000	2	26.000
Researcher	1	4.200	34	142.800
Researcher	1	4.200	12	50.400
Postdoc	1	2.600	12	31.200
TOTAL PERSONNEL COSTS				250.400
Other direct Costs				
Description				Cost
Consumables				50.000
Travels				13.800
Equipment				30.000
Publications				5.000
TOTAL OTHER DIRECT COSTS				98.800

SISSA budget for Consumables and Equipment will be used as following:

Consumables		
Amount	Type	Purpose
12,000 €	Animals: 100 leeches per year	30 Euro x leech + 1.000 Euro for shipping, maintenance and feeding
6,000 €	Chemicals	For preparing solutions and for blocking and modulating the ongoing activity (NMDA blockers, K+ blockers,...) Costing approx. 2.000 Euro/year
5,000 €	Redesign of amplifiers	Task 2.1: need to redesign the amplifiers and rebuild the head-stages.

500 €	10 low noise Lemo-Lemo Cables	Consumables and small items for electrophysiological recordings
2,500 €	Bi-phasic Stimulator Unit	
1,500 €	4-Axis Micromanipulator	
2,000 €	Dissecting tools and minipins for preparing ganglia and leech dissection	
2,000 €	4 Teflon Valves for perfusion and tubing	
1,300 €	Temperature Controller for Objective	
4,000 €	Mechanical stimulator	Task 2.3: upgrade of a mechanical stimulator previously used and acquisition of some new mechanical and electronic components.
7,000 €	Software upgrade	3.000 Euros: 2 Matlab licences and some associated Tools 4.000 Euros for the implementation of changes in the present software for data analysis coming from the new algorithms developed within SI-CODE
1,200 €	Small mechanical items (tubing, wires, pipette,..)	
1,000 €	Electronic components (resistors, amplifiers, portable hard disks	
4,000 €	Electrode holders + platinum wires	200 EUR/each: Use of about at least 15 electrode holder Reasonable supply of platinum wires
Equipment		
Amount	Type	
5,000 €	Digidata from Axon (A/D converter)	
6,000 €	Puller from Sutter	
10,000 €	Axon Amplifier for intracellular recording	
5,000 €	Antivibrating top	
4,000 €	Hydraulic microdriver	

2.4.3 MPI-BC

Personnel Costs				
Role	Number	Salary/month	Man months	Cost
Post-Doc (RTD)	2	5.407	45	243.315
TOTAL PERSONNEL COSTS				243.315

Other direct Costs	
Description	Cost
Consumables	73.000
Travel	13.500
TOTAL OTHER DIRECT COSTS	86.500

MPI-BC budget for Consumables and Equipment will be used as following:

Purpose	Amount
25.000 €	MRI experiments
2.250 €	Homemade stimulation electrodes
6.000 €	Electrode arrays
7.500 €	Miscellaneous Supplies
2.250 €	Rats
30.000 €	Monkeys

2.4.4 UZH

Personnel Costs				
Role	Number	Salary/month	Man months	Cost
Post-Doc (RTD)	1	6.665	36	239.940
Full Professor (RTD)	1	11.046	3	33.138
TOTAL PERSONNEL COSTS				273.078
Other direct Costs				
Description	Cost			
Equipment	15.000			
Consumables	65.200			
Meeting (travel)	9.200			
Dissemination/conference travel	3.500			
TOTAL OTHER DIRECT COSTS	92.900			

UZH budget for Consumables and Equipment will be used as following:

Consumables		
Amount	Type	Purpose
15,380 €	Fabrication and packaging of first 12mm ² prototype neuromorphic chip using the AMS 0.18um technology via the Europractice service. The basic fabrication cost is of 1100 EUR/mm ² for registered academic institutes (such as UZH). The additional expenses cover chip packaging costs, shipping expenses, etc.	D6.2
36,620 €	Fabrication and packaging of second 30mm ² neuromorphic chip	D6.5

9,200 €	Printed circuit boards (PCBs), electronic material and assembly costs for hosting and controlling the fabricated chips. As each chip fabrication run returns 10 samples, we plan to build several PCBs, allowing multiple project partners to work with the custom VLSI devices (e.g. 2xtesting setups, 6xapplication-ready PCBs).	D6.3, D6.6, D6.7
4,000 €	Software licenses, electronic components and other consumables	For the (already existing) instrumentation that will be used to design and test the custom VLSI.
Equipment		
Amount	Type	Purpose
6,500 €	VLSI design flow and simulation server	For running the circuit and layout design tools as well as the CPU intensive SW simulations of Task 6.1. The server will also manage version management repositories, on-line wikis, etc.
4,500 €	CAD design station	Including a dual-screen setup for circuit schematic and layout design (for D6.2 and D6.5)
4,000 €	VLSI chip evaluation and testing setup	Comprising a PC and interfacing boards for acquiring signals from the custom VLSI devices and characterizing their computational properties (for Task 6.3 and D6.7)

2.4.6 Subcontracting

Subcontracting is used only to cover the costs related to the obligation of submitting audit certificates by three partners. The total amount for subcontracting will be 15.200€, divided as following:

IIT: 11.000€

SISSA: 5000€

MPI-BC: 2200€

B3. Impact

B 3.1 Strategic impact

Impact on Science

From the scientific point of view, the most prominent impact is that the results of SI-CODE will force neuroscientists to rethink radically about the variables to measure when studying neural activity, its role in coding and brain function in general. SI-CODE will show that understanding neural computations not only requires the study of the “driver” signals

reflecting neural processing of sensory or cognitive information, but also of the ongoing dynamics of the neural circuit. This knowledge will elucidate the role of ongoing activity on neural information coding, thereby leading to a paradigm shift in studies of neural coding and of brain function in general, which previously considered state dependence mostly as noise or nuisance rather than an integral part of the neural messages.

SI-CODE will provide a completely novel set of neural coding principles leading to a new set of explicit professionally coded open source algorithms which decode neural activity by using state information to discount state-induced variability. These algorithms, and ways to quantify neural responses, depart substantially both from previous studies decoding algorithms (which ignored the network state) and from previous studies of state-dependence of neurons (which did not explore the implications for decoding). The created algorithms will have a profound short-term impact on the analysis of all levels of neurophysiological investigations: high density recordings from cultures and slice; multiple-site recordings from invertebrate and vertebrates; and intracranial recordings performed in human patients to test the functionality of the brain tissue or to detect the focus of epilepsy. During these experiments the scientists will not only measure the response to the stimulus, but they will also measure it together with the “internal state” parameters that SI-CODE will find to be informative.

Brain research is about to undergo a transformation to a new phase where scientists investigate brain functions by interacting and dialoguing with the nervous system and probing and manipulating it, rather than simply observing and characterizing neural function.

SI-CODE will lead to a paradigm shift in developing bidirectional communication with the brain. Information and communication technologies (ICT) will play a key role in this transition. The research of SI-CODE, and in particular the research leading to a state dependent bidirectional BMI prototype, will deeply influence the way neuroscientists will use ICT to probe brain functions by interacting with brain. As the variables likely to express the network state (such as the balance of excitation and inhibition, reflected in membrane potential or LFP fluctuations) are spatially coherent over large distances, neurons communicating within a brain’s network do not only have access to their own output, but also to the state variables (i.e. the biophysical “context”) that generated the response. Most likely, this sharing of information within a common knowledge of biophysical context is a key rule of within-brain communication. Yet, current plans and prototypes to probe brain function with dialoguing ICT systems do not include the crucial “brain context” variable in the communication yet. SI-CODE will provide the crucial breakthroughs that will make this possible. Moreover, without the development of efficient coding/decoding schemes to dialogue with brain signalling, the implementation of efficient and truly miniaturized BMIs for brain-prosthetics requiring the implementation of optimized technological solution for managing critical issues related to power consumption, packaging and device sizes, will remain unclear and of limited efficiency.

In summary, our expectation is that SI-CODE will deeply impact on the way that investigators will interface the nervous system to probe it for basic scientific research and for developing BMIS. Extraction of information from neural activity will be greatly facilitated because the knowledge of state dependence of neural responses will enable discounting the very (likely considerable) fraction of neural variability which is due to ongoing changes of cortical excitability. The research of SI-CODE is also likely to have a large impact upon our

ability to give feedback information to the nervous system by means of electrical or optogenetic stimulation. While optogenetic stimulation provides unique cellular/population specific selectivity, the combination with electrical stimulation will enable the consortium to fine tune the stimulation of neuronal responses. This unique exploratory experimental framework will contribute in refine the current knowledge on how to effectively evoke responses, with direct implications in both selectivity and safety with respect to currently used stimulation techniques. The idea of injecting information into the nervous system relies on the being able to evoke by means of stimulation a given pattern of neural activity that in turn elicits a certain sensation or percept that the subject needs to experience in order to perform a task (for example, information about the relative distance from a target to be reached or information about the temperature or weight of an object to be grasped). The possibility to generate selective percepts by local electrical stimuli to the somatosensory cortex has been demonstrated by numerous studies both in humans and in non-human primates. However, despite some success in eliciting and controlling percepts by brain stimulation, our current ability of eliciting a desired, behaviorally relevant pattern of neural activity is limited. One problem is that little is yet known about how microstimulation works and how the evoked activity propagates in the nervous system and is processed by it. The unique technology of Prof. Logothetis (MPI) puts the SI-CODE consortium in the position to track down and measure (by combining electrophysiology and fMRI) the propagation and effect of microstimulation in the brain. Indeed, the new experiments of WP4 will provide a large bulk of new knowledge about how specific patterns of neural activity are elicited by different types of stimulation (electrical or optogenetic); how the elicited patterns related to both the cortical state and the stimulation parameters; and how the elicited patterns are processed through the brain. Moreover, the discoveries to be made within SI-CODE about the dependence of elicited neural response patterns upon both cortical state and stimulation parameters will also likely lead to improvements in specificity and safety of providing feedback information to the brain. Electrical brain stimulation is already common in clinical practice, both in the acute exploration of cortical responses for surgical procedures involving the ablation of brain tissue and in chronic implant of deep brain stimulation electrodes for treatment of Parkinson's disease. Stimulation of the visual cortex is being tested by other groups (for example those working in visual prostheses, such as the Eye Clinic in Tuebingen). However, the stimulation of somatosensory areas by chronically implanted electrode arrays has not yet been attempted in humans. Current electrical stimulation protocols in various preparations (from cultures to in vivo) usually try to elicit a “good” (i.e. sustained) neural response by injecting relatively strong currents. The knowledge gained by SI-CODE about state dependence of responses to stimulation could be used to minimize (or more generally, optimize) the amount of current needed to elicit a desired neural response, thereby increasing the safety of the stimulation and minimizing its potential long-term impact on the nervous system. For example, if we determine that the cortical region under consideration is in a “highly excitable” state then we will know that we can inject less current than when cortex is in a “less excitable” state and yet obtained a desired response. It is also conceivable the knowledge of SI-CODE could help us improving the specificity of the feedback information to be injected, because SI-CODE proposes to use the additional knowledge of state dependence to fine tune stimulation and optimize the match between desired and elicited neural response.

Impact on society

SI-CODE seeks to lay the foundations for a long-term impact on society.

One of the key societal challenges that ICT tries to overcome is the provision of innovative intelligent systems solutions to support healthcare, especially for facing demographic ageing and social inclusion. Some key solutions include:

- The development of services, social robotics and highly intelligent systems in support of the ageing population, enabling people to live in a more autonomous way, with improved independent living and quality of life, and ensuring at the same time a higher efficiency of care.
- The development of smart and customized solutions based on human-machine interfaces to improve social inclusion of people who are suffering of social exclusion as a consequence of a major motor-related disability and in need of constant assistance.

In both cases, such systems could be used not only for increasing the independence of the above mentioned target groups but also for a better delivery of healthcare, especially for motor rehabilitation, both on site and remotely, thanks to systems allowing a more accurate diagnosis followed by a better targeted support.

Brain-Machine Interfaces (BMIs) can play a key role. They are widely considered as one of the means by which ICT can contribute to alleviate some specific and otherwise untreatable types of motor-related disabilities and the social exclusion that these diseases bring upon the patient. While current BMIs prototypes tested with animals are undisputed milestones in neural engineering, their current performance is so limited that they need to be radically improved before they can be used and make a significant impact on human healthcare. Indeed, SI-CODE aims at making significant breakthroughs to address the three major problems in the current state-of-the-art BMIs, as follows.

The first problem is that the high variability of neural responses limits severely the bandwidth of communication with the brain, and this small bandwidth in turn limits both the number and complexity of the tasks that a BMI can execute, as well as the accuracy and reliability of execution. SI-CODE lays the scientific foundations for eradicating this problem, firstly by understanding the very origin of this variability (the dependence of neural responses on network states), secondly by using this knowledge to discount this variability, and finally by enlarging the number and quality of functions that a BMI can be programmed to execute.

The second limit of current BMIs that guide movement of artificial limbs for motor prostheses are based only on visually-based feedback task execution. Purely visual feedback is not enough, because it cannot convey to the brain important non-visual information that is important for compliant execution of virtually everyday tasks. For example, planning grasping requires tactile or temperature information about the object to be grasped, as well as proprioceptive signals originating from the joints, tendons and muscles. SI-CODE will provide important progress to this limitation, by providing a BMI prototype that executes a motor command by decoding neural activity and at the same time communicates to the brain non-visual information about the progress of task execution.

A third problem concerns the need for BMI users to dedicate undivided attention to the instantaneous performance of a task. This is, again, unlike movement physiology. When we open a door, we do not need to focus our minds on the motions of our fingers wrapping

around the handle. But we can do just that, if we so choose. In order to assist people with motor impairment in the execution of tasks and help them towards an autonomous existence in a social context, it is important that BMIs can voluntarily and consciously access the motor programme that automatically executes a task, rather than having to focus the attention on the execution of even the simplest motor command. Our final ICT prototype, the bidirectional state-dependent BMI, provides a novel way to establish a programmable, automatic motor behaviour, which is inspired by the mechanisms by which the spinal cord operates the translation of cortical commands into motor behaviour. The possibility of a selection of this automated behaviour by a voluntary movement will begin to be explored by the final stage of SI-CODE, in which the awake animal will learn to use the bBMI. The inclusion of state dependency is likely to play a crucial role in the success of the implementation of a volitional selection mechanism, as volitional commands may act by modulate the state of the neural network which communicates with the interface.

Taken together, these considerations indicate that the state-dependent bBMI of SI-CODE will lay the foundation for the integration of voluntary commands and pre-programmed automatic responses, so as to generate dynamically stable movements.

The challenging idea of restoring motor function for patients with disabilities has driven the research of an increasing number of scientists in multidisciplinary fields worldwide. This interest is also related to the fact that in ageing societies, people that suffer of diseases related to the nervous system represent an increasing social cost. The development of Brain Machine Interfaces with the goal of improving the quality of life of these patients has to face two main issues. First of all the first fundamental issue is whether a BMI system is safe. In particular for invasive BMIs for clinical applications the researchers and the neurosurgeons need to take in account the half-life of the device and how the patient responds histologically to the implant. The other issue is the performance-related factors that we need to consider before a clinical application of an invasive BMI. For these two reasons, nowadays patients that may benefit by an invasive BMI are a subpopulation of the large community of people with motor disabilities. In particular we can restrict the possible clinical applications to people with “severe” motor disorders as people suffering of midbrain stroke with locked-in syndrome, ASL, cerebral palsy and spinal cord injury with severe paralysis. This is a small fraction of a vast population of patients with movement disorders that counts 2 million of people in U.S and among them 700.00 with a disability involving the spinal cord, with a lifetime medical cost that can range from US\$624,000 to US\$2.8 million (source: World Health Organization). One of the main goals of the SI-CODE project is to restore the natural sensory-motor interaction by developing a closed-loop system and by improving the decoding and encoding algorithms. This objective, related to the performance-related issue of the actual BMIs, could open up the user community to people with limbs amputated that need to restore the lost sensory channels. Moreover, if SI-CODE succeeds in improving the “giving feedback” part of the BMIs, the breakthroughs made by our research could in principle be used for improving purely sensory BMIs such as cochlear or retinal implants.

In order to have the above described long-term impact on healthcare, SI-CODE results need further development and integration. Nevertheless, the consortium considers it key for a more long-term exploitation approach and as potential field for seeking commercial application and support.

Impact on technology

From the technological point of view, our better understanding of the state dependency of neural responses will have a direct and immediate impact on the development of BMIs which are currently being developed with the aim of restoring motor function to people whose brain can still deliver motor commands or intentions but the connection with actuators is non-functional. It is in fact commonly accepted that the most serious bottleneck in performance of the current BMIs stems from the limited amount of information that can be extracted from each electrode. This relatively small amount of information limits at the source the reliability and the number of the different operations that can be accomplished by such devices. Our project will deliver a specific set of algorithms that can enhance this “information bottleneck” in all cases in which the state dependent information is much higher than the information obtained without taking into account the network state. Researchers in BMI will be able to use these open-source algorithms immediately and so to improve the performance and the reliability of such devices.

Furthermore, the implementation of new computing paradigms in low power VLSI networks of spiking neurons will dramatically enhance the impact of the project: it will lead to the first hardware spiking neural network applying neural-context/state depended processing for a real-time task (in this case, online bidirectional communication with the brain). Current digital technologies are rapidly approaching the resolution limit of the silicon technologies and it is believed by many that the massively parallel, low-power, asynchronous spike based technology offered by neuromorphic VLSI provides a potential way forward. An increasing number of research institutions and government agencies are indeed beginning to recognize that the neuromorphic VLSI technology is likely to be a key technology for the future (e.g. see the NSF-funded Centers for Neuromorphic Systems Engineering and the EU life-like perception systems stock-taking report). However, key for the success of the technology is the development of a theory and methodology to guide the design of large-scale multi-chip networks of spiking neurons.

Therefore, SI-CODE is clearly positioned to contribute to a major technological challenge.

The entire system will be implemented using a real-time hardware platform composed of biologically realistic neuromorphic spike based processing elements. By tightly constraining solutions, we are forced to take seriously the processing constraints and requirements faced by biological systems. This is a difficult problem, and as yet there are no neuromorphic solutions. A key aspect of this technological solution is that it should support flexible reconfiguration of representations “on the fly” in order for the system to adapt and to model dynamic changes in brain state and in neural recording conditions. The development of a system architecture which supports high-level context dependent responses responding autonomously to such changes will be a major and highly impactful step forward.

In the short term, neuromorphic engineering has a clear path to become part of the silicon industry. This can be achieved by building convincing prototypes, distributing them widely to potential users, and by convincingly solving difficult problems. The ideas for how to use spikes effectively for computation in application scenarios will probably come from outside users who will not even think about spike based computation until they get their hands on the devices, and systems, interfaced to conventional digital architectures. SI-CODE can accelerate this process by teaching techniques of neuromorphic computation with practical exercises at

university courses carried out by some partners, and by continuing to encourage the use of the systems produced by SI-CODE in application scenarios to expose their weaknesses and at the same time to learn the necessary steps for improvement.

In addition to neuromorphic technology, SI-CODE will provide an efficient and powerful event, by processing hardware infrastructure that is programmable, re-configurable and adaptive. The consortium's experience with previous projects shows that the construction of distributed systems with multiple cortical-like event processing layers is feasible. Each layer can be very sophisticated using many modules. Such cortical-like structures present very powerful computing capabilities and offer an extremely high processing speed. Currently it is known how such systems can be built in hardware, but there is still little knowledge on how such systems should be autonomously assembled and configured. SI-CODE can contribute to increase this knowledge by providing efficient spike-based computing infrastructure to the research community at large, which, together with meaningful examples (like this project's demonstrator), will most likely give life to new applications. These in turn will support the development of sophisticated computational neuroscience models and provide additional means to make progress in theoretical neuroscience, which will trigger the development of novel neuromorphic hardware systems, and so on, making this new field to range over many disciplines, industries, and society at large.

The SI-CODE consortium has also carried out a preliminary competitive intelligence analysis, in order to perform a first general assessment of the most relevant R&D investments in the fields of interest of the project. This has been done on the basis of the number of patent applications issued every year in the last 10 years. This analysis confirmed the interest towards this technology by some of the most influential industries in the ICT sector (e.g. the Japanese multinational Matsushita Electric Industrial Co (Panasonic Corporation), Siemens, IBM and Sony) as well as the realistic expectation for breakthrough innovations with high potential impact on the manufacturing of electronic and computing industry. By bringing innovative solutions in this field, there are very high chances for SI-CODE to attract the interest of industry towards its results, ensuring a very strong technological impact. The consortium will take all the necessary steps to make sure that the projects results reach these stakeholders and are taken on board for future technological development.

Finally, SI-CODE contribution to substantial innovations at the European industrial level will be given by enhancing exploitation opportunities for the SI-CODE results through a well-balanced IPR strategy. The consortium has chosen an 'open systems' approach with regards to datasets and algorithms. At the same time, licensing and spin-off policies will be promoted since the beginning of the project in order to ensure that all innovative and marketable technologies arising from SI-CODE will find a way in the market of BMIs, especially in the area of robotics interfaces and applications, where strong commercial development opportunities can be forecasted.

European dimension

Realizing the aims of SI-CODE requires making a series of breakthroughs in basic empirical neuroscience (to measure relationships between neural responses to stimuli and ongoing activity), in mathematical neuroscience (to develop state dependent information extraction algorithms, and to develop mathematical models of state dependency), in VLSI engineering, and finally in the interdisciplinary field of Brain Machine Interfaces, which sits at the

interfaces between all the previously mentioned disciplines. It is clear that achieving such goals is far beyond what could be achieved by research groups located in a single country. The necessary expertise to carry out this project is unique and scattered among different research groups in different European countries. A coordinated and truly European effort is therefore the only solution to reach the ambitious objectives set out by SI-CODE. Here is a non-exclusive list of this unique expertise: the techniques of simultaneous colocalized recordings of neural activity and fMRI signals together with electrical and optogenetic stimulation (necessary to monitor whole brain impact of stimulation) developed by Logothetis (MPI Germany) are totally unique and unparalleled in the world; the state dependent information extractions algorithms of Panzeri and the neural network models explicitly relating information coding to network dynamics (IIT) could not conceivably be carried out in other European laboratories, because they require a high level of specialized expertise which builds upon a bulk of previous research that is only available in such laboratory; similarly, the high density recording techniques developed by Berdondini (IIT) and the low power VLSI neuromorphic technology of Indiveri (UZH) are world class expertise that are not available at other sites in the SI-Code consortium; the expertise on bidirectional BMIs of Mussa-Ivaldi (Northwestern University, Chicago), which is unquestionably a pioneer in this field and will participate in this project as IIT-RBCS visiting Professor, and the analytical studies of oscillations and state changes in recurrent network of Brunel (University of Chicago), which is a world leader in such studies and will participate in this project as IIT-CNCS visiting Professor further adds to a level of skills and expertise that can only be gathered at the worldwide level and cannot be reached by a single-nation research programme.

Expected impacts listed in the work programme

In this paragraph we compare the expected FET Open Work Programme targeted outcomes against the expected targeted outcomes of SI-CODE.

Outcome #1: “Foundational breakthroughs as crucial steps towards new forms and uses of information and information technologies within a clear long-term vision that is far beyond the state-of-the-art.”

Our foundational breakthrough will consist in the discovery of a new way to conceptualize and interpret neuronal responses in terms of the state of the network that generated them. This is a genuine breakthrough from the neuroscientific point of view, and it is a result which demands the rethinking of the interfaces for communication between ICT devices and nervous systems, which never included state dependence in all realizations and prototypes developed or conceived so far.

Our long-term plan builds on this breakthrough with a clear vision. After individuating the key problem for the communication between brains and ICT devices to be neural variability, we devised the following phases to eradicate the problem: firstly to characterize the neurophysiological basis of this variability; secondly to predict, by means of rigorous and neuroscientifically grounded mathematical models, neural response variability from previously ignored neural variables; and finally to implement these predictions in low-power hardware for real time discounting of variability.

Outcome #2: “Ambitious proof-of-concept and its supporting scientific foundation, where novelty comes from new, high-risk ideas rather than from the refinement of current ICT approaches”

Our work proposes to realize an ambitious proof-of-concept of an ICT prototype which can communicate with the brain in two ways. The adopted approach departs substantially from any other previous approach to the problem of communicating with the brain as it is based on the radically new idea that bandwidth of communication with the brain can be substantially improved once we understand how to predict the origin of neural response variability which are intrinsic to the brain and we can test discount it. The project lays the scientific foundations for such a novel idea by: firstly, studying the interplay between ongoing network states and stimulus-evoked responses in various nervous systems of different complexity; secondly by developing advanced algorithms and models of network dynamics to determine the network state variables best predicting and discounting neural variability; finally by constructing optimal state-dependent rules to decode neural activity. We will implement these algorithms in a new “state-dependent bidirectional BMI” prototype using low-power neuromorphic VLSI circuits that extract in real time network state information and use it to produce outputs optimally suited for both decoding of recorded signals and delivering electrical stimulation to a neural tissue in a given state. This BMI will be tested in a benchmark experiment in rats to guide an external device with closed loop control.

Outcome #3: “New inter-disciplinary collaborations, possibly with prominent and internationally recognized non-EU research teams where these can provide a significant added value”

SI-CODE gathers a highly interdisciplinary team composed of: neuroscientists working on variety of different and complementary systems scale, from invertebrates to in-vitro and in-vivo mammalian cortical networks; mathematical neuroscientists; theoretical physicists VLSI engineers. The consortium includes a unique blend of expertises that are both fully complementary and are represented at the highest level of competence within their own discipline.

The consortium will establish a new and key research project on state-dependent bidirectional BMIs (WP8) with Prof. F.A. Mussa-Ivaldi of Northwestern University, USA). Mussa Ivaldi is a pioneer of the development of bidirectional BMIs and he is a recognized world leader in this. This link will allow the consortium to benefit from knowledge which is currently unavailable in Europe and at the same time to open a link outside Europe for further dissemination of the project’s results.

Relevance to more general FET Objectives.

SI-CODE is also very close to the more general FET objectives, e.g. the objectives of FET proactive on Brain inspired ICT, and synergies with already funded projects under these objectives will be further explored (see section on dissemination).

B 3.2 Plan for the use and dissemination of foreground**3.2.1 Dissemination plans**

SI-CODE will have a strong dissemination strategy, which is viewed as a crucial forerunner for further exploitation of the project outcomes. This is reflected in the fact that a whole work-package devoted to dissemination and exploitation activities has been included in the

project's work plan (see section 1.3), and that all the partners are committed to the success of this work package.

SI-CODE will strongly encourage interactivity amongst the different researchers, in specialized workgroups and in general meetings as well as between the consortium and the EC. The development and dissemination of knowledge and results will be promoted within the members of the project, and extended to the entire scientific community after appropriate validation.

The Project Coordinator, in cooperation with the Project Steering Committee, will be responsible for designing, organising and coordinating dissemination activities for all project outcomes, facilitating partners in the dissemination of their discipline and task specific results, fostering the collaboration among partners for the dissemination of interdisciplinary results. Due to SI-CODE strong interdisciplinary nature, this is crucial for an efficient management and dissemination of its results. The dissemination effort will provide contacts, stimulate interest, enhance cooperation and concertation and build trust among the consortium and the research and academic communities that it addresses.

SI-CODE dissemination strategy will be deployed along the following axes:

1. dissemination channels (e.g. internet, mass-media, scientific publications)
2. dissemination events (e.g. conferences, workshops, symposia, lectures, debates)
3. open-access strategies (e.g. open-source tools, public releases of data)

SI-CODE will rely on a number of different dissemination channels for promoting knowledge and technology sharing and raising awareness of the project outcomes.

Internet will be one of the main dissemination channels that will be used in the following ways:

• ***creation of a project website***

A project website will be set up at the very beginning of the project. It will function as a communication tool for the consortium and as a means for reaching the wider public as well as the research and academic community. The structure and the design of the website will reflect the different types of audience targeted by the project, i.e. there will be a restricted area for sharing documents, data and tools within the consortium as well as a public area where scientific publications, announcements on academic events, access to data, tools and other resources will be made available to the research community. *Ad hoc* sections can be developed during the project to better reply to its dissemination needs. The website will have some default pages which will provide an overview of the project and its objectives, using a language understandable also by a non-scientific public. They will include related publications for the general public (leaflets, posters, articles in popular science magazines), examples of the technology that will be developed and its potential uses, etc. A “feedback and expression of interest” section will allow interested parties to interact with the consortium members.

• ***contribution of editorials and audiovisual presentations to online science and technology portals***

Project news, views and findings/results will be reported and posted in international online science and technology portals such as AlphaGalileo.org and EurActiv.com. Whenever possible, short videos presenting the experiments and giving an overview of the work carried out within the project will also be created and posted in online scientific film and video

distribution channels such as AthenaWeb. All these portals reach thousands of broadcasters worldwide as well as the general public interested in science and technology, and the broader research community.

• ***links within related research and development project websites and use of mailing lists***

The collaboration with other projects (both EU and non-EU funded) as well as links to the project's website will be advertised in external websites and portals. A general presentation could be hosted by CORDIS but also by more specialized websites. Links to other major research projects as well as technology platforms and/or other international initiatives will be exploited to guarantee a large dissemination of the results. Dissemination of the project's news and findings will also be done through corresponding mailing lists (e.g. the media mailing list of the European Commission which reaches over 3000 journalists, but also scientific mailing lists).

A clear ***project image*** will be developed at the very first beginning of the project and will be directly linked to the website. The consortium is convinced that a project image with a pleasant and a strong graphical characterization will represent an important tool for strengthening the project's identity amongst participants, as well as towards external stakeholders. A set of guidelines concerning the project logo and letterheads, as well as templates for documents, reports, presentations and diagrams will be produced and distributed among the project partners.

Printed publications will play a complementary role to the above-mentioned dissemination activities which target mainly the general public. Printed publications are meant both for the general and the scientific public. Brochures/leaflets, posters, publications in science and technology magazines and articles in newspapers will support the more general dissemination done through the website. On the contrary, the vast majority of printed items, mainly scientific publications in international journals and conferences, are meant to target the scientific community. SI-CODE partners will publish in journals and conferences that pertain to their specific disciplines as well as to more interdisciplinary ones.

Given the paradigm shifts in understanding of brain function targeted by SI-CODE, we will target publication of such results in the main discovery journals in biology (such as Science, Nature, Current Biology, PNAS, PLoS Biology, PLoS Computational Biology), as well as in the highest impact journals that report original discoveries in both empirical and mathematical Neuroscience (Neuron, Nature Neuroscience, Journal of Neuroscience, Cerebral Cortex, Journal of Neurophysiology, Frontiers in Neuroscience, Neural Computation,). We will take a particular effort in trying to bring all the PIs in the consortium (and if not possible, as many as them as possible) to write together a review paper in some of the highest profiles review journals (such Nature Reviews Neuroscience, Trends in Neurosciences, Current Biology, Frontiers in Neuroscience) that target the wider neuroscience or the biology community, as well as in publications aimed at the general public. The tem possesses a very considerable track record in publishing regularly in all these type of highly respected journals, which ensure a wide dissemination and maximize the impact that our results would have in the Neuroscience Community.

Workshops, special sessions in conferences and other similar events will complete the dissemination plan. These tools will be mainly used to foster and promote collaborations with other related projects. Joint events could indeed be organized to showcase the

milestones/results of the participating projects and explore synergies for future research cooperation. Those events will be open to the public and will feature invited speakers in order to maximize the exposure and facilitate also the cooperation with other scientific communities. The possibility of hosting public debates on topics related to the proposed research and round-table discussions with experts will also be explored, as a new and highly-interactive way of brainstorming.

Targeted international conferences are: : annual meeting of the US Society for Neuroscience, Neural Information Processing (NIPS) conference; AREADNE biannual conference on neural coding; Computational Neuroscience (CNS) meeting; Capocaccia Workshop on Neuromorphic Engineering (organized by Giacomo Indiveri).

SI-CODE partners will also organize an open event in conjunction with the final review meeting in order to facilitate to dissemination of the project's results to a wider public. Main speakers will be SI-CODE Principal Investigators and other invited speakers.

Training actions will also be organized by SI-CODE in order to assure that the project's concepts, methods and results are not only as widely known and understood as possible but also highly influential in the target audiences. SI-CODE partners will participate in related summer schools and conferences by providing lectures and tutorials on topics related to the project. Training material resulting from the project will be gathered and used by the academic partners as part of their course materials at postgraduate level. The research activities within the project might also lead to PhD or/and post-doctoral degrees for some of the participants.

The dissemination of the project outcomes will be facilitated through the adoption of an **"open-access"** sharing which enabling the research community to build rapidly on SI-CODE's achievements. We believe that the generation, distribution and access to open research content is crucial for promoting continuous state-of-the-art innovation in the European research landscape. The main experimental datasets as well as the new state-dependent information extraction algorithms developed by SICODE will be made available to the public through pre-existing open source portals, such as IIT's www.ibtb.org and <http://code.google.com/p/pyentropy/> or additional open source channels (for example, the CARMEN neuroscience data sharing portal) that will be identified and selected during the project development.

Dissemination activities will start from the very first month of the project and will continue up until the end of the project. In order to guarantee a smooth and complete dissemination of the results, a dissemination plan will be developed during the first months of the projects and will address the dissemination for the whole duration.

Dissemination will cover the whole lifecycle of SI-CODE, starting from raising awareness on the objectives and research directions of the project, passing through the dissemination of intermediate results/findings and technologies to the presentation of the final outcomes of the project. SI-CODE results are meant to reach not only European audiences but also to have a more international echo thanks to the involvement of Prof. Mussa-Ivaldi. This will allow SI-CODE to acquire visibility in the international research landscape, giving the opportunity also to European research in general to strengthen its presence outside Europe.

Each WP team will be responsible for the dissemination of the knowledge resulting from its WP, subject to the guidelines of the WP leader. The Project Coordinator will monitor the frequency of the publication of results.

Finally, SI-CODE will contribute to the portfolio and concertation activities at FET-Open level. In order to support scientific cooperation at the FET-Open level and broad public awareness of project achievements, consortium members will ensure within the areas of interest of the project:

- a. Project results shall be published throughout the duration of the project in widely accessible science and technology journals, as well as through conferences and through other channels, including the Web, reaching audiences beyond the academic community.
- b. Beneficiaries shall deposit an electronic copy of the published version or the final manuscript accepted for publication of a scientific publication relating to foreground published before or after the final report in an institutional or subject-based repository at the moment of publication.
- c. Beneficiaries are required to make their best efforts to ensure that this electronic copy becomes freely and electronically available to anyone through this repository:
 - immediately if the scientific publication is published “open access”, i.e. if an electronic version is also available free of charge via the publisher, or
 - within 6 months of publication.
- d. Periodic press releases shall be issued, and other means of disseminating project progress to a wider audience e.g. via video.
- e. Participation in FET-organized events, for example conferences, dedicated workshops & working groups, consultation meetings, summer schools, online fora, etc.
- f. International Co-operation - contribution to relevant national and international activities (ex. Joint workshops, calls, etc. for example with NSF).

The above activities will be reported in the project’s Dissemination Plan and in periodic progress reports. In addition, the consortium agrees to include the following reference in all project-related publications, activities and events: “The project SI-CODE acknowledges the financial support of the Future and Emerging Technologies (FET) programme within the Seventh Framework Programme for Research of the European Commission, under FET-Open grant number: FP7-284553.”

3.2.2 Dual use/misuse of research results

The SI-CODE consortium is aware of potential risks to participants and society as a whole from inappropriate dissemination of the research results of the project. In order to be prepared to face potential dual use/misuse, the Project Steering Committee will be responsible for continuously control the dissemination of results and for identifying potential risks. The Committee will report periodically to the EC on potential issues too, if applicable. It will also be responsible of developing an appropriate strategy to deal with issues of informed consent and risk management for participants and for society where sensitive information is concerned.

As a general rule, the results will be made freely available as this is considered critical for the advancement of science in this field. Nevertheless, the consortium remains committed to

dealing responsibly and effectively with potential issues that may be raised by papers submitted for publication. SI-CODE scientists involved in publications should consider each time the appropriate level of risk to accomplish effective review of papers that raise such dual use/misuse issues and refer first to the already in place procedures that journals might have to reduce those risks. Each time that a risk is identified, the consortium will evaluate the best means to disseminate the results in order to maximize the scientific dissemination and minimize the societal negative impact. Papers should be modified or not be published only if an editor, after evaluating the risks, concludes that the potential harm of publication outweighs the potential societal benefits. This is considered an extremely rare exception and should be avoided as much as possible. The same procedure will apply to the research results published on the project website and any other information published in a written form.

3.2.3 Exploitation of results

A specific effort of SI-CODE dissemination and exploitation tasks will be concentrated to ensure the potential exchange of information with other research projects that focus on a similar or a complementary subject, in order to increase the cooperation among the EU or FP7 research projects in a same sector and to use synergies in the projects. SI-CODE partners have identified other research initiatives that could be potentially linked to the project a series of different joint research and dissemination initiatives. In particular already financed FP projects, such as the FACETS Project (<http://facets.kip.uni-heidelberg.de/>) will be considered. Moreover the Consortium plans to seek strong collaboration with projects funded under *Objective ICT-2009.8.9: Coordinating Communities, Plans and Actions in FET Proactive Initiatives*. The above mentioned clustering actions will represent the starting point for ensuring that the results of the project are properly prepared for exploitation.

SI-CODE project will progress new technological development as far as a final demonstrator to be used in the control of an autonomous robot with a neuromimetic chip able to exploit the new algorithms developed. The project will also prepare a sound technological basis for further research incorporating the new framework for unified experimental platform on neuronal networks, on the one side, as well as the new models of networks to be used for developing new neuromorphic devices.

Future exploitation plans include therefore a raft of further research projects emerging from the project to build on the success of the results implemented in the medium term.

In addition, whenever possible, an integrated exploitation approach will be pursued by the following activities:

- Transfer of research results into actual developments, devices and services;
- Gain feedback on economic benefits and impact of the research projects especially through surveys and interviews;
- Market examinations for the best use of research results and for creating medium and long-term business opportunities.

This general exploitation process will be achieved through the promotion of academia-industry collaborations, licensing and spin-offs. Such forms of cooperation, going beyond the project development in itself, will be sought from the beginning of the project, also with the support of professional IP service and consultants.

3.2.4 Management of Intellectual Property Rights

A consortium agreement is under preparation and will be elaborated prior to the start of the project execution. The agreement will take into account the different interest of the partners as related to pre-existing knowledge, ownership of results and new equipment and intellectual property rights. The Agreement will be based on the provision of the *Commission Recommendation on the management of intellectual property in knowledge transfer activities and Code of Practice for universities and other public research organisations* published by the EC, Directorate-General for Research in 2008.

In general terms the SI-CODE Consortium agreed on adopting an 'Open Access' approach to new knowledge generated by the project, both as far as experimental datasets and algorithms are concerned. As already mentioned, the partners will archive the main experimental datasets with a clean metadata format suitable for sharing in repositories such as CARMEN (www.carmen.org.uk) and STA toolkit (<http://neuroanalysis.org/>). There is currently no gold standard of metadata format for neuroscience sharing; therefore SI-CODE shall evaluate the proper metadata format for the shared data after discussion with the partners, with other key experts in the systems neuroscience and neuroinformatics community, and with the organizations running the neuroscience data sharing portals. Open source neural data analysis software portals will be used to promote the dissemination of the new state-dependent information extraction algorithms developed in this proposal.

This choice has been taken in order to maximize the medium and long term impact of the projects results in a field of research which is still very open to substantial innovation and where high level of interdisciplinarity is involved.

The SI-CODE Consortium is made of five research institutions without any industrial player being represented: the partners believe therefore that this kind of approach to IPR issues creates new opportunity for a better exploitation of the project results by establishing the foundations for successful research partnerships with industry and public bodies. It will moreover enhance the prospect for continued top-quality research and education in the areas of interest of the project.

On the other hand, as far as exploitation of the project results is concerned, SI-CODE consortium is aware of the fact that public research organizations need to more actively engage in the exploitation of publicly-funded research results, for instance through academia-industry collaborations, licensing and spin-offs. Therefore, if the EU evaluates the project positively, the Consortium will seek professional IP support in order to identify the most effective licensing and venture partnering solution for the medium and long term exploitation of the project's results.

B4. Ethical issues

Members of SI-CODE are aware of and will fully conform to the International, European and National legislations regulating research. The participants will take into consideration the advices given by the European and national ethics committees and in particular the advices given by the European Group of Adviser on the Ethical Implication of Biotechnology (1991-1997) and the European group on Ethics in Science and Nex Technologies (as from 1998).

4.1 General information about Animal experiments

In accordance with the Amsterdam protocol on animal protection and welfare, animal experiments will be replaced with alternatives wherever possible. Suffering by animals must be avoided or kept to a minimum. This particularly applies (pursuant to Directive 86/609/EEC) to animal experiments involving species which are closest to human beings. Altering the genetic heritage of animals and cloning of animals will be considered only if the aims are ethically justified and the conditions are such that the animals' welfare is guaranteed and the principles of biodiversity are respected. The work will follow the national laws and the Council Directive 86/609/EEC of 24 November 1986 (http://ec.europa.eu/comm/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf) on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. The consortium is aware of the opinion given to the European Commission by the "European Group on Ethics in Science and New Technologies" on "Ethical aspects of genetic modification of animals" (Number 7, 21st of May, 1996 - http://ec.europa.eu/comm/european_group_ethics/gaieb/en/opinion7.pdf). The SI-CODE consortium values the 3R principles very much, and indeed it takes a particular effort to find unifying modelling principles that could be used to rationalize and limit the experimental work (WP5). Part of the project (see WP4) is devoted to develop statistically efficient methods to reduce the number of data (and thus animals) needed to measure the quantities of interest, something on which some of the applicants) have extensive and well documented experience in this statistical work. Further partner -specific details about Ethics in animal are reported in the following.

Partner 1: IIT

For IIT, safety issues regarding care of rats/mice are taken care by the local animal facility by following safety and ethical procedure authorizations issued by national authorities, strictly obeyed in the laboratory work. The IIT Animal Facility responsible for the care, welfare and health of laboratory animals adheres to the standards of FELASA and is designated as research facility by Italian Ministry of Health (D.M.S. n. 44/94-A). License for use of animals for the research purposes included in the IIT unit is granted by the Italian Animal Experiments Inspectorate. Animal housing and caretaking, including housing of transgenic mice in a special facility according to Italian regulations, is supervised by a registered veterinarian at the IIT Animal Facility of Genova in the respect of the national current regulations regarding the protection of animals used for scientific purpose (D.L.vo 27/01/1992, n. 116). Research protocols are at first reviewed and approved by an IIT Ethical Committee and then specific authorizations are requested to the Italian Ministry of Health. In the case of approval of the proposed research project, research protocols involving animals will be formally submitted to the Institutional Ethical Committee for reviewing and consideration for approval. For in-vivo experiments planned to be carried out in IIT, during the overall project duration, 75 rats (Long-Evans, Charles River Italy) will be used as subjects following the research protocol already approved by the IIT Ethical Committee and the Italian Ministry of Health. The estimated number of animals has been reduced merging different experimental phases (e.g. by planning of using the same data collected by running the bBMI also to better understand how the ongoing activity influence information transmission).

Initially to understand the role of the ongoing activity, acute experiments will be performed according to the experimental protocol already approved. The rats will be anesthetized with a mixture of Zoletil (30 mg/kg) and Xylazine (5 mg/kg) delivered intraperitoneally and for the entire duration of the experiment anaesthesia will be maintained with supplementary doses of anaesthetic. Every 15 minutes the state of anaesthesia will be checked pinching paws strongly to attempt to elicit a withdrawal reflex. Cardiopulmonary function and body temperature will be monitored during the entire surgery procedure. To test the ability of awake rats' brain to control the bBMI just a small number of selected subjects (n=20) will be chronically implanted with microwire arrays specifically build to be kept by the animals with minimum discomfort. Ophthalmic ointment on eyes to keep them from drying out and protect them from accidental spills, local anaesthetic and pre/post operation antibiotic treatment to prevent infection will be used to permit a complete recovering from the surgery. After the surgery the rats will be monitored until they are fully awake, moving around and aware of their surroundings. During the whole experimental period the healthy condition of the subjects will be monitored and they will be kept in an enriched environment. At the end of the experimental sessions, both for acute and chronic experiments, euthanasia will be performed using an adequate dose of Tanax in deeply anesthetized animals and the local animal facility will take care of animal carcasses. For in-vitro experimentation on neuronal networks over the 36 month of the project, a total of 45 pregnant mice (i.e. *Mus Musculus*, C57B6J, from Charles River Italy) and 55 pregnant rats (*Rattus*, Sprague-Dawley, from Charles River Italy) are planned. Briefly, neuronal cultures for embryos at E18 will be prepared. However, throughout the project, the definition of the protocols for experimental validation, of the number of animals, and the choice of the animal species will be based on ethical issues that will be addressed following the 3R principles of Reduction, Refinement, and Replacement. However, it is currently impossible to replace the use of animals in this project and the mouse and the rat are the lowest vertebrate that can be used for the proposed research. Every effort has been made to reasonably estimate the number of animals that will be required for each phase of this project. We will carefully monitor our data by using a Power Analysis statistical method to ensure the lowest possible number of animals. As already stated, we will try to reduce numbers of animals by combining more experimental procedures (i.e. in-vitro and in-vivo) to be carried out and we will actively focus on avoiding unnecessary duplication of experiments among partners by regularly coordinating our experimental activities.

Partner 2: SISSA

Work at SISSA will require the use of leeches. Adult leeches *Hirudo medicinalis* or *Hirudo verbana* obtained from Ricarimpex (Eysines, France) will be kept at 5°C in tap water dechlorinated by previous aeration for 24 h. Before every experiment, animals will be anesthetized with an 8% ethanol solution at room-temperature for 15-20 minutes. Leeches will be extended and the skin will be dried carefully and then moved to the Petri dish covered with sylgard elastomere. The leeches will be immersed in 150-200 ml chilled normal ringer solution. Leeches still under anaesthesia will be pinned with fine needles in their mid-body. Animals will be dissected so to expose two central ganglia. During the dissection, the temperature will be maintained at 6-8°C using a cold chamber. At the end of the dissection, animals will be left to recover from anaesthesia and left to adapt at room temperature for 30 minutes. At present, in Italy, experiments on leeches do not require any approval from an

Ethics Committee. After behavioural experiment leeches are left to recover and are moved back to their tank. Leeches dissected for electrophysiological experiments are rapidly killed at the end of the experiment and their body transferred to an appropriate container. We plan to perform on average two experiments per week (approx. 100 leeches/year).

Partner 3: MPI-BC

Partner MPI-BC will investigate induced or spontaneous network-states in monkeys and rats using *in vivo* stimulation and recording. There is no experimentally induced pain or distress to the animals. According to German law the protocols of all animal studies performed at partner MPG will be approved by the Regierungspräsidium of Tübingen which itself carefully checks each approach for the three Rs (Replacement, Reduction and Refinement). This approval also covers the approval of the listed scientific hypotheses, the scientific objectives and the scientific measures being used. The procedures will be conducted in accordance with the latest revised version (April 2001) of the German Animal Protection Law, take into account NIH regulations as well as fully consider the regulations of Council Directive 86/609/EEC on the “Protection of animals used for experimental and other scientific purposes”. All animal experiments will be performed using procedures that are similar to those already in use at the partner site and that have already been approved by the local authorities. Experiments with animals are part of numerous ongoing experiments in the laboratories of the participants. These experiments are conducted strictly according to the legal and ethical requirements demanded by law, and have been approved by local ethical committees. All the necessary permits are available, or will be applied for as soon as the project is granted and contract is signed with the EU and will be applied to before prior to the start of the RTD activities that involve animals. Potential discomfort (‘suffering’) will be evaluated at the level of the individual animal. Animal discomfort clearly must be minimised for ethical and experimental reasons. The housing of the animals will be in full compliance with the revised Appendix A (2006) of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes – Guidelines for accommodation and care of Animals”. Suffering of the animals before, during and after the experimental sessions is reduced to a minimum. The approval by the Regierungspräsidium of Tübingen will assure that the data analysis and statistics are planned in a way to reduce the number of animals used to the minimum. When looking at the publication list there cannot be any fear that any experiments will be a duplication of already existing knowledge as the principal investigator of partner MPG is fully aware of the state of the art and only publishing in the highly ranked scientific journals. The technical setting of animal husbandry at the partner site is above the levels expected by the local authority. Animals are maintained in accordance with the Animal Welfare Act and the DHHS (Guide for the Care and Use of Laboratory Animals). All the regulations of the laboratory have been written in detail by the primary investigator in form of a Standard Operating Procedures (SOP) book that is made available to every person joining the laboratory. *Estimates of number of animals for LGN stimulation experiments.* Experiments will be conducted in rats and in the non-human primate (macaque monkeys) under anaesthesia. The project will require 20 rats and 2 monkeys per year, whereby the rats and the first two monkeys will be used for the development and optimization of the techniques. These numbers are evaluated based upon extensive previous experience of Partner MPI-BC. *Estimate of number of animals to be used for LC stimulation.* We estimated the sample size

with within-factors repeated measures ANOVA. Given a medium effect size $f=0.5$, in order to achieve the test power of 0.8 with significance level of $p<0.005$, the minimal number is 10 animals. Based on this calculation, we expect to obtain the data from 10 rats with completed series of recordings/stimulations per each experimental condition. The experiments planned for this project are technically extremely demanding, and from previous experience on similar setups we expect a 40% cumulative failure of single day experiment including 1) failure to implant electrodes in target areas – 20%; 2) failure to obtain stable cortical recordings – 10%; 3) complications due to surgery and anaesthesia – 10%. Thus, the maximal number of 14 rats per experimental condition is planned. The cases with electrode implantation outside of the LC nucleus will be used as additional control condition. Also, we will plan up to 10 animals for technical optimization of all stages of experiments. Total maximal number: 66 rats.

4.2 Requirements of the Ethical Review

This subsection reports the information needed to comply with the requirements requested by the Ethical Review Report.

4.2.1. “Justification for the use of non-human primates is needed.”

Partner MPI-BC:

In the proposed project we will be investigating the thalamo-cortical system in the non-human primate as the Logothetis laboratory has the greatest expertise in electrophysiological investigation of the primate visual system. Rodents and carnivores cannot be used because the hierarchical organization of the visual inputs as well as the organization of direct afferent connections to visual cortex are much more elaborate in primates. Furthermore, primates have many more visual cortical areas and parallel input through the old (s.c. + pulvinar) and new (LGN) pathway allowing for differentiated function which is of importance for pathological conditions like blind sight. Thus, the primate model is inevitable if the basics of human brain function need to be understood in order to provide new therapeutic options.

4.2.2. “Experimental procedures have to be further detailed.”

Partner IIT:

Experimental procedure for preparing rat/mice embryonic neuronal cultures (Berdondini).

Over the 36 months of the project, a total of 45 pregnant mice (i.e. *Mus Musculus*, C57B6J, from Charles River Italy) and 55 pregnant rats (*Rattus*, Sprague-Dawley, from Charles River Italy) are planned. Primary neuronal cultures from mice and rat will be prepared from embryos at between E15 and E19. Pregnant mothers are bought directly from a SPF provider (i.e. Charles River Italy) and delivered to the IIT animal facility at the age of E14, to avoid any risk of delivery during animal transportation. Successively, pregnant mothers will be maintained in the IIT facility until the day of the cell culture preparation, for a maximum stay of 5 days. For the dissection, the pregnant rat/mice is anesthetized by carbonarcosis and sacrificed by cervical dislocation. Under sterile conditions, embryos are extracted by caesarean section and by cutting the uterine segment. After having isolated each single

embryo, each of them is decapitated and cortex and hippocampi are extracted. The brain tissue is dissociated by enzymatic digestion in Trypsin 0.125% –20 min. at 37 C – and finally triturated with a fire-polished Pasteur pipette. The obtained cellular suspension is used for seeding a controlled number of neurons over previously coated (i.e. with poly-D-lysine and laminin) MEA devices. Primary cultures are Incubated over 2-4 weeks with 1% Glutamax, 2% B-27 supplemented Neurobasal Medium (Invitrogen), in a humidified atmosphere 5% CO₂, 95% air at 37 C; and by changing 50% of the medium every week. In order to reduce the number of needed animals, we seed between 10 to 20 MEA devices for each sacrificed pregnant animal and we are also developing a cryo-preservation protocol for primary neuronal cells. Carefully monitor of our data to ensure the lowest possible number of animals will be performed by using a Power Analysis statistical method. In order to enable optogenetic modulation of network activity state, the expression of optogenetic bio-molecular probes, e.g. ChannelRhodopsin2 (ChR2), HaloRhodopsin (HR) and ArcheoRhodopsin (Arch, Mac), will be performed by viral infection (at about 7 days-in-vitro) or by plasmid transfection of the cellular suspension.

Experimental procedure for acute and chronic experiments by using multielectrode arrays (Vato)

Two different series of in-vivo experiments will involve rats and will use multielectrode arrays to record the neural activity and to deliver patterns of intracortical microstimulation: i) in the first series we will record and stimulate the neural activity of anesthetized rats during acute experimental sessions; ii) in the second series the rats will be chronically implanted with multielectrode arrays placed in different regions of the brain. These experiments will share most of the following experimental procedures.

Over the whole duration of the project, experiments will be carried out on 90 adult rats (Long-Evans, weighting 250-350 g., Charles River Italy) initially anaesthetized with a mixture of Zoletil (30 mg/kg) and Xylazine (5 mg/kg) delivered intraperitoneally. For the entire duration of the experiment, anaesthesia will be maintained with supplementary doses of anaesthetic (intra-peritoneal or intra-muscular) and the anaesthesia status will be checked with tail or toe pinch test, checking for responses with muscle tones, righting reflex, hand clap startle, ear brush or corneal reflex. The body temperature will be maintained at 36–38°C with a thermostatically controlled heating pad. After placed the anesthetized animal in a stereotaxic apparatus (Myneurolab, St. Louis, MO) a craniotomy will be made, using a micro drill, over the primary somatosensory cortex (S1) and primary motor cortex (M1) whisker representations of the ipsilateral hemisphere identified according to vascular landmarks and stereotaxic coordinates.

For both acute and chronic experiments we will use commercially available multielectrode arrays: i) TDT microwires arrays formed by 16/32 polyimide-insulated tungsten electrodes (50µm wire diameter, 250µm electrode spacing and 375µm rows separation, Tucker-Davis Technologies, Gainesville, FL) ii) silicon-based Michigan multielectrode arrays formed by 16/32 microelectrode sites patterned on different shanks (NeuroNexus, AnnArbor, MI) iii) multichannel, high-density Utah array consisted of up to 100 three dimensional sharpened silicon needles coated with platinum and insulated with polyimide (Blackrock, Saltlake City, UT).

The dura mater will not be removed since the electrodes are sufficiently rigid to pass through it and will be kept moist with a 0.9% saline solution. The arrays will be lowered through the cortical surface using a hydraulic microdrive (Kopf, 2650) and the placement of the electrodes will be tested and confirmed by recording the neuronal responses to manual whisker stimulation. The intracortical microstimulation (ICMS) will consist of trains of biphasic pulses and each stimulation train will be delivered throughout two electrodes of the stimulation array using a programmable 8 channel stimulus generator (Stg4008, Multichannel Systems, Reutlingen, Germany) built with a stimulus isolation unit for each output channel. Extracellular neuronal discharges will be amplified, digitalized and recorded using a multichannel recording system (Map system, Plexon Inc, Dallas TX) with a sampling frequency of 40 KHz per channel.

Before and after the surgery, the rats will be treated with an anti-pain and antibiotic therapy (ketorolac or ketoprofene, 5-7,5 mg/kg or 2-5 mg/kg IP or SC, respectively and enrofloxacin 5-10 mg/kg SC/12-24 hrs or 100mg/l PO in drinking water) to ensure their welfare and minimize the possible infections after performing a chronic surgery. To obtain long-lasting chronic implants, when the proper depth is reached, the melectrode array will be secured with biocompatible dental cement, and after letting the cement dry, the wound will be closed with surgical sutures. To prevent infection, a small amount of antibacterial ointment will be placed on the surgical site. After the surgery, the rats will be monitored for recovery, stable respiration and temperature making sure that the rats are awake before returning it to animal housing.

Partner MPI-BC:

General Surgery and Anesthesia. MRI-compatible skull-form-specific head holders and chambers made out of PEEK (polyether etherketone; TecaPEEK, Ensinger, Inc., Nufringen, Germany) will be implanted stereotaxically on the cranium of each animal using aseptic technique. The implants were secured with custom-made ceramic screws (zirconium oxide Y2O3-TPZ 5x1, Pfannenstiel, Germany). Post-operatively, the animals will be held in large, specially designed recovery chairs for 3 days, during which they will be taken out for walks by the care persons 2-3 times a day. The chairs allow the animals to freely move body and hands, but prevent them from touching the implants. As a prophylactic measure, antibiotics (enrofloxacin; Baytril™) and analgesics (Flunixin; Finadyne® vet.) will be administered for 3 to 5 days. All surgical procedures will be carried out under general balanced anesthesia, the induction and maintenance of which will be performed by trained personnel.

Anesthesia for Physiology and fMRI Experiments. During the fMRI experiments, anesthesia will be maintained with remifentanyl (0.5-2 µg/kg/min) in combination with a fast acting paralytic, mivacurium chloride (5-7mg/kg/hr). Because the BOLD signal is very sensitive to changes in body temperature, oxygenation, pH, and blood pressure, the physiological state of the animal will be monitored continuously and maintained tightly within normal limits. Body temperature will be strictly maintained at 38.5-39.50C, and end-tidal CO2 and oxygen saturation kept constant at 33 mm Hg and over 95%, respectively. Acidosis will be prevented by the administration of lactated Ringer's solution with 2.5% glucose, infused at 10 ml/kg/hr, and intravascular volume maintained by the additional administration of colloids (hydroxyethyl starch, 20-30ml over 1-2 minutes or 20 ml/kg/hr). The absence of a "typical"

anesthetic, e.g. isoflurane, desflurane, or propofol, does not cause stress to the animal, as we measured catecholamines and optimized dosages to ensure unaffected physiological responses at normal catecholamine concentrations.

Positioning of the Electrodes. Recording and stimulation hardware, including electrodes and microdrives, are developed at the Max Planck Institute for Biological Cybernetics. Recording chambers and head holders will be positioned stereotaxically on the basis of individual, high-resolution MRI. The chambers for LGN will be placed at the approximate coordinates AP=8, ML=12. Stimulation sites will be selected so as to ensure reliable stimulation-evoked BOLD activation on the operculum of the brain. Once the electrode is at the desired location in LGN, we align the visual projector and plot the receptive field of the multiple units.

Visual Stimulation. Visual stimuli will be presented binocularly, initially with an SVGA fiber-optic system (AVOTEC, Silent Vision SV-7021, Stuart, FL) and later with our own custom-made MR-compatible visual stimulator. Both have a spatial resolution of 800x600 pixels and a frame rate of 60 Hz.

Optogenetic stimulation. LGN stimulation will follow the methodology implemented in two recent publications (Diester et al., 2011; Han et al., 2009). Briefly, ChR2 packaged into adeno-associated virus (AAV) will be injected into the primate LGN based on electrophysiological and MRI guidance. Access to LGN will be gained via a chronically implanted recording chamber or permanently implanted guide cannulas. Prior to injection microelectrodes will be advanced to the LGN guided by MRI reconstructions of the brain. LGN neurons will be identified using well-established electrophysiological criteria for ocularity and magno- vs parvocellular systems. Subsequently a small micro-cannula will be lowered to the target area. A volume of 0.5 μ l will be injected per site at a rate of < 0.5 μ l / minute using a Hamilton syringe and microprecision pumps. We will wait at least 10 minutes between injections. We will perform up to 5 injections (2.5 μ l total volume) for a given LGN trajectory ensuring complete coverage of all layers. We will apply similar procedures up to 5 further injection trajectories with the aim to transfect a large part of LGN. The injection agent will consist of a light sensitive opsin (ChR2, Halo, Arch) in combination with a fluorescent protein (GFP, YFP) packaged into an adeno-associated virus (AAV) by the University of North Carolina Genetherapy Center. The viruses we will be using have been cultured to be non-infectious in several generations. More than 2/3 of their genome has been deleted and they are not self-replicating. After opsin expression has reached its maximum and steady state approximately 4 weeks post-injection we will start experiments. Thalamo-cortical projection neurons will be excited using the stimulation of blue light generated by a laser and transmitted to LGN using small (100-200 μ m diameter) fiberoptic bundles. We will measure the impact of this manipulation on neural populations using electrophysiological or fMRI methods. Histological and histochemical analyses in some of the animals will corroborate the electrophysiological and fMRI measures. There is no evidence that a repeated induction of opsin expression will be required.

The fMRI experiment will consist of visual or optogenetic stimulation, or trials of alternating or combined visual and optogenetic stimulation. The trials start with a 10s blank period followed by 6s stimulation (rotating checkerboard or stimulation, randomized within a scan) and a 14s blank epoch. This sequence will be repeated 10 times during an MR scan of 5min

duration. A number of different stimulation parameters will be tested to optimize optogenetic stimulation.

MRI in Anesthetized Monkeys. Experiments in anesthetized animals will be conducted in a vertical 4.7 Tesla scanner with a 40 cm diameter bore (BioSpec 47/40v, Bruker BioSpin, Ettlingen, Germany). The system has a 50mT/m (180 μ s rise time) actively shielded gradient coil (Bruker, BGA26) with an inner diameter of 26 cm. We use a custom-made chair to position the monkey in the magnet, and customized radiofrequency (RF) coils: an 85mm diameter surface coil or a quadrature volume coil combining a Helmholtz coil and a surface coil on the operculum of the monkey. With the quadrature coil one can image deep brain structures like thalamus while still maintaining high SNR in the primary visual cortex.

Typically, 12 slices will be acquired, covering visual cortex and thalamus, at a temporal resolution of 2 seconds with four-shot GE-EPI images (TR/TE=500/15ms, bandwidth=100 kHz, FA=30o, FOV=96x96mm, matrix=96x96, 2mm slice thickness). T1-weighted, high-resolution anatomical scans will be obtained using 3D-MDEFT with FOV 96x96x128 mm, matrix 192x192x128 or 256x256x128, TE of 4.5 ms, TR of 15-25 ms, BW 50 kHz, FA of 20 deg, and 4 segments.

The anesthesia level and animal state is closely monitored throughout the experiment and until complete recovery from the anesthesia. Two weeks of rest period is given to the animal after each experiment to reassure complete recovery from possible stress related to the anesthetised state.

Partner SISSA:

Three preparations will be used: the first to quantify skin deformation and the second to analyze motoneuron firing during local bending. Both preparations will be kept in a Sylgard-coated dish at room temperature (20–24°C) and bathed in Ringer's solution (in mM: 115 NaCl, 1.8 CaCl₂, 4 KCl, 12 glucose, and 10 Tris maleate buffered to pH 7.4 with NaOH)

The first preparation consists of a hemisection of leech skin (approximately three segments in length, isolated from the rest of the body). The skin will be flattened and fixed with pins to the bottom of the recording chamber but will be allowed to deform during muscle contraction.

The middle segment will be kept innervated by its ganglion.

The second preparation will be an isolated leech segmental ganglion with exposed nerve roots. Up to 8 suction pipettes will be used to perform parallel extracellular recordings from the anterior-anterior (AA), anterior-medial (MA), and posterior-posterior (PP) roots and from the two bifurcations of the dorsal posterior root (DP:B1 and DP:B2). Spikes recorded from these roots will be classified according to dimension and shape and will be identified by impaling each motoneuron with a sharp intracellular microelectrode (input resistance, 30 M Ω ; filled with 4 Mpotassium acetate), as previously described (Pinato et al., 2000; Arisi et al., 2001). In this way, it will be possible to characterize the firing activity of a large fraction of all leech motoneurons: the excitatory motoneurons of longitudinal muscles (cells 3, 4, 5, 6, 8, 107, 108, and L), the excitor of flattener muscles (cell 109), the annulus erector, and two inhibitory motoneurons of longitudinal muscles (cells 102 and 119).

In both preparations, local bending will be initiated by intracellular stimulation of mechanosensory dorsal or ventral P cells, ipsilateral to the recorded roots. In some experiments, a mechanical stimulus will be delivered to the first preparation by rapidly

pressing a nylon filament driven by a solenoid on the skin, as previously described (Pinato and Torre, 2000). Skin deformations will be quantified by computing the optical flows from image sequences of the contracting leech skin.

The third preparation will consist of a semi-intact leech preparation, which will be obtained in the following way: adult leeches *Hirudo medicinalis* or *Hirudo verbana* obtained from Ricarimpex (Eysines, France) will be kept at 5°C in tap water dechlorinated by previous aeration for 24 h. Before every experiment, animals will be anesthetized with an 8% ethanol solution at room-temperature for 15-20 minutes. Leeches will be extended and the skin dried carefully. Beads of 5 mm diameter will be glued on the dorsal side of the leech with Nexaband S/C tissue adhesive (Abott Labs, Chicago, USA) near their head and tail. Once beads are correctly glued, leeches will be moved to the Petri dish covered with sylgard elastomere (Corning corp., U.S.A.). The leeches will be immersed in 150-200 ml chilled normal ringer solution (in mM: 115 NaCl, 1.8 CaCl₂, 4 KCl, enriched with 10 glucose and buffered with 10 Tris-maleate pH 7.4 with NaOH). Leeches still under anaesthesia will be pinned with fine needles in their mid-body. Animals will be dissected so to expose two central ganglia. During the dissection, the temperature will be maintained at 6-8°C using a cold chamber. In some experiments, a complete segment (skin from mid dorsal to mid ventral) will be left innervated by roots from one side, recording contralateral electrical signals and the changes of the skin area will be analyzed. At the end of the dissection, animals will be left to recover from anaesthesia and left to adapt to room temperature for 30 minutes. Experiments will be performed at room's temperature (19-22° Celsius) and semi-intact leeches will be illuminated using a white light lamp without abrupt spatial and/or temporal gradients (Olympus Highlight 3100, Europe). At the end of the dissection, animals will be left to recover from anaesthesia and left to adapt at room temperature for 30 minutes. After behavioural experiment, leeches will be left to recover and will be moved back to their tank. Leeches dissected for electrophysiological experiments will be rapidly killed at the end of the experiment and their body transferred to an appropriate container. We plan to perform on average two experiments per week (approx. 100 leeches/year).

4.2.3. "The applicant must demonstrate awareness of the new Protection of Animals for Scientific Purposes Directive, 2010/63/EC and how it will be implemented: a) in the interim until 2013, and b) how it will be implemented when legally enforced by the countries where the studies will be done."

Partner IIT:

The IIT animal facility is fully aware of the new Protection of Animals for Scientific Purposes Directive, 2010/63/EC and is already modifying its internal procedures and animal housing environment to fulfil these rules. In particular, standard environmental enrichment as described in Directive, 2010/63/EC are under implementation in our Facility (almost complete), an internal animal-welfare body (see Article 26) was instituted and experimental protocols including methods of killing animals are under review by the animal-welfare body. In particular, the new Directive introduces specific regulations for foetal forms of mammals in the last third of the period of their development that must be satisfied. The objective of our Facility is to maintain all animals in a SPF and enriched environment at the highest standards.

Animals are regularly controlled responding to the FELISA list of pathogens. Already now, only competent persons can access the animal facility, perform experiments on living rodents and kill the animals.

Therefore,

- in the interim until the adoption of the Directive 2010/63/EC in Italy, the Animal Facility of IIT has already started to implement procedures for housing and for animal experimentation fulfilling the new regulation. Currently, standard enriched environments have already been introduced in the rodents cages (i.e. GLP Semi-detached Dome Home, 14 x 9 x 6h cm from biological instruments S.N.C., Besozzo (VA) Italy), an internal animal-welfare body has been instituted and experimental procedures are under internal revision by the animal-welfare body of the Animal Facility. A specific animal experimentation protocol for the SI-CODE project will be requested in Italy since the project start and will implement the new directive. A copy of this protocol will be provided to the European Commission.
- when legally enforced, the new directive will be applied in all the projects involving the use of animals and will undergo the approval by the Italian Animal Experiments Inspectorate; animal housing and caretaking, including housing of transgenic mice in a special facility, will have to fulfil the updated Italian regulations, and will be supervised by a registered veterinarian at the IIT Genoa Animal Facility in the respect of the national regulations regarding the protection of animals used for scientific purposes. If required, the experimental protocol will be re-submitted to fulfil the directive changes imposed by the Italian Animal Experiments Inspectorate.

Partner MPI-BC:

The Max-Planck Institutes work in close interaction with local authorities (Regierungspraesidium) and continuously try to fulfil all necessary requirements to animal housing. The local authorities have regular visits to both primate and rodent facilities in order to reassure welfare of the animals. The MPG employees responsible for animal research are informed and aware of the new Protection of Animals for Scientific Purposes Directive, 2010/63/EC. The non-human primate facility in our institute has been built at highest standards and last year was additionally extended to fulfill the latest regulations. In particular, standard environmental enrichment as described in Directive, 2010/63/EC are implemented, an internal animal-welfare body (see Article 26) was instituted and experimental protocols including methods of killing animals are approved by the animal-welfare body.

The objective of our both non-human primate and rodent facilities is to maintain all animals in a SPF and enriched environment at the highest standards. Animals are regularly controlled responding to the FELISA list of pathogens. The access to animal facilities is restricted to only competent persons. The animal experiments are performed exclusively by trained personnel or under supervision of an experienced investigator. All these points, including list of the employees involved in each experimental procedure, are described in the experimental protocol and approved by the local authorities (Veterinäramt Tübingen and Regierungspräsidium Tübingen).

The current German regulations are already at the highest standards and the Animal Facility of our MPI entirely fulfills the latest requirements. Therefore, only minor changes can be

expected with the new Directive in 2013 that can be easily implemented in the interim until 2013.

According to the official publication (Official Journal of the European Union), one of the main official changes for macaque monkeys is the space they can be kept in. Our facility already provides larger stables than suggested by the new regulations due to the originally-built large stables that we currently use. Another very serious change will be the retrospective assessment of all primate projects (“All projects using non-human primates and projects involving procedures classified as ‘severe’, including those referred to in Article 15(2), shall undergo a retrospective assessment.”). In the past, although no explicit retrospective assessment was legally enforced, the ethics commission at the Regierungspräsidium (§15 of the German law for the protection of animals) always judged the performance of our previous approved projects and no concerns were raised with respect to the quality and quantity of published research results.

Standard enriched environments have already been introduced in the rodent cages (i.e. plastic tubes and nest-building material). The rats are typically housed in groups of four. Recently, we have introduced two-level housing cages for rats allowing more rich sensory-motor behavioral repertoire within a cage. An internal animal-welfare body has been instituted and experimental procedures are under internal revision by the animal-welfare body of the Animal Facility.

A specific animal experimentation protocol for the SI-CODE project will be submitted to local authorities since the project start and will implement the new directive. A copy of this protocol will be provided to European Commission.

When legally enforced, the new directive will be applied in all ongoing projects involving the use of animals and will undergo the approval by the local authorities. If necessary, animal housing and caretaking will be further modified to fulfill the updated regulations, and will be supervised by our veterinarian in respect to the directives for protection of animals used for scientific purpose.

Partner SISSA:

Professor Vincent Torre and all his collaborators are aware of the new Protection of Animals for Scientific Purposes Directive 2010/63/EC. However, at present - to the best of our knowledge - experiments on leeches do not require any approval from any Ethics Committee neither in Italy, nor in the USA, France, Germany and all other nations where investigators of leeches operate. Nevertheless, we will take all possible precautions to avoid putting the leeches in critical conditions or under any noxious stimulation.

4.2.4. The applicant must reassure the Commission that the monkeys have been obtained ethically - have been bred in captivity for research purposes (2010/63/EC #19 and 20).

Partner MPI-BC:

We use exclusively bred non-human primates, which can be documented by the CITES papers for each animal and we get documentation by the primate centers (German Primate Center, Göttingen; Primate Center of the Université Louis Pasteur, Strasbourg) or companies

(Bioprim, RCS Toulouse B 433 791 548, Parc de Lantarese 31450 BAZIEGE) where we buy our animals.

4.2.5. The applicant must reassure the Commission that animal facilities are of the highest standard and include a positive social enrichment environment to minimise distress and meet the monkeys' needs for social interaction and to comply with housing requirements in table 6.3 for Macaque monkeys in the new Directive.

Partner MPI-BC:

Our facilities for both non-human primates and rodents are of the highest standards and entirely fulfil the latest regulations of the German law. We group non-human primates of mixed gender and there are sufficient possibilities for social interactions and an opportunity to retreat from too much social stress. The space requirements in our facilities clearly exceed the values in table 6.3.

The animal facilities are constantly populated by our animal-care persons during working hours including all weekends and holidays. Furthermore, our veterinarians visit every animal at least once a day. In addition, representatives of local authorities make their regular inspections of our Animal Facility.

4.2.6. When applying for ethical approval from the competent local/national Ethics/legal bodies, detailed information should be provided on why living animals have to be used and why that species has been chosen. In addition, information should be given on the numbers of animals to be used in experiments, the nature of the experiments, the procedures that will be carried out and their anticipated impact (e.g. potential for pain, suffering, stress) and how that has been minimized. Furthermore, details should be provided on what procedures have been implemented to ensure the welfare of the animals during their lives (e.g. husbandry, minimizing harms, criteria for humane endpoints, inspection protocols). The applicant should provide evidence of awareness of relevant European legislation and regulations covering animal experimentation and that the Principle of the Three Rs will be rigorously applied.

Partner MPI-BC

All of the points raised above are covered by the "Tierversuchsantrag" (experiment protocol application) and approved by local authorities.

The proposed experiments can be only performed in vivo. Most of the experiments will be performed in rodents. The primate model, however, is essential if the basics of human brain function need to be understood in order to provide new therapeutic options, which is the ultimate target of the entire project.

The experiments planned for this project are technically extremely demanding, which is predefined by several factors: 1) first of all, difficulty to target deep brain structures like LGN or LC; 2) simultaneous targeting of multiple brain regions; 3) multiple phases of each experiment (e.g. electrode placement and adjustment, MRI scanning, electrophysiological recording); 4) longitudinal character of the study in case of non-human primates only (e.g. chamber implantation, repeated MRI scanning, recovery from anesthesia). Each of these factors seriously challenges the successful data collection. For obtaining the reliable results

and of publishable quality, we need at least 3 to 5 successful repetitions of each experimental condition in rats and at least 2 repetitions for non-human primates. To maximize the efficiency of data collection and minimize the number of experimental animals, we apply multiple strategies including MRI-guided electrode placement, using of multi-electrode arrays, applying maximum number of the experimental conditions in each animal, and most importantly, using the highest-level expertise existing in the Logothetis lab. We have already justified the number of animals we are planning to use (see Technical Annex, page 66 and also in point 8 of the present document).

By using non-human primates we are working on 2 of the 3Rs, refinement and reduction, by improving implantation techniques, increasing efficiency of data acquisition by using multichannel recordings, using functional imaging for the improvement of electrode implantations, and testing maximal number of experimental conditions in the same animal. To some extent, we use also replacement as some experiments are done in rats (e.g. the analysis algorithms can be first tested on the rat data and later validated for monkey recordings). Our experimental protocols have been designed to minimize potential pain, suffering and stress of the animal. In particular, all experiments in both primates and rodents will be performed under deep anesthesia. The level of anesthesia is continuously monitored by a fully qualified and formally approved person. The opiate anesthesia remifentanyl (0.5-2 $\mu\text{g}/\text{kg}/\text{min}$) in combination with a fast acting paralytic, mivacurium chloride (5-7 $\text{mg}/\text{kg}/\text{hr}$) is used for non-human primates; this type of anesthesia is known to affect so-called “pain matrix” and therefore maximally reduce any nociception, while preserving activity in primary sensory cortices. Body temperature, oxygenation, pH, and blood pressure, the physiological state of the animal are continuously monitored and maintained tightly within normal limits. Body temperature is strictly maintained at 38.5-39.50C, and end-tidal CO₂ and oxygen saturation kept constant at 33 mm Hg and over 95%, respectively. Acidosis is prevented by the administration of lactated Ringer’s solution with 2.5% glucose, infused at 10 ml/kg /hr, and intravascular volume maintained by the additional administration of colloids (hydroxyethyl starch, 20-30ml over 1-2 minutes or 20 ml/kg/hr). The absence of a “typical” anesthetic, e.g. isoflurane, desflurane, or propofol, does not cause stress to the animal, as we measured catecholamines and optimized dosages to ensure unaffected physiological responses at normal catecholamine concentrations.

Urethane (1.5g/kg), a commonly used anesthetic, is used for rat experiments. Rectal temperature, heart rate, CO₂ and SpO₂ levels are monitored and kept constant throughout the experiment. A supplement of anesthetic drug is given when necessary. Rodents are killed by lethal dose of sodium pentobarbital (250 mg/kg i.p.; Narcoren®, Merial GmbH, Germany). Safety issues regarding care of both non-human primates and rats are taken care by strictly following regulations issued by local authorities. The MPI Animal Facility is responsible for the care, welfare and health of laboratory animals corresponding to the standards of the Veterinäramt Tübingen and Regierungspräsidium Tübingen. License for use of animals for the research purposes is provided (Aktuelle Haltungsgenehmigung 07-2011).

Animal housing and caretaking is supervised by the animal care personnel, a veterinarian, and local authorities in respect of the current regulations regarding the protection of animals used for scientific purposes. Research protocols are at first reviewed and approved by the internal animal-welfare body and then specific authorizations are requested from the

Regierungspräsidium. In the case of approval of the proposed research project, research protocols involving animals will be formally submitted to the animal-welfare body for reviewing and consideration for approval.

As regards animal experimentation, MPI strictly obey the German law for the protection of animals and is fully aware of the Directive 2010/63/EU.

The experiments are planned not to cause any animal suffering. Suffering of animals will be avoided or kept at minimum. Maintenance of animals will be carried out at the highest standard in carefully controlled and monitored conditions to prevent any animal stress.

Partner IIT:

Over the 36 month of the project, a total of 45 pregnant mice (i.e. *Mus Musculus*, C57B6J, from Charles River Italy) 55 pregnant rats (*Rattus*, Sprague-Dawley, from Charles River Italy) and 90 adult rats (Long-Evans from Charles River) are planned. As the project objective requires the experimental study of the functional properties of neuronal networks, currently no alternative to animal experimentation exists. Rodents (mice/rats) represent an experimental model with minimal complexity to successfully achieve the tasks planned in IIT contribution to the project. Indeed, rodents enable sufficiently complex behavioural studies to be correlated with in-vivo electrophysiological studies and in-vitro investigation. In particular, in-vitro neuronal networks offer a facilitated access to structural and functional network properties. Our experimental protocols have been designed to target the minimization of potential pain, suffering and stress. In particular, for in-vitro experiments, neuronal cultures are prepared from wild-type rat/mice embryos that are killed following the procedures indicated in Annex IV of the Directive 2010/63/EU (22.09.2010). Pregnant mothers are killed by carbonarcosis (gradual fill of CO₂, 5% until saturation) and cervical dislocation. After extraction and separation, the embryos are sacrificed by decapitation given their small size and the rapidity of this procedure. Other methods for killing the embryos are not possible and might affect the viability of brain tissue. Animals for in-vitro cell culture preparations are bought from Charles River Italy and transported by the same company to our Animal Facility. These animals stay up to a maximum of 5 days in our Animal Facility, within cages fulfilling the size requirements indicated in the Directive 2010/63/EU.

For in-vivo experiments, Long-Evans rats have been chosen because of their better visual acuity with respect to albino rat. To minimize the number of animals, we designed our experimental sessions to maximize the amount of collected data performing more than one procedure for each animal. At the end of the experimental sessions, after being anaesthetized, rodents are killed with anaesthetic overdose according to the Annex IV of the Directive 2010/63/EU.

In the IIT Animal Facility, rodents benefit from cages fulfilling the size requirements and by an enriched environment implemented by GLP Semi-detached Dome Homes (14 x 9 x 6h cm from biological instruments S.N.C., Besozzo (VA) Italy) in each cages.

Safety issues regarding care of rats/mice are taken care by the animal facility by following safety and ethical procedure authorizations issued by national authorities, strictly obeyed in the laboratory work. The IIT Animal Facility responsible for the care, welfare and health of laboratory animals adheres to the standards of FELASA and is designated as research facility by Italian Ministry of Health (D.M.S. n. 44/94-A). License for the use of animals for the

research purposes included in the IIT unit is granted by the Italian Animal Experiments Inspectorate. Animal housing and caretaking, including housing of transgenic mice in a special facility according to Italian regulations, is supervised by a registered veterinarian at the IIT Genoa Animal Facility in the respect of the national current regulations regarding the protection of animals used for scientific purposes (D.L.vo 27/01/1992, n. 116). Research protocols are at first reviewed and approved by the internal animal-welfare body and then specific authorizations are requested to the Italian Ministry of Health. At the beginning of the SI-CODE project, research protocols involving animals will be formally submitted to the animal-welfare body for reviewing and consideration for approval. As regards animal experimentation, IIT obeys to the directive 2003/65/EC of 22 July 2003, amending Council Directive 86/609/EEC, on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Concerning the protection of animals, IIT obeys the Amsterdam Protocol on Animal Protection and Welfare and is fully aware of the Directive 2010/63/EU. Suffering of animals will be avoided or at least kept to a minimum. The experiments are planned not to cause any animal suffering. Breeding and maintenance of animals will be carried out in carefully controlled and monitored conditions to prevent any animal stress. The IIT Animal Facility is managing the animal housing, breeding and animal experimentation using a dedicated management software tool (Pyrat, from Scionics Computer Innovation, Dresden, Germany) that provides extensive reports on animals and cages.

The planned animal experimentation of our project is based on the consideration of the 3R principles of Reduction, Refinement, and Replacement. The estimated number of animals has been reduced merging different experimental phases (e.g. by planning of using the same data collected by running the bBMI also to better understand how the ongoing activity influences the information transmission). We will try to refine numbers of animals by combining more experimental procedures (i.e. in-vitro and in-vivo) to be carried out and we will actively focus on avoiding unnecessary duplication of experiments among partners by regularly coordinating our experimental activities. To do so, we will carefully monitor our data by using a Power Analysis statistical method to ensure the lowest possible number of animals. Furthermore, to reduce the number of needed animals and replace the need of animal killing, for in-vitro experimentation on primary neuronal cultures we are developing (team of Dr. Berdondini) cryo-preservation protocol of neuronal cells, a method that will be evaluated for our experimental activities on in-vitro preparations.

Partner SISSA:

As already mentioned above, at present - to the best of our knowledge - experiments on leeches do not require any approval from any Ethics Committee neither in Italy, nor in the USA, France, Germany and all other nations where investigators of leeches operate. Nevertheless, we will take all possible precautions to avoid putting the leeches in critical conditions or under any noxious stimulation.

4.2.7. Copies of ethical approvals by the competent local/national ethical/legal bodies, together with copies of relevant authorizations for animal experiments must be forwarded to the European Commission prior to the commencement of the research.

The partners undertake the obligation to forward to the European Commission prior to the commencement of the research copies of ethical approvals by the competent local/national ethical/legal bodies, together with copies of relevant authorizations for animal experiments.

4.2.8. Regulations regarding experimentation regarding non-human primates must be addressed. This refers not only to experimental conditions but also to license [see above], animal handling and exact procedures. Numbers need to be justified and supported by a statistical analysis.

Partner MPI-BC:

All animal experiments will be performed using procedures that are similar to those already in use at the partner site and that have already been approved by the local authorities (see documents attached). The planned experiments with animals are part of numerous ongoing experiments in the MPI. These experiments are conducted strictly according to the legal and ethical requirements demanded by law, and have been approved by local ethical committees (see documents attached). All the necessary permits are available, or will be applied for as soon as the project is granted and the contract is signed with the European Commission and will be applied prior to the start of the RTD activities that involve animals. Potential discomfort ('suffering') will be evaluated at the level of the individual animal. Animal discomfort is clearly minimized for ethical and experimental reasons. The housing of the animals is in full compliance with the revised Appendix A (2006) of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes – Guidelines for accommodation and care of Animals". Suffering of the animals before, during and after the experimental sessions is reduced to a minimum. The approval by the Regierungspräsidium of Tübingen will assure that the data analysis and statistics are planned in a way to reduce the number of animals used to the minimum. The technical setting of animal husbandry at the MPI is above the levels expected by the local authority. Animals are maintained in accordance with the Animal Welfare Act and the DHHS (Guide for the Care and Use of Laboratory Animals). All the regulations of the laboratory have been written in detail by the primary investigator in form of a Standard Operating Procedures (SOP) book that is made available to every person joining the laboratory. The lab personnel and the scientists are trained for correct animal handling. The animals are trained for procedures like approaching the animal care person, climbing into the primate chair, sitting still in the setup, performing behavioural task.

Estimates of number of animals for LGN stimulation experiments. Experiments will be conducted in rats and in the non-human primate (macaque monkeys) under anesthesia. The project will require 20 rats and 2 monkeys per year, whereby the rats and the first two monkeys will be used for the development and optimization of the optogenetic stimulation techniques. The numbers for non-human primates are justified, but no statistical analysis is available for a pilot project of this kind. In primate research, typically very small numbers (N=2) are accepted and usually sufficient to prove a finding.

Estimate of number of animals to be used for LC stimulation. We estimated the sample size with within-factors repeated measures ANOVA. Given a medium effect size $f=0.5$, in order to achieve the test power of 0.8 with significance level of $p<0.005$, the minimal number is 10

animals. Based on this calculation, we expect to obtain the data from 10 rats with completed series of recordings/stimulations per each experimental condition. The experiments planned for this project are technically extremely demanding, and from previous experience on similar setups we expect a 40% cumulative failure of a single day experiment including: 1) failure to implant electrodes in target areas – 20%; 2) failure to obtain stable cortical recordings – 10%; 3) complications due to surgery and anesthesia – 10%. Thus, the maximal number of 14 rats per experimental condition is planned. The cases with electrode implantation outside of the LC nucleus will be used as additional control condition. Also, we will plan up to 10 animals for technical optimization of all stages of experiments. Total maximal number: 66 rats. These numbers are evaluated based upon extensive previous experience of MPI. Animal numbers and the exact procedures will be additionally detailed in the experimental protocol application for the SI-CODE project.

4.2. 9. As a requirement and in line with the precautionary principle, the periodic report to be submitted to the EC must cover aspects of research findings with the potential of dual use, if applicable. See communication from the Commission on the Precautionary Principle from February 2000.

http://europa.eu/legislation_summaries/consumers/consumer_safety/l32042_en.htm

The consortium is committed to include in the periodic report to the European Commission cases of dual use of the research results. A specific task on this has been included in WP9.

4.3 ETHICAL ISSUES TABLE

	YES	PAGE
Informed Consent		
Does the proposal involve children?		
Does the proposal involve patients or persons not able to give consent?		
Does the proposal involve adult healthy volunteers?		
Does the proposal involve Human Genetic Material?		
Does the proposal involve Human biological samples?		
Does the proposal involve Human data collection?		
Research on Human embryo/foetus		
Does the proposal involve Human Embryos?		
Does the proposal involve Human Foetal Tissue / Cells?		
Does the proposal involve Human Embryonic Stem Cells?		
Privacy		

Does the proposal involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political, religious or philosophical conviction)		
Does the proposal involve tracking the location or observation of people?		
Research on Animals		
Does the proposal involve research on animals?	X	p. 13-18 and 26-27
Are those animals transgenic small laboratory animals?	X	p. 13-15
Are those animals transgenic farm animals?		
Are those animals non-human primates?	X	p.16-18
Research Involving Developing Countries		
Use of local resources (genetic, animal, plant etc)		
Benefit to local community (capacity building ie access to healthcare, education etc)		
Benefit to local community (capacity building, access to healthcare, education etc)		
Dual Use		
Research having potential military / terrorist application		

B5. Gender aspects

According to an initial diagnosis of the current state of women's participation in the science disciplines represented by the SI-CODE consortium, it seems necessary to include discussion of several actions that would be undertaken within SI-CODE to improve the current low involvement. The SI-CODE consortium fully supports the European initiative to eliminate gender inequalities and promote gender equality throughout the European Community in accordance with Articles 2 and 3 of the EC Treaty (gender mainstreaming) as well as Article 141 (equality between women and men in matters of employment and occupation) and Article 13 (sex discrimination within and outside work place). The consortium already includes some female researchers among the key scientists involved in delivering important parts of the research, but being them still underrepresented, the consortium is committed to further promote their involvement within the project and beyond. Whenever possible, gender balance will be used when assigning leadership roles within the partnership.

The consortium is also committed to take the following specific actions in order to guarantee equal opportunities:

- Recruitment of new researchers: SI-CODE will ensure an open and impartial selection procedure, by using mixed selection panels, with panel members trained on gender

bias; by advertising posts widely and explicitly encouraging women to apply; by accommodating atypical career patterns.

- Working conditions and culture: SI-CODE partners will ensure that a working culture that fosters equal working conditions (pay, opportunities for training, access to grants and funding, flexi-time, home working, etc.) is pursued within their institutions. This working culture will try to accommodate private commitments or different career structures and will be aware of different possibilities in terms of geographical mobility (e.g. offering help to the researcher's partner to find a job in the new region of occupation)
- Monitoring of research to exclude gender bias: SI-CODE partners will appoint among them a gender equality officer who will make sure that bias and discrimination on a gender basis are not happening at the partnership level and on the contrary, will take the necessary steps to reduce them in cooperation with all the stakeholders involved in the project.
- Career development: this will be promoted for both male and female researchers but the partners are committed to support initiatives promoting women in decision-making positions.
- Training on gender issues: whenever possible, SI-CODE partners are committed to participate in specialized training on gender issues in EU projects in order to develop know-how to be used also beyond SI-CODE cooperation and in the framework of other research projects.

Although gender is not relevant for and within the subject matter of this project, it will be taken into account when analyzing the more long term impacts of the projects results, especially in the case of their exploitation.

Finally, IIT can already count on employees who have been trained on gender issues and will make this expertise available to the whole consortium.

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Baden-Württemberg
REGIERUNGSPRÄSIDIUM TÜBINGEN

Regierungspräsidium Tübingen · Postfach 26 66 · 72016 Tübingen

Max-Planck-Gesellschaft zur Förderung der
Wissenschaften e.V. München
z. H. Herrn Prof. Dr. Nikos Logothetis
Max-Planck-Institut für
Biologische Kybernetik
Spemannstraße 38
72076 Tübingen

Tübingen 26.07.2011
Name Dr. Saskia Hogreve
Durchwahl 07071 757-3384
Aktenzeichen 35/9185.46
(Bitte bei Antwort angeben)

Kassenzeichen: 1105151051095

Bitte bei Zahlung angeben!

Betrag: 150,00 EUR

nachrichtlich:

Herrn
Dr. Christoph Kayser
Max-Planck-Institut für
Biologische Kybernetik
Spemannstraße 38
72076 Tübingen

 Tierschutzgesetz in der Fassung der Bekanntmachung vom 18.05.2006 (BGBl I S. 1206) TierSchG;
Erlaubnis zur Zucht und Haltung von Wirbeltieren zu wissenschaftlichen Zwecken
nach § 11 Abs. 1 Nr. 1 a und b TierSchG
Erweiterungsantrag vom 30.05.2011; Posteingang: 21.06.2011 mit Ergänzung vom
12.07.2011; Posteingang: 14.07.2011

Anlagen
1 Zahlschein

Sehr geehrter Herr Prof. Logothetis,

auf Ihren Antrag ergeht folgende

I. Entscheidung

1. Dem Max-Planck-Institut für Biologische Kybernetik der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e. V. wird die Erlaubnis erteilt, in den Tierhaltungsräumen auf dem

**Campus der Max-Planck-Institute Tübingen,
Spemannstraße 38, 41 und 42 in 72076 Tübingen,**

Wirbeltiere zu wissenschaftlichen Zwecken zu halten und zu züchten.

Die für die Tierhaltung vorgesehenen Räumlichkeiten sind:

- Spemannstraße 38 : Raum L009, Raum L012 (Aufwachraum)
- Spemannstraße 41: Raum -1A17 (Suncus), Raum -1B02 (bis zu 24 Ratten für max. je 4 Wochen)
- Spemannstraße 42: Raum L007, Raum L008, Raum L026, Raum L020 („Kinderzimmer“), Raum L022 sowie Raum L023 (Quarantäne)

Die **Haltung** von Wirbeltieren zu wissenschaftlichen Zwecken umfasst:

Mäuse - Gesamthöchstzahl: **500**, davon bis zu 250 gentechnisch verändert

Ratten - Gesamthöchstzahl: **200**, davon bis zu 30 gentechnisch verändert

Suncus etruscus - Gesamthöchstzahl: **50**

Callithrix jacchus jacchus - Gesamthöchstzahl: **90** (davon max. 30 Tiere Nachwuchs, max. 10 Tiere pro Gruppenkäfig)

Macaca mulatta - Gesamthöchstzahl: **56** bei Haltung in nicht gemischt-geschlechtlichen Gruppen
85 bei Haltung in gemischt-geschlechtlichen Gruppen

Macaca fascicularis - Gesamthöchstzahl: **4** für jeweils max. 10 Wochen

Die **Zucht** von Wirbeltieren zu wissenschaftlichen Zwecken umfasst:

Mäuse - Gesamthöchstzahl: **500** pro Jahr, davon max. 400 gentechnisch verändert

Ratten, gentechnisch verändert - Gesamthöchstzahl: **100** pro Jahr

Suncus etruscus - Gesamthöchstzahl **30** pro Jahr

Callithrix jacchus jacchus - Gesamthöchstzahl: **30** pro Jahr

2. Für die Tätigkeit nach Ziffer I. 1. **verantwortlich** im Sinne des § 11 Abs. 1 Satz 2 Nr. 2 TierSchG ist in beiden Gebäuden **Herr Prof. Dr. N. K. Logothetis**, seine **Stellvertreter** sind **Herr PD Dr. Matthias Munk** (für Makaken), **Dr. Christoph Kayser** (für Nager) und **Frau Tierärztin Cornelia Stamm** (für Callithrix).

3. Die vorgelegten Pläne sind Bestandteil dieser Entscheidung.
4. Außengehege sind vorzusehen.
5. Die dem Max-Planck-Institut für Biologische Kybernetik früher erteilten Erlaubnisse nach § 11 TierSchG oder gemäß § 21 S. 1 TierSchG vorläufig als erteilt geltende Erlaubnisse werden durch die vorliegende Entscheidung aufgehoben.
6. Für die Entscheidung wird eine Gebühr von 150,00 Euro festgesetzt.

II. Nebenbestimmungen

1. Änderungen, die die Voraussetzungen der Erlaubnis betreffen, sind dem Regierungspräsidium Tübingen unverzüglich mitzuteilen.
2. Das Regierungspräsidium behält sich vor, Nebenbestimmungen nachträglich aufzunehmen, zu ändern oder zu ergänzen oder die Erlaubnis zu widerrufen, soweit dies aus tierschutzrechtlichen Gründen erforderlich ist.
3. Die Haltung/Zucht von gentechnisch veränderten Wirbeltieren ist nur dann zulässig, wenn dies unter I. für die jeweilige Tierart ausdrücklich aufgeführt ist.
4. Für Betreuung, Pflege, Transport sowie die fachgerechte Tötung von Tieren ist sachkundiges Personal in ausreichender Anzahl einzusetzen. Die Versorgung der Tiere muss auch an Wochenenden und Feiertagen gewährleistet sein und ist erforderlichenfalls durch einen Dienstplan zu regeln sowie zu dokumentieren.
5. Da das Wohlbefinden der Tiere i.d.R. von der Funktion technischer Einrichtungen (z. B. Klima- und/oder Filteranlagen) abhängig ist, sind geeignete Maßnahmen zur Sicherstellung der Funktion zu ergreifen. Erforderlichenfalls sind Alarmanlagen und/oder automatisch bzw. manuell zuschaltbare Notstromaggregate zu installieren sowie ein Alarmplan aufzustellen.
6. Über die Versorgung und Betreuung der Tiere sind nach Vorgabe der Überwachungsbehörde Aufzeichnungen zu führen. Dies gilt insbesondere für Tiere, die aufgrund von genetischen oder anderen Defekten erheblich belastet sind oder bei denen eine solche Belastung nicht auszuschließen ist.
7. Die nach § 11 a Abs. 1 TierSchG vorgeschriebenen Aufzeichnungen sind laufend zu aktualisieren, drei Jahre lang aufzubewahren und den zuständigen Behörden auf Verlangen vorzulegen.

8. Die Käfige oder sonstige Einrichtungen, in denen Wirbeltiere zu Versuchszwecken gezüchtet und gehalten werden, sind - soweit nicht mit der Überwachungsbehörde anders vereinbart - mindestens mit folgenden Angaben zu kennzeichnen:
 - a) Anzahl, Rasse/Stamm/Genotyp und Geschlecht der Tiere
 - b) Kennzeichnung der Tiere, soweit vorgeschrieben
 - c) Datum der Aufnahme in die Tierhaltung bzw. Geburtsdatum
 - d) verantwortliche Person / Versuchsleiter
 - e) ggf. Versuchsnummer eines genehmigten oder angezeigten Tierversuches.
9. Anfallende tote Tiere sind getrennt ordnungsgemäß zu lagern und unschädlich zu beseitigen.
10. Das Regierungspräsidium behält sich vor, Nebenbestimmungen nachträglich aufzunehmen, zu ändern oder zu ergänzen oder die Erlaubnis zu widerrufen, soweit dies aus tierschutzrechtlichen Gründen erforderlich ist.

Sachverhalt:

Das Max-Planck-Institut für biologische Kybernetik hat mit Schreiben vom 30.05.2011 die Erweiterung der vorhandenen Erlaubnis zur Haltung bzw. Zucht bestimmter Wirbeltiere beantragt. Die Änderungen hinsichtlich der Nutzung der Räumlichkeiten machen eine überarbeitete und konsolidierte Fassung der Erlaubnis erforderlich. Anlässlich einer Vor-Ort-Besichtigung am 06.07.2011 durch Herrn Dr. Bauer (Landratsamt Tübingen - Veterinäramt) und Frau Dr. Hogreve (Regierungspräsidium Tübingen) wurde festgestellt, dass die Voraussetzungen für die Erteilung der Erlaubnis erfüllt sind.

Begründung:

Rechtsgrundlage der Erlaubnispflicht ist § 11 Abs. 1 Nr. 1a und 1b TierSchG. Die Erlaubnis ist erforderlich für die Haltung und Zucht von Wirbeltieren zu den in § 9 Abs. 2 Nr. 7, § 4 Abs. 3, § 6 Abs. 1 Satz 2 Nr. 4, § 10 Abs. 1 sowie § 10a TierSchG näher bestimmten wissenschaftlichen Zwecken.

Nach § 1 der Verordnung des Ministeriums Ländlicher Raum über Zuständigkeiten nach dem Tierschutzrecht vom 25. März 1999 (GBl. S. 166) ist das Regierungspräsidium für die Erteilung der Erlaubnis zuständig.

Die Nebenbestimmungen finden ihre Rechtsgrundlage in § 11 Abs. 2 a TierSchG in Verbindung mit Ziffer 12.2.5.2. der Allgemeinen Verwaltungsvorschrift zur Durchführung des Tierschutzgesetzes.

Die Bestimmungen unter Ziffer I. 1. und 4. sowie unter II. 1. bis 9. sind erforderlich, um sicherstellen zu können, dass die der Tätigkeit dienenden Räume und Einrichtungen auch eine artgemäße und bedarfsgerechte Ernährung, Pflege und Unterbringung der Tiere ermöglichen (§ 11 Abs. 2 Nr. 3 TierSchG).

IV.

Hinweise:

1. Eingriffe und Behandlungen zu wissenschaftlichen Zwecken an Wirbeltieren unterliegen der Anzeige-/Genehmigungspflicht. Für die Durchführung von Experimenten sind insbesondere die Bestimmungen der §§ 4 bis 11b TierSchG zu beachten.

Sofern Eingriffe oder Behandlungen im Sinne von § 9 Abs. 2 Nr. 7, § 4 Abs. 3, § 6 Abs. 1 Satz 2 Nr. 4, § 10 Abs. 1 sowie § 10a TierSchG durchgeführt werden, ist die Einrichtung verpflichtet, einen oder mehrere Tierschutzbeauftragte zu bestellen. Die Tätigkeiten des Tierschutzbeauftragten - insbesondere dessen Stellung und Befugnisse - sind gemäß § 8 b Nr. 6 TierSchG schriftlich durch Satzung oder innerbetriebliche Anweisung anhand der Vorgaben des § 8 b TierSchG zu regeln.

2. Wenn die Auflage nicht erfüllt wird, müssen Sie mit dem Widerruf der Erlaubnis rechnen (§ 49 Abs. 2 Nr. 2 Landesverwaltungsverfahrensgesetz - LVwVfG).
3. Die Erlaubnis muss widerrufen werden, wenn die Anlage nicht mehr den tierschutzrechtlichen Bestimmungen entspricht.
4. Da die Antragstellung in der Vergangenheit von unterschiedlichen Personen erfolgte, richtet das Regierungspräsidium diesen Bescheid und zukünftige weitere Bescheide an Sie als Verantwortlichen im Sinne von § 11 Abs. 1 Satz 2 Nr. 2 TierSchG.

Es wird empfohlen, allen an der Zucht und Haltung von Wirbeltieren zu Versuchszwecken beteiligten Personen diese Erlaubnis zur Kenntnis zu geben.

Gebührenfestsetzung:

Die Gebühr wird mit der Bekanntgabe dieses Bescheides fällig, § 18 LGebG. Sie ist innerhalb eines Monats nach Fälligkeit an die Landesoberkasse Baden-Württemberg (Konto-Nr. 7 495 530 102, Baden-Württembergische Bank, BLZ: 600 501 01) unter Verwendung des beigefügten Zahlscheins bzw. Angabe des Kassenzeichens zu überweisen. Nach Ablauf der Zahlungsfrist müssen Säumniszinsen nach § 20 LGebG erhoben werden.

V.

Rechtsbehelfsbelehrung:

Gegen diesen Bescheid kann innerhalb eines Monats nach Bekanntgabe beim Verwaltungsgericht Sigmaringen, Karlstraße 13 in 72488 Sigmaringen, schriftlich oder zur Niederschrift des Urkundsbeamten der Geschäftsstelle des Gerichts Klage gegen das Land Baden-Württemberg erhoben werden.

Mit freundlichen Grüßen



Dr. Conrad Maas

Über den
Tierschutzbeauftragten
der Einrichtung

An das
Regierungspräsidium Tübingen
- Referat 35 -
Konrad-Adenauer-Straße 20

72072 Tübingen

Antrag auf Genehmigung von Versuchsvorhaben

Alle Paragraphenangaben beziehen sich auf das Tierschutzgesetz in der Fassung der Bekanntmachung vom 25. Mai 1998 (BGBl. I S. 1105)

N a m e u n d A n s c h r i f t des Antragstellers, sowie Tel./Fax-Nr

Prof. NIKOS LOGOTHETIS
Max Planck Institut für biologische Kybernetik
Spemannstr. 38
72076 Tübingen
Tel. 07071/ 601 650
Fax. 07071/ 601 652

Der Unterzeichnende beantragt die Genehmigung zur Durchführung von Tierversuchen nach § 8 Abs.1 des Tierschutzgesetzes für folgendes Versuchsvorhaben:

1. Angaben zum Versuchsvorhaben
 - 1.1 Bezeichnung des Versuchsvorhabens (einschließlich der internen Kurzbezeichnung)

Funktion und Konnektivität von neuromodulatorischen Kernen

- 1.2 Ort, beabsichtigter Beginn (Datum) und voraussichtliche Dauer des Versuchsvorhabens (§ 8 Abs. 2 Satz 3 i.V.m. § 8a Abs. 2 Nr. 4):

Max Planck Institut für biologische Kybernetik, Tübingen, Deutschland
1. April 2009 - 31. März 2011

2. Personen, die im Rahmen der Versuchsdurchführung Eingriffe und Behandlungen an Tieren durchführen (§ 9 Abs. 1)

- 2.1 Leiter des Versuchsvorhabens

- 2.1.1 Name und Anschrift (inkl. Tel./Fax-Nr. und E-mail-Adresse):

Prof. Nikos K. Logothetis
Max Planck Institut für biologische Kybernetik
Spemannstr. 38, 72076 Tübingen
Tel. 07071/ 601650, Fax. 07071/ 601652. email. nikos.logothetis@tuebingen.mpg.de

2.1.2 Berufsbezeichnung:

Direktor am Max Planck Institut für biologische Kybernetik

2.1.3 Fachliche Eignung mit Darstellung der tierexperimentellen Erfahrung:

Der Antragsteller absolvierte ein Biologiestudium, Fachrichtung Humanbiologie, und hat über fünfundzwanzig Jahre Erfahrung sowohl mit psychophysischen, elektrophysiologischen und Läsionsexperimenten, als auch mit bildgebenden MR-Verfahren an Affen und Ratten.

ist bereits mit Antrag vom 8. Januar 1997 dargelegt worden

2.2 Stellvertretender Leiter des Versuchsvorhabens

2.2.1 Name und Anschrift:

Dr. Oxana Eschenko
Max Planck Institut für biologische Kybernetik
Spemannstr. 38, 72076 Tübingen
Tel. 07071/ 601 1679 email. Oxana.eschenko@tuebingen.mpg.de

2.2.2 Berufsbezeichnung:

Wissenschaftlerin am Institut für biologische Kybernetik.

2.2.3 Fachliche Eignung mit Darstellung der tierexperimentellen Erfahrung:

Frau Eschenko studierte Biologie (Master 1993) mit anschließender Doktorarbeit in Neurowissenschaften (PhD 1999, Universität Moskau). Anschließend arbeitete sie als Wissenschaftlerin in Finnland (1999-2000), den USA (2001-2003) und Frankreich (2003-2006) und ist seit 2006 als Wissenschaftlerin an unserem Institut tätig. Die meisten ihrer Arbeiten beruhen auf Nagetieren als Modellsystem, an welchen sie sowohl die neuronalen Grundlagen des Schlafes als auch von verschiedenen Verhaltensmustern untersuchte. In ihrer Arbeit lernte und kombinierte sie verschiedenste Techniken, von Verhaltenstraining, über elektrophysiologische Messungen bis hin zu histologischen Untersuchungen von Gewebe. Frau Eschenko hat mehr als 10 Jahre Erfahrung in der Arbeit mit Ratten, dem Training der Tiere auf Verhaltensaufgaben, steriler Chirurgie und der Überwachung von Tieren unter Narkose sowie elektrophysiologischen Ableitungen.

2.3 Sonstige Personen, die im Rahmen der Versuchsdurchführung Eingriffe oder Behandlungen an Tieren vornehmen

2.3.1 Namen der Personen und deren Tätigkeit (ausgenommen Betäubung, siehe Nr. 3.2):

- Dr. Henry Evrard
- Dr. Christoph Kayser
- Dr. Ulrich Schridde
- Dr. Alexander Rauch
- Dr. Santiago Canals
- Herr Michael Beyerlein, TA

Aufgaben: chirurgische Operationen, die präoperative und postoperative Versorgung der Tiere, sowie elektrophysiologische Messungen

2.3.2 Qualifikation (§ 9 Abs. 1 Satz 2 und 3) und tierexperimentelle Erfahrung; ggf. Hinweis auf eine erteilte Ausnahmegenehmigung:

- Dr. Henry Evrard: Studium der Neurowissenschaften, PhD in Neurowissenschaften, 6 Jahre Erfahrung in steriler Chirurgie und Elektrophysiologie an Ratten und Affen.
- Dr. Christoph Kayser: Neurowissenschaftler, 8 Jahre Erfahrung mit elektrophysiologischen und bildgebenden MR-Verfahren an Ratten, Katzen und Affen. Aus- und fortgebildet zur Durchführung und Leitung von Tierversuchen in der Schweiz nach FELASA Kat. B&C.
- Dr. Ulrich Schridde: PhD in Neurowissenschaften, 7 Jahre Erfahrung mit Narkose von Ratten.
- Dr. Alexander Rauch: Studium der Humanmedizin, Dr. med, 6 Jahre Erfahrung im Umgang mit Ratten, steriler Chirurgie und der Überwachung von Tieren unter Narkose.
- Santiago Canals: Studium der Biologie und Neurowissenschaften, PhD in Neurowissenschaften, 11 Jahre Erfahrung in der Arbeit mit Ratten, der Ausführung steriler Chirurgie und Elektrophysiologie und der Überwachung von Tieren unter Narkose.
- Michael Beyerlein: Studium der Biomedizintechnik, 4 Jahre Erfahrung in der Arbeit mit narkotisierten Ratten, steriler Chirurgie, Durchführung von Mikroinjektionen und MRT. Ausbildung zur Durchführung von Tierversuchen nach FELASA Kat. B.

2.4.1 Gegebenenfalls Namen der Personen, die die Betäubung durchführen oder die Durchführung der Betäubung beaufsichtigen:

- Prof. Nikos Logothetis
- Dr. Christoph Kayser
- Dr. Henry Evrard
- Dr. Oxana Eschenko
- Dr. Ulrich Schridde
- Dr. Alexander Rauch
- Dr. Santiago Canals
- Herr Michael Beyerlein, TA
- Herr Mark Augath, TA

2.4.2 Qualifikation (§ 9 Abs. 2 Nr. 4 Satz 2) und tierexperimentelle Erfahrung:

Herr Mark Augath arbeitet seit vielen Jahren bei uns als Technischer Mitarbeiter und Experte für MRT; außerdem hat er viele Jahre Erfahrung in der Überwachung von Narkosen. Die Qualifikationen der weiteren Personen wurden oben aufgeführt. Alle haben mehrere Jahre Erfahrung in der Einleitung und Aufrechterhaltung von Narkosen.

3. Berechtigung der Personen zur Benutzung der Einrichtung, in der die Tierversuche durchgeführt werden (§ 8 Abs. 6)

3.1 Sind die unter Abschnitt 2 genannten Personen bei der Einrichtung beschäftigt?

ja

3.2 Wenn nein, sind sie mit Zustimmung des verantwortlichen Leiters der Einrichtung zur Benutzung der Einrichtung befugt?

4. Erfüllung der Voraussetzungen des § 8 Abs. 3 und 4

4.1 Name, Anschrift und Qualifikation der für die Pflege und Betreuung der Tiere verantwortlichen Person:

Frau Tania Carbrera und Herr Marcel Henni. Beide arbeiten am Max Planck Institut für Biologische Kybernetik als Tierpfleger. Frau Carbrera ist zusätzlich ausgebildete Tierärztin.

4.2 Name, Anschrift und Qualifikation der für die medizinische Versorgung der Tiere verantwortlichen Person

Dr. Cornelia Fritz, Approbierte Tierärztin am Institut für Biologische Kybernetik.
Vertretung: Dr. Steidel und Hartmann, Tierärztliche Klinik, Jurastr. 23, 72072 Tübingen

5. Verfahren am Versuchsende

Die Tiere werden mit einer Überdosis Pentobarbital (100mg/kg) getötet. Gegebenenfalls wird das Gehirn für histologische Untersuchungen fixiert und entnommen. Hierzu wird das Tier unter tiefer Anästhesie mit einer heparinhaltigen, physiologischen Kochsalzlösung und anschließend mit 4% Paraformaldehyd in Phosphatlösung perfundiert. Zur histologischen Untersuchung wird das Gehirn entnommen und mittels eines Mikrotoms in Schnitte zerlegt. Diese werden mit etablierten Methoden gefärbt um die verschiedenen Areale zu identifizieren.

5.1 Name und Anschrift des Tierarztes, dem nach Abschluß des Versuchs die überlebenden Tiere der in § 9 Abs. 2 Nr. 8 genannten Arten vorgestellt werden:

Alle Tiere werden am Ende der Studie getötet.

6. Verpflichtungserklärung

Mit der Unterschrift verpflichtet sich der Leiter und sein Stellvertreter, die Verantwortung für die Einhaltung der Vorschriften nach § 9 Abs. 1 und 2, sowie ggf. von Auflagen nach § 8 zu übernehmen und die Aufzeichnungspflicht gemäß § 9a zu beachten.

Gleichzeitig wird die Kenntnis des Tierschutzgesetzes bestätigt.

Tübingen, 28.02.2009

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Unterschrift des Leiters des Versuchsvorhabens

.....
Unterschrift des stellvertretenden Leiters des Versuchsvorhabens

7. Angaben zum Versuchsvorhaben

7.1 Bezeichnung des Versuchsvorhabens (einschließlich der internen Kurzbezeichnung)

Funktion und Konnektivität von neuromodulatorischen Kernen

7.2 Zweck und Unerläßlichkeit des Versuchsvorhabens (§ 7 Abs. 2)

7.2.1 Zweck des Versuchsvorhabens und wissenschaftlich begründete Darlegung, daß dieser einem der in § 7 Abs. 2 Satz 1 genannten Zwecke zuzuordnen ist:

Die geplanten Experimente an Ratten dienen der Grundlagenforschung und dem Verständnis der neuromodulatorischen Strukturen im Gehirn.

Während das Großhirn vor allem mit der Verarbeitung von sensorischen Reizen, höheren kognitiven Leistungen und der Planung von motorischen Reaktionen beschäftigt ist, gibt es eine Reihe von Kernen im Hirnstamm und Mittelhirn welche die ‚Arbeit‘ des Großhirns modulieren. Diese neuromodulatorischen Neurone beeinflussen die Aktivität im Großhirn durch ihre weitgefächerten anatomischen Projektionen und durch eine Reihe von spezifischen Transmittern, den so genannten Neuromodulatoren, wie zum Beispiel Dopamin, Serotonin oder Noradrenalin (Foote and Morrison, 1987). Der genaue Einfluss der Neuromodulatoren im Zielgebiet und das Zusammenspiel verschiedener Neuromodulatoren sind bis jetzt nicht verstanden. Klar ist allerdings, dass viele psychologisch-neuronale Zustände von der Ausschüttung von Neuromodulatoren abhängen. So spielen diese eine wichtige Rolle bei der Kontrolle von kognitiven Leistungen wie Aufmerksamkeit, Motivation, Lernen und Gedächtnis (Aston-Jones and Cohen, 2005; Boulougouris and Tsaltas, 2008; Gonzalez-Burgos and Feria-Velasco, 2008). Fehlfunktionen in diesen Systemen können die Funktion des Großhirns stark beeinträchtigen, was zu einer Reihe von psychopathologischen Zuständen führt. Dazu gehören Änderungen des Gemütszustandes, der Motivation und der Affektion, wie sie zum Beispiel bei Parkinson, Depression, Schizophrenie und bipolaren Störungen eine Rolle spielen (Aston-Jones and Cohen, 2005; Jacobs and Azmitia, 1992; Schultz, 1998). Entsprechend ist ein Verständnis dieser neuromodulatorischen Systeme nicht nur von Interesse für ein grundlegendes Verständnis der Hirnfunktion, sondern betrifft auch medizinisch-psychologische äußerst relevante Themen.

Das schwierige an der Erforschung der Neuromodulatoren ist, dass die entsprechenden Kerne mehrere kortikale Areale gleichzeitig beeinflussen, untereinander wechselwirken und für einen bestimmten klinischen Zustand der relative Einfluss auf verschiedene Areale genau so wichtig ist wie der spezifische Einfluss auf ein gezieltes Areal (Foote and Morrison, 1987). Hinzu kommt, dass die Aktivität der neuromodulatorischen Kerne auch durch reziproke Verbindungen des Großhirns kontrolliert werden kann, und verschiedene neuromodulatorische Systeme sich gegenseitig beeinflussen bzw. gegensätzliche Wirkung auf das Großhirn ausüben können. Um diese Systeme besser zu verstehen planen wir daher systematische Untersuchungen ihrer funktionellen Verbindungen sowie ihrer getrennten bzw. kombinierten Einflüsse auf Zellen im Großhirn. Um ein komplexes System zu verstehen, ist es notwendig möglichst kontrollierte Bedingungen zu erhalten, weshalb die geplanten Versuche unter Narkose erfolgen sollen. Zudem werden wir die in vorhergehenden Versuchen entwickelten Methoden der kombinierten elektrophysiologischen Messungen, elektrischen Mikrostimulation und Magnetresonanztomographie verwenden, was es uns erlaubt, den Einfluss der neuromodulatorischen Systeme auf verschiedenen Skalen zu untersuchen.

Ratten bieten sich für alle diese Fragen als ideales Modellsystem an. Es lassen sich alle für diese Versuche relevanten invasiven Methoden anwenden und die relative Größe verschiedener Hirnstrukturen ist sehr konstant über Individuen, was einen großen Vorteil für die Studie von sehr kleinen Strukturen wie den neuromodulatorischen und integrativen Kernen bietet.

Die geplanten Versuche

Das Hauptziel dieses Projektes ist es die Wechselwirkung zwischen den drei wichtigsten Neuromodulatoren Serotonin, Dopamin und Noradrenalin zu verstehen. Jedes dieser Systeme wurde mit psychopathologischen Zuständen wie Schizophrenie, Depression oder Parkinson in Verbindung gebracht und alle beeinflussen Fähigkeiten wie Aufmerksamkeit, Lernen und Gedächtnis. Entsprechend zielen viele der momentan auf dem Markt befindlichen Psychopharmaka auf diese drei Systeme. Die neuromodulatorischen Neurone dieser Systeme befinden sich für das serotonerge System im Raphe Kern (RK), für das dopaminerge System vor allem in der Ventral Tegmental Area (VTA) und der Substantia Nigra, und für das noradrenerge System im Locus Coeruleus (LC). Jedes dieser Systeme unterhält diffuse Projektionen in weite Teile des Großhirns und erhält von diesem eine Menge an afferenten Verbindungen. Zudem können die verschiedenen Systeme untereinander wechselwirken, so dass einzelne Systeme unter natürlichen Bedingungen kaum getrennt verstanden werden können, bzw. selten allein auf das Großhirn wirken.

1 – Funktionelle Verbindungen der noradrenergen, dopaminergen und serotonergen Systeme

Wir möchten daher den Einfluss der verschiedenen Systeme und ihrer Wechselwirkung auf das Großhirn unter gut kontrollierten Bedingungen untersuchen. Dazu planen wir Experimente mit in Tieren unter Narkose, da sich so am besten sämtliche sensorischen Reize kontrollieren lassen, und man mit einer Kombination von elektrischer Stimulation und lokaler Applikation von Neuromodulatoren die Interaktionen der verschiedenen Systeme genau charakterisieren kann. Die hier geplanten Experimente basieren wesentlich auf vorhergehenden Studien unseres Labors, welche die Techniken entwickelt haben, welche hier zur Anwendung kommen (Canals et al., 2008b). Wichtig für diese Versuche ist vor allem die Kombination von Magnetresonanztomographie (MRT) und elektrischer Mikrostimulation, welche es erlaubt die funktionellen Verbindungen der einzelnen neuromodulatorischen Systeme zu bestimmen. Dies ist notwendig da klassische Anatomische Studien neuronaler Verbindungen keine Aussage über den funktionellen Einfluss einzelner Projektionen machen können und daher nicht bekannt ist, inwiefern sich die Einflussgebiete der drei neuromodulatorischen Systeme überlappen, und welche Areale sie konkret beinhalten.

2 – Elektrophysiologie des neuromodulatorischen Einfluss auf neuronale Aktivität im Großhirn

Komplementär zu diesen Versuchen möchten wir in elektrophysiologische Ableitungen den funktionellen Einfluss der Neuromodulatoren auf Neurone im Großhirn charakterisieren. Dazu werden wir mittels moderner elektrophysiologischer Methoden die Aktivität einzelner Zellen und ganzer Zellpopulationen in verschiedenen Arealen des Großhirns messen, und gleichzeitig die neuromodulatorischen Systeme durch elektrischer Stimulation oder direkte Applikation von Neuromodulatoren steuern. Ziel ist es die Interaktion der verschiedenen neuromodulatorischen Systeme untereinander, und mit Aktivität ausgelöst durch Sinnesreize, zu charakterisieren, um zu verstehen wie die verschiedenen Systeme zusammenspielen und die Arbeit des Großhirns beeinflussen.

7.2.2 Wissenschaftlich begründete Darlegung der Unerläßlichkeit des Versuchsvorhabens unter Berücksichtigung des jeweiligen Standes der wissenschaftlichen Erkenntnisse (§ 7 Abs. 2 Satz 2 erster Halbsatz):

Das Ziel unserer Untersuchungen ist das Verständnis der physiologischen Mechanismen, die der Sinneswahrnehmung, dem Lernen und höherer kognitiver Leistungen zugrunde liegen. In den hier beantragten Studien konzentrieren wir uns auf neuromodulatorische Strukturen. In den letzten Jahren ist klar geworden, dass sowohl die einfache Verarbeitung von Sinnesreizen, wie auch höhere Kognitive Leistungen wie Lernen und Aufmerksamkeit durch Neuromodulatoren beeinflusst werden. Dies zeigt sich nicht nur in Experimenten mit Tieren, sondern auch in Patienten. Viele psychopathologische Ausfälle, wie zum Beispiel Parkinson, Depression, bipolare Störungen, Aufmerksamkeitsdefizite oder Schizo-

phrenie hängen kausal mit Veränderungen der neuromodulatorischen Systeme zusammen. Leider sind weder diese genauen Zusammenhänge, noch die genaue Rolle einzelner Systeme verstanden. Ein besseres Verständnis der neuromodulatorischen Kerne kann nicht nur neue Erkenntnisse für die Grundlagenforschung bringen, sondern betrifft direkt Themen welche klinisch relevant sind. Es gibt bereits Modellsysteme an Ratten oder Primaten welche zur Studie von den genannten psychopathologischen Ausfällen dienen, und an welchen Medikamente und andere Therapien entwickelt werden. Entsprechend lässt sich die Erkenntnis aus den hier geplanten Studien später gegeben falls direkt einsetzen um diese Therapieansätze weiter zu verbessern. So können diese Untersuchungen zur langfristigen Verbesserung einer Vielzahl von medizinischen und praktischen Anwendungen beitragen.

7.2.3 Wissenschaftlich begründete Darlegung, daß der Versuchszweck nicht durch andere Methoden oder Verfahren als den Tierversuch erreicht werden kann (§ 7 Abs. 2 Satz 2 zweiter Halbsatz):

Die Gehirnforschung ist der Versuch, eine der kompliziertesten Strukturen im bekannten Universum zu verstehen. Der massive Vernetzungsgrad und die unterschiedlichen hierarchischen Organisationsstufen dieses Systems sind kaum für eine realistische Simulation durch künstliche informationsverarbeitende Systeme geeignet. Während künstliche neuronale Netzwerke, mathematische Algorithmen und theoretische Methoden uns im allgemeinen mit exzellenten Möglichkeiten versorgen, um die Anforderungen einer gegebenen Informationsverarbeitungsaufgabe zu erfüllen, sind sie kaum ausreichend selbst für einfache biophysikalische Simulationen. Durch das Erforschen des menschlichen oder tierischen Verhaltens können die sensorischen Kapazitäten und die Entscheidungsfähigkeit des Organismus gemessen werden, aber es reicht nicht aus, um die strukturellen und funktionalen Architekturtypen des Gehirns aufzuzeigen, die solches Verhalten ermöglichen. Die Komplexität solcher Prozesse, die von einem gegebenen Reiz zu einer bestimmten Reaktion führen, kann unmöglich in Zellkulturen, sondern nur am lebenden Tier untersucht werden. Eine integrative, interdisziplinäre Methode ist die einzige Möglichkeit in der Gehirnforschung. Nur durch eine kombinierte Studie des Gehirnaufbaus und seiner Verschaltung, sowie auch der Gehirnaktivität im gleichen Subjekt, kann schlussendlich die Funktionsweise dieses komplexen Steuerzentrums des menschlichen Körpers verstanden werden.

7.3 Ausschöpfung zugänglicher Informationsmöglichkeiten (§ 8 Abs. 3 Nr. 1 Buchstabe b)

7.3.1 Welche Informationsmöglichkeiten wurden genutzt?

- Studium der internationalen Fachliteratur
- Besuch von wissenschaftlichen Kongressen im In- und Ausland
- Erfahrungsaustausch und persönlicher Kontakt mit Wissenschaftlern und Zusammenarbeit im In- und Ausland

7.3.2 Wissenschaftlich begründete Darlegung, daß das angestrebte Versuchsergebnis nicht hinreichend bekannt ist bzw. daß die Überprüfung des hinreichend bekannten angestrebten Versuchsergebnisses durch einen Doppel- oder Wiederholungsversuch unerlässlich ist.

Die in diesem Antrag beschriebenen Experimente sind völlig neuartig und sowohl für die Grundlagen als auch für die klinische Forschung wichtig. Unsere Publikationen in wissenschaftlichen Zeitschriften beweisen den innovativen und originellen Charakter dieser Forschung. Keines der Experimente ist repetitiv oder ist schon von anderen Wissenschaftlern durchgeführt worden. Einige der hier genannten Methoden wollen wir bei uns neu etablieren, um sie auch später in anderen Projekten anwenden zu können. Diese Methoden versprechen neuartige und verfeinerte Erkenntnisse, was es in späteren Experimenten vielleicht auch dazu dienen kann die Anzahl der Tiere langfristig zu reduzieren.

7.4 Art und Anzahl der vorgesehenen Tiere

(§ 8 Abs. 2 Satz 3 i.V.m. § 8a Abs. 2 Nr. 2 und § 9 Abs. 2)

7.4.1 Vorgesehene Tierarten und Begründung für die Wahl der Tierart

(§ 9 Abs. 2 Nr. 1):

Es werden für alle Experimente Ratten (z.B. Wistar, Sprague Dawley, Fischer, Long Evans) eingesetzt, da sie weltweit am meisten für Experimente verwendet werden, speziell für Tierexperimente gezüchtet sind, und schnell zur Verfügung stehen. Dies erlaubt einen minimalen Aufenthalt in unseren Tierställen. Im Vergleich zur Maus sind Ratten für diese Experimente besser geeignet, da sie ausgeprägtere Sinnessysteme besitzen und deutlich besser Lerneigenschaften haben als Mäuse. Außerdem ist das Gehirn der Ratte größer als das von Mäusen, was für die MR basierenden und elektrophysiologischen Methoden von Vorteil ist.

7.4.2 Vorgesehene Anzahl und Begründung für die Anzahl der Tiere einschließlich Angaben zur biometrischen Planung (§ 9 Abs. 2 Nr. 2):

1 – F funktionelle Verbindungen der noradrenergen, dopaminergen und serotonergen Systeme

Ziel dieser Versuche ist es den funktionellen Einfluss dieser neuromodulatorischen Systeme auf verschiedene Areale des Großhirns zu bestimmen. Da verschiedene solche Systeme in die gleichen Areale des Großhirns projizieren, ist es wichtig ihr Zusammenspiel zu charakterisieren. Zusätzlich werden weite Teile des Großhirns durch externe sensorische Reize aktiviert, weshalb die Wirkung der neuromodulatorischen Zentren abhängig vom ‚Kontext‘ durch sensorische Reize ist. Daher planen wir diese verschiedenen Zentren elektrisch zu stimulieren und die resultierenden Aktivitätsmuster im Großhirn mittels MRT zu messen (Canals et al., 2008b; Tolia et al., 2005). Dies geschieht sowohl während nur ein neuromodulatorisches System stimuliert wird, als auch wenn mehrere Systeme stimuliert werden, und wenn Kombinationen von neuromodulatorischen und Sinnessystemen stimuliert werden.

1.1 Optimierung der Stimulationsparameter

Als Vorbereitung müssen einige der Stimulationsparameter optimiert werden, um möglichst gute MRT Bilder zu erhalten. Ein Parameter ist z.B. die Stärke der Mikrostimulation, welche so bestimmt wird, das für jedes System gute MRT Bilder mit minimal notwendiger Stromstärke erzielt werden.

Biometrie: Das Hauptproblem bei diesen Versuchen ist es Mikroelektroden in den neuromodulatorischen Zentren zu platzieren, da diese Kerne sehr klein sind (z.B. LC ~0.3-0.8 mm Durchmesser). Aufgrund unserer (Eschenko and Sara, 2008) und der Erfahrung Anderer (Aston-Jones and Bloom, 1981; Wu et al., 1999) gehen wir davon aus, dass nur 30-50% der Versuche erfolgreich sind; dies gilt insbesondere für den LC, welcher die kleinste dieser Strukturen ist. Die Größe dieser Struktur ist auch in der Abbildung in Anhang 3 illustriert. Daher ist es bei allen diesen Versuchen notwendig genügend Tiere als Sicherheit einzuplanen. Zur Bestimmung der optimalen Stimulationsparameter benötigen wir 3 Tiere pro System. Mit der entsprechend vorsichtigen Planung:

- Etablierung der Methode 3*3 Tiere (50% Erfolgsquote) 18 Tiere

TOTAL 18 Ratten

1.2 Vergleich der Aktivierung durch Stimulation verschiedener Neuromodulatoren

Ziel ist es die MRT Aktivierung im Großhirn während der Stimulation eines oder mehrerer Neuromodulatoren zu messen und die Areale welche durch verschiedenen Neuromodulatoren aktiviert werden zu vergleichen; insbesondere ist es hierbei von Interesse, welche Areale nur durch ein, bzw. welche durch mehrere Systeme aktiviert werden. Die kombinierte Stimulation mehrerer Kerne ist technisch äußerst schwierig und wird vorerst nur für eine Kombination geplant.

Biometrie: Der Vergleich der Aktivierungen durch verschiedene Modulatoren erfolgt aufgrund einer statistischen Auswertung der MRT Bilder. Dieser Vergleich erfolgt mittels eines t-tests. Leider gibt es kaum Daten von Experimenten mit neuromodulatorische Aktivierungen, weshalb eine genaue Voraussage der Effektgröße schwierig ist. Wir gehen davon aus, dass die Effekte ähnlich zu denen durch sensorische Stimulation sind, und planen daher einen Vergleich zwischen verschiedenen Modulatoren mit 5 Tieren. Wie oben (1.1) beschrieben hängt die Erfolgsquote von der Größe des Kerns ab.

- Stimulation des noradrenergen Systems (LC): 5 Tiere (40% Erfolg) 12 Tiere
- Stimulation des dopaminergen Systems (VTA): 5 Tiere (60% Erfolg) 8 Tiere
- Stimulation des serotonergen Systems (RK): 5 Tiere (60% Erfolg) 8 Tiere
- Kombinierte Stimulation des LC und RK: 5 Tiere (30% Erfolg) 16 Tiere

TOTAL 44 Ratten

1.3 Kombinierte elektrische und pharmakologische Stimulation von Neuromodulatoren

Ziel dieser Versuche ist es die Spezifität der elektrischen Mikrostimulation eines neuromodulatorischen Systems zu bestimmen. Diese Versuche sind komplementär zu der Stimulation verschiedener Zentren unter 1.1.2 und kombinieren die elektrische Stimulation eines Zentrums mit der direkten Applikation pharmakologischer Agonisten eines anderen Systems. Durch die elektrische Stimulation eines Kernes werden sämtliche Projektionen dieses Kernes aktiviert, was ein Vorteil ist, da verschiedene Rezeptoren im Großhirn aktiviert werden. Allerdings hat dieses den Nachteil, dass nicht nur die neuromodulatorischen Neurone im jeweiligen Kern aktiviert werden, sondern auch andere, deren Einfluss unbekannt ist. Diese Nachteile werden durch die direkte Applikation von neuromodulatorischen Substanzen umgangen; allerdings hat dies wiederum den Nachteil nur einen bestimmten Rezeptortyp zu aktivieren (je nach Agonist). Deswegen liefern diese Experimente wichtige komplementäre Daten zu 1.2. Die elektrische Stimulation bleibt in diesen Versuchen auf den LC beschränkt, da dieser am homogensten ist, und daher bei seiner Stimulation hauptsächlich noradrenerge Fasern und wenige andere Neurone aktiviert werden.

Biometrie: Die Planung der Versuche ist wie unter 1.2.

- Mikrostimulation des LC, Applikation von Dopamin: 5 Tiere (40% Erfolg) 12 Tiere
- Mikrostimulation des LC, Applikation von Serotonin: 5 Tiere (40% Erfolg) 12 Tiere

TOTAL 24 Ratten

1.4 Kombinierte Stimulation von Neuromodulatoren und Sinnessystemen

Ziel ist es die Interaktion von externer sensorischer Stimulation von Neuromodulatoren zu untersuchen. Dazu wird die MRT Aktivierung im Großhirn während Stimulation eines Neuromodulators und eines Sinnessystems (Seh- und Tastsinn) gemessen und verglichen.

Biometrie: Die Planung der Versuche ist wie unter 1.2.

- Stimulation des LC, Stimulation des Tastsinns: 5 Tiere (40% Erfolg) 12 Tiere
- Stimulation des LC, Stimulation des Sehsinns: 5 Tiere (40% Erfolg) 12 Tiere

TOTAL 24 Ratten

1.5 Abbildung der Verbindungen der neuromodulatorischen Zentren mittels MRT-basierender Kontrastmittel

Ziel ist es die unter 1.2 bestimmten Zielgebiete der verschiedenen Neuromodulatoren mit einer anderen Methode zu validieren. In vorhergehenden Studien haben wir die Methoden der MRT-basierenden Kontrastmittel entwickelt (Canals et al., 2008a), welche es erlaubt die Verbindungen eines bestimmten Systems in vivo mittels MRT darzustellen. Dazu wird das MRT-Kontrastmittel Mangan-Chlorid ($MnCl_2$, 1M Lösung in NaCl) in den neuromodulatorischen Kern injiziert. Das Kontrastmittel verteilt sich entlang der Nervenbahnen des modulatorischen Systems, was anschließend (nach 24 Stunden) in einer

MRT Messung abgebildet wird. Um einen direkten Vergleich der verschiedenen Systeme zu erhalten ist es wichtig die Projektionen von mindestens zwei Systemen am gleichen Tier zu bestimmen.

Eingriffe: An jedem Tier werden folgende Messungen vorgenommen (alle unter Narkose):

Baseline MRT Messung – 48h Pause – Eingriff mit Injektion und anschl. MRT Messung – 24 h Pause – MRT Messung - 3 Wochen Pause, um einen Abbau des Kontrastmittels zu gewährleisten - Eingriff mit Injektion und anschl. MRT Messung – 24 h Pause – MRT Messung ohne aufwachen.

Biometrie: Die Verteilung der Kontrastmittel im Anschluss an die Injektion wird mit einer Kontrollmessung am gleichen Tier vor der Injektion verglichen (t-test). Um statistisch valide Bilder der Verteilung zu erhalten machen wir entsprechend unserer Erfahrung folgende Annahmen: 2-seitiger Test, p-wert 0.05, statistische Power des Tests 0.8 und erhalten daraus eine minimale Anzahl von 5 Tieren. Wie oben gehen wir davon aus, dass Injektionen in neuromodulatorischen Kerne nur bei 40% gelingen.

- Injektion in LC und anschließend in VTA: 5 Tiere (40% Erfolg) 12 Ratten
- Injektion in LC und anschließend in RK: 5 Tiere (40% Erfolg) 12 Ratten

TOTAL 24 Ratten

2 – Elektrophysiologie des neuromodulatorischen Einflusses auf neuronale Aktivität im Großhirn

Ziel dieses Projektes ist es die Auswirkung der Stimulation eines Neuromodulators auf die Aktivität einzelner Neurone und ganzer Neuronen-Population im Großhirn zu messen. Zum Beispiel ist bekannt, dass serotonerge und noradrenerge Systeme die Antwortigenschaften von Zellen in sensorischen Arealen beeinflussen können, während andere Kombinationen die Antworten im Frontallappen beeinflussen (Hurley et al., 2004). Leider lassen sich die genauen Auswirkungen von Neuromodulatoren auf neuronale Antworten nicht aus der Verteilung von entsprechenden Rezeptoren oder afferenten Projektionen ableiten, weshalb eine direkte Messung des Einflusses auf neuronale Antworten notwendig ist (Eickhoff et al., 2007). Wir planen hier den Einsatz so genannter Multikontakt-Elektroden, welche es erlauben die neuronale Aktivität in einzelnen Schichten des Großhirns zu messen. Zunächst wird der Einfluss von sensorischer, neuromodulatorischer und kombinierter Stimulation gemessen. Anschließend wird das neuromodulatorische System durch eine systemische Injektion von dem entsprechenden Antagonisten deaktiviert, und die Messung wiederholt. Dadurch lassen sich die sensorischen Antworten in Abwesenheit von modulatorischen Einflüssen charakterisieren.

Die Zielgebiete unsere elektrophysiologischen Ableitungen ergeben sich teilweise aus den Ergebnissen in 1, da wir solche Gebiete untersuchen möchten, welche von mehreren Neuromodulatoren beeinflusst werden. Momentan scheinen die präfrontalen, visuellen oder somatosensorischen Areale die besten Kandidaten, da sie sowohl noradrenerge als auch serotonerge Projektionen aufweisen.

2.1 Etablierung der elektrophysiologischen Ableitungen

Aufgrund der technischen Herausforderung dieser Experimente, planen wir einige Experimente um den genauen Ablauf, die relevanten Parameter und den besten Umgang mit Multikontakt-Elektroden zu lernen. Insbesondere ist es wichtig die richtigen Koordinaten für die Platzierung der Mikroelektroden zu bestimmen. Die Position der einzelnen neuromodulatorischen Kerne im Rattenhirn kann zwar dem Atlas des Rattenhirns entnommen werden, allerdings zeigt die Erfahrung, dass die genauen Koordinaten von Alter und der genauen Art abhängt.

TOTAL 15 Ratten

2.2 Kombination von neuromodulatorischer und sensorischer Stimulation

Ziel dieser Versuche ist es den Einfluss von neuromodulatorischen Systemen auf die sensorischen Antworten in zwei verschiedenen Sinnessystemen zu messen. Die Aktivität der modulatorischen Systeme

wird sowohl durch elektrische Stimulation der entsprechenden Kerne als auch durch systemische Injektion von entsprechenden Agonisten kontrolliert.

Biometrie: Wie bei vielen elektrophysiologischen Studien ist es schwierig die genauen Effekte vorherzusagen und entsprechend Aussagen über die Anzahl der Tiere zu machen. Dies liegt daran, dass die Aktivität einer Anzahl von Neuronen gemessen werden muss, allerdings a priori nicht bekannt ist, welche Anzahl Neurone an einer Stelle gemessen werden können. Wir gehen davon aus, dass 6 Tiere pro Bedingung notwendig sind. Allerdings gibt es wie bei 1.1 eine Anzahl von Schwierigkeiten in diesen Experimenten (Stimulation der Kerne, Stabile elektrophysiologische Ableitung). Um den bestmöglichen Gewinn zu erzielen, versuchen wir in jedem Experiment eine Stimulationselektrode in LC und RK zu platzieren, wobei rein technisch die Trefferquote für RK höher liegen wird. Durch die Kombination der Faktoren (Stimulation, stabile Ableitung) gehen wir von einer Gesamterfolgsquote von 30% aus.

- Stimulation in LC und / oder RK und Stimulation des Tastsinns: 6 Tiere (30% Erfolg) 20 Tiere
- Stimulation in LC und / oder RK und Stimulation des Sehsinns: 6 Tiere (30% Erfolg) 20 Tiere

TOTAL 40 Ratten

2.3 Gleichzeitige elektrophysiologische Ableitungen im Großhirn und neuromodulatorischen Kernen

Ein komplementärer Ansatz die funktionelle Kopplung verschiedener neuronaler Systeme zu untersuchen ist die simultane elektrophysiologische Ableitung in diesen. Aus solchen paarweisen Ableitungen kann man anschließend mit modernen Methoden der Signalauswertung, direktionale und kausale Interaktionen bestimmen. Zwar sind diese Methoden noch nicht perfekt, aber vorhergehende und laufende Arbeiten von uns (Kayser 2009, Salazar 2004) und anderen zeigen, dass diese Methoden schon durchaus in der Lage sind Interaktionen zwischen verschiedenen Arealen zu charakterisieren. Hier werden diese modernen Methoden der Datenanalyse mit direkten kausalen Methoden der Mikrostimulation und der Applikation von neuromodulatorischen Agonisten kombiniert.

Es ist bekannt, dass alle drei monoaminergen Systeme untereinander Wechselwirken können (Aston-Jones and Cohen, 2005) und die reziproken Verbindungen der verschiedenen Systeme wurden mit der Effektivität von Antidepressiva bei Angstzuständen in Verbindung gebracht (Blier et al., 2001). Entsprechend wird z.B. der Einfluss von Serotonin auf das Großhirn nicht nur durch die Projektion dieses serotonergen Systems ins Großhirn bestimmt, sondern auch durch die reziproke Wechselwirkung von serotonergem und noradrenergem System. Das Ziel ist es daher, die Wechselwirkungen der neuromodulatorischen Systeme untereinander, und mit Arealen des Großhirns zu verstehen. Dazu möchten wir gleichzeitig in zwei neuromodulatorischen Zentren und einem Areal des präfrontalen Kortex (PFC) oder dem Hippocampus (HC) ableiten. Beide Strukturen spielen eine wichtige Rolle in der Kontrolle von Handlungen, Gedächtnis und Aufmerksamkeit, und sind daher bei Störungen der neuromodulatorischen Systeme am wahrscheinlichsten betroffen. Zuerst wird die Wechselwirkung dieser Areale unter spontanen Bedingungen (keine sensorische Stimulation, keine Stimulation eines Neuromodulators) untersucht. Anschließend wird eines dieser Systeme durch Mikrostimulation aktiviert und die Wirkung auf Großhirn bzw. andere modulatorische Systeme gemessen und die gleiche Messung wird während sensorischer Stimulation wiederholt. Zu letzt wird in separaten Experimenten eines der Systeme durch Applikation von einem Antagonisten ausgeschaltet, um die kausale Wechselwirkung in der Abwesenheit der modulation durch ein bestimmtes System messen zu können.

Biometrie: Wie bei 2.2 gehen wir von 6 Tieren pro experimenteller Bedingung aus.

Ableitungen unter spontanen Bedingungen und während sensorischer Stimulation:

- Ableitung in LC, RK und in HC, PFC: 6 Tiere (40% Erfolg) 15 Tiere
- Ableitung in LC, VTA und in HC, PFC: 6 Tiere (40% Erfolg) 15 Tiere
- Ableitung in RK, VTA und in HC, PFC: 6 Tiere (40% Erfolg) 15 Tiere

TOTAL 45 Ratten

Übersicht

Die folgende Tabelle fasst die geplanten Experimente, insbesondere die Zahl der Tiere und die Anzahl der Eingriffe pro Tier zusammen.

Nr.	1.1	1.2	1.3	1.4	1.5	Ratten
# Tiere	18	44	24	24	24	134
	Narkose/kein aufwachen	Narkose/kein aufwachen	Narkose/kein aufwachen	Narkose/kein aufwachen	Mehrere Narkosen, 2 Eingriffe mit 3 Wochen Pause	
Nr.	2.1	2.2	2.3			
# Tiere	15	40	45			100
	Narkose/kein aufwachen	Narkose/kein aufwachen	Narkose/kein aufwachen			

7.4.3 Handelt es sich um eigens für Tierversuche gezüchtete Tiere (§ 9 Abs. 2 Nr. 7)?

ja

Wenn nein, ist - soweit nach § 9 Abs. 2 Nr. 7 gefordert - ein Antrag auf Zulassung einer Ausnahme nach § 9 Abs. 2 Nr. 7 Satz 2 mit Begründung erforderlich, warum nicht eigens für Tierversuche gezüchtete Tiere verwendet werden sollen (auf gesondertem Blatt!)

Gegebenenfalls Begründung, warum eine Entnahme aus der Natur für erforderlich gehalten wird (§ 9 Abs. 2 Nr. 1 Satz 2)

7.4.4 Die vorgesehenen Tiere wurden bereits in einem Versuchsvorhaben im Sinne des § 9 Abs. 2 Nr. 5 verwendet

Nein

wenn ja, Beschreibung der Art und Dauer der bislang erfolgten Eingriffe an den betreffenden Tieren:

7.5 Beschreibung der Art, Durchführung und Dauer der vorgesehenen Eingriffe und Behandlungen (§ 8 Abs. 2 Satz 3 i.V.m. § 8a Abs. 2 Nr. 3) Die Angaben in Abschnitt 7.5 sind zusätzlich in einer dem Genehmigungsantrag beizufügenden Belastungstabelle (Anl. 1 zum Antrag) einzutragen.

7.5.1 Welche Eingriffe oder Behandlungen sollen durchgeführt werden? (Detaillierte Darstellung sämtlicher Maßnahmen mit zeitlichem Ablauf)

1. Elektrische Stimulation der neuromodulatorischen Systeme und gleichzeitige MRT Messung (Projekte 1.1 – 1.4)

Das gesamte Experiment findet unter Narkose statt, und das Tier wird am Ende mit einer Überdosis Pentobarbital getötet; gegeben falls wird das Gehirn zur histologischen Untersuchung entnommen.

Nach Einleitung der Narkose (*) wird das Tier in einem stereotaktischen Halter positioniert. Der Kopf wird rasiert und desinfiziert und der Schädel zwischen Bregma und Lambda entlang der sagittalen Sutura freigelegt. An der beabsichtigten Injektionsstelle wird eine Kraniotomie (\varnothing 2mm) durchgeführt und ein kleiner Einschnitt in die Dura gemacht. Anschließend wird eine (oder mehrere) Mikroelektroden zur elektrischen Stimulation der neuromodulatorischen Kerne implantiert. Die Mikroelektrode besteht dabei aus einer Glaspipette und einem dünnen Iridiumdraht (\varnothing .1mm) und wird von einem stereotaktischen Halter positioniert. Zusätzlich wird eine Plastikschraube im Schädel befestigt, um die Fixierung der Elektroden durch Knochenzement zu gewährleisten.

Wird bei dem gleichen Experiment noch eine pharmakologische Stimulation von Neuromodulatoren vorgenommen (1.1.3), so wird vor der MRT Messung eine Mikroinjektion wie folgt vorgenommen. An der beabsichtigten Injektionsstelle wird eine Kraniotomie (\varnothing 2mm) durchgeführt und ein kleiner Einschnitt in die Dura gemacht. Anschließend wird eine Mikropipette, welche die entsprechend neuromodulatorisch wirksame Substanz enthält, mittels eines Mikromanipulators positioniert. Die Injektion findet typischerweise 1mm unter der Cortex-Oberfläche mit einer Infusionsrate von 0.5 nl/min statt. Die Osmolarität und der pH der Injektionslösung wurden vorausgehend den physiologischen Bedingungen angepasst. Die Injektionsmenge kann bis zu 200nl betragen. Die 32G Injektionsnadel verbleibt nach der Injektion noch für ca. 10min an der Injektionsstelle und wird dann langsam entfernt.

Wird bei dem gleichen Experiment noch eines der Sinnessysteme stimuliert (1.1.4), so wird der entsprechende Stimulator angebracht. Im Falle des Sehsystems handelt es sich hierbei um eine LED, welche vor den Augen positioniert wird. Im Falle des Tastsinns, handelt es sich um zwei Nadelelektroden, welche an der Vorderpfote unter der Haut zwischen der zweiten und fünften Zehe eingesteckt werden. Zur Stimulation wird ein Strom von 1.5mA mit 0.3ms Pulsdauer bei 3Hz angewandt, welcher auf der Basis von maximalem zerebralem Blutfluss optimiert ist ohne jedoch den mittleren arteriellen Blutdruck zu verändern, was auf Schmerzempfinden schließen lassen würde.

Im Anschluss an diese Eingriffe wird das Tier in den Magnetresonanztomographen eingebracht, und die MRT Messung durchgeführt. Das ganze Experiment dauert ca. 6-12 Stunden.

(*) Als Narkose verwenden die Experimente entweder einer Inhalationsnarkose mit Isofluran (je nach Tier zwischen 1.7% und 2%) da diese Narkose gut verträglich ist und die Tiefe der Narkose individuell anhand der Reaktion auf sensorische Stimuli sehr gut angepasst werden kann. Das Narkosegas wird dabei als Luft-Sauerstoff Gemisch bzw. als Lachgas-Sauerstoff Gemisch verabreicht. Alternativ verwenden wir eine Injektionsanästhesie mit Urethan, welches bei uns sehr häufig bei MRT Messungen verwendet wird, da es einen sehr stabilen kardiovaskulären Zustand erzeugt. Die Narkose wird dabei mit einer i.p. Injektion von 1.5g/kg Urethan in 30% Lösung initialisiert. Bei Bedarf können jederzeit weitere Dosen gegeben werden.

Die elektrische Mikrostimulation erfolgt mit folgenden Parametern: 200-500 μ s langen biphasischen Pulse, Wiederholrate von 100-300Hz und periodischen an/aus-Phasen von 200ms. Die Stromstärke variiert zwischen 10 μ A bis 1000 μ A und wird an das jeweilige Zielgebiet angepasst. Ähnliche Stimulationsprotokolle werden auch zur Behandlung von neurologisch bedingten Erkrankungen wie Parkinson oder bei chronischen Schmerzen an Patienten angewandt und führen korrekt angewandt zu keiner Beeinträchtigung des Gehirns.

Zusätzlich kommen folgende Medikamente im Bedarfsfall zur Anwendung:

- Glycopyrrolat (Robinol, 0.05mg/kg) wird direkt nach der Narkoseeinleitung gegeben, reduziert die Sekretion während der Narkose.

- Pancuroniumbromid. Bei funktionellen Versuchen mit künstlicher Beatmung als Muskelrelaxans (0.2mg/kg/h i.v.). Bei herkömmlichen Narkosen gegebenenfalls zusätzlich lokal zur Inhibition der Wirkung des Nervus Vagus (0.2 mg/kg i.p. lokal hinter den Ohren).
- 2% Lydocain-Lösung. Bei durchzuführenden operativen Eingriffen wird nach erfolgter Narkose um das Operationsfeld zusätzlich Lydocain als Lokalanästhetikum subkutan verabreicht.
- Bepanthen oder Regepithel Augensalbe Schützt bei durchgeführten Anästhesien vor dem Austrocknen der Augen.

2. Messungen der Verteilung von MRT Kontrastmitteln

(Projekt 1.5)

Die MRT Messungen und Injektion finden unter Narkose (Inhalationsnarkose Isofluran 1.7% - 2%) statt.

Die Injektion des Kontrastmittel erfolgt wie unter (1) oben beschrieben mittels einer Mikropipette. Sobald das Tier unter Narkose ist wird eine Basismessung mit dem MRT gemacht, um einen Vergleichswert vor der Injektion zu erhalten. Im Anschluss an die Injektion wird der Knochen mit Knochenwachs verschlossen und Muskel und Haut vernäht. Das Tier erhält Antibiotika (Baytril, 5 mg/kg; s. c.) und Schmerzmittel (Finadyne, 2.5 mg/kg; s. c). Anschließend wird das Tier in den MRT gebracht und dort die genaue Positionierung der Injektion gemessen. Die ganze Prozedur dauert ca 3-4 Stunden. Anschließend wacht das Tier auf.

Nach einem Zeitraum von 24 Stunden wird die Verteilung des Kontrastmittels gemessen. Dazu wird das Tier unter Narkose wieder in den MRT gebracht und eine Messung durchgeführt. Im Anschluss an diese Messung wacht das Tier auf. Über einen Zeitraum von 3 Wochen erfolgt keine weitere Messung oder Eingriff.

Nach 3 Wochen wird erneut eine Injektion in ein anderes Gebiet durchgeführt. 24 Stunden später erfolgt eine weitere MRT Messung, an deren Ende das Tier getötet wird.

Injektion & MRT Messung unter Narkose	24 h Ruhe	MRT Mes- sung unter Narkose	3 Wochen Ruhe	Injektion & MRT Mes- sung unter Narkose	24 h Ruhe	MRT Messung unter Narkose – Ohne aufwach- chen
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3. Kombinierte Stimulation von Kernen und elektrophysiologische Messungen

(Projekte 2.1-3)

Das gesamte Experiment findet unter Narkose (Urethan, siehe (1) oben) statt, und das Tier wird am Ende mit einer Überdosis Pentobarbital getötet; gegeben falls wird das Gehirn zur histologischen Untersuchung entnommen.

Nach Einleitung der Narkose wird das Tier in einem stereotaktischen Halter positioniert. Der Kopf wird rasiert und desinfiziert und der Schädel zwischen Bregma und Lambda entlang der sagittalen Sutur freigelegt. An den beabsichtigten Ableitungstellen wird eine Kraniotomie (Ø 2mm) durchgeführt und ein kleiner Einschnitt in die Dura gemacht. Anschließend werden Mikroelektroden zur elektrophysiologischen Ableitung bzw. Stimulation von einem stereotaktischen Halter positioniert.

Wird bei dem gleichen Experiment noch eines der Sinnessysteme stimuliert (1.2.2), so wird der entsprechende Stimulator angebracht, wie unter (1) oben beschrieben.

Zunächst werden sämtliche Messungen mit sensorischer und elektrischer Stimulation durchgeführt. Im Anschluss wird eines der neuromodulatorischen Systeme durch eine Injektion des entsprechenden Antagonisten ausgeschaltet. Die Injektion erfolgt systemisch, z.B. in die Bauchfalte. Das ganze Experiment dauert ca. 6-12 Stunden.

7.5.2 Welche Eingriffe oder Behandlungen sollen unter Betäubung durchgeführt werden und welche Betäubungsverfahren sind dabei vorgesehen?

Sämtliche Eingriffe, MRT oder elektrophysiologische Messungen erfolgen unter Anästhesie (Isofluran, Urethan, siehe 7.5.1).

7.5.3 Sind schmerzhafte Eingriffe ohne Betäubung vorgesehen?

nein

7.5.4 Sollen an einem nicht betäubten Tier mehrere erheblich schmerzhafte Eingriffe oder Behandlungen durchgeführt werden?

nein

7.5.5 Welchen Belastungen (Intensität und Dauer von Schmerzen oder Leiden) werden die Tiere voraussichtlich ausgesetzt oder welche Schäden werden ihnen voraussichtlich zugefügt?

Alle Versuche laufen unter Narkose ab. Für Projekte 1.5 wachen die Tiere nach einem Eingriff auf. Hierbei kann einmaliger post-operativer Wundschmerz auftreten, welcher mit der Gabe von schmerzstillenden Mitteln nach der Operation abgeschwächt wird (siehe 7.5.1). Dies stellt eine geringe Belastung dar. Nach den anderen Versuchen wachen die Tiere nicht auf.

Für die Tiere welche nach einem Eingriff wieder aufwachen, legen wir folgende Abbruchkriterien fest.

Abbruchkriterien: Wir verwenden einen Belastungs-score, welcher auf der Beobachtung von Gewicht, Spontanverhalten, Allgemeinzustand und klinischen Befund beruht, und wie er zum Beispiel von der Uni Würzburg publiziert wird (siehe Anhang 2). Tiere werden sofort getötet wenn sich ihre Belastung als schwer ergibt. Bei mittlerer Belastung der Tiere werden gegeben falls Analgetika (falls nicht schon der Fall) verabreicht, und sollte sich der Zustand binnen 48 Stunden nicht bessern, werden die Tiere getötet. Aufgrund aller uns zur Verfügung stehenden Informationen ist allerdings nicht davon auszugehen, dass die geplanten Läsionen zu starken Beeinträchtigungen der Tiere führen.

7.5.6 Vorgesehene Maßnahmen zur Schmerzlinderung nach Abklingen der Betäubung:

Unmittelbar postoperativ sowie in den auf die Operation folgenden zwei Tagen werden ein Antibiotikum (Baytril, 5 mg/kg; s. c.) und ein Analgetikum (Finadyne 2.5 mg/kg; s. c.) verabreicht. Diese Substanzen werden routinemäßig an Ratten angewendet.

7.5.7 Vorgesehene Maßnahmen und Kontrollen im Rahmen der medizinischen Versorgung (inkl. Angaben zu speziellen Haltungsbedingungen aufgrund hygienischer Anforderungen oder Erkrankungsneigungen der vorgesehenen Tiere):

Die Gesundheit der Tiere durch Beobachtung des Verhaltens der Tiere, des Appetits, der Fezes, der Haut und der Haare sowie des Gesamteindrucks überwacht. Nach einem Eingriff oder einer Narkose werden die Tiere zusätzlich täglich gewogen. Falls irgendein Anzeichen von Krankheit oder Unwohlsein auftreten sollte, wird das Tier isoliert und von einem qualifizierten Veterinär untersucht. In dem Fall, dass das Tier chronische Schmerzen oder eine schwere Krankheit erleidet, wird es sofort und vor Beendigung des Versuchs eingeschläfert.

7.6 Ethische Vertretbarkeit des Versuchs (§ 7 Abs. 3)

7.6.1 Wissenschaftlich begründete Darlegung, daß die zu erwartenden Schmerzen, Leiden oder Schäden der Versuchstiere im Hinblick auf den Versuchszweck ethisch vertretbar sind (§ 7 Abs. 3 Satz 1):

Unsere Versuche erforschen die Sinnessysteme mittels natürlicher Stimulation über die Sinnesorgane. Experimentell wird den Tieren kein Schmerz und kein Leiden zugefügt. Wie in jedem anderen Tierversuch auch kann das Tier jedoch unter den folgenden Bedingungen Schmerz oder Leid erfahren: Infektionen oder Krankheiten und postoperativer Schmerz. Bei den geplanten Experimenten mit Ratten verweilen die Tiere nur kurze Zeit in unserem Tierstall. Daher ist die Wahrscheinlichkeit einer Krankheit oder Infektion gering. Der postoperative Schmerz kann als Folge des Eingriffes entstehen und wird schon vor, während und nach dem Eingriff durch geeignete Analgetika behandelt. Durch unsere große Erfahrung können die nötigen Eingriffe rasch und mit minimaler Traumatisierung des Gewebes durchgeführt werden. Alles in allem verursachen unsere Verfahren deutlich weniger Schmerz und Leid als so mancher Arztbesuch es nach sich ziehen kann.

Rechtfertigung für die in ihrem Ausmaß begrenzten Beschwerden, die den Ratten zugefügt werden, ist der Erwerb von enormem Wissen über die Funktion und über Funktionsstörungen des Gehirns. Dieses Wissen kann dazu beitragen, Störungen des menschlichen Gehirns besser zu verstehen und irgendwann auch zu behandeln.

Wir sind der Ansicht, dass die Forschung an Tieren von entscheidender Bedeutung für viele Gebiete der medizinischen und der Gesundheitswissenschaften war, ist und sein wird. Am wichtigsten ist aber vielleicht, dass die Forschung mit Tieren nicht nur die Lebenszeit der Menschen verlängert, sondern dass sie auch die Lebensqualität wesentlich verbessert hat. Die Beiträge der Tierforschung können kaum alle aufgezählt werden und würden viele Zeilen füllen. Die Tierforschung der Gegenwart gibt sehr vielen Leuten Hoffnung, ob sie nun an Krebs, Diabetes, Alzheimer, verschiedenen Infektionskrankheiten, Mukoviszidose oder AIDS leiden. Der Nutzen der neurowissenschaftlichen Forschung reicht vom besseren Verständnis der Mechanismen des Lernens, der Aggression, der Gefühle und ganz allgemein aller menschlichen Interaktionen bis hin zu einer langzeitigen Verbesserung einer ganzen Reihe von medizinischen und klinisch-praktischen Anwendungen. Humane, verantwortungsvoll durchgeführte Studien an Tieren sichern uns nicht nur eine gesündere Umwelt und sicherere Produkte für den Konsum, sondern sie lassen uns auch hoffen, die Natur des Tiers oder des Menschen selbst und die Beschaffenheit der Beziehungen von Individuen in komplexen Gesellschaften verstehen zu können.

7.6.2 Bei länger anhaltenden oder sich wiederholenden erheblichen Schmerzen oder Leiden, wissenschaftlich begründete Darlegung, daß das angestrebte Versuchsergebnis vermutlich für wesentliche Bedürfnisse von Mensch und Tier einschließlich der Lösung wissenschaftlicher Probleme von hervorragender Bedeutung ist (§ 7 Abs. 3 Satz 2):

Entfällt.

8. Verfahren am Versuchsende

8.1 Beabsichtigter Verbleib der Tiere:

X Tötung während des Versuchs oder vor dem Erwachen aus der Narkose

Tötung nach Beobachtungszeit von .

Weiterleben der Tiere ohne Beeinträchtigung des Wohlbefindens

8.2. Falls die Tiere getötet werden sollen, welches Tötungsverfahren ist vorgesehen?

Die bereits anästhesierten Tiere werden durch eine Überdosis Pentobarbital (100mg/kg) getötet. Dies entspricht den Richtlinien der "American Veterinary Association Panel on Euthanasia" und den Vorschlägen des „Guide for Care and Use of Laboratory Animals of the National Institute of Health in United States“

9. Wird die Anonymisierung des Antrages gewünscht?

nein

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Anhang 2 - Abbruchkriterien

Beobachtung	Punktwertung
I Körpergewicht - unbeeinflusst oder Anstieg - Änderung < 5% - Gewichtsreduktion 5-10% - Gewichtsreduktion 11-20% - Gewichtsreduktion > 20%	0 1 5 10 20
II Allgemeinzustand - Fell glatt, glänzend; Körperöffnungen sauber; Augen klar, glänzend - Felldefekte (verminderte oder übersteigerte Körperpflege) - Fell stumpf, ungeordnet, ungepflegte Körperöffnungen, Augen trüb; erhöhter Muskeltonus - Schmutziges Fell, verklebte oder feuchte Körperöffnungen, unnormale Haltung, Augen trüb; hoher Muskeltonus - Verkrampfungen, Lähmungen (Rumpfmuskulatur, Extremitäten); Atemgeräusche; Tier fühlt sich kalt an	0 1 5 10 20
III Spontanverhalten - normales Verhalten (Schlafen, Reaktion auf Anblasen und Berührung, Neugier, Sozialkontakte) - geringe Abweichungen vom Normalverhalten - ungewöhnliches Verhalten, eingeschränkte Motorik oder Hyperkinetik - Selbstisolation, Lethargie; ausgeprägte Hyperkinetik bzw. Verhaltensstereotypen; Koordinationsstörungen - Schmerzlaute beim Ergreifen; Selbstamputation (Autoaggression)	0 1 5 10 20
IV Klinischer Befund - Temperatur, Atmung und Puls normal, Extremitäten warm, Schleimhäute gut durchblutet - geringe Abweichungen von der Normalsituation - Temperaturabweichung 1 - 2°C, Atmung und Puls + 30% - Temperaturabweichung > 2°C, Atmung und Puls + oder - 50%; Durchmesser eines tastbaren Tumors >2 cm (Kleinnager)	0 1 10 20
Bewertung, Maßnahmen	Punktsumme
Belastungsgrad 0 = keine Belastung Belastungsgrad 1 = geringe Belastung Belastungsgrad 2 = mittelgradige Belastung; tierärztliche Versorgung (Analgesie) Belastungsgrad 3 = hochgradige Belastung; das Tier einschläfern.	0 1-9 10-19 20 oder höher



Abbildung: Anatomischer Schnitt durch eine Rattenhirn. Der Locus ceruleus wurde dunkel eingefärbt (innerhalb der roten Ellipse links). Die weiße Region innerhalb des LC auf der rechten Seite zeigt die Größe einer typischen Elektrode relativ zum LC.



Oxana Eschenko

Baden-Württemberg
REGIERUNGSPRÄSIDIUM TÜBINGEN

Regierungspräsidium Tübingen · Postfach 26 66 · 72016 Tübingen

Herrn
Prof. Dr. Nikos K. Logothetis
Max-Planck-Institut
für biologische Kybernetik
Spemannstraße
72076 Tübingen

Tübingen 17.03.2009

Name Dr. Cornelia Jäger

E-Mail cornelia.jaeger@rpt.bwl.de

Durchwahl 07071 757-3384; Fax: -93384

Aktenzeichen 35/9185.81-2

Versuchs-Nr. KY 2/09

(Bitte bei Antwort angeben)

nachrichtlich:

Herrn
Prof. Dr. Christian Wehrhahn
Tierschutzbeauftragter
MPI für biologische Kybernetik
Postfach 21 69
72012 Tübingen

Tierschutzgesetz in der Fassung der Bekanntmachung vom 18.05.2006

(BGBl. I S. 1206) - TierSchG;

Tierversuch Nr. KY 2/09

Antrag vom 28.02.2009; Posteingang: 02.03.2009

Sehr geehrter Herr Prof. Logothetis,

aufgrund Ihres o.g. Antrages zur Genehmigung von Versuchen an Wirbeltieren, ergeht folgende

Entscheidung

1. Die Verwendung von Wirbeltieren für Eingriffe oder Behandlungen zu Versuchszwecken im Rahmen des

Versuchsvorhabens: **Funktion und Konnektivität von neuromodulatorischen Kernen**

Tierart: Ratte

Tierzahl: 234

wird genehmigt.

2. Als verantwortlicher Leiter der Versuche wird **Herr Prof. Nikos K. Logothetis**, als dessen Stellvertreterin **Frau Dr. Oxana Eschenko** benannt. Jeder Wechsel in der Person des Leiters des Versuchsvorhabens oder seines Stellvertreters ist umgehend hierher anzuzeigen.
3. Jede beabsichtigte Änderung der Versuchsdurchführung ist rechtzeitig vorher mitzuteilen und darf erst nach Bestätigung der Genehmigungsfreiheit oder Erteilung der Genehmigung durchgeführt werden.
4. An den Käfigen, Boxen oder sonstigen, der Tierhaltung dienenden Behältnissen muss die Versuchs-Nr., der Name des Leiters des Versuchsvorhabens und der Name des Experimentators angebracht sein.
5. Die vorgelegten Antragsunterlagen sind Bestandteil der Genehmigung.
6. Die Genehmigung wird befristet bis zum **31.03.2011**.

Gründe:

I. Sachverhalt

Mit o.g. Antrag wurde die Genehmigung eines Tierversuchsvorhabens beantragt. Dieser Antrag wurde der Kommission nach § 15 TierSchG vorgelegt. Auf der Grundlage des Votums der Kommission und ggf. nach Klärung offener Fragen wird dem Antrag zugestimmt. Die Zustimmung erfolgt nach Abwägung der zu erwartenden Belastungen für die Tiere, der möglichen Ergebnisse und der Unerlässlichkeit im Hinblick auf den Versuchszweck.

II. Begründung

Nach § 2 Nr. 2 der Verordnung des Ministeriums für Ernährung und Ländlichen Raum über Zuständigkeiten nach dem Tierschutzrecht vom 08. Januar 2007 (GBl. 2007, S. 2) ist das Regierungspräsidium für die Erteilung der Erlaubnis zuständig. Rechtsgrundlage für die Erteilung der Genehmigung ist § 8 Abs. 1 und 3 TierSchG. Nach § 8 Abs. 4 TierSchG sind im Genehmigungsbescheid der Leiter und sein Stellvertreter anzugeben. Diese Vorschrift ermächtigt die Behörde ausdrücklich zu der unter Ziff. 2 formulierten Nebenbestimmung. Die Bestimmung soll sicherstellen, dass

zu jedem Zeitpunkt des Tierversuches eine zuverlässige und sachkundige Person das Versuchsvorhaben begleitet und betreut.

Rechtsgrundlage für die Ziffern 3. bis 5. ist § 36 LVwVfG.

Ziffer 3. dient der Prüfung der Genehmigungspflicht nach § 8 Abs. 7 Sätze 1 und 2 TierSchG sowie der Einhaltung von § 9 TierSchG. Ziffer 4. und 5. dienen der Einhaltung der §§ 7 Abs. 2-5, 9 Abs. 2 und 9a TierSchG.

Die Befristung des Tierversuches laut Ziffer 6. erfolgt nach § 8 Absatz 5 Satz 1 TierSchG. Dabei hält sich das Regierungspräsidium an Ziffer 6.4.3 der für die Genehmigungsbehörde bindenden Allgemeinen Verwaltungsvorschrift zur Durchführung des Tierschutzgesetzes (AVVTSchG).

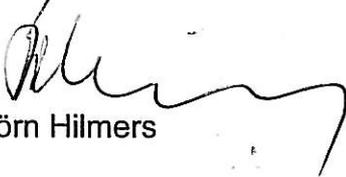
Hinweise:

1. Die allgemeinen Vorschriften des Tierschutzgesetzes in bezug auf Haltung, Betreuung, Ernährung und Pflege der Versuchstiere (§§ 1 und 2) und die besonderen Vorschriften für die Durchführung der Tierversuche (§§ 9 und 9a) sind einzuhalten.
2. Wechsel von Personen, die unmittelbar an der Versuchsdurchführung beteiligt sind, sind mitzuteilen.
3. Die nach § 16 Abs. 1 Nr. 3a des Tierschutzgesetzes vorgeschriebene Überwachung wird vom zuständigen Veterinäramt durchgeführt. Da hierbei die nach § 9a Abs. 1 des Tierschutzgesetzes zu fertigenden Aufzeichnungen eingesehen werden, müssen diese im Tierlabor aufbewahrt werden bzw. muss ein Hinweis vorhanden sein, wo diese eingesehen werden können. Die Aufzeichnungen sind 3 Jahre lang nach Abschluss des Versuchsvorhabens aufzubewahren. Der Abschluss des Versuchsvorhabens ist dem Regierungspräsidium Tübingen anzuzeigen.
4. Die Aufstallung der Tiere in die vorgesehenen Versuchstierhaltungen ist im Hinblick auf die mögliche Belegung und Versorgung mit dem Tierschutzbeauftragten abzustimmen.

Rechtsbehelfsbelehrung:

Gegen diesen Bescheid kann innerhalb eines Monats nach Bekanntgabe beim Verwaltungsgericht Sigmaringen, Karlstraße 13, 72488 Sigmaringen, schriftlich oder zur Niederschrift des Urkundsbeamten der Geschäftsstelle des Gerichts Klage erhoben werden.

Mit freundlichen Grüßen


Dr. Jörn Hilmers



Oxana Eschenko

Baden-Württemberg
REGIERUNGSPRÄSIDIUM TÜBINGEN

Regierungspräsidium Tübingen · Postfach 26 66 · 72016 Tübingen

Herrn
Prof. Dr. Nikos K. Logothetis
Max-Planck-Institut
für biologische Kybernetik
Spemannstraße
72076 Tübingen

Tübingen 17.03.2009

Name Dr. Cornelia Jäger

E-Mail cornelia.jaeger@rpt.bwl.de

Durchwahl 07071 757-3384; Fax: -93384

Aktenzeichen 35/9185.81-2

Versuchs-Nr. KY 2/09

(Bitte bei Antwort angeben)

nachrichtlich:

Herrn
Prof. Dr. Christian Wehrhahn
Tierschutzbeauftragter
MPI für biologische Kybernetik
Postfach 21 69
72012 Tübingen

Tierschutzgesetz in der Fassung der Bekanntmachung vom 18.05.2006

(BGBl. I S. 1206) - TierSchG;

Tierversuch Nr. KY 2/09

Antrag vom 28.02.2009; Posteingang: 02.03.2009

Sehr geehrter Herr Prof. Logothetis,

aufgrund Ihres o.g. Antrages zur Genehmigung von Versuchen an Wirbeltieren, ergeht folgende

Entscheidung

1. Die Verwendung von Wirbeltieren für Eingriffe oder Behandlungen zu Versuchszwecken im Rahmen des

Versuchsvorhabens: **Funktion und Konnektivität von neuromodulatorischen Kernen**

Tierart: Ratte

Tierzahl: 234

wird genehmigt.

2. Als verantwortlicher Leiter der Versuche wird **Herr Prof. Nikos K. Logothetis**, als dessen Stellvertreterin **Frau Dr. Oxana Eschenko** benannt. Jeder Wechsel in der Person des Leiters des Versuchsvorhabens oder seines Stellvertreters ist umgehend hierher anzuzeigen.
3. Jede beabsichtigte Änderung der Versuchsdurchführung ist rechtzeitig vorher mitzuteilen und darf erst nach Bestätigung der Genehmigungsfreiheit oder Erteilung der Genehmigung durchgeführt werden.
4. An den Käfigen, Boxen oder sonstigen, der Tierhaltung dienenden Behältnissen muss die Versuchs-Nr., der Name des Leiters des Versuchsvorhabens und der Name des Experimentators angebracht sein.
5. Die vorgelegten Antragsunterlagen sind Bestandteil der Genehmigung.
6. Die Genehmigung wird befristet bis zum **31.03.2011**.

Gründe:

I. Sachverhalt

Mit o.g. Antrag wurde die Genehmigung eines Tierversuchsvorhabens beantragt. Dieser Antrag wurde der Kommission nach § 15 TierSchG vorgelegt. Auf der Grundlage des Votums der Kommission und ggf. nach Klärung offener Fragen wird dem Antrag zugestimmt. Die Zustimmung erfolgt nach Abwägung der zu erwartenden Belastungen für die Tiere, der möglichen Ergebnisse und der Unerlässlichkeit im Hinblick auf den Versuchszweck.

II. Begründung

Nach § 2 Nr. 2 der Verordnung des Ministeriums für Ernährung und Ländlichen Raum über Zuständigkeiten nach dem Tierschutzrecht vom 08. Januar 2007 (GBl. 2007, S. 2) ist das Regierungspräsidium für die Erteilung der Erlaubnis zuständig. Rechtsgrundlage für die Erteilung der Genehmigung ist § 8 Abs. 1 und 3 TierSchG. Nach § 8 Abs. 4 TierSchG sind im Genehmigungsbescheid der Leiter und sein Stellvertreter anzugeben. Diese Vorschrift ermächtigt die Behörde ausdrücklich zu der unter Ziff. 2 formulierten Nebenbestimmung. Die Bestimmung soll sicherstellen, dass

zu jedem Zeitpunkt des Tierversuches eine zuverlässige und sachkundige Person das Versuchsvorhaben begleitet und betreut.

Rechtsgrundlage für die Ziffern 3. bis 5. ist § 36 LVwVfG.

Ziffer 3. dient der Prüfung der Genehmigungspflicht nach § 8 Abs. 7 Sätze 1 und 2 TierSchG sowie der Einhaltung von § 9 TierSchG. Ziffer 4. und 5. dienen der Einhaltung der §§ 7 Abs. 2-5, 9 Abs. 2 und 9a TierSchG.

Die Befristung des Tierversuches laut Ziffer 6. erfolgt nach § 8 Absatz 5 Satz 1 TierSchG. Dabei hält sich das Regierungspräsidium an Ziffer 6.4.3 der für die Genehmigungsbehörde bindenden Allgemeinen Verwaltungsvorschrift zur Durchführung des Tierschutzgesetzes (AVVTSchG).

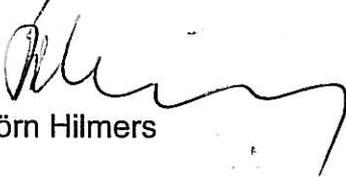
Hinweise:

1. Die allgemeinen Vorschriften des Tierschutzgesetzes in bezug auf Haltung, Betreuung, Ernährung und Pflege der Versuchstiere (§§ 1 und 2) und die besonderen Vorschriften für die Durchführung der Tierversuche (§§ 9 und 9a) sind einzuhalten.
2. Wechsel von Personen, die unmittelbar an der Versuchsdurchführung beteiligt sind, sind mitzuteilen.
3. Die nach § 16 Abs. 1 Nr. 3a des Tierschutzgesetzes vorgeschriebene Überwachung wird vom zuständigen Veterinäramt durchgeführt. Da hierbei die nach § 9a Abs. 1 des Tierschutzgesetzes zu fertigenden Aufzeichnungen eingesehen werden, müssen diese im Tierlabor aufbewahrt werden bzw. muss ein Hinweis vorhanden sein, wo diese eingesehen werden können. Die Aufzeichnungen sind 3 Jahre lang nach Abschluss des Versuchsvorhabens aufzubewahren. Der Abschluss des Versuchsvorhabens ist dem Regierungspräsidium Tübingen anzuzeigen.
4. Die Aufstallung der Tiere in die vorgesehenen Versuchstierhaltungen ist im Hinblick auf die mögliche Belegung und Versorgung mit dem Tierschutzbeauftragten abzustimmen.

Rechtsbehelfsbelehrung:

Gegen diesen Bescheid kann innerhalb eines Monats nach Bekanntgabe beim Verwaltungsgericht Sigmaringen, Karlstraße 13, 72488 Sigmaringen, schriftlich oder zur Niederschrift des Urkundsbeamten der Geschäftsstelle des Gerichts Klage erhoben werden.

Mit freundlichen Grüßen


Dr. Jörn Hilmers



Baden-Württemberg
REGIERUNGSPRÄSIDIUM TÜBINGEN

Regierungspräsidium Tübingen · Postfach 26 66 · 72016 Tübingen

Herrn
Dr. Michael C. Schmid
Max-Planck-Institut
für biologische Kybernetik
der Max-Planck-Gesellschaft e.V.
Spemannstraße 38
72076 Tübingen

Tübingen 13.12.2010
Name Dr. Saskia Hogreve
Durchwahl 07071 757-3384
E-mail saskia.hogreve@rpt.bwl.de
Aktenzeichen 35/9185.81-5 /
Versuch-Nr. KY 8/10
(Bitte bei Antwort angeben)

nachrichtlich:

Frau
Prof. Dr. Almut Schüz
Tierschutzbeauftragte
Max-Planck-Institut
für biologische Kybernetik
Postfach 21 69
720712 Tübingen

 Tierschutzgesetz in der Fassung der Bekanntmachung vom 18.05.2006
(BGBl I S. 1206) - TierSchG
Tierversuch-Nr. KY 8/10
Antrag vom 28.10.2010; Posteingang: 29.10.2010 mit letzter Ergänzung vom
24.11.2010 (E-Mail)

Sehr geehrter Herr Dr. Schmid,

aufgrund Ihres o.g. Antrages zur Genehmigung von Versuchen an Wirbeltieren, er-
geht folgende

Entscheidung

1. Die Verwendung von Wirbeltieren für Eingriffe oder Behandlungen zu Versuchs-
zwecken im Rahmen des

Versuchsvorhabens: **Thalamo-kortikale Mechanismen des Blindsehens - Optogenetik**

Tierart: Rhesusaffe

Tierzahl: **4 (Teilgenehmigung für Untersuchungsziel 1 und 2)
wird genehmigt.**

Es gelten die nachfolgend aufgeführten Nebenbestimmungen:

2. Als verantwortlicher Leiter der Versuche wird **Herr Dr. Michael C. Schmid**, als dessen Stellvertreter **Herr Prof. Dr. Nikos Logothetis** benannt. Jeder Wechsel in der Person des Leiters des Versuchsvorhabens oder seines Stellvertreters ist umgehend hierher anzuzeigen.
3. Für die Tiere, die in den Versuch übernommen werden, sind Daten über die bisherige Verwendung wie auch einzeltierbezogene Daten vorzulegen, die mindestens Angaben zu Tierart, Alter, Geschlecht, Tätowierungsnummer, Art und Anzahl der zuvor durchgeführten Eingriffe sowie Ergebnisse einer klinischen Untersuchung enthalten, die den einwandfreien Gesundheitszustand des Tieres belegen.
4. Zudem ist für jedes Tier, das in den Versuch genommen wird, vorab ein repräsentatives individuelles Profil zur spontanen Flüssigkeitsaufnahme ohne Restriktion zu erstellen und der Behörde zusammen mit dem geplanten Flüssigkeitsregime vorzulegen.
5. Es ist durch geeignete Maßnahmen dafür Sorge zu tragen, dass die Tiere freiwillig, d. h. ohne Zwangsmaßnahmen, in den so genannten Primatenstuhl einsteigen.
6. Der Gesundheitsstatus der Tiere ist unter Einbeziehung des Tierarztes der Einrichtung regelmäßig zu erheben und zu dokumentieren.
 - 6.1 Vor Beginn operativer Eingriffe ist mindestens eine Allgemeinuntersuchung durchzuführen sowie erforderlichenfalls ein Blutstatus zu erstellen.
 - 6.2 Im Anschluss an operative Eingriffe sowie Ableitungen ist das Wohlbefinden der Tiere anhand von Verhaltensparametern (Spontanverhalten, z. B. artspezifische tagesperiodische Aktivitätsmuster, Komfortverhalten, Explorationsverhalten, Spielverhalten, ggf. auch Stereotypien und Apathie, Einschränkung des Körperpflegeverhaltens) zu erfassen.

- 6.3 Die jeweiligen Ergebnisse sind dem Regierungspräsidium unverzüglich vorzulegen sowie zur Einsicht für die Überwachungsbehörde bereitzuhalten.
7. Beträgt die Dauer der Bewegungseinschränkung nach der Operation im sog. Aufwachstuhl mehr als drei Tage, ist das operierte Tier täglich aus der Fixationseinrichtung herauszunehmen und unter Berücksichtigung seines individuellen Verhaltens- und Aktivitätsprofils zu bewegen. Über die Bewegungshäufigkeit, deren Zeitpunkte und deren Dauer sind Aufzeichnungen zu führen.
 8. Bei Komplikationen wie beispielsweise einer Infektion im Bereich eines Implantats ist das Ableiten auszusetzen und mit geeigneten medizinischen Maßnahmen nach Antibiotogramm zu behandeln. Sollte die Infektion mit konservativen Maßnahmen nicht zu eliminieren sein, ist eine Explantation vorzunehmen, um ein Ausheilen zu ermöglichen, oder eine Euthanasie in Betracht zu ziehen.
 9. Eine Reimplantation oder Implantation weiterer Kammern darf nur nach vollständiger Wiederherstellung des Gesundheitszustandes des Tieres erfolgen.
 10. Die Untersuchungen zur Feststellung der Wiederherstellung des Gesundheitszustandes und deren Ergebnisse sind zu dokumentieren.
 11. Ein tierärztliches Gutachten zum Gesundheitsstatus jedes verwendeten Tieres ist am Ende des Versuchsvorhabens zu erstellen und dem Landratsamt Tübingen - Veterinäramt - sowie nachrichtlich dem Regierungspräsidium Tübingen vorzulegen.
 12. Jedes verendete oder euthanasierte Versuchstier ist einer pathologisch-anatomischen Untersuchung durch eine hierzu geeignete staatliche bzw. staatlich getragene oder universitäre Einrichtung zuzuführen. Der hierüber zu fertigende tierärztliche Sektionsbericht soll
 - Anhaltspunkte auf mögliche Schädigungen und Gesundheitsbeeinträchtigungen zu Lebzeiten des Tieres,
 - im Falle eines verendeten Tieres die Todesursache und
 - ggf. Möglichkeiten zur Verbesserung der Implantationstechnik aufzeigen. Der Bericht ist unverzüglich dem Regierungspräsidium sowie dem Landratsamt Tübingen zu übersenden.

13. Jede beabsichtigte Änderung der Versuchsdurchführung ist rechtzeitig vorher mitzuteilen und darf erst nach Bestätigung der Genehmigungsfreiheit oder Erteilung der Genehmigung durchgeführt werden.
14. An den Käfigen, Boxen oder sonstigen, der Tierhaltung dienenden Behältnissen muss die Versuchs-Nr., der Name des Leiters des Versuchsvorhabens und der Name des Experimentators angebracht sein.
15. Die vorgelegten Antragsunterlagen sind verbindlicher Bestandteil der Genehmigung.
16. Wechsel von Personen, die unmittelbar an der Versuchsdurchführung beteiligt sind, sind dem Regierungspräsidium mitzuteilen.
17. **Die Genehmigung weiterer Tiere kann nach Zwischenbericht der Ergebnisse und im Bedarfsfall mit neuer biostatistischer Berechnung der Tierzahl in einem Gutachten erfolgen.**
18. Die Genehmigung wird befristet bis zum **31.12.2013**.

Auflagenvorbehalt:

Die Festlegung weiterer Auflagen wird vorbehalten.

Dies gilt insbesondere für die im Rahmen der Antragsbearbeitung angesprochene zukünftige Schaffung eines regelmäßigen Auslaufs in einem Außengehege. Hierzu wird auf verschiedene Besprechungen zwischen Regierungspräsidium und Max-Planck-Institut für biologische Kybernetik verwiesen.

Begründung:

Mit o. g. Antrag wurde die Genehmigung eines Tierversuchsvorhabens beantragt. Dieser Antrag wurde der Kommission nach § 15 TierSchG vorgelegt.

Auf der Grundlage des Votums der Kommission und nach Klärung offener Fragen kann nach Beurteilung und Abwägung der zu erwartenden Belastungen für die Tiere, der möglichen Ergebnisse, der Unerlässlichkeit im Hinblick auf den Versuchszweck sowie der sonstigen in den §§ 8 ff. TierSchG aufgeführten Voraussetzungen die Genehmigung erteilt werden.

Nach § 2 Nr. 2 der Verordnung des Ministeriums für Ernährung und Ländlichen Raum über Zuständigkeiten nach dem Tierschutzrecht vom 08. Januar 2007 (GBl. 2007 S. 2) ist das Regierungspräsidium für die Erteilung der Genehmigung zuständig. Rechtsgrundlage für die Erteilung der Genehmigung ist § 8 Abs. 1 und 3 TierSchG.

Nach § 8 Abs. 4 TierSchG sind im Genehmigungsbescheid der Leiter und sein Stellvertreter anzugeben. Diese Vorschrift verpflichtet die Behörde ausdrücklich zu der unter Ziffer 1 formulierten Nebenbestimmung. Die Bestimmung soll sicherstellen, dass zu jedem Zeitpunkt des Tierversuches eine zuverlässige und sachkundige Person das Versuchsvorhaben begleitet und betreut.

Rechtsgrundlage für die Ziffern 2 bis 18 ist § 36 Landesverwaltungsverfahrensgesetz (LVwVfG).

Ziffer 2 gibt der Genehmigungsbehörde eine Überprüfungsmöglichkeit auch hinsichtlich der zum Zeitpunkt der Genehmigungserteilung noch nicht im Bestand befindlichen Tiere.

Die Ziffern 3 bis 12 dienen der Erfassung und Bewertung der Belastung des Einzeltieres sowie dem Bemühen, diese im Hinblick auf den zu erwartenden Erkenntnisgewinn so gering wie möglich zu halten.

Ziffer 13 dient der Prüfung der Genehmigungspflicht nach § 8 Abs. 7 Sätze 1 und 2 TierSchG sowie der Einhaltung von § 9 TierSchG und stellt sicher, dass vor einer Entscheidung des Regierungspräsidiums die Änderung nicht durchgeführt werden darf.

Ziffer 14 und 15 dienen der Einhaltung der §§ 7 Abs. 2-5, 9 Abs. 2 und 9a TierSchG.

Die Befristung des Tierversuches laut Ziffer 18 erfolgt nach § 8 Abs. 5 Satz 1 TierSchG. Dabei legt das Regierungspräsidium Ziffer 6.4.3. der die Genehmigungsbehörde bindenden Allgemeinen Verwaltungsvorschrift zur Durchführung des Tierschutzgesetzes (AVVTierSchG) zugrunde.

Die beigefügten Nebenbestimmungen sind im Übrigen erforderlich, angemessen und geeignet, die Einhaltung der Bestimmungen der §§ 7 ff. TierSchG zu gewährleisten.

Der Auflagenvorbehalt für das Verlangen nach Schaffung eines Auslaufs in einem Außengehege beruht auf der Erwägung, dass aus tierschutzrechtlichen Gründen für die Versuchstiere diese Möglichkeit bestehen muss, da es sich bei Primaten um hoch entwickelte Tiere handelt, die eine ihren komplexen Bedürfnissen angemessene angereicherte Umgebung benötigen. Gemäß den Empfehlungen der Europäischen Kommission vom 18.06.2007 mit Leitlinien für Unterbringung und Pflege von Tieren,

die für Versuche und andere wissenschaftliche Zwecke verwendet werden, sollten Primaten nach Möglichkeit Zugang zu Außenbereichen haben, um von Merkmalen natürlicher Umgebung profitieren zu können. Dies gilt insbesondere für Tiere, deren Bewegungsmöglichkeit versuchsbedingt eingeschränkt ist.

Da die Einzelheiten noch nicht abschließend geklärt sind, wurde davon abgesehen, bereits zum jetzigen Zeitpunkt eine verbindliche und konkrete Festlegung vorzunehmen. Jedoch muss sich der Antragsteller darauf einstellen, dass u. U. bereits für dieses Vorhaben zu gegebener Zeit ein entsprechendes Verlangen erfolgt.

Hinweise:

1. Die allgemeinen Vorschriften des TierSchG in Bezug auf Haltung, Betreuung, Ernährung und Pflege der Versuchstiere (§§ 1 und 2) und die besonderen Vorschriften für die Durchführung der Tierversuche (§§ 9 und 9a) sind einzuhalten.
2. Die nach § 16 Abs. 1 Nr. 3a des TierSchG vorgeschriebene Überwachung wird vom zuständigen Landratsamt Tübingen - Veterinäramt - durchgeführt. Da hierbei die nach § 9a Abs. 1 des TierSchG zu fertigenden Aufzeichnungen eingesehen werden, müssen diese im Tierlabor aufbewahrt werden bzw. muss ein Hinweis vorhanden sein, wo diese eingesehen werden können. Die Aufzeichnungen sind 3 Jahre lang nach Abschluss des Versuchsvorhabens aufzubewahren. Der Abschluss des Versuchsvorhabens ist dem Regierungspräsidium Tübingen anzuzeigen.
3. Die Aufstallung der Tiere in die vorgesehenen Versuchstierhaltungen ist im Hinblick auf die mögliche Belegung und Versorgung mit dem Tierschutzbeauftragten abzustimmen.
4. Die Genehmigung kann u. a. dann zurückgenommen bzw. widerrufen werden, wenn im Antrag unrichtige oder unvollständige Angaben erfolgten oder wenn Auflagen nicht eingehalten worden sind.
5. Ein etwaiger Antrag auf Verlängerung des vorliegenden Versuchsvorhabens ist dem Regierungspräsidium mindestens drei Monate vor Ablauf der Genehmigungsfrist einzureichen, um eine sachgerechte Prüfung unter Beteiligung der Kommission zu gewährleisten.

6. Auf Nr. 6.4.3. der AVVTierSchG (Genehmigungshöchstdauer) wird verwiesen. Darüber hinaus wird auf § 9 Abs. 2 Nr. 5 TierSchG (Wiederverwendung) hingewiesen.
7. Für diesen Bescheid wird eine Gebühr erhoben. Hierfür ergeht ein gesonderter Gebührenbescheid.

Rechtsbehelfsbelehrung:

Gegen diesen Bescheid kann innerhalb eines Monats nach Bekanntgabe beim Verwaltungsgericht Sigmaringen, Karlstraße 13, 72488 Sigmaringen, schriftlich oder zur Niederschrift des Urkundsbeamten der Geschäftsstelle des Gerichts Klage erhoben werden.

Mit freundlichen Grüßen



Dr. Jörn Hilmers

Über den

**Tierschutzbeauftragten
der Einrichtung**

**An das
Regierungspräsidium Tübingen
Referat 35
Konrad-Adenauer-Straße 20
72072 Tübingen**

Antrag auf Genehmigung von Versuchsvorhaben

(Version Mai 2009)

Alle Paragraphenangaben beziehen sich auf das Tierschutzgesetz in der Fassung der Bekanntmachung vom 18. Mai 2006 (BGBl. I S. 1206); geändert durch Gesetz vom 21. Dezember 2006 (BGBl. I S. 3294)

Name und Anschrift des Antragstellers, sowie Tel./Fax-Nr./E-Mail-Adresse:

Dr. rer. nat. Michael C. Schmid
Max-Planck-Institut für biologische Kybernetik
Spemannstraße 38
72076 Tübingen
michael.schmid@tuebingen.mpg.de
Tel.: 07071/601-651
Fax: 07071/601-652

Der Unterzeichnende beantragt die Genehmigung zur Durchführung von Tierversuchen nach § 8 Abs.1 des Tierschutzgesetzes für folgendes Versuchsvorhaben:

1. Angaben zum Versuchsvorhaben

1.1 Bezeichnung des Versuchsvorhabens (einschließlich der internen Kurzbezeichnung)

Thalamo-kortikale Mechanismen des Blindsehens
Optogenetik

1.2 Ort der Versuchsdurchführung, Ort der Tierhaltung, beabsichtigter Beginn (Datum) und voraussichtliche Dauer des Versuchsvorhabens (§ 8 Abs. 2 Satz 3 i.V.m. § 8a Abs. 2 Nr. 4):

Max-Planck-Institut für biologische Kybernetik, Tübingen, 1.12.2010 bis 31.11.2013

2. Personen, die im Rahmen der Versuchsdurchführung Eingriffe und Behandlungen an Tieren durchführen (§ 9 Abs. 1)

2.1 Leiter des Versuchsvorhabens

2.1.1 Name und Anschrift (inkl. Tel./Fax-Nr. und E-Mail-Adresse):

Dr. rer. nat. Michael C. Schmid
Max-Planck-Institut für biologische Kybernetik
Spemannstraße 38
72076 Tübingen
michael.schmid@tuebingen.mpg.de
Tel.: 07071/601-651
Fax: 07071/601-652

2.1.2 Berufsbezeichnung:

Arbeitsgruppenleiter am Max-Planck-Institut für biologische Kybernetik
Arbeitsgruppenleiter am Ernst Strüngmann Institut, Frankfurt a. M.

2.1.3 Fachliche Eignung mit Darstellung der tierexperimentellen Erfahrung:

Herr Dr. Schmid hat eine Ausbildung in chirurgischen Verfahren, speziell in der Chirurgie an Primaten. Er verfügt über umfangreiche Erfahrung in der Durchführung von Läsionierungen und Untersuchungen (funktionelle Kernspintomographie, Elektrophysiologie, Pharmakologie, Verhalten) des visuellen Systems von nicht-humanen Primaten. Er hat als Diplomand und Doktorand am Max-Planck-Institut für biologische Kybernetik unter Professor Logothetis (von 2002 bis 2006), sowie als Post-Doktorand an den National Institutes of Health, Bethesda, USA (von 2006 bis 2010) eine ausgiebige Ausbildung in diesen Verfahren erhalten.

siehe beantragte Ausnahmegenehmigung, sowie Antrag vom 28.02.2006,
Projektnummer KY2/06

2.2 Stellvertretender Leiter des Versuchsvorhabens

2.2.1 Name und Anschrift:

Prof. Dr. N. K. Logothetis
Max-Planck-Institut für biologische Kybernetik
Spemannstraße 38
72076 Tübingen
Tel: 07071-601-651
Fax: 07071-601-652
E-Mail: nikos.logothetis@tuebingen.mpg.de

2.2.2 Berufsbezeichnung:

Direktor, Max-Planck-Institut für biologische Kybernetik

2.2.3 Fachliche Eignung mit Darstellung der tierexperimentellen Erfahrung:

ist bereits mit Antrag vom 8. Januar 1997 dargelegt worden

2.3 **Sonstige Personen, die im Rahmen der Versuchsdurchführung Eingriffe oder Behandlungen an Tieren vornehmen**

2.3.1 Namen der Personen, deren Berufsbezeichnung und deren Tätigkeit im beantragten Projekt (ausgenommen Betäubung, siehe Nr. 2.3.3):

Behnaz Jarrahi, M.Sc., Doktorandin

2.3.2 Qualifikation (§ 9 Abs. 1 Satz 2 und 3) und tierexperimentelle Erfahrung (incl. Projekt-Nummern früherer Tierversuche, Funktion in früheren Tierversuchen); ggf. Hinweis auf eine erteilte Ausnahmegenehmigung (Datum):

Sie wird im Rahmen des Vorhabens von Dr. Schmid (siehe beiliegender Ausnahmeantrag) und Prof. Dr. Logothetis eine Ausbildung erhalten.

2.3.3 Gegebenenfalls Namen der Personen, die die Betäubung durchführen oder die Durchführung der Betäubung beaufsichtigen:

- PD Dr. med. Matthias Munk
- Dr. Richard Saunders
- Dr. med. Alexander Rauch
- Dr. Christoph Kayser
- Dr. Henry Evrard
- Dipl. Biol. Matthias Valverde
- Frau Cornelia Stamm, Tierärztin
- Herr Dr. Pedro Douay, Tierarzt
- Herr Thomas Steudel, TA
- Herr Mark Augath, TA
- Herr Mirko Lindig, TA
- Frau Deniz Ipek, TA

2.3.4 Qualifikation (§ 9 Abs. 2 Nr. 4 Satz 2) und tierexperimentelle Erfahrung:

Die Qualifikationen aller unter 2.3.3 gelisteten Personen sind bekannt bzw. handelt es sich um ausgebildete Tierärzte (Fritz und Douay). Richard Saunders von den National Institutes of Health, Bethesda, USA ist der weltweit führende Experte für Neurochirurgie in Affen (mehr als 50 Publikationen mit Läsionen, siehe zB beiliegende Nature Publikation). Alle, einschließlich des Antragstellers, haben mehrere Jahre Erfahrung in der Einleitung und Aufrechterhaltung von Narkosen. Die technischen Assistenten (TA) wurden eingehend für die Überwachung der Narkose während der Durchführung von Experimenten mit betäubten Affen ausgebildet, wie in früheren Anträgen bereits dargelegt.

3. Berechtigung der Personen zur Benutzung der Einrichtung, in der die Tierversuche durchgeführt werden (§ 8 Abs. 6)

3.1 Sind die unter Abschnitt 2 genannten Personen bei der Einrichtung beschäftigt?

nein (Arbeitgeber: Ernst Strüngmann Institut in Kooperation mit der Max Planck Gesellschaft, Frankfurt)

3.2 Wenn nein, sind sie mit Zustimmung des verantwortlichen Leiters der Einrichtung zur Benutzung der Einrichtung befugt?

ja, Prof. Dr. Nikos Logothetis ist stellvertretender Leiter des Versuchsvorhabens (siehe 2.2)

4. Erfüllung der Voraussetzungen des § 8 Abs. 3 und 4

4.1 Name, Anschrift und Qualifikation der für die Pflege und Betreuung der Tiere verantwortlichen Person, sowie Darstellung der Regelung im Vertretungsfall und an Wochenenden bzw. Feiertagen:

Herr Marcel Henni, Herr Hannes Göhring, Herr Alexander Sigle und Herr Jan Laucken sind ausgebildete Tierpfleger mit mehrjähriger Erfahrung Alle sind angestellt am Max-Planck-Institut für biologische Kybernetik, Spemannstraße 38, 72076 Tübingen.

4.2 Name, Anschrift und Qualifikation des/der mit der veterinärmedizinischen Versorgung der Tiere beauftragten Tierarztes/Tierärztin, sowie Darstellung der Regelung im Vertretungsfall und an Wochenenden bzw. Feiertagen

Frau Cornelia Stamm, approbierte Tierärztin und
Herr Dr. Pedro Douay, Tierarzt und Assistent,
Max-Planck-Institut für biologische Kybernetik
Spemannstraße 38, 72076 Tübingen
Stellvertreter (z.B. während des Urlaubs) sind: Dr. Hartmann / Dr. Steidel,
Tierärztliche Klinik, Jurastraße 23, 72072 Tübingen

5. Verfahren am Versuchsende

5.1 Name und Anschrift des Tierarztes, dem nach Abschluss des Versuchs die überlebenden Tiere der in § 9 Abs. 2 Nr. 8 genannten Arten vorgestellt werden:

Frau Cornelia Fritz, approbierte Tierärztin und Herr Dr. Pedro Douay, Tierarzt

6. Verpflichtungserklärung

Mit der Unterschrift verpflichtet sich der Leiter und sein Stellvertreter, die Verantwortung für die Einhaltung der Vorschriften nach § 9 Abs. 1 und 2, sowie ggf. von Auflagen nach § 8 zu übernehmen und die Aufzeichnungspflicht gemäß § 9a zu beachten.

Mit der Unterschrift wird bestätigt, dass die Genehmigungsvoraussetzungen nach § 8 Abs. 3 sichergestellt sind. Gleichzeitig wird die Kenntnis des Tierschutzgesetzes bestätigt.

Tübingen, den 28. Oktober, 2010



Unterschrift des Antragstellers



Unterschrift des Leiters des Versuchsvorhabens

Unterschrift des stellvertretenden Leiters des Versuchsvorhabens

7. Angaben zum Versuchsvorhaben

7.1 Bezeichnung des Versuchsvorhabens (einschließlich der internen Kurzbezeichnung)

Thalamo-kortikale Mechanismen des Blindsehens - Optogenetik

7.2 Zweck und Unerlässlichkeit des Versuchsvorhabens (incl. Literaturangaben) (§ 7 Abs. 2)

7.2.1 Zweck des Versuchsvorhabens und wissenschaftlich begründete Darlegung, dass dieser einem der in § 7 Abs. 2 Satz 1 genannten Zwecke zuzuordnen ist:

Das Ziel unserer Experimente ist zu verstehen, wie die thalamischen Kerne des Zwischenhirns mit der Großhirnrinde kommunizieren, um das Blindsehen zu ermöglichen. Blindsehen ist ein scheinbar paradoxes Phänomen, das bei Menschen und Affen nach Läsionierung der primären visuellen Großhirnrinde V1 auftaucht: Im betroffenen Sehfeld kann das Auftreten visueller Ereignisse zuverlässig angezeigt werden, obwohl ein bewusstes Erleben weitgehend, wenn nicht sogar vollständig, fehlt^[1, 2]. Unsere bisherigen Arbeiten mit der Kernspintomographie haben gezeigt, dass die Assoziationsareale der Großhirnrinde unabhängig von V1 arbeiten und Blindsehen vermitteln können^[3, 4]. Es ist uns außerdem gelungen, mit dem Corpus geniculatum laterale den thalamischen Kern zu identifizieren, der visuelle Informationen an V1 vorbei direkt in die Assoziationsfelder der Großhirnrinde übermittelt^[3]. Ziel des aktuellen Versuchsvorhabens ist zu verstehen 1) wie die elektrophysiologisch gemessenen neuronalen Antworten in einem speziellen visuellen Assoziationsareal MT mit dem Blindsehen korrelieren, und 2) welches der drei bekannten Nervenprojektionssysteme innerhalb des Corpus geniculatum laterale für die elektrophysiologischen Antworten im kortikalen Areal MT während des Blindsehens zuständig ist. Die Ergebnisse der Studie werden zu neuen fundamentalen Erkenntnissen über die neuronalen Mechanismen des visuellen Bewusstseins führen und darüber hinaus wichtige Hinweise für die Behandlung neurologischer Patienten liefern.

7.2.2 Wissenschaftlich begründete Darlegung der Unerlässlichkeit des Versuchsvorhabens unter Berücksichtigung des jeweiligen Standes der wissenschaftlichen Erkenntnisse (zum Stande der wissenschaftlichen Erkenntnisse ist die Angabe einer Schlüsselpublikation zweckdienlich, sie sollte dem Antrag beigelegt werden) (§ 7 Abs. 2 Satz 2 erster Halbsatz):

Blindsehen, visuelle Sehleistungen, die vom bewussten Erleben entkoppelt sind, ist seit seiner erstmalig dokumentierten Erkundung im Jahre 1917 bei der Untersuchung von Kriegsverletzten immer wieder Gegenstand der Forschung gewesen^[5, 6]. Das Phänomen ist gleichermaßen interessant für Ärzte, die sich für Regenerationsvorgänge im Gehirn interessieren, für Biologen und Psychologen, die den neuronalen Gesetzen des Sehens auf der Spur sind, wie auch für Philosophen, die schon seit langer Zeit das Bewusstsein zu erklären suchen. Dabei liegen die neuronalen Grundlagen des Blindsehens noch weitgehend im Dunkeln. Hoffnung kam auf, als es Alan Cowey und Petra Stoerig im Jahr 1995 gelang, ein zuverlässiges Tiermodell des Blindsehens in Makaken vorzustellen, das den Weg zu einem besseren Verständnis seiner neuronalen Grundlagen ebnen sollte^[1]. Letztlich wurden aber bis dato fast sämtliche Untersuchungen unter Vollnarkose durchgeführt, so dass die Verbindung zwischen Verhalten und neuronalen Antworten weitestgehend unbekannt ist. Trotzdem haben diese Untersuchungen einige wichtige Informationen geliefert, die im Folgenden kurz erläutert werden.

Es wird allgemein angenommen, dass das Blindsehen über eine Reihe subkortikaler und kortikaler Strukturen, die parallel und zum Teil unabhängig zur Projektion über V1 operieren, zustande kommt. Die Evidenz hierfür ist allerdings sehr durchwachsen. Positive Ergebnisse werden von negativen in Frage gestellt, was zum Teil an methodischen Limitierungen liegen mag. Es ist zum Beispiel unklar, ob die positiven Ergebnisse im Humanbereich evtl. auf unspezifische Blutflusseffekte der Kernspintomographie zurückzuführen sind. Andererseits könnte es auch sein, dass die negativen Resultate im Tierbereich durch die Anästhesie beeinflusst sind. Außerdem besteht die Möglichkeit, dass die Kernspintomographie Signale (z.B. elektrische Feldpotentiale) abgebildet hat, die mit traditioneller elektrophysiologischen Einzelzelleitungen meist nicht erfasst wurden. Unter den Assoziationsarealen im visuellen Kortex herrscht die größte Übereinstimmung für das Areal MT: Sowohl in der Kernspintomographie, also auch in elektrophysiologischen Untersuchungen wurden V1- unabhängige Aktivierungen gefunden^[3, 7-10](siehe aber^[11]). Allerdings ist unklar, inwieweit die neuronale Aktivierung dieses Areals mit dem Blindsehen einhergeht. Eine weitere Untersuchung dieser Frage ist allerdings wünschenswert, da die Antworten von Neuronen in MT im normalen Gehirn eng an die Wahrnehmung von Bewegung gekoppelt sind^[12-15]. In den hier vorgestellten Experimenten wollen wir herausfinden, ob diese Eigenschaft von MT auch unabhängig von V1 operiert und somit die Fähigkeit von Patienten erklärt, bewegende Reize zu erkennen. Dabei wird entscheidend sein, mit modernen elektrophysiologischen Methoden die Antworten mit und ohne Eingangssignal von V1 zu untersuchen. Durch systematische Untersuchung der Einzelzellaktivierung sowie der elektrischen Feldpotentiale erhoffen wir uns wichtige Ergebnisse hinsichtlich der Eingangs- und Ausgangssignale von MT. Außerdem erlauben moderne experimentelle und analytische Methoden, von vielen Zellen gleichzeitig abzuleiten und ihre Kommunikationsmuster zu untersuchen. Schließlich wollen wir durch eine systematische Erhebung der Zellaktivierung vor und nach der V1-

Läsion untersuchen, inwieweit es zu Reorganisationsprozessen in MT kommt, die mit Verhaltensverbesserungen einhergehen. Evidenz für eine solche Rehabilitation des Verhaltens wurde sowohl im Tierversuch [16] also auch mit Patienten [17] erbracht. Noch unveröffentlichte Daten unserer Gruppe, die mittels Kernspintomographie gewonnen wurden, unterstützen diese Befunde ebenfalls und deuten darauf hin, dass sich Assoziationsareale wie MT innerhalb der ersten 2 Monate nach der Läsionierung von V1 reorganisieren. Ob ein direkter Zusammenhang zwischen Rehabilitation der Neuronen und Einsetzen des Blindensehens besteht, ist bisher noch nicht bekannt und daher ein wichtiger Bestandteil unserer Untersuchungen: Wir planen Verhalten und elektrophysiologische Aktivierung von MT longitudinal vor der Läsionierung, unmittelbar danach, bis in die Monate nach dem Eingriff zu erheben.

Wie nun die visuellen Informationen an V1 vorbei in die Assoziationsareale wie MT kommen, ist auch noch nicht vollständig verstanden. Klar ist, dass der oberen Vierhügelplatte (Colliculus superior) im Dach des Mittelhirns eine zentrale Bedeutung zukommt. Das Blindsehen von Makaken wird eliminiert, wenn zusätzlich zu V1 auch der Colliculus zerstört wird [16]. Außerdem scheinen V1-unabhängige Antworten im Areal MT über den Colliculus vermittelt zu werden [18]. Wie nun die Information vom Colliculus nach MT übersetzt wird, war lange Zeit Teil vieler Spekulationen. Unsere eigenen Untersuchungen haben nun gezeigt, dass auf dem Weg zu MT dem Corpus geniculatum laterale eine entscheidende Bedeutung zukommt [3]. War dieser Kern inaktiviert, so konnten kernspintomographisch keine Signale mehr in MT gemessen werden, und die Affen waren für den Zeitraum der Inaktivierung komplett blind. Diese Ergebnisse zeigten also, dass das Corpus geniculatum laterale für das Blindsehen von zentraler Bedeutung ist. Es bleiben jedoch entscheidende Fragen bestehen: Kommt das Eingangssignal für das Corpus geniculatum laterale aus dem Colliculus oder direkt aus der Retina? Im Geniculatum gibt es drei verschiedene kortikale Projektionssysteme: Welche dieser Systeme erklären die Antworten in MT und das Blindsehen? Projektionen vom Corpus geniculatum laterale zu MT sind ja auch im gesunden Gehirn vorhanden [19]. Welche Rolle kommt dieser Projektion für normale Sehleistungen zu? Da diese Projektion ja den Weg über V1 spart, ist es attraktiv zu spekulieren, dass sie schnelle Aufmerksamkeitsprozesse während des Sehens vermittelt. Im Folgenden wollen wir erläutern, wie neue optogenetische Verfahren helfen können, diese Fragen zu beantworten.

Der optogenetische Mechanismus wurde im Jahr 2003 von Forschern des Max-Planck-Instituts für Biophysik in Frankfurt/Main entdeckt [20] und fand schnell vielversprechende Anwendungen in den Neurowissenschaften. Die lichtempfindlichen Proteine Channelrhodopsin-2 (ChR2) der *Chlamydomonas reinhardtii* und Halorhodopsin aus *Natronomonas pharaonis* (NpHR) ändern das Membranpotential einer Zelle [21, 22]. Beim ChR2 führt das Auftreffen von Licht mit einer Wellenlänge von 470 nm zu einem Einstrom der Kationen Na^+ , H^+ und Ca^{2+} durch die Öffnung des Kanals und depolarisiert so die Membran. Beim Halorhodopsin ist der Effekt ein anderer: nach Lichteinfall mit einer Wellenlänge von 580 nm wird Cl^- in die Zelle gepumpt und die Membran damit hyperpolarisiert. Da die beiden Kanäle auf unterschiedliche Teile des Lichtspektrums antworten, können sie unabhängig voneinander manipuliert werden und Aktionspotentiale sowohl hervorrufen als auch unterdrücken. Um ChR2 und NpHR im gewünschten Hirnareal exprimiert zu bekommen, wird entweder ein Lentivirus oder ein Adeno-assoziiertes Virus (AAV) lokal injiziert. Im Vektor ist gewöhnlich auch ein fluoreszierendes Protein (z.B. GFP) enthalten, das eine mikroskopische Analyse der Transfektion ermöglicht. Die erste Anwendung von Optogenetik in Neuronen wurde im Jahr 2005

erstmalig von unserem Projekt-Partner Ed Boyden und Kollegen an neuronalen Zellkulturen durchgeführt^[21]. Seit jener Publikation haben Dr. Boyden und eine Reihe weiterer Gruppen die Methode in verschiedenen Tierspezies erfolgreich angewandt, so auch in der Großhirnrinde von Makaken^[23]. Die hier beschriebenen Experimente mit optogenetischer Methodik im Corpus geniculatum laterale des Affen werden die ersten ihrer Art sein. In einem ersten Schritt sollen Geniculatum-Zellen nicht selektiv mit ChR2 und NpHR infiziert werden. In einem zweiten Schritt sollen die unterschiedlichen Zelltypen des Geniculatum selektiv manipuliert werden, was auf Grund ihrer unterschiedlichen chemischen Eigenschaften möglich ist, so dass spezifische Vektoren konstruiert werden können. Nach einer methodischen Analyse der optogenetischen Methodik im Geniculatum vor Ort, ist die optogenetische Manipulation des Geniculatum und ihr Einfluss auf Zellen im Areal MT von Tieren mit und ohne V1 Läsion geplant. Der Ansatz ermöglicht deshalb, die Rolle der geniculären Zellsysteme für das Blindsehen und für Aufmerksamkeitsprozesse im Kortex zu verstehen. Das primäre Ziel unseres Antrags ist: 1) den direkten Zusammenhang zwischen neuronaler Aktivierung von MT und Blindsehen zu beschreiben 2) die V1 unabhängigen thalamischen Projektionen nach MT und deren Bedeutung für Blindsehen zu verstehen.

7.2.3 Darstellung der eigenen wissenschaftlichen Vorarbeiten zum Thema des Versuchsvorhabens (einschließlich der Angabe einer eigenen Schlüsselpublikation, die dem Antrag beigelegt werden sollte).

In der Forschung unserer Gruppe wurden Methoden der fMRI und Elektrophysiologie angewendet, um neuronale Plastizität im visuellen Kortex von Primaten in der Folge von retinalen^[24] und nach kortikalen Verletzungen^[3, 4] zu untersuchen. Diese Arbeiten^[4, 24] wurden mit der Genehmigung des Regierungspräsidiums am Max-Planck-Institut für biologische Kybernetik durchgeführt. Speziell, was das Blindsehen angeht, haben wir in der Folge von Läsionierungen von V1 gezeigt, dass trotz fehlender Eingangssignale aus V1 der Assoziationskortex von Primaten etwa einen Monat nach der Läsionierung wieder visuell moduliert werden kann^[3, 4]. Wir haben außerdem gezeigt, dass unter den visuellen thalamischen Kernen das Corpus geniculatum laterale kritisch ist, um visuelle Signale an V1 vorbei zum Kortex zu senden und das Blindsehen zu ermöglichen. Unser Kollege Ed Boyden vom Massachusetts Institute of Technology ist einer der beiden weltweit führenden Wissenschaftler auf dem Gebiet der Optogenetik. Er hat mit seinem Team diese Methode auch schon im Kortex von Makaken erfolgreich angewandt^[23].

7.2.4 Wissenschaftlich begründete Darlegung, dass der Versuchszweck nicht durch andere Methoden oder Verfahren als den Tierversuch erreicht werden kann (§ 7 Abs. 2 Satz 2 zweiter Halbsatz):

Es gibt keine Möglichkeit, die gewünschte Information aus Computersimulationen oder theoretischen Analysen abzuleiten. Die einzige Möglichkeit, zu verstehen, wie dieses biologische System arbeitet, ist das Experiment. Untersuchungen an Menschen bieten keinen brauchbaren Ersatz für die hier vorgeschlagenen Versuche an Makaken, da sie 1) keine physiologische Information liefern können, da die letztere zahlreiche invasive Aufzeichnungen von Gehirnaktivität mittels Mikroelektroden erfordert, 2) Methoden um die

Aktivität von Zellen selektiv zu manipulieren (Optogenetik) nicht verfügbar sind, und sie 3) schlecht kontrollierbar sind (Experimente der Natur), was es schwierig, wenn nicht sogar unmöglich macht, genaue Schlüsse über die Mechanismen zu ziehen.

7.3 Ausschöpfung zugänglicher Informationsmöglichkeiten (§ 8 Abs. 3 Nr. 1 Buchstabe b)

7.3.1 Welche Informationsmöglichkeiten wurden genutzt?

Studium der internationalen Literatur, Teilnahme an wissenschaftlichen Kongressen im In- und Ausland, Austausch von Erfahrungen und persönlicher Kontakt mit Wissenschaftlern im In- und Ausland.

7.3.2 Wissenschaftlich begründete Darlegung, dass das angestrebte Versuchsergebnis nicht hinreichend bekannt ist bzw. dass die Überprüfung des hinreichend bekannten angestrebten Versuchsergebnisses durch einen Doppel- oder Wiederholungsversuch unerlässlich ist.

Siehe auch Abschnitt 7.2.2. Es ist derzeit noch wenig über den Mechanismus des Blindsehens und seiner thalamo-kortikalen Mechanismen bekannt. Bis zur jüngeren Arbeit unserer Gruppe war nicht daran zu denken, dass Areale des Assoziationskortex unabhängig von V1 visuell moduliert werden können [3, 4]. Es gilt nun, genauer zu verstehen, wie die elektrophysiologischen Signale der einzelnen Areale mit dem Blindsehen zusammenhängen und wie sie sich reorganisieren. Bevor unsere jüngste Arbeit den Fokus im Thalamus auf das Korpus geniculatum laterale gelenkt hat, war eine weitverbreitete Annahme in unserem Forschungsfeld, dass ein anderer thalamischer Kern, das Pulvinar das Blindsehen ermöglichen könnte. Das Genuculatum besteht allerdings aus einer Reihe unterschiedlicher Zell- und Projektionssysteme. In unserer aktuellen Forschung wollen wir diese isolieren und ihre Funktion für Blindsehen und Aufmerksamkeit erforschen.

7.4 Art und Anzahl der vorgesehenen Tiere; Haltungsform (§ 8 Abs. 2 Satz 3 i.V.m. § 8a Abs. 2 Nr. 2 und § 9 Abs. 2)

7.4.1 Vorgesehene Tierarten und Begründung für die Wahl der Tierart (§ 9 Abs. 2 Nr. 1):

Es ist von entscheidender Bedeutung, dass wir die Experimente an Tieren durchführen, da uns dadurch Zugang zu invasiven Techniken wie der Elektrophysiologie und der Optogenetik ermöglicht wird, um die Mechanismen von Blindsehen und Aufmerksamkeit zu untersuchen.

Wesentlich hierfür ist es, Experimente an einer Tierart durchzuführen, die in Neuroanatomie und Physiologie dem Menschen so nah wie möglich kommt: Rhesusaffen haben eine sehr ähnliche Neuroanatomie und Neurophysiologie und ähnliche

Verhaltensmuster wie Menschen. Es hat sich umfangreiches Wissen über kortikale Elektrophysiologie des visuellen Systems dieser Spezies angehäuft, welches die Interpretation unserer Befunde unterstützt. Kortikale Organisation und Verhalten bei Makaken sind sehr ähnlich wie beim Menschen, daher ist es sehr wahrscheinlich, dass wir unsere Befunde direkt auf den Menschen übertragen können.

7.4.2 Vorgesehene Anzahl und Begründung für die Anzahl der Tiere einschließlich präziser Angaben zur biometrischen Planung (hilfreich ist die Beilegung eines biometrischen Gutachtens) (§ 9 Abs. 2 Nr. 2):

Auf der Grundlage unserer Erfahrung aus vorausgegangener Arbeit und der allgemein akzeptierten Standards, die sich auf dem Gebiet der kortikalen Elektrophysiologie etabliert haben, gehen wir davon aus, dass sich 2 Tiere zur Untersuchung des spezifischen Ziels Nr. 1 (Korrelation von elektrophysiologischen Antworten in MT mit Blindsehen) im allgemeinen als ausreichend erweisen werden. Dies wird natürlich von der zu überprüfenden Übereinstimmung der Ergebnisse zwischen den Tieren abhängen.

Das spezifische Ziel Nr. 2 sieht die funktionale Isolierung der thalamischen Projektionssysteme mittels Optogenetik vor, die Erforschung ihrer Auswirkung auf elektrophysiologische MT- Antworten mit und ohne Läsion in V1 und ihre Rolle beim Blindsehen. Die Methode der Optogenetik verspricht sehr gut kontrollierbare Versuchsbedingungen und die Entdeckung fundamentaler Gehirnmechanismen. Da sie erstmalig in unserem Labor angewendet wird, ist von einiger Entwicklungsarbeit auszugehen. Um eine rasche und erfolgreiche Anwendung sicher zu stellen, werden wir für dieses Ziel mit Prof. Dr. Ed Boyden vom MIT, einem der beiden weltweit führenden Optogenetiker zusammenarbeiten. Ein erster Schritt wird sein, die Effizienz der Transfektion mikroskopisch zu analysieren. Hierfür werden die thalamischen Genuculata von 2 Tieren mit dem Virus infiziert. Nach etwa 1 bis 2 Monaten werden die Tiere euthanasiert und ihre Gehirne mikroskopisch untersucht. Sollte die Transfektion erfolgreich gewesen sein, werden die Genuculata von 2 weiteren Tieren infiziert. In diesen Tieren soll dann der Effekt der Optogenetik physiologisch, zunächst im Genuculatum, und anschliessend im Areal MT untersucht werden. Um den Zusammenhang zwischen Blindsehen und der Kommunikation zwischen dem Genuculatum und MT zu untersuchen, werden dann die Tiere aus den spezifischen Zielen zusammengenommen. Die Optogenetiktiere erhalten eine Läsion in V1, die V1-lädierten Tiere die Optogenetik.

Die Mindestzahl von Tieren, die für das vorgeschlagene Projekt benötigt werden, ist demnach 6 für einen Zeitraum von 3 Jahren. Angesichts der Unsicherheiten, die Bestandteil des Experimentierens sind, halten wir es für ratsam, im Voraus und in angemessenem Rahmen mögliche Probleme zu berücksichtigen, welche die teilweise nicht vorherzusehende Variabilität des Experimentierens mit sich bringen könnte. So ist es beispielsweise vorstellbar, dass es notwendig wird, zusätzliche Tiere in die Untersuchung aufzunehmen, wenn bei, sagen wir, 1-2 Tieren das Ausmaß der experimentellen Manipulation (Läsion, Transfektion) nicht genau wie vorgesehen ausfällt oder wenn wir in unseren Daten Tendenzen beobachten, die zusätzliche Untermuerung durch ein drittes Tier erfordern, um eine statistische Absicherung zu erzielen. Um dieser Möglichkeit Rechnung zu tragen, beantragen wir für die hier beschriebenen Fälle, zwei zusätzliche

Tiere in den Untersuchungen verwenden zu können. Die Gesamtzahl der für dieses Vorhaben beantragten Tiere über die nächsten 3 Jahre wäre demnach 8.

7.4.3 Vorgesehene Haltungsform (Tierzahl/Gruppe, Einzelhaltung, Käfiggröße, ggf. Ausstattung und kurze Begründung)

Die Versuchstiere werden in unserer Tierhaltung in Gruppen gehalten. Einzelhaltung erfolgt nur unmittelbar nach operativen Eingriffen, wobei dann nach Möglichkeit Sicht- und Hörkontakt zur Gruppe hergestellt wird. Im Zuge der Umstellung unserer Primatenhaltung auf gemischtgeschlechtliche Gruppen versuchen wir derzeit, möglichst natürliche Gruppenstrukturen einzurichten. Bisher konnten wir erfolgreich jeweils ein ausgewachsenes männliches Tier mit 3 weiblichen Tieren vergesellschaften und haben dadurch ruhigere Interaktionen in den Gruppen beobachten können. Selbstverständlich werden antikonzeptive Maßnahmen, vorzugsweise Vasektomien oder seltener hormonelle Antikonzeptiva in Form von Depotpräparaten, durch unsere Tierärzte angewendet. Wir sind bemüht, diese Umstellung fortzusetzen, insbesondere auch vor dem Hintergrund, dass wir dadurch ein natürlicheres Sozialverhalten und damit eine artgerechtere Haltung verwirklichen können.

7.4.4 Handelt es sich um eigens für Tierversuche gezüchtete Tiere (§ 9 Abs. 2 Nr. 7)?

ja

Wenn nein, ist – soweit nach § 9 Abs. 2 Nr. 7 gefordert – ein Antrag auf Zulassung einer Ausnahme nach § 9 Abs. 2 Nr. 7 Satz 2 mit Begründung erforderlich, warum nicht eigens für Tierversuche gezüchtete Tiere verwendet werden sollen (auf gesondertem Blatt!)

Gegebenenfalls Begründung, warum eine Entnahme aus der Natur für erforderlich gehalten wird (§ 9 Abs. 2 Nr. 1 Satz 2)

entfällt

7.4.5 Die vorgesehenen Tiere wurden bereits in einem Versuchsvorhaben im Sinne des § 9 Abs. 2 Nr. 5 verwendet

nein

wenn ja, Beschreibung der Art und Dauer der bislang erfolgten Eingriffe an den betreffenden Tieren:

entfällt

7.4.6 Herkunft der Tiere - Bezugsquelle

Bioprim Toulouse
Primatenzentrum Strasbourg (ULP)
Deutsches Primatenzentrum (DPZ)

7.5 **Beschreibung der Art, Durchführung und Dauer der vorgesehenen Eingriffe und Behandlungen** (§ 8 Abs. 2 Satz 3 i.V.m. § 8a Abs. 2 Nr. 3)

Die Angaben in Abschnitt 7.5 sind zusätzlich in einer dem Genehmigungsantrag beizufügenden Belastungstabelle (Anl. 1 zum Antrag) einzutragen. Der Antragsteller wird gebeten, auch eine persönliche Einschätzung der Gesamtbelastung anzugeben zum Beispiel entsprechend den Belastungskategorien des Bundesamtes für Veterinärwesen der Schweiz (s. „Einteilung von Tierversuchen nach Schweregraden vor Versuchsbeginn (Belastungskategorien)“ www.bvet.admin.ch/themen/tierschutz/00777/00778/index.html?lang=de).

7.5.1 Welche Eingriffe oder Behandlungen sollen durchgeführt werden? (Detaillierte Darstellung sämtlicher Maßnahmen mit zeitlichem Ablauf)

Die folgende Kurzbeschreibung stellt die allgemeine Zeitachse für ein typisches Experiment dar.

Erstes Ziel (Korrelation von Blindsehen und Elektrophysiologie des Areal MT):

Sobald die Verhaltensleistung der Tiere bewegte Reize zu erkennen stabil ist, werden unter Narkose fMRT-Karten vom Areal MT gewonnen, um die gezielte Implantation einer Kammer für elektrophysiologische Messungen in MT sicherzustellen. Sobald die Kammer implantiert ist, werden die Verhaltensleistungen des Tieres mit elektrophysiologischen Signalen korreliert. Im weiteren Verlauf der Experimente wird dann ein Teil des Areal V1 eine Läsion erhalten. Diese Läsionierung wird als getrennter chirurgischer Eingriff unter allgemeiner Anästhesie erfolgen. Nach der Läsionierung werden weitere elektrophysiologische und Verhaltensmessungen durchgeführt.

Zweites Ziel (Optogenetik im Thalamus, Blindsehen und Elektrophysiologie des Areal MT):

Zunächst wird die Effektivität der Optogenetik mit mikroskopischen und elektrophysiologischen Methoden gemessen. Sollte die Optogenetik wider Erwarten nicht erfolgreich zu implementieren sein, werden wir an dieser Stelle auf traditionelle Methoden der temporären pharmakologischen Inaktivierung zurückgreifen, mit denen wir viel Erfahrung haben [3]. In jedem Fall, wird anschließend eine Kammer für das Areal MT implantiert und Verhalten und elektrophysiologische Aktivierung während der thalamischen Inaktivierung korreliert. Diese Experimente werden nach einer Läsionierung von V1 wiederholt werden.

Im Folgenden stellen wir Schritt für Schritt die verschiedenen Verfahren dar, die auf der oben beschriebenen Zeitachse durchgeführt werden:

A. Allgemeine chirurgische Verfahren und postoperative Behandlung:

Dem Affen wird subkutan Robinul (0.01 mg/kg) oder Atropin (0.05 mg/kg) injiziert, um Sekretionen zu reduzieren, innerhalb von 10-15 Minuten gefolgt von einer Dosis Ketamin (10-15mg/kg, i.m.). Ein intravenöser Katheter wird aseptisch in die Vena saphena platziert. Der Katheter wird am umgebenden Gewebe befestigt und mit einem trockenen Verband fixiert. Der Larynx wird mit Lidocain besprüht und der Affe intubiert, um die Atemwege freizuhalten. Mit einer elektrischen Schere werden die Haare von der Operationsstelle entfernt. Das Tier wird in einem stereotaktischen Apparat auf dem Operationstisch platziert, um während der Operation mechanische Stabilität sicherzustellen. Isofluran-Anästhesie wird zu diesem Zeitpunkt begonnen und während des gesamten Eingriffs beibehalten.

Die Tiefe der Anästhesie wird durch Auslösen von Reflexen (pinch reflex, Kornealreflex, Tonus der Kaumuskulatur) sowie Monitoring von Atmung, Herzschlag und -rhythmus, endexpiratorisches CO₂, Sauerstoffsättigung, Blutdruck und Färbung der Schleimhäute überwacht. Während des gesamten chirurgischen Eingriffs werden dem Tier intravenös 2.5% Dextrose in Ringer-Laktat-Lösung mit einer Rate von ungefähr 10-15 ml/kg*h verabreicht. Die Körpertemperatur wird mithilfe eines thermostatisch kontrollierten Heizkissens oder einer Warmluftdecke bei 37 °C gehalten. Im Fall von intrakranieller Chirurgie wird Dexamethason intramuskulär 48 Stunden vor der Operation (0.5 mg/kg/Tag) verabreicht, um den intrakraniellen Druck zu reduzieren. Baytril (5 mg/kg/Tag) wird 24 Stunden vor der Operation verabreicht, um das Risiko von Infektionen zu minimieren. Eine angemessene Vorbereitung der Haut für ein steriles Verfahren wird daraus bestehen, das Fell des Tieres zu rasieren, nachdem es mindestens drei Mal mit Betadine gescheuert wurde, gefolgt von 70% Alkohol. Alle weiteren chirurgischen Prozeduren werden unter aseptischen Bedingungen durchgeführt.

Am Ende des chirurgischen Eingriffs wird das Tier extubiert. Initiale Dosen von Antibiotika (Baytril, 5 mg/kg, IM) und Analgetika werden verabreicht während das Tier noch narkotisiert ist. Wenn der Affe das Bewusstsein wiedererlangt, wird er in einen Erholungskäfig in einem speziell für das Aufwachen eingerichteten Raum gebracht, wo er kontinuierlich überwacht wird, bis er in der Lage ist zu sitzen. Eine Heizlampe oder ein Heizofen wird bereitgestellt und bei Bedarf verwendet. Hautklammern oder Nähte werden je nach Heilungsverlauf nach ca 10 Tagen entfernt. Antibiotika werden i.d.R. weitere 5 bis 7 Tage lang verabreicht. Analgetika werden ebenfalls 5 weitere Tage lang verabreicht, um sicherzustellen, dass das Tier schmerzfrei bleibt.

B. Implantation eines Kopfhalters:

Dies ist notwendig, um den Kopf des Tieres dauerhaft ruhigzustellen, bevor das Verhaltenstraining, die physiologischen oder fMRI-Messungen durchgeführt werden können. Vor allen größeren chirurgischen Eingriffen und Allgemeinnarkosen wird den Tieren mindestens 8 Stunden, meist über Nacht kein Futter gegeben. Bis zu einer Stunde vor dem Eingriff werden sie Wasser zur freien Verfügung haben. Direkt vor der Operation werden Antibiotika (Baytril, 50 mg/kg, i.m) verabreicht. Zusätzlich werden wir Analgetika (Buprenex, 0.01 mg/kg, i.m und Banamin, 1 mg/kg, i.m.) verabreichen. Zuerst werden die Versuchstiere anästhesiert, wobei eine intramuskuläre Injektion von Ketamin, 15 mg/kg, angewendet wird. Um übermäßige Sekretionen zu vermeiden, werden wir darüber hinaus

entweder Robinul 0.01 mg/kg oder Atropin 0.05 mg/kg injizieren. Nach der endotrachealen Intubation (unter Verwendung lokaler Anästhetika wie Xylocain auf der Spitze der Intubationsröhre) und ggf unter Verwendung eines Bolus Propofol (7.5-12.5 mg/kg i.v. zur Induktion) wird die Anästhesie durch die Verabreichung von Isofluran (1-2.5%, 100/0 O₂/N₂O, bei 0.8-1.0 l/min) aufrechterhalten. Während der Anästhesie werden wir EKG, Respirationsrate, Rektaltemperatur, Sauerstoffgehalt, Blutdruck und endexpiratorisches pCO₂ überwachen. Die Haut wird geöffnet, um den Schädel freizulegen. Annähernd zehn bis fünfzehn Löcher (Durchmesser zwischen 1 und 3mm) werden nach genauer stereotaktischer Planung in den Schädel gebohrt (die Dura nicht penetrierend), um mithilfe von keramischen Kortikalisschrauben die Implantate am Schädel zu befestigen. Wenn der Kopfhalter auf dem Schädel platziert ist, werden die Schraubenköpfe mit geringsten Mengen Dentalacrylzement versiegelt, um Eintrittspforten für Keime zu verschließen. Im Anschluss daran werden Muskel, Faszie und Haut mit entsprechend resorbierbaren Nähten verschlossen, wobei insbesondere eine intrakutane Hautnaht ein frühes Zurücksetzen der Tiere in den Käfig bzw die Gruppe ermöglicht. Die Affen werden in ihren Käfig zurückgebracht sobald sie ohne Hilfe aufrecht sitzen können. Normalerweise werden diese Kopfhalter an ihrem Platz bleiben bis die Affen eingeschläfert werden. Allerdings kann ein neuer Kopfhalter implantiert werden (mit demselben Verfahren wie bei dem ersten Kopfhalter), wenn der Affe seinen Kopfhalter zerstört oder verliert.

Nachdem der Affe sich erholt hat, werden postoperativ mindestens 3-5 Tagen lang Analgetika (Buprenex, 0.01 mg/kg, BID, i.m. und Banamine, 1mg/kg, i.m., QD) verabreicht. Antibiotika (Baytril 5mg/kg QD) werden prophylaktisch 7-10 Tage lang postoperativ verabreicht.

Auf der Mittellinie des Schädels wird ein Schnitt gemacht, und die Haut wird zurückgezogen und in Saline-getränkten Verbandsmull eingewickelt. Alternativ wird ein halbkreisförmiger Einschnitt um die Implantationsstelle des Kopfhalters gemacht. Dann wird der Schädel vom Periost befreit. Die Bodenplatte des Kopfhalters (eine passgenaue Maßanfertigung), die aus einem MRI-kompatiblen Tecapeek hergestellt ist, wird dann am Schädel angebracht. Die Bodenplatten haben Löcher mit regelmäßigen Abständen für keramische Schrauben. Löcher werden in den Schädel gebohrt und Gewinde geschnitten. Je nach Form und individueller Knochenstabilität werden die Implantate mit ungefähr 12 Schrauben befestigt. Sobald die Platten sicher befestigt sind, werden die Drähte der Augenspule subkutan vom Jochbeinbogen weg durch eine vorgefertigte Bohrung im Kopfhalter geführt, die an einer Position im Implantat endet, die außerhalb der geschlossenen Kopfhaut liegen wird, damit später Messgeräte angeschlossen werden können. Dann wird die Kopfhaut um den Kopfhalter herum gelegt, der an der Stelle, an der er von der Haut umschlossen wird, ungefähr 1 cm Durchmesser hat, zurechtgelegt und vernäht. Alternativ kann Dentalzement benutzt werden, um den Kopfhalter am Schädel zu befestigen, und die Haut wird in diesem Fall um den Dentalzement herum verschlossen. Wenn alle Prozeduren abgeschlossen sind, wird das Tier aus dem stereotaktischen Halter herausgenommen und die Anästhesie beendet. Alternativ werden formspezifische Kopfhalter maßgefertigt, die zusammen mit den Füßchen aus einem Teil sind, und die ausgezeichnet zwischen Implantat und den darunterliegenden Schädel passen. Diese Implantate sind dafür entworfen, um mit hoch auflösender anatomischer MR-Bildgebung kompatibel zu sein.

C. Implantation von Ableitkammern:

Diese Implantate sind notwendig, um häufigen Zugang zum Gehirn für elektrophysiologische Aufzeichnungen und elektrische Stimulationsexperimente zu ermöglichen. Dieses Verfahren kann gleichzeitig mit der Platzierung eines Kopfhalters oder als unabhängiger Eingriff durchgeführt werden. Nach der (initialen) Vorbereitung wird der Affe in einem stereotaktischen Apparat platziert und eine Hautklappe (skin flap) angelegt, um die Stelle freizulegen, an der die Tecapeek-Kammer angebracht wird. Das Bindegewebe wird von der Muskelummantelung getrennt, Haut und Bindegewebe werden zurückgezogen und unter Saline-getränkten Verbandsmull gelegt. Formspezifische Kammern mit Füßen werden aus einem Stück maßgefertigt und passen einwandfrei zwischen Implantat und darunterliegenden Schädel. Diese Implantate werden auf der Grundlage von hoch aufgelösten anatomischen MR-Aufnahmen mit CAD entworfen und gefertigt, damit sie an der vorgesehenen Stelle optimal zur Oberfläche des Schädels passen. Die Kammer (tecapeek, MRI-kompatibel) wird dann an den gewünschten Koordinaten im gewünschten Winkel platziert, und ihre Position wird markiert. Die Kammer wird zunächst wieder entfernt, um eine Kraniotomie durchzuführen.

Anschließend wird die Kammer an ihrem Platz angeschraubt. Dazu werden, wie bei der Implantation des Kopfhalters, Löcher durch die Füße der Kammer gebohrt, dann wird sie mit 8-16 Keramikschauben im Schädel festgeschraubt. Die Kammer wird dann mit einem maßgefertigten Deckel verschlossen. Das Bindegewebe und die Haut werden vernäht, wobei für das Bindegewebe resorbierbare Nähte und für die Haut entweder nicht-resorbierbare Nähte (Nylon monofilament) oder rostfreie Stahlklammern verwendet werden. Der Affe wird dann aus dem stereotaktischen Instrument herausgenommen und aus der Narkose geweckt. Danach kann er sich mehrere Tage lang erholen, bis das Training wiederaufgenommen wird. Alternativ zur Einpassung eines formgefertigten Implantats kann eine Kammer gelegentlich auch mittels antibiotikahaltigem Acryl-Knochenzement (Palacos) am Knochen und entsprechenden Schrauben in der unmittelbaren Nähe verankert werden. Dazu werden einige (~8-12) 6-8mm lange Keramikschauben um die Kammer herum im Knochen befestigt. Die Kammergröße variiert zwischen 15 und 30 mm innerem Durchmesser, je nachdem welche Areale abgeleitet werden sollen. Die maximale Zahl von Kammerimplantaten, die ein Tier jemals erhalten wird, ist zwei. Es wird davon ausgegangen, dass das Grundlagenexperiment (basic experiment) mit grundsätzlich zwei Kammerimplantationen pro Tier durchgeführt werden kann, einer in jeder Hemisphäre, aber gelegentlich kann es zur Kammerreparatur erforderlich sein, zusätzliche Implantationen durchzuführen, sollten Kammern sich aus irgendwelchen Gründen lockern. Gelegentlich werden Kammern angebracht, und das Tier wird 5-10 Tage Zeit haben, um sich zu erholen, und erst dann wird unmittelbar vor dem Beginn der Aufzeichnungen mittels Trephine oder Kugelbohrer eine Kraniotomie durchgeführt werden, um eine frische unberührte Dura für das Ableiten zu gewährleisten.

D. Kraniotomien und Durapräparation für elektrophysiologische Ableitungen

Am Vortag des Aufzeichnungsbeginns wird innerhalb der Kammer unter allgemeiner Anästhesie entweder eine kleine Kraniotomie (2-4mm) oder eine vollständige Kraniotomie (bis zu 20 mm) durchgeführt. Ein verzögertes Anlegen einer Kraniotomie ermöglicht es, mehr Daten zu sammeln, weil die Dura mater durch die mechanische Belastung nach wenigen Tagen anfängt zu proliferieren und das neugebildete Narbengewebe so stabil wird, dass eine Penetration mit Mikroelektroden immer schwieriger und schließlich unmöglich ist. Dies geschieht meist innerhalb von 3-6 Wochen. Wenn die Dura undurchdringlich geworden ist, entfernen wir entweder das Granulationsgewebe ggf

einschließlich des nachgewachsenen Knochens (das Abtragen des Knochens wird weiter unten beschrieben) oder führen eine neue Kraniotomie unter Allgemeinnarkose durch. Dieses Verfahren wird unter sterilen Bedingungen durchgeführt, unter Verwendung sterilisierter Trephine oder eines Dentalbohrers. Während der Durchführung von Experimenten kann es notwendig sein, vom Rand der Kraniotomie im Inneren der Aufzeichnungskammer Granulationsgewebe oder neu gewachsenen Knochen zu entfernen. Da es sich hierbei um ein invasives Verfahren handelt, wird es unter einer geringen Dosis Ketamin (5-15 mg/kg, i.m.), eventuell kombiniert mit Medetomidin (antagonisierbar!) und unter sterilen Bedingungen durchgeführt.

E. Kortikale Läsionierungen:

Wir planen, Teil-Läsionierungen der primären visuellen Sehrinde V1 von Makaken durchzuführen, um ein Modell des Blindsehens in Makaken zu generieren und die Grundlagen bewussten visuellen Erlebens zu beschreiben. Läsionierungen von V1 werden keine motorischen Defizite verursachen, und die foveale Repräsentation wird verschont werden, sodass die Tiere normale Augenbewegungen und Fixationen durchführen können. Wir gehen nicht davon aus, dass die kortikalen Läsionierungen oder irgendein anderes von uns angewendetes Verfahren sich in sichtbaren Defiziten niederschlagen wird, die die Gewohnheiten der Nahrungsaufnahme oder des Sozialverhaltens beeinträchtigen könnten. Begrenzte visuelle Defizite werden auftreten, aber sie werden nur durch spezifische Tests zu erkennen sein und werden das Verhalten des Affen im Alltag nicht beeinträchtigen.

Die Läsionsoperation wird von den Professoren Nikos Logothetis und von Dr. Richard Saunders, dem weltweit führenden Neurochirurgen für Primaten der National Institutes of Health, durchgeführt. Wir haben diesen Ansatz erfolgreich in unseren beiden bisherigen Publikationen [3, 4] angewandt. Die Operation wird unter sterilen Bedingungen und unter Vollnarkose durchgeführt. Für Verfahren der Läsionierung, die weniger als 2h dauern, werden wir eine Injektionsnarkose mit Ketamin/Xylazin durchführen. Für längere Verfahren werden stattdessen Intubation und Anästhesie durch Remifentanyl oder Isofluran angewandt. Nach der (initialen) Vorbereitung wird der Affe in dem stereotaxic Apparat platziert, und es wird ein Einschnitt gemacht, um an der gewünschten Stelle den Schädel freizulegen. Das Bindegewebe wird zurückgezogen und unter Saline-getränktem Verbandsmull platziert/mit Saline-getränktem Verbandsmull bedeckt. Ein Dentalbohrer oder Trephine wird verwendet, um an der gewünschten Stelle einen Teil des Schädels zu entfernen und Zugang zum Gehirn zu erhalten. Die Dura Mater wird über V1 mit einem Schnitt geöffnet. Anschließend wird die piale Blutversorgung von V1 koaguliert, die Pia wird abgezogen und die oberen Schichten der grauen Substanz werden mit einem Katheter sorgfältig abgesaugt. Nach der Läsionierung wird der Knochen mit Nylonnähten wieder an seinen Platz genäht. Dann werden das Bindegewebe und die Haut vernäht, wobei resorbierbare Nähte für das Bindegewebe und für die Haut entweder nicht-verdaubare Nähte (nylon monofilament) oder nicht rostende Stahlklammern verwendet werden. Einen Tag vor der Operation wird mit der Gabe von Dexamethason (4mg/kg/Tag auf drei Dosen aufgeteilt) begonnen, sie wird bis 5 Tage nach der Läsionierung fortgesetzt und dann allmählich abgebaut, um das Risiko von Entzündungsreaktionen/einer Entzündungsreaktion zu reduzieren. Nach dem Eingriff können sich die Tiere mehrere Tage erholen. In Anbetracht der minimalen Invasivität des Läsionsverfahrens gehen wir davon aus, dass die Tiere sich rasch erholen und innerhalb von 1-4 Tagen nach der Läsionierung zur Wiederaufnahme von Verhaltenstests in der Lage sein werden.

G. Entfernen einer Ableitkammer:

Nach dem Einschnitt in die Haut und der Freilegung des Knochens werden um die Kammer herum aller Dentalzement und alle Schrauben, die die Kammer mit dem Knochen verbinden, entfernt. Dann wird auch die Kammer selbst entfernt. Danach wird meistens die Haut wieder zusammengenäht. Bevor die Haut zusammengenäht wird, wird eine dünne Hautschicht entfernt, um durch den frischen Schnitt die Heilung der Haut, die vernäht werden muss, zu beschleunigen. Wenn wir während dieses Verfahrens feststellen, dass der darunterliegende Knochen nicht sehr gesund ist (manchmal dringen Infektionen bis zum Knochen vor), werden wir auch künstliche Knochenplatten hinzufügen, um die Stelle der Kraniotomie zu versiegeln. Dies ist genau das gleiche Verfahren, das auch manchmal bei Operationen am Menschen angewandt wird. Die Platte wird mit speziellen Schrauben am Knochen befestigt. In manchen Fällen wird die Platzierung der Kammer geändert. Typischerweise wird die bereits vorhandene Kammer entfernt wie oben beschrieben, und während derselben Operation wird eine neue Kammer implantiert, wie im obigen Abschnitt D bereits beschrieben. In diesem Versuchsvorhaben ist die Implantation einer einzigen Kammer (für das Areal MT) geplant.

H. Allgemeine Methoden des Verhaltenstrainings:

Unsere Experimente verlangen, dass die Tiere trainiert werden, sensomotorische Aufgaben auszuführen, bei denen sie über Augenbewegungen oder mittels der Betätigung von Hebeln über ihre visuelle Wahrnehmung einen Bericht abgeben. Die Dauer der Trainingsphase hängt von der Aufgabe sowie von den Eigenschaften des Tieres ab. Es dauert normalerweise ungefähr 4 bis 6 Monate, wobei die Tiere typischerweise an 5-6 Tagen pro Woche trainiert werden. Die Tiere werden zuerst konditioniert, in einen Primatenstuhl ein- und auszusteigen und eine entspannte, normale Haltung beizubehalten, solange sie sich im Stuhl befinden. Die langsame Gewöhnung der Tiere an das Sitzen in ihren Stühlen wird durch die begleitende großzügige Gabe von Saft und Obst verstärkt. Die Zeit, die das Tier in seinem Stuhl verbringt, wird allmählich verlängert. Die Tiere lernen schnell, den Stuhl mit Belohnung und der Situation der Ausführung einer visuell gesteuerten Aufgabe in Verbindung zu bringen, und gewöhnlich setzen sie sich nach den ersten paar Tagen der Gewöhnung von selbst in den Stuhl. Während dieses Verfahrens des Stuhltrainings wird allmählich eine Flüssigkeitsbeschränkung eingeführt.

Jedes Tier trägt einen losen, Halskragen mit festem Durchmesser aus Metall (eloxiertes Aluminium) oder Kunststoff, der unter einer leichten Kurznarkose angelegt wird. Für Trainingssitzungen wird das Tier in einen Primatenstuhl geführt, indem man eine Metallstange verwendet, die man an seinem Kragen einhakt. Nach wenigen Trainingstagen lernen die Affen, selbstständig in den Stuhl zu klettern, und sie bleiben dabei so ruhig, dass sie nach kurzem den Hals mit dem Ring zum Einhaken anbieten, damit das Manöver schnell erledigt wird. Natürlich werden diese Schritte zumindest anfangs durch Belohnung verstärkt. Wenn das Tier im Stuhl Platz genommen hat, werden über der offenen Seite des Stuhls Kunststofftüren angebracht, und das Tier ist somit vollständig im Primatenstuhl eingeschlossen. Dieser wird dann in den Trainingsraum oder das Labor geschoben. Jedes Tier verfügt über seinen eigenen Primatenstuhl, der in fast allen Richtungen verstellbar ist, so dass eine bequeme Sitzhaltung individuell und dauerhaft ermöglicht werden kann. In seinem Wohnkäfig kann sich jedes Tier

uneingeschränkt bewegen. Für die Konditionierungen benutzen wir ausschließlich positive Verstärkungsmethoden (reinforcement), die standardmäßig angewandt werden.

Für die hier vorgesehene Verhaltensexperimente, bei denen Bewegungsreize erkannt werden sollen, werden die darauf trainierte ihre Antworten per Augenbewegungen (Sakkaden) anzuzeigen. Während des Trainings befindet sich das Tier in einem geschlossenen, abgeschirmten Raum und wird fortwährend mit einer Videokamera überwacht. Wenn das Tier zur Beantwortung seiner Aufgabe einen Hebel betätigen soll, trainieren wir diese Fähigkeiten normalerweise und andere grundlegende Aspekte der Verhaltensaufgabe, bevor eine sklerale Augenspule und ein Kopfhalter implantiert wird. Diese Trainingsperiode dauert typischerweise 2-4 Monate. Wenn das Tier als Antwort eine Augenbewegung machen soll, müssen natürlich Kopfhalter und sklerale Augenspule vor jeglichem Aufgabentraining und unmittelbar nach dem Stuhlgewöhnungsverfahren implantiert werden.

Training und Flüssigkeitseinschränkung werden 7-10 Tage nach der Implantation wieder aufgenommen, vorausgesetzt, das Tier hat sich gut erholt. Der Kopfhalter wird an einem entsprechenden Stativteil entweder am Primatenstuhl oder am Rahmen des Setups befestigt. Dieses kann jedoch frühestens 3 Wochen nach der Operation belastet werden, damit das Einheilen der Implantate nicht gestört wird. Die sehr stabile Kopfhalterung wird benötigt, um die Augenposition zu überwachen und die Mikroelektrodenableitungen durchzuführen. Die Dauer der Kopffixation ist anfänglich sehr kurz (wenige Minuten) und wird im Lauf des Trainingsprozesses allmählich erhöht. Die Tiere passen sich der Kopfbefestigung ohne Weiteres an, u.a. weil der Kopfhalter direkt mit dem Schädel verbunden wird und so keinen Druck auf Weichteile wie die Haut erzeugen kann. Sobald das Tier an die Fixation des Kopfes gewöhnt ist und die Augenposition zuverlässig überwacht werden kann, wird es trainiert, seine Aufgabe auszuführen, während es typischerweise seinen Blick auf einen kleinen Punkt in der Mitte des Videobildschirms (Fixationspunkt) gerichtet hält. Durch diese Kontrolle der Fixierung wissen wir, wo die visuellen Reize auf die Retinae des Tieres fallen, und wir schaffen auf diese Weise kontrollierte, wiederholbare Reize. Das Training einer Fixationsaufgabe dauert gewöhnlich 1-2 Monate. Ist das Tier einmal in dieser grundlegenden Aufgabe erfahren, beginnen wir, neurophysiologische Daten zu sammeln.

Trockenfrüchte werden gewöhnlich am Ende einer Sitzung bereitgestellt. Während des Trainings und der Aufzeichnungen werden Flüssigkeitsaufnahme, Körpergewicht und Arbeitsleistung täglich aufgezeichnet. Die Hydrierung wird inspektorisch und über die Hautspannung (Hautturgor) beurteilt. Neben der ohnehin erfolgenden täglichen tierärztlichen Inspektion (Zustand des Fells (Stumpfheit), Fäkalien und Urinproduktion) wird auch das Spontanverhalten beobachtet und bei Abweichungen sofort eine Konsultation der Tierärzte vorgenommen. Tiere mit eingeschränktem Zugang zu Wasser werden nicht übergangslos wieder unbegrenzten Zugang zu Wasser erhalten, um zu vermeiden, dass sie sich in zu kurzer Zeit zu viel Wasser zu sich nehmen (gorging). Wenn ein Tier wieder freien Zugang zu Wasser erhalten soll, wird es normalerweise am Ende einer Arbeitseinheit in seinem Wohnkäfig Wasser in definierter Menge bereitgestellt bekommen. Wenn das nicht möglich ist, wird Wasser in Abständen von 1-2 Stunden in 100 ml Portionen gegeben bis der Durst des Tiers gestillt ist, dann wird wieder freier Zugang zu Wasser gewährt. Falls Zeichen von übermäßigem Entzug auftreten sollten, werden wir den Wasserbeschränkungsplan sofort entsprechend anpassen. Tiere arbeiten grundsätzlich bis sie ihren Durst gestillt haben, gewöhnlich konsumieren sie zwischen 200 und 400 ml über einen Zeitraum von 2-4 Stunden. Jedem Tier, das sich nicht

(mindestens) 20 ml/kg Wasser innerhalb einer Arbeitseinheit erarbeitet hat, wird zusätzlich Wasser gegeben bis zu dieser Mindestmenge oder bis sein Durst gestillt ist. Dieses Wasser wird typischerweise am Ende des Tages gegeben, sodass das Tier nicht unmittelbar dafür belohnt wird, dass es die Arbeitssitzung vorzeitig beendet hat.

J. MRT/fMRT-Messungen ("Scannersitzungen"):

Dieses Verfahren wird unter Vollnarkose durchgeführt wie oben beschrieben. Ziel der Experimente ist: 1) Anatomische MRT-Aufnahmen ermöglichen hochaufgelöste Darstellungen des Gehirns, die eine dreidimensionale Rekonstruktion von Schädel und Gehirn ermöglichen. Außerdem soll in diesen Sitzungen die Position der Elektroden verifiziert werden. 2) in funktionellen MRT Aufnahmen werden bewegte visuelle Reize gezeigt, die das Areal MT besonders gut aktivieren. Diese Aufnahmen werden dazu dienen das Areal MT funktionell zu lokalisieren, um so eine genauere Implantation der Kammer für die elektrophysiologischen Ableitungen zu ermöglichen. Für diese Messungen werden wir eine intravenöse Injektion von MION (Monocrystalline Iron Oxide Nanocolloid) in die Vena saphena (d.h. eine Injektion von ~1-2 ml MION, 10 mg/kg pro Tag Maximum) vornehmen, um eine höhere Signalstärke gegenüber dem traditionellen körperinhärenten BOLD sicherzustellen. Die Kanüle wird anschließend mit einem zusätzlichen Bolus von 1 ml einer sterilen Ringerlösung gespült. Unmittelbar danach wird die Kanüle aus der Vene entfernt. Die Radiofrequenzpulse innerhalb des Scanners produzieren ein tiefes und sich wiederholendes „Klopf“- Geräusch, und menschliche MR-Versuchspersonen tragen normalerweise einen Gehörschutz, um den Geräuschpegel dieser RF-Pulse zu reduzieren. Wir verwenden üblicherweise einen zweifachen Schutz mit zwei „Schichten“ von Schaumstöpseln, die mindestens eine Reduktion des RF-Pulsgeräusches um 20-40 dB bewirken.

K. Reversible Inaktivierung unter Verwendung pharmakologisch wirksamer und optogenetischer Substanzen:

In einigen Experimenten werden wir den Thalamus lokal inaktivieren, indem wir kleine Injektionen von Muscimol oder THIP (GABA-Rezeptor-Agonisten) oder Lidocain (Lokalanästhetikum) verabreichen oder die lokale kortikale Aktivität erhöhen, indem wir den GABA-Antagonisten Bicucullin injizieren. Die Injektionen erfolgen MR-geführt durch eine zuvor unter Vollnarkose (wie oben beschrieben) permanent implantierte Kanüle [3]. Diese Agenzien wirken lokal und reversibel und können ohne Beeinträchtigung des Wohlbefindens der Tiere und ganz sicher schmerzfrei in das Gehirn von wachen Tieren injiziert werden. Auch sind dadurch keine bleibenden Verhaltensdefizite zu erwarten. Möglicherweise wird es vorübergehende Veränderungen der Verhaltensleistung geben (z.B. räumlich beschränkte visuelle Defizite, sogenannte temporäre Skotome, siehe [3, 16]).

Die Kappe, die die Kanüle überdeckt, wird zuerst entfernt. Dann werden wir unter Anwendung steriler Techniken die GABA-Antagonisten (Bicucullin) oder GABA-Agonisten (Muscimol, THIP) mittels einer sterilen Hamilton-Spritze durch die Kanüle injizieren. Dann erfolgen kleine Injektionen (1-3 µl Volumen bei einer Rate von 0.5-1 µl/Minute). Unsere bisherigen Messungen im Thalamus haben ergeben, dass eine Injektion Gewebe bis in etwa 2 mm Entfernung temporär wirkt (Schmid et al., 2010). In verschiedenen Sitzungen werden wir entweder Muscimol/THIP oder Bicucullin injizieren. In Kontrollsitungen

werden wir alternativ gleiche Mengen steriler Kochsalzlösung injizieren. Die Effekte von Muscimol halten bis ungefähr 3-4 Stunden nach der Injektion an. Die Effekte von Bicucullin, THIP und Lidocain halten weniger als 2 Stunden an. Nach der Injektion wird der Zugang zur Kanüle geschlossen und die elektrophysiologischen und Verhaltensmessungen beginnen.

Die Optogenetik ist eine neue Methode zur lichtabhängigen Manipulation von Gehirnaktivität. Es wird dafür lokal ein Gen injiziert, das ein licht-sensitives Protein enkodiert. Schwache Lichtpulse können dann lokal die Neuronen mit einer Präzision im Millisekundenbereich an- und ausschalten. Sofern wir diese Methode erfolgreich implementieren können, wird sie ein wesentlicher Fortschritt sein gegenüber den herkömmlichen Methoden, die wir zur Gehirnmanipulation benutzen. Ein grosser Vorteil liegt in der zeitlichen Präzision der Optogenetik, die letztendlich auch zu einer geringeren Belastung der Tiere beiträgt. Um die Effekte pharmakologischer Manipulation zu ermitteln, muss die Wirksamkeit der Substanzen beachtet werden, weshalb Tests mehrere Stunden dauern, und wiederholte Messungen einige Tage auseinander liegen. Die Optogenetik bietet dagegen die Möglichkeit, die Effektivität der Manipulation innerhalb einer Testsession durch einfache Stimulation mit Licht mit hoher zeitlicher Präzision, spezifisch für bestimmte Zelltypen, gleich mehrfach zu untersuchen. Für unser Versuchsvorhaben kann also der Beitrag bestimmter thalamischer Neuronen für das Blindsehen einfach innerhalb einer Sitzung von Testdurchgang zu Testdurchgang bestimmt werden, indem in einer Reihe von Durchgängen mit Licht stimuliert wird, in den anderen nicht. Außerdem lässt sich auch die Zeit genau bestimmen, mit der stimuliert wird. Man kann die Lichtstimulation also vor, während oder nach der Präsentation eines visuellen Reizes durchführen, wodurch wichtige Informationen hinsichtlich der temporalen Struktur der Gehirnverarbeitung erhalten werden. Für unsere simultanen elektrophysiologischen Ableitungen ist darüber hinaus entscheidend, dass die elektrische Interferenz, die der Methode der elektrischen Mikrostimulation inhärent ist, in der Optogenetik komplett vermieden wird.

Die Gene, die die lichtsensitiven Proteine enkodieren, werden, wie oben auch für die Pharmakologie beschrieben, als Virus über eine Hamilton-Spritze und eine permanent implantierte Kanüle im wachen, kopf-fixierten oder Ketamin-anästhesierten Tier in den Thalamus injiziert. Die Viren Adeno-assoziiertes Virus (AAV) und Lentivirus werden von unserem Kollegen Dr. Ed Boyden vom MIT in Boston bzw. von der University of Pennsylvania Gene Therapy Vector Core geliefert. Diese Vektoren der jüngsten Generation haben eine ausgezeichnete Sicherheitshistorie (Gene Therapy Program, 2007a, 2007b). Replikationsinkompetente AAV und Lentiviren wurden in mehreren Generationen gezüchtet, um nicht mehr infektiös zu sein. Mindestens 2/3 ihres viralen Genoms wurden entfernt, damit sie nicht selbstreplizierend sind und diese Eigenschaft durch Mutationen auch nicht wieder zurückerhalten. Der Vektor, den wir benutzen, wurde bereits erfolgreich am Gehirn von Nagern und im Affen am MIT, an den National Institutes of Health und anderen Laboren getestet [25-28]. Die Viruspartikel werden in einer physiologischen Salinelösung langsam (ungefähr 0.2 µl pro Minute) injiziert bis ein Volumen von maximal 10-12 µl erreicht ist. Wir rechnen nicht damit, dass die Viren oder die lichtsensitiven Gene, die sie enkodieren, zu irgendwelchen Veränderungen der Gesundheit der Affen führen. Der Virus wird nur lokal injiziert, die neu exprimierten Proteine können nur durch helles Licht einer bestimmten Wellenlänge aktiviert werden. Wir werden trotzdem sehr genau das Verhalten der Affen hinsichtlich unerwarteter Nebeneffekte wie Schmerz oder Stress beobachten. Sollten Nebeneffekte auftreten, die

nicht mit den Standardprozeduren der Tierärzte behoben werden können, werden die Tiere euthanasiert und histologisch examiniert.

Die ersten beiden Affen werden ca. 3-4 Wochen nach der Injektion euthanasiert und histologisch analysiert, um sicherzustellen, dass die Transfektionsmethode erfolgreich ist. Die anderen 4 Affen werden ca. 3-4 Wochen nach erfolgreicher Injektion, elektrophysiologisch und im Verhalten getestet. Dafür werden optische Glasfasern und Mikroelektroden durch die permanent implantierte Kanüle (die vorab für die Injektion genutzt wurde) zum Thalamus geführt. Die Methodik hierfür ist vergleichbar zu den oben beschriebenen elektrophysiologischen Ableitungen. Die Glasfasern bestehen aus sicherem, biokompatiblen Glas und haben einen Durchmesser von ca. 100 – 200 µm. Sie übertragen Licht, das mit einer Laserquelle Computer kontrolliert erzeugt wurde. Die Lichtintensitäten werden von uns gemessen werden und werden weniger als 10 mW/mm² betragen.

7.5.2 Welche Eingriffe oder Behandlungen sollen unter Betäubung durchgeführt werden und welche Betäubungsverfahren sind dabei vorgesehen?

Alle operativen Eingriffe, Kraniotomien und Durapräparation, kortikale Läsionierungen, anatomische Messungen im MR-Tomographen, gelegentlich elektrophysiologische Ableitungs- und Mikrostimulationsexperimente und Experimente mit pharmakologischer Manipulation des neuronalen Substrats sowie Terminalversuche.

7.5.3 Sind schmerzhaft Eingriffe ohne Betäubung vorgesehen?

nein

Wenn ja, Begründung:

7.5.4 Sollen an einem nicht betäubten Tier mehrere erheblich schmerzhaft Eingriffe oder Behandlungen durchgeführt werden?

nein

Wenn ja, Begründung:

7.5.5 Welchen Belastungen (Intensität und Dauer von Schmerzen oder Leiden) werden die Tiere voraussichtlich ausgesetzt oder welche Schäden werden ihnen voraussichtlich zugefügt?

Im Anschluss an einen chirurgischen Eingriff besteht die Möglichkeit des postoperativen Wundschmerzes. Dieser wird (möglichst) durch (präventive) Gabe von Analgetika reduziert oder verhindert. Im Verlaufe der Experimentierzeit kann es gelegentlich zu leichten, lokalen Infektionen im Bereich des Überganges von Haut und Implantat kommen. Es ist eine lokale Säuberung in diesem Bereich und die lokale und selten auch eine systematische Gabe von Antibiotika erforderlich, die nach klinischen und bakteriologischen Kriterien ausgewählt werden. Falls die Reinigung für die Tiere unangenehm ist, werden Lokalanästhetika (z.B. Lidocain 2%) vor und während der Reinigungsprozedur verwendet. Im Rahmen der Trainingsarbeit wird es in einigen Abschnitten des Trainings notwendig sein, dass die Tiere ihren Wasserbedarf ausschließlich im Trainingssetup decken dürfen, was dazu führt, dass sie nach wenigen Tagen zunehmend und oft ihren gesamten Flüssigkeitsbedarf erarbeiten. Es ist selbstverständlich, dass der Experimentator sicherstellen muss, dass das Tier nicht wegen z.B. eines zu hohen Schwierigkeitsgrads der Aufgabe sich keine oder keine ausreichende Flüssigkeitsmenge erarbeiten kann, wenn es will. Uns ist klar, dass es sich hierbei um eine erzieherische Zwangsmaßnahme handelt, die wir nur dann einsetzen und mit höchster Aufmerksamkeit überwachen, wenn es keine andere Möglichkeit gibt, das Tier in einem angemessenen Zeitraum zum Arbeiten zu motivieren. In einzelnen Fällen gelingt es, durch vorübergehende Umstellung des Verstärkers z.B. von Saft auf Wasser aber auch umgekehrt, eine bessere Motivation zu erzielen. Wir schätzen die Belastung durch die kontrollierte Flüssigkeitszufuhr als deutlich geringer ein als die Belastungen durch sozialen Stress oder Einzelhaltung, die in vielen anderen Primatenhaltungen noch immer praktiziert wird. Unsere umfangreiche Studie zum Flüssigkeitshaushalt unserer derzeitigen Versuchstiere hat u.a. auch ergeben, dass eingeschränkte Nierenfunktion ganz selten vorkommt und fast immer auf andere Noxen zurückzuführen ist als auf die trainingsbedingte Wasserrestriktion. Schließlich werden für manche Versuche zwecks Messung der Augenbewegungen subkonjunktival implantierte Messspulen benötigt, was gelegentlich mit Fremdkörpergefühl und seltener mit einer Konjunktivitis einhergehen kann, die umgehend behandelt wird.

7.5.6 Vorgesehene Maßnahmen zur Schmerzlinderung (z.B. nach Abklingen der Betäubung):

Der postoperative Schmerz hängt stark von der Lokalisierung des Eingriffs ab. Unsere chirurgischen Verfahren führen zu somatischen Schmerzen. Diese bestehen aus Oberflächenschmerzen durch Verletzung der Haut und Tiefenschmerzen durch Verletzung von Fascien und Muskulatur. Viszerale Schmerzen treten nicht auf. Aus diesem Grund bevorzugen wir den Einsatz von peripheren Analgetika gegebenenfalls kombiniert mit Opioiden in niedriger Dosierung. Die Opiode werden vor allem während der Eingriffe eingesetzt (Buprenorphin 0.01 - 0.03mg/kg, intra-muskulär) um starke Schmerzen zu vermeiden. Periphere Analgetika werden in allen Fällen 3 Tage postoperativ zugeführt (Paracetamol 10mg/kg, oder Flunixin-Meglumin 5 mg/kg). Diese kurzzeitige Zufuhr vermindert die Nebenwirkungen und ist ausreichend, um Wundschmerzen (2 Tage postoperativ) zu behandeln.

7.5.7 Vorgesehene Maßnahmen und Kontrollen im Rahmen der medizinischen Versorgung (inkl. Angaben zu speziellen Haltungsbedingungen aufgrund hygienischer Anforderungen oder Erkrankungsneigungen der vorgesehenen Tiere):

Die Gesundheit der Tiere wird während des gesamten Versuchs sehr sorgfältig durch Beobachtung des Verhaltens der Tiere, des Appetits, der Fezes, der Haut und der Haare sowie des Gesamteindrucks überwacht. Die Beobachtung des Verhaltens spielte schon bisher eine große Rolle in unserer Tierhaltung, wird aber in Zukunft noch einen wesentlich größeren Stellenwert erfahren, um das Wohlergehen der Versuchstiere möglichst umfassend zu überwachen und zu dokumentieren. Wir verweisen diesbezüglich auf folgende Parameter: artspezifisches zirkadianes Aktivitätsmuster, Komfort-, Explorations-, Spiel- und Sexualverhalten, Stereotypien und Apathie (Baum et al., 1998), wobei die Sensitivität dieser Parameter für die Beeinträchtigung des Verhaltens bei Makakenaffen unseres Wissens bisher nicht quantitativ untersucht wurde.

An unserem Institut steht eine Tierärztin zur Verfügung, die die Tiere täglich inspiziert und regelmässig untersucht. Insbesondere werden die Tiere regelmässig gewogen, um eventuelle Stoffwechselstörungen zu entdecken. Falls irgendein Anzeichen von Krankheit oder Unwohlsein auftreten sollte, wird das Tier gegebenenfalls isoliert und entsprechend behandelt. Im Falle, dass ein Tier chronische Schmerzen oder eine schwere Krankheit erleidet, die unter zumutbarer Belastung nicht kurativ behandelt werden kann, wird das Tier vor Beendigung des Versuchs eingeschläfert. Alle Beobachtungen und Behandlungsmaßnahmen werden im Datenbanksystem unserer Tierärztin aufgezeichnet und stehen allen Entscheidungsträgern jederzeit zur Verfügung.

7.5.8 Abbruchkriterien, falls erforderlich.

Die folgenden Abbruchkriterien stellen einen Leitfaden dar, wann

- 1) Tiere aus dem Versuch zu nehmen und sofort medizinischer Diagnostik und Behandlung zuzuführen sind(*),
 - 2) intensive medizinische Behandlung erfolgen muss (^),
 - 3) konkret darüber entschieden werden muss, Tiere zu euthanasieren (†).
- Bei der folgenden Aufstellung dürfen nicht nur einzelne Kriterien betrachtet werden, obwohl einzelne Kriterien ausschlaggebend sein können.

I Körpergewicht (es ist der Zeitraum der Änderung zu berücksichtigen, insbesondere im Zusammenhang mit dem Trainingsverlauf zu beurteilen)

- Änderung < 5%
- Gewichtsverlust 5-10% ²
- Gewichtsverlust 11-20% (*)
- Gewichtsverlust > 20% (†)

² hier schon Herausnahme aus dem Versuch erwägen, wenn andere Kriterien auch dafür sprechen, vor allem wenn Gewichtsverlust von <10% innerhalb weniger (2-3) Tage erfolgt.

II Allgemeinzustand

- Gut, normale Mobilität, klarer Blick,
- Fell struppig, nicht glänzend (Alopezie kann auch durch zu heftige Fellpflege bedingt sein), vorübergehendes Unwohlsein, das sich z.B. in der Sitzhaltung ausdrückt (in Kombination mit anderen Kriterien ggf schon Zeitpunkt für Abbruch des Trainings)
- reduzierte Mobilität, unnatürliche Haltungen, (*,^)
- kalte Glieder (nicht Präshock), schwere Fehlhaltung, Atemprobleme, schwere klinische Ausfälle . (†)

III Spontanverhalten

- Normales Verhalten: Schlafen, Reaktion auf Ansprache oder ggf auf Anbieten von Futter oder Flüssigkeit, Neugier, Sozialkontakte, Drohen oder Unterwerfung,
- reduziertes Neugierverhalten, erhöhte Aggressivität gegen Artgenossen , fehlendes Drohverhalten, eingeschränkte Motorik oder Hyperkinetik: Diagnostik und bei Befund weitere Maßnahmen,
- Selbstisolation; Lethargie oder ausgeprägte Hyperkinetik oder Stereotypien; Koordinationsstörungen; Schmerzlaute beim Ergreifen, Verminderte Wasser und Futteraufnahme, (*,^)
- schwer autoaggressives Verhalten, andauernde Anorexie oder Apathie (†)

IV Erlerntes Verhalten (entsprechend dem bereits erfolgten Training)

- Erlerntes in den Käfig kommen und in den Stuhl steigen, regelmäßige Arbeit,
- Verweigerung zur Mitarbeit oder reduzierte Arbeitsleistung: auch hier schon genauere Prüfung einleiten,
- lässt sich nicht absperren oder einhaken,
- Zeichen von Panik, die ein geordnetes Arbeiten andauernd unmöglich macht (*).

V Klinischer Befund

- Temperatur, Atmung, HF und Puls normal, Extremitäten warm, Schleimhäute gut durchblutet , Hautturgor normal, kein Exsudate,
- geringe Abweichungen von der Normalsituation, leichte Abweichungen im Blutbild /klinische Chemie, Temperaturabweichung 1,5 °C (Referenz), Atmung und Puls im physiologischen Bereich entsprechend Körpergröße und Geschlecht, Hautturgor leicht vermindert, keine auffällige Veränderungen im Muskeltonus, keine nässenden Wunden,
- pathologische Abweichungen im Blutbild/kl.Ch., pathologisch veränderte Reflexe, sichtbare Schädigungen des ZNS im MRT, Hautturgor stark vermindert, pathologische Atem- und Herzgeräusche (*,^).
- Therapieresistente, chronische Entzündungen ohne andere Symptome bedürfen einer äußerst sorgfältigen engmaschigen Überwachung. Die dabei erforderlichen Diskussionen werden immer irgendwann an einen Punkt kommen, an dem trotz fehlender klinischer Symptomatik eine Entscheidung für die Beendigung des Versuchs fallen muss. Den Zeitpunkt für diese schwierige Entscheidung kann man im Voraus nicht sinnvoll festlegen. Hierbei wird die Einschätzung der Tierärzte und der Tierschutzbeauftragten ausschlaggebend sein. Die Grundlagen für diese Entscheidungen werden durchgehend dokumentiert, damit diese jederzeit nachvollziehbar sein werden. (*,^/†)
- Therapieresistente, chronische Entzündungen mit klinischer Symptomatik (Gewichtsverlust > 10%, oder reduzierte Mobilität und unnatürliche Haltungen oder zentralnervöse Symptome oder epileptische Anfälle oder stark verminderte Wasser und Futteraufnahme), therapieresistente Atem- oder Kreislaufprobleme, schwere irreversible Nierenfunktionsstörungen, fehlende zentrale Reflexe, Exsikkose (†).

7.6 Ethische Vertretbarkeit des Versuchs (§ 7 Abs. 3)

7.6.1 Wissenschaftlich begründete Darlegung, dass die zu erwartenden Schmerzen, Leiden oder Schäden der Versuchstiere im Hinblick auf den Versuchszweck ethisch vertretbar sind (§ 7 Abs. 3 Satz 1):

Wir werden uns bemühen, den Schmerz und die Bedrängnis für die Tiere so gering wie möglich zu halten, und wir gehen nicht davon aus, dass die Tiere während unserer Experimente eine längere Zeit lang Schmerz oder Bedrängnis erfahren werden. Während aller operativen Eingriffe wird das Tier unter genereller Anästhesie gehalten, entweder mit Isofluran/Remifentanyl oder (bei kurzen Eingriffen) Ketamin und Xylazin (im Einzelnen beschrieben unter Methoden). Antibiotika und Analgetika werden sowohl peri operativ verabreicht (Methoden) als auch weitere 7 oder mehr Tage lang (postoperativ) verabreicht, sollte das Tier Zeichen einer Infektion oder des Unbehagens zeigen. Alle Tiere werden die Prozeduren, die unter Methoden aufgeführt sind, durchlaufen. Da das Gehirn keine Schmerzrezeptoren besitzt, werden die Tiere keinen Schmerz als direkte Folge der Experimente mit Läsionen, die wir hier beantragen, erfahren.

Die Defizite, die die Tiere als Folge der von uns beantragten V1-Läsionierung erfahren werden, werden räumlich begrenzte Defizite des visuellen Erkennens sein (Skotome). Da wir in unseren Experimenten ausdrücklich die Repräsentation der Fovea aussparen, werden sämtliche Tiere die Fähigkeit behalten, ihre Umwelt mit hoher Auflösung zu sehen. Wir haben bereits Erfahrung mit dem Verhalten ähnlich läsionierter Tiere (Schmid et al., 2009, 2010) und können daher versichern, dass nicht davon auszugehen ist, dass die Tiere unter motorischen oder somatosensorischen Defiziten leiden müssen, und dass das Defizit des visuellen Feldes, das wir verursachen werden, die Tiere nicht daran hindern wird, sozial zu interagieren und sich normal zu ernähren, da sie das Defizit problemlos durch Augenbewegungen ausgleichen können.

Während des Verhaltenstrainings und der elektrophysiologischen Experimente werden die Affen zu jedem Zeitpunkt während der Einschränkung überwacht. Um ihr Unbehagen oder mögliche Beschwerden zu minimieren, wird die Bewegungseinschränkung allmählich eingeführt während Wasser und Futterbelohnungen eingesetzt werden, um sie erträglicher zu machen. Die Grade des Wasserentzugs während der Verhaltensexperimente werden genauestens überwacht, und die Affen werden täglich (an allen Trainingstagen) gewogen. Freier Zugang zu Wasser wird an mindestens einem Tag pro Woche gewährt.

Der erwartete potentielle Nutzen dieser Experimente ist beträchtlich sowohl im Hinblick darauf, dass sie uns dabei helfen, den Mechanismus der visuellen Wahrnehmung sowie der Plastizität in vivo im Primatenhirn zu verstehen. Die Befunde werden wichtige Hinweise für die Rehabilitation menschliche Patienten mit kortikalen Verletzungen erbringen.

7.6.2 Bei länger anhaltenden oder sich wiederholenden erheblichen Schmerzen oder Leiden, wissenschaftlich begründete Darlegung, dass das angestrebte Versuchsergebnis für wesentliche Bedürfnisse von Mensch und Tier einschließlich

der Lösung wissenschaftlicher Probleme von hervorragender Bedeutung ist (§ 7 Abs. 3 Satz 2):

entfällt

8. Verfahren am Versuchsende

8.1 Beabsichtigter Verbleib der Tiere:

Betäubung während des gesamten Versuchs mit Tötung vor Erwachen aus der Narkose

Tötung nach Beobachtungszeit von

Weiterleben der Tiere ohne Beeinträchtigung des Wohlbefindens

8.2. Falls die Tiere getötet werden sollen, welches Tötungsverfahren ist vorgesehen?

Überdosis Barbiturat, Perfusion und anschließende histologische Aufarbeitung des Gehirns ist für dieses Projekt von fundamentaler Bedeutung, um die Vollständigkeit der V1-Läsionen zu überprüfen und ihre Ausdehnung zu bestimmen. Außerdem ist eine histologische Analyse der Optogenetik geplant.

9. Wird die Anonymisierung des Antrages gewünscht?

ja nein

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UNIVERSITA' DEGLI STUDI DI FERRARA
DIPARTIMENTO DI SCIENZE BIOMEDICHE
E TERAPIE AVANZATE
SEZIONE DI FISIOLOGIA UMANA
Via Fossato di Mortara, 17/19
Tel. 0532.291241 - 291238
Telefax 0532.291242
44100 FERRARA

AL MINISTERO DELLA SALUTE
DIPARTIMENTO DELLA SANITÀ PUBBLICA VETERINARIA,
LA NUTRIZIONE E LA SICUREZZA DEGLI ALIMENTI
DIREZIONE GENERALE DELLA SANITA' ANIMALE E DEL FARMACO
VETERINARIO
"UFFICIO VI-BENESSERE ANIMALE"
PIAZZALE G. MARCONI, 25
00144 ROMA

Oggetto: **Richiesta di autorizzazione ad effettuare esperimenti su animali senza anestesia, ai sensi dell'art. 9, comma 1 del D. Lg. n. 116/1992.**
Richiesta di autorizzazione ad effettuare esperimenti su ratti, ai sensi della normativa regionale n° 20 del 2002.

Il sottoscritto Prof. Luciano Fadiga, Professore Ordinario di Fisiologia umana presso la Facoltà di Medicina e Chirurgia dell'Università di Ferrara,

chiede, ai sensi dell'art. 9, comma 1 del D.L. n. 116/1992 e della normativa regionale n° 20 del 2002, l'autorizzazione ad effettuare nell'ambito della propria struttura, in deroga all'art. 3, commi 2 e 3 del D.L. citato, esperimenti su ratti da svolgersi nell'ambito del progetto scientifico di cui sotto.

Chiede inoltre, ai sensi dell'art. 9, comma 1, del D.Lg. n. 116/1992, l'autorizzazione ad effettuare nell'ambito della propria struttura esperimenti sugli animali senza anestesia, in deroga all'art. 4, comma 3, del D.L. citato, per il seguente motivo: l'anestesia è incompatibile con il fine dell'esperimento.

L'obiettivo del progetto "**Studio di matrici di multielettrodi per la derivazione dell'attività unitaria neuronale dalla corteccia sensorimotoria del ratto**" e' quello di utilizzare un modello animale molto usato e studiato sia dal punto di vista fisiologico che comportamentale quale il ratto, per lo sviluppo di una interfaccia cervello-macchina bidirezionale, in cui il segnale neurale registrato dalla corteccia motoria venga utilizzato per controllare un oggetto esterno (per esempio la posizione di un cursore su uno schermo o il movimento di un braccio robotico). Simultaneamente si vuole esplorare la possibilità di trasferire informazioni circa lo stato dell'oggetto esterno direttamente al cervello utilizzando protocolli di microstimolazione elettrica applicati alla corteccia somato-sensoriale.

Tale studio può essere eseguito solo in vivo, sia perché l'ottimizzazione delle tecniche di registrazione neurofisiologica richiede una complessità di segnale presente solo nel tessuto nervoso in toto, sia perché è necessario dimostrare la presenza di correlazioni tra la registrazione



UNIVERSITA' DEGLI STUDI DI FERRARA
DIPARTIMENTO DI SCIENZE BIOMEDICHE
E TERAPIE AVANZATE
SEZIONE DI FISIOLOGIA UMANA

Via Fossato di Mortara, 17/19

Tel. 0532.291241 - 291238

Telefax 0532.291242

44100 FERRARA

neuronale e il comportamento dell'animale e, in ultimo, perché occorre studiare la reazione tissutale all'impianto del sistema nervoso.

Il tipo di animale scelto, cioè il ratto, rappresenta la specie di minor complessità nervosa in cui eseguire tali esperimenti, inoltre questa scelta è giustificata dall'abbondanza di dati in letteratura che possono fornire una base alla sperimentazione proposta senza richiedere la ripetizione di pratiche precedentemente già testate in altri laboratori.

In questa ricerca si è deciso di utilizzare ratti appartenenti alla specie Long Evans poiché nella fase di addestramento verrà utilizzato un task visuo-motorio ed è stato dimostrato da vari studi comportamentali che questa particolare specie ha migliori caratteristiche, rispetto ad altri tipi di ratti, sia per quanto riguarda l'acutezza visiva sia per le capacità motorie.

Il valore potenziale di questa ricerca consiste nel portare conoscenze nuove nel campo dello sviluppo di sistemi di interfaccia cervello-macchina e si propone di dare un contributo significativo nella comprensione del codice neurale usato dal cervello nei processi motori e sensoriali. Questa ricerca potrebbe portare anche significativi vantaggi alle attuali tecniche di riabilitazione che utilizzano protesi robotiche in pazienti che hanno perso la funzionalità di arti superiori o inferiori esplorando il ruolo del feedback sensoriale nel controllo motorio.

Il sottoscritto allega alla presente domanda:

- 1) Protocollo sperimentale
- 2) Relazione tecnico-scientifica relativa alla procedura chirurgica e sperimentale, alle condizioni di stabulazione degli animali ed elenco delle pubblicazioni più rilevanti il progetto
- 3) Curriculum del sottoscritto con elenco delle sue principali pubblicazioni

Con ossequi,

Il responsabile del progetto
(Prof. Luciano Fadiga)

Ferrara, 2 novembre 2007

PROGETTO DI RICERCA SPERIMENTALE ANIMALE IN REGIME DI "AUTORIZZAZIONE IN DEROGA"

(AI SENSI DEGLI ARTICOLI 8 E 9 DEL D.LGS 27 GENNAIO 1992, N. 116)

PROTOCOLLO SPERIMENTALE

- AL MINISTERO DELLA SALUTE

Dipartimento della Sanità Pubblica Veterinaria, la Nutrizione e
la Sicurezza degli Alimenti
Direzione Generale della Sanità Animale e del Farmaco
Veterinario
"Ufficio VI - Benessere animale"
Piazzale G. Marconi, 25
00144 Roma

e, p.c.

- ALLA REGIONE EMILIA ROMAGNA
- ALLA PREFETTURA DI FERRARA
- AL COMUNE DI FERRARA
- AZIENDA UNITA' SANITARIA LOCALE DI
FERRARA - Servizio Veterinario
- AL MEDICO VETERINARIO SORVEGLIANTE

LORO SEDI

A. PERSONALE RESPONSABILE

1. RESPONSABILE DEL PROGETTO DI RICERCA

Cognome:	FADIGA
Nome:	LUCIANO
Titolo di studio:	LAUREA IN MEDICINA E CHIRURGIA
Codice Fiscale:	FDGLCN61M08A944R
Qualifica:	PROFESSORE DI RUOLO I FASCIA
Residenza:	VIA FOSSATO DI MORTARA 17/19, FERRARA
Dipartimento:	DIPARTIMENTO S.B.T.A, SEZIONE FISILOGIA UMANA

2. RESPONSABILE DELLA ESECUZIONE DELL'ESPERIMENTO

Cognome:	FADIGA
Nome:	LUCIANO
Titolo di studio:	LAUREA IN MEDICINA E CHIRURGIA
Codice Fiscale:	FDGLCN61M08A944R
Qualifica:	PROFESSORE DI RUOLO I FASCIA
Residenza:	VIA FOSSATO DI MORTARA 17/19, FERRARA
Dipartimento:	DIPARTIMENTO S.B.T.A, SEZIONE FISILOGIA UMANA

3. RESPONSABILE DELLO STABULARIO DELL'UNIVERSITA' DI FERRARA

Cognome:	FADIGA
Nome:	LUCIANO
Titolo di studio:	LAUREA IN MEDICINA E CHIRURGIA
Codice Fiscale:	FDGLCN61M08A944R
Qualifica:	PROFESSORE DI RUOLO I FASCIA
Residenza:	VIA FOSSATO DI MORTARA 17/19, FERRARA
Dipartimento:	DIPARTIMENTO S.B.T.A, SEZIONE FISILOGIA UMANA

3a. RESPONSABILE DELLO STABULARIO DELL'ISTITUTO NAZIONALE PER LA RICERCA SUL CANCRO-GENOVA

Cognome:	SANGUINETI
Nome:	MARINA
Titolo di studio:	LAUREA IN SCIENZE BIOLOGICHE
Codice Fiscale:	SNGMRN53B42C621N
Qualifica:	BIOLOGA, RESPONSABILE STABULARIO
Residenza:	LARGO R. BENZI 10, GENOVA
Dipartimento:	ISTITUTO NAZIONALE PER LA RICERCA SUL CANCRO-GENOVA

4.PERSONALE CHE ATTENDE ALLE ESECUZIONI DEGLI ESPERIMENTI E/O AL CONTROLLO DEGLI ANIMALI (compreso il Responsabile degli esperimenti)

ESPERIMENTI PRESSO L'UNIVERSITA' DI FERRARA

Cognome e Nome	Titolo di studio	Qualifica	Dipartimento
FADIGA LUCIANO	LAUREA IN MEDICINA E CHIRURGIA	PROFESSORE DI RUOLO I FASCIA	DIPARTIMENTO S.B.T.A, SEZIONE FISILOGIA UMANA
CRAIGHERO LAILA	LAUREA IN PSICOLOGIA SPERIMENTALE	PROFESSORE DI RUOLO II FASCIA	DIPARTIMENTO S.B.T.A, SEZIONE FISILOGIA UMANA
FRANCHI GIANFRANCO	LAUREA IN MEDICINA E CHIRURGIA	RICERCATORE DI RUOLO	DIPARTIMENTO S.B.T.A, SEZIONE FISILOGIA UMANA
SQUARZONI PAOLO	LAUREA IN MEDICINA VETERINARIA	VETERINARIO	UNIVERSITA' DI FERRARA
VERONESI CARLO	LAUREA IN BIOLOGIA	ASSEGNISTA	DIPARTIMENTO S.B.T.A, SEZIONE FISILOGIA UMANA

ESPERIMENTI PRESSO L'ISTITUTO NAZIONALE PER LA RICERCA SUL CANCRO DI GENOVA

Cognome e Nome	Titolo di studio	Qualifica	Dipartimento
FADIGA LUCIANO	LAUREA IN MEDICINA E CHIRURGIA	PROFESSORE DI RUOLO I FASCIA	DIPARTIMENTO S.B.T.A, SEZIONE FISILOGIA UMANA
MAGGIOLINI EMMA	LAUREA IN BIOLOGIA	CONTRATTISTA	FONDAZIONE IIT GENOVA
VATO ALESSANDRO	LAUREA IN BIOINGEGNERIA	CONTRATTISTA	FONDAZIONE IIT GENOVA
GYTIS BARANAUSKAS	DOTTORE DI RICERCA IN BIOFISICA	CONTRATTISTA	FONDAZIONE IIT GENOVA
CILLI MICHELE	LAUREA IN MEDICINA VETERINARIA	VETERINARIO	IST.NAZ. RICERCA SUL CANCRO-GENOVA

B. STABILIMENTO UTILIZZATORE

UNIVERSITA' DEGLI STUDI DI FERRARA

Via Savonarola, n. 9 - Codice fiscale: UNIV 80007370382

(autorizzazione rilasciata con D.M. dell'8 settembre 2000 prot. n. 42/2000-A)

Medico veterinario sorvegliante (Responsabile dei controlli - D.Lgs 116/92 – Art. 6)

Cognome:	DOTT. SQUARZONI
Nome:	PAOLO
Recapito:	AMBULATORIO VETERINARIO SQUARZONI TAZZARI VIA DELL' UNITA', 12 - MOLINELLA (BO)

ISTITUTO NAZIONALE PER LA RICERCA SUL CANCRO DI GENOVA

Via Largo R. Benzi, n.10 - Codice fiscale: IST 80100850108

(autorizzazione rilasciata con D.M. del 24 marzo 1994 prot. n. 44/94-A)

Medico veterinario sorvegliante (Responsabile dei controlli - D.Lgs 116/92 – Art. 6)

Cognome:	DOTT. CILLI
Nome:	MICHELE
Recapito:	LARGO R. BENZI 10, GENOVA

C. PROGETTO DI RICERCA

Titolo del progetto:	STUDIO DI MATRICI DI MULTIELETTRODI PER LA DERIVAZIONE DELL'ATTIVITA' UNITARIA NEURONALE DALLA CORTECCIA SENSORIMOTORIA DEL RATTO
Obiettivo del progetto:	L'OBIETTIVO DI QUESTO PROGETTO E' QUELLO DI UTILIZZARE UN MODELLO ANIMALE MOLTO USATO E STUDIATO SIA DAL PUNTO DI VISTA FISIOLGICO CHE COMPORTAMENTALE QUALE IL RATTO, PER LO SVILUPPO DI UNA INTERFACCIA CERVELLO-MACCHINA BIDIREZIONALE IN CUI IL SEGNALE NEURALE REGISTRATO DALLA CORTECCIA MOTORIA VENGA UTILIZZATO PER CONTROLLARE UN ATTUATORE ESTERNO (PER ESEMPIO LA POSIZIONE DI UN CURSORE SU UNO SCHERMO O IL MOVIMENTO DI UN BRACCIO ROBOTICO). SIMULTANEAMENTE SI VUOLE ESPORARE LA POSSIBILITÀ DI TRASFERIRE INFORMAZIONI CIRCA LO STATO DELL'OGGETTO ESTERNO DIRETTAMENTE AL CERVELLO UTILIZZANDO PROTOCOLLI DI STIMOLAZIONE ELETTRICA APPLICATI ALLA CORTECCIA SOMATO-SENSORIALE. QUESTA RICERCA POTREBBE PORTARE SIGNIFICATIVI VANTAGGI ALLE ATTUALI TECNICHE DI RIABILITAZIONE CHE UTILIZZANO PROTESI ROBOTICHE IN PAZIENTI CHE HANNO PERSO LA FUNZIONALITÀ MOTORIA, ANCHE ESPORANDO IL RUOLO DEL FEEDBACK SENSORIALE NEL CONTROLLO MOTORIO.
Durata della sperimentazione (max 36 mesi):	36 MESI

D. ANIMALI UTILIZZATI

Specie e tipo:	RATTI (RATTUS NORVEGICUS) LONG-EVANS
Numero previsto:	50 ANIMALI PER ANNO
Fornitore:	CHARLES RIVER ITALIA, HARLAN ITALIA E ALTRI STABILIMENTI ALLEVATORI NAZIONALI
Ubicazione dello stabulario:	LOCALI DELLA SEZIONE DI FISILOGIA UMANA DELL' UNIVERSITA' DI FERRARA E PRESSO LO STABULARIO DELL' ISTITUTO NAZIONALE PER LA RICERCA SUL CANCRO DI GENOVA
Sede dell'esperimento:	LOCALI DELLA SEZIONE DI FISILOGIA UMANA DELL' UNIVERSITA' DI FERRARA E PRESSO LO STABULARIO DELL' ISTITUTO NAZIONALE PER LA RICERCA SUL CANCRO DI GENOVA

E. METODOLOGIA E TECNICA DELL'ESPERIMENTO

Descrizione dell'esperimento (D.Lgs. 116/92 - Art. 3):	SCOPO DELL'ESPERIMENTO E' L'OTTIMIZZAZIONE DELLA REGISTRAZIONE DELL'ATTIVITA' NERVOSA MEDIANTE MICROELETTRODI IMPIANTATI CRONICAMENTE NELLA CORTECCIA CEREBRALE. ALLO SCOPO VERRANNO UTILIZZATE MATRICI DI MICROELETTRODI SIA COMMERCIALI (CYBERKINETIC TUCKER AND DAVIS) SIA REALIZZATI IN PROPRIO. DURANTE GLI ESPERIMENTI GLI ANIMALI SARANNO LASCIATI LIBERI DI SVOLGERE LE NORMALI ATTIVITA'. IN ALCUNI CASI VERRANNO UTILIZZATI ANIMALI ALLENATI PRIMA DELL'IMPIANTO A SVOLGERE TASKS SENSORIMOTORI, COME PREMERE UN PULSANTE ALL'ACCENSIONE DI UNA LUCE, PER STUDIARE LA CORRELAZIONE TRA ATTIVITA' NERVOSA E COMPORTAMENTO. ALLA FINE DEL PERIODO DI IMPIANTO, VERRANNO ANALIZZATE LE REAZIONI TISSUTALI (GLIOSI, ECC.) MEDIANTE TECNICHE ISTOLOGICHE STANDARD.
Tipo di sofferenza che si ritiene di infliggere agli animali: (barrare la casella)	<input checked="" type="checkbox"/> Poca o nessuna sofferenza <input type="checkbox"/> Alcune sofferenze (di breve durata) <input type="checkbox"/> Estreme ed intollerabili sofferenze (in animali coscienti) <input type="checkbox"/> Forti privazioni, mutilazioni o altri traumi
Esecuzione di anestesia: (barrare la casella)	<input checked="" type="checkbox"/> SI <input type="checkbox"/> NO
Tipo di anestesia:	INIEZIONE INTRAPERITONEALE DI ANESTETICO ZOLETIL (TILETAMINA+ZOLAZEPAM)
Riutilizzazione degli animali post- esperimento: (barrare la casella)	<input type="checkbox"/> SI <input checked="" type="checkbox"/> NO
Modalità di soppressione degli animali:	GLI ANIMALI SONO SOPPRESSI TRAMITE INIEZIONE LETALE (TANAX) DOPO INDUZIONE DI ANESTESIA GENERALE

F. DOCUMENTAZIONE A CORREDO (pubblicazioni, bibliografia, ecc.)

Andersen RA, Musallam S, Pesaran B. Selecting the signals for a brain-machine interface. Curr Opin Neurobiol 2004; 14(6): 720-6.

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Butovas S, Schwarz C. Spatiotemporal effects of microstimulation in rat neocortex: a parametric study using multielectrode recordings. J Neurophysiol 2003; 90(5): 3024-39.

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Strata F, dePolvi AR, Chang EF, Bonham BH, Nakahara H, Liu RC, Merzenich MM (2005) Perinatal anoxia degrades auditory system function in rats. *Proc. Natl. Acad. Sci. USA* 102(52):19156-19161.

Strata F., Coq J.O., Byl N., Merzenich M.M. (2004) Sensorimotor restriction more than anoxia disrupts gait and motor cortex organization: a rodent model of cerebral palsy? *Neuroscience* 129:141-156.

Tehovnik EJ, Tolia AS, Sultan F, Slocum WM, Logothetis NK. Direct and indirect activation of cortical neurons by electrical microstimulation. *J Neurophysiol* 2006;96(2):512-21.

Van Camp N, Verhoye M, Van der Linden A. Stimulation of the rat somatosensory cortex at different frequencies and pulse widths. *NMR Biomed* 2006;19(1):10-7.

VandenBerg PM, Hogg TM, Kleim JA, Whishaw IQ. Long-Evans rats have a larger cortical topographic representation of movement than Fischer-344 rats: a microstimulation study of motor cortex in naive and skilled reaching-trained rats. *Brain Res Bull* 2002;59(3):197-203.

G. DICHIARAZIONE

Riferita alle prescrizioni di cui all'art. 4 (Inevitabilità del ricorso ad esperimenti su animali e necessità del ricorso ad una specie determinata ed al tipo di esperimento) e all'art. 5 (Rispetto delle condizioni) del D.LGS. 116/92.

<p>Inevitabilità del ricorso ad esperimenti su animali e necessità del ricorso ad una specie determinata ed al tipo di esperimento</p>	<p>TALE STUDIO PUO' ESSERE ESEGUITO SOLO IN VIVO, SIA PERCHE' L'OTTIMIZZAZIONE DELLE TECNICHE DI REGISTRAZIONE NEUROFISIOLOGICA RICHIEDE UNA COMPLESSITA' DI SEGNALE CHE E' SOLO PRESENTE NEL TESSUTO NERVOSO IN TOTO, SIA PERCHE' E' NECESSARIO DIMOSTRARE LA PRESENZA DI CORRELAZIONI TRA LA REGISTRAZIONE NEURONALE E IL COMPORTAMENTO DELL'ANIMALE, SIA, IN ULTIMO, PERCHE' OCCORRE STUDIARE LA REAZIONE TISSUTALE ALL'IMPIANTO DEL SISTEMA NERVOSO.</p> <p>IL TIPO DI ANIMALE SCELTO (RATTO) DA UN LATO RAPPRESENTA LA SPECIE DI MINOR COMPLESSITA' NERVOSA IN CUI ESEGUIRE TALI ESPERIMENTI, DALL'ALTRO E' GIUSTIFICATA DALL'ABBONDANZA DI DATI IN LETTERATURA CHE POSSONO FORNIRE UNA BASE ALLA SPERIMENTAZIONE PROPOSTA SENZA RICHIEDERE LA RIPETIZIONE DI PRATICHE PRECEDENTEMENTE GIA' TESTATE IN ALTRI LABORATORI. GLI ESPERIMENTI VERRANNO CONDOTTI SU RATTI ACQUISTATI PRESSO STABILIMENTI FORNITORI IN POSSESSO DELL'AUTORIZZAZIONE PREVISTA DALLE VIGENTI NORME DI LEGGE.</p>
<p>Rispetto delle condizioni</p>	<p>GLI ANIMALI VERRANNO STABULATI E MANTENUTI NELL'IDONEA STRUTTURA PRESENTE NELL'ISTITUZIONE ED ALLOGGIATI NELLE COMUNI GABBIE IN MATERIALE PLASTICO DOTATE DI GRIGLIA E BOTTIGLIA DI ABBEVERAGGIO. I RATTI SARANNO MANTENUTI IN PICCOLE COLONIE ED ALLOGGIATI IN NUMERO DI 2-3 PER GABBIA AL FINE DI PREVENIRE L'ISOLAMENTO SOCIALE O CONDIZIONI DI STRESS DA SOVRAFFOLLAMENTO. I RATTI SARANNO ALLOGGIATI IN GABBIE PIU' AMPIE (80X60X40 CM) DURANTE GLI ESPERIMENTI. OGGETTI DI VARIE DIMENSIONI E CONSISTENZA SARANNO COLLOCATI NELLE GABBIE SPERIMENTALI AL FINE DI FORNIRE UN ARRICCHIMENTO AMBIENTALE. OGNI ANIMALE UTILIZZATO, SIN DALLE FASI IMMEDIATAMENTE SUCCESSIVE ALL'INTERVENTO CHIRURGICO O AL TERMINE DI OGNI SEDUTA DI OSSERVAZIONE SPERIMENTALE, VERRA' RIPORTATO NELLA PROPRIA GABBIA DI STABULAZIONE.</p>

Il Responsabile del Progetto

Il Responsabile dell'esperimento

Prof.
(nome e
cognome)

LUCIANO FADIGA

Prof.
(nome e
cognome)

LUCIANO FADIGA

Firma

Firma

Ferrara, 2 novembre 2007

CURRICULUM VITAE DI LUCIANO FADIGA ED ELENCO DELLE PUBBLICAZIONI

Nasce a Bologna l'8/8/1961. Si laurea con lode in Medicina e Chirurgia presso l'Università degli Studi di Bologna nell'anno accademico 1989/1990 discutendo una tesi dal titolo: "*Aspetti neurochimici della regolazione ipotalamica del ciclo di sonno rivelati dallo studio di un secondo messaggero: l'adenosin-monofosfato ciclico*". Conseguisce con il massimo dei voti l'abilitazione all'esercizio della professione di medico chirurgo. Nel 1995 consegue il titolo di Dottore di Ricerca in Neuroscienze.

Curriculum accademico e attività scientifica.

1985-1990: Studente interno e successivamente medico frequentatore presso l'Istituto di Fisiologia umana dell'Università di Bologna.

Attività scientifica: studio con tecniche neurochimiche e neurofisiologiche dei meccanismi di regolazione del ciclo di sonno e delle relazioni tra sonno e regolazioni neurovegetative (1-4). Affinamento della preparazione neurofisiologica e tecnica, in particolare nel campo dell'elettrotecnica e dei linguaggi di programmazione per elaboratore elettronico.

1990-1995: Vincitore del concorso per l'ammissione al 6° ciclo del Dottorato di Ricerca in Neuroscienze presso l'Università di Parma. Nel 1995 consegue il titolo di Dottore di Ricerca in Neuroscienze discutendo la tesi "*Corteccia premotoria ventrale e suo possibile ruolo nel riconoscimento di gesti manuali*".

Attività scientifica: Studio dell'organizzazione funzionale della corteccia motoria e premotoria della scimmia sia durante la programmazioni di azioni finalistiche manuali (7,15), sia nella codifica a livello neuronale dello spazio peripersonale (5,11,17). Studio nei primati della cinematica dei movimenti di raggiungimento-prensione in condizioni normali e dopo inattivazione temporanea con muscimolo (18,30). Fornisce contributi essenziali alla scoperta e allo studio, nell'area F5 della corteccia premotoria del macaco, di neuroni che si attivano sia quando l'animale esegue azioni di afferramento con la mano, sia quando egli osserva altri individui eseguire azioni simili (neuroni *mirror*, 6,9,10,16,23). E' stato proposto che questi neuroni possano costituire un substrato anatomico-funzionale al meccanismo che permette la comprensione delle azioni altrui. Tali osservazioni sperimentali hanno prodotto notevole risonanza a livello nazionale ed internazionale come attestato da articoli di revisione su riviste internazionali di prestigio ed articoli di stampa nazionale a larga diffusione.

1995-1999: vincitore di premio di ricerca Human Frontier Science Program e successivamente classificato al primo posto per l'assegnazione di una Borsa di Studio della European Neuroscience Association. Nel dicembre 1996 è risultato vincitore del Concorso per un posto di Ricercatore Universitario (settore scientifico disciplinare

E06A) e nell'aprile 1997 ha preso servizio presso l'Istituto di Fisiologia umana dell'Università di Parma.

Attività scientifica: oltre alla prosecuzione degli esperimenti di elettrofisiologia sulla corteccia premotoria della scimmia (21,26,27), si dedica allo studio sull'uomo delle funzioni corticali utilizzando nuove tecniche di indagine come la tomografia ad emissione di positroni e la risonanza magnetica funzionale (12,13,19,29, esperimenti svolti in collaborazione con il gruppo CNR di Milano presso l'Ospedale S. Raffaele, con S. Grafton e M.A. Arbib a Los Angeles, con il gruppo di K. Zilles a Juelich, Germania) e la stimolazione magnetica transcranica (8,22), mettendo a punto la tecnica in prima persona e coordinando il lavoro sperimentale presso l'Istituto di Fisiologia umana di Parma. In particolare, grazie all'integrazione di queste tecniche con i dati emersi dallo studio elettrofisiologico della corteccia premotoria della scimmia, rivolge la propria attività scientifica alla dimostrazione (anche con tecniche di psicofisica) delle influenze che le rappresentazioni motorie corticali possono esercitare sia nella modulazione della soglia percettiva (24,25) sia nel riconoscimento di azioni eseguite da altri individui (34). Dimostra tra l'altro che, nell'uomo, la semplice immaginazione o l'osservazione di azioni manuali induce una facilitazione (che può essere rivelata mediante la stimolazione magnetica transcranica) dei circuiti corticospinali responsabili del controllo di movimenti congruenti con quelli osservati (8,22). In collaborazione con F. Baldissera e P. Cavallari dell'Università di Milano, dimostra che peculiari cambiamenti di eccitabilità del sistema motorio si verificano anche a livello spinale (29). Studia, nell'uomo e nella scimmia, l'influenza esercitata dall'osservazione di oggetti manipolabili sul sistema motorio deputato al controllo dei movimenti manuali (14,20).

2000- : Nel novembre 2000 viene chiamato dalla Facoltà di Medicina e Chirurgia dell'Università di Ferrara come Professore associato di Fisiologia umana. Mette a punto i laboratori necessari a proseguire lo studio neurofisiologico nel primate inferiore (registrazione extracellulare di singoli neuroni e registrazione cinematica del movimento dell'arto superiore, 31) e nell'uomo (stimolazione magnetica transcranica, psicofisica).

Attività scientifica: (i) caratterizzazione funzionale della porzione ventrale della corteccia premotoria e parietale della scimmia (32), messa a punto di sistema computerizzato per la realizzazione tridimensionale di impianti ossei in titanio necessari alla registrazione di singoli neuroni. (ii) Registrazione dell'attività di singoli neuroni della corteccia premotoria della scimmia volta alla caratterizzazione funzionale della regione F5, sede dei neuroni mirror. Lo studio, supportato dal contratto con la Commissione Europea MIRROR IST 2000-20159, ha permesso finora di definire il ruolo dei neuroni premotori dell'area F5 nel controllo visivo a feedback dell'esecuzione di azioni manuali. (iii) Studio con tematiche psicofisiche dei meccanismi alla base dell'attenzione visuospatiale. Tali indagini hanno permesso di dimostrare che limitazioni periferiche della motilità oculare, quali lesioni del 6° nervo cranico (33) o posture dell'occhio in lateralità (40), influiscono sulla capacità di orientamento attenzionale nonostante il compito cui vengono sottoposti i soggetti non richieda movimenti oculari. (iv) Studio mediante stimolazione magnetica transcranica

dell'eccitabilità della corteccia motoria primaria durante l'ascolto di stimoli verbali. Lo studio è volto alla comprensione dei meccanismi nervosi che consentono la comprensione del linguaggio parlato, è supportato da fondi COFIN e ESF (CNR) ed ha finora dimostrato che durante l'ascolto di parole il sistema motorio dell'ascoltatore viene facilitato in maniera congruente con gli stimoli presentati (35). (v) Studio con tecniche di brain imaging del ruolo svolto dal lobo frontale nella comprensione delle azioni, incluse quelle comunicative. Il contributo fornito in tal senso spazia dalla collaborazione ad esperimenti di mappatura somatotopica del giro prefrontale (eseguiti a Juelich, Germania) durante l'osservazione di azioni eseguite con vari effettori ad un più recente interesse sul ruolo del giro frontale inferiore nella comprensione di azioni comunicative (anche non verbali) portato a termine presso la Royal Holloway University di Londra. (vi) Studio con stimolazione magnetica transcranica dell'eccitabilità corticospinale durante l'autosomministrazione di stimoli dolorosi (in collaborazione con C. Porro, Università di Udine) ed in pazienti affetti da emiplegia perinatale (in collaborazione con E. Olivier, Università di Lovanio, Belgio). (vii) Registrazione di singoli neuroni nella corteccia umana, durante interventi neurochirurgici su pazienti svegli e collaboranti, allo scopo di caratterizzare elettrofisiologicamente il bordo funzionale tra tessuto nervoso sano e tessuto invaso da tumori a basso grado (in collaborazione con M. Skrap, Neurochirurgia di Udine)

2005- : In data 1/4/2005 prende servizio in qualità di Professore di ruolo di prima fascia(Fisiologia) presso la Facoltà di Medicina e Chirurgia dell'Università di Ferrara. Nell'anno 2006 inizia il coordinamento di un progetto di ricerca sulle interfacce cervello-macchina presso la Fondazione Istituto Italiano di tecnologia di Genova.

Attività organizzativa.

Luciano Fadiga è stato chiamato dalla Facoltà di Medicina e Chirurgia dell'Università degli Studi di Ferrara il 1° novembre 2000. Nonostante gli stretti rapporti di collaborazione scientifica con l'Istituto di Fisiologia di Parma da cui proveniva, preso atto della massima disponibilità locale, ha di conseguenza trasferito subito la sua residenza a Ferrara ed ha iniziato la sua attività di ricerca dedicandosi al reperimento dei fondi, del personale e delle risorse in genere, necessari all'allestimento dei laboratori per lo svolgimento della sua attività. In dettaglio, ha provveduto personalmente a realizzare le seguenti strutture:

1) Completamento del laboratorio di elettrofisiologia sul primate inferiore, acquisto di animali, ottenimento di autorizzazione ministeriale in deroga, messa a punto di una nuova metodica di impianto biocompatibile e totalmente tollerata dagli animali da esperimento.

2) Allestimento di un laboratorio per la stimolazione magnetica transcranica sull'uomo, con progettazione e realizzazione di un sistema per la registrazione ad alta sensibilità dei potenziali motori evocati. Realizzazione di un sistema

hardware/software per la localizzazione sul singolo soggetto del sito corticale di stimolazione.

3) Allestimento di un laboratorio per esperimenti di psicofisica allo scopo di studiare l'orientamento dell'attenzione visuospatiale, la capacità predittiva durante l'osservazione di movimenti biologici e per eseguire misurazioni cinematiche sull'arto superiore durante azioni di raggiungimento-prensione. Per realizzare quest'ultimo obiettivo ha reperito i fondi necessari all'acquisto di una sofisticata apparecchiatura per la misura del movimento in tre dimensioni alla frequenza di campionamento di 1000 Hz.

Gruppo di ricerca.

Nel corso dell'ultimo triennio, grazie ai finanziamenti ricevuti, Luciano Fadiga ha potuto bandire quattro concorsi per borse di ricerca biennali, ha instaurato otto contratti annuali di collaborazione coordinata e continuativa, ha ottenuto il finanziamento di due borse di Dottorato in Neuroscienze. L'attribuzione di queste posizioni ha interessato ed interessa collaboratori italiani e di vari paesi europei.

Coordinamento di progetti scientifici in corso.

- Responsabile scientifico di Contratto CE (Mirror, 5° framework) sui meccanismi per il riconoscimento delle azioni (2001-2004).
- Responsabile scientifico di Contratto CE (Neurobotics, 6° framework) sulla creazione di sistemi per aumentare le potenzialità motorie (2003-2007).
- Responsabile scientifico di Contratto CE (RobotCub, 6° framework) dedicato allo studio del modo in cui il sistema cognitivo si sviluppa in un robot umanoide mediante la sua interazione con il mondo circostante e gli altri (2004-2009).
- Responsabile scientifico di Contratto CE (CONTACT, 6° framework) dedicato allo studio dei meccanismi nervosi che permettono il riconoscimento del linguaggio (2005-2008).
- Responsabile scientifico di progetto OMLL della European Science Foundation (finanziato tramite il C.N.R.) sul riconoscimento del linguaggio parlato (2002-2005).
- Responsabile scientifico di progetto COFIN sul coinvolgimento del sistema mirror nella comunicazione interindividuale (2002-2004).
- Responsabile scientifico di progetto FIRB sulla registrazione elettrofisiologica di neuroni nell'uomo (2002-2006).
- Responsabile scientifico di progetto Ministero della Salute sulla neurofisiologia/neurochirurgia della malattia di Parkinson (2002-2006).
- Responsabile scientifico di fondi dell'Università di Ferrara (ex 60%) (2003).
- Responsabile scientifico di finanziamento Fondazione Cassa di Risparmio di Ferrara per una borsa di Dottorato di Ricerca in Neuroscienze (2003-2009).

Riconoscimenti e attività accademiche.

Luciano Fadiga ha ricevuto il Premio della Società Italiana di Fisiologia per l'anno 1997.

E' stato molte volte invitato come relatore a Congressi internazionali e a tenere seminari e letture presso Centri di ricerca internazionali.

E' membro dell'Advisory Council di "Attention and Performance".

E' revisore delle principali riviste internazionali di Neuroscienze tra cui Brain, Experimental Brain Research, Psychological Research, European Journal of Neuroscience, Clinical Neurophysiology, European Journal of Applied Physiology, Neuron, NeuroImage.

E' stato nominato più volte revisore di progetti dalla Commissione Europea.

Ha in atto proficue collaborazioni scientifiche con molti Centri di ricerca di livello internazionale.

Nel 2000 ha pubblicato su invito, assieme a G. Rizzolatti, il capitolo "Controllo corticale del movimento" nell'opera "Frontiere della Vita" curata dall'Istituto Enciclopedico Treccani e recentemente tradotta in inglese dall'editore Academic Press.

Ha collaborato attivamente ad attività di divulgazione scientifica partecipando a trasmissioni radiofoniche ed interviste su periodici e ospitando troupes televisive presso i suoi laboratori per la realizzazione di documentari scientifici (Televisione di Stato Belga e BBC inglese).

E' autore di capitoli di libro per l'insegnamento delle Neuroscienze ed ha curato la revisione scientifica della traduzione italiana dei testi "Fundamentals Neuroscience, Zigmond M.J., Bloom F.E., Landis S.C., Roberts J.L., Squire L.R., Academic Press, 1999" e "Fisiologia umana" di Germann e Stanfield, per i tipi della EDISES di Napoli.

La produzione scientifica di Luciano Fadiga è attestata da un totale di oltre 60 pubblicazioni, 50 delle quali sottoposte a revisione per la pubblicazione. L'impatto delle ricerche svolte da Luciano Fadiga è attestato dalle oltre 4000 citazioni dei suoi lavori su Riviste internazionali.

Pubblicazioni sottoposte a revisione:

1) Zamboni G., Perez E., Amici R., Fadiga L., Calasso M., Parmeggiani P.L. The influence of the light-dark and wake-sleep cycles on preoptic cAMP concentration in the rat. *Boll.Soc.It.Biol.Sper.*, Napoli, 1989; 65; 37.

2) Perez E., Zamboni G., Amici R., Fadiga L., Calasso M., Parmeggiani P.L. The effect of propranolol on cAMP concentration in the rat preoptic region during the wake-sleep cycle. *Boll.Soc.It.Biol.Sper.*, Napoli, 1989; 65; 41.

- 3) Amici R., Fadiga L., Perez E., Zamboni G., Parmeggiani P.L. Relationship between anterior hypothalamic-preoptic cAMP and the stages of the ultradian wake-sleep cycle. *J. Autonomic Nervous System*, Amsterdam, 1990; 30: S5.
- 4) Perez E., Zamboni G., Amici R., Fadiga L., Parmeggiani P.L. Ultradian and circadian changes in the cAMP concentration in the preoptic region of the rat. *Brain Res.*, Amsterdam, 1991; 551: 132.
- 5) Fogassi L., Gallese V., di Pellegrino G., Fadiga L., Gentilucci M., Luppino G., Matelli M., Pedotti A., Rizzolatti G. Space coding by premotor cortex. *Exp. Brain Res.*, New York, 1992; 89: 686.
- 6) di Pellegrino G., Fadiga L., Fogassi L., Gallese V., Rizzolatti G. Understanding motor events: a neurophysiological study. *Exp. Brain Res.*, New York, 1992; 91: 176.
- 7) Fadiga L. Le aree motorie corticali e la programmazione dei movimenti. *Riabilitazione oggi*, Milano, 1994, XI(6): 23.
- 8) Fadiga L., Fogassi L., Pavesi G., Rizzolatti G. Motor facilitation during action observation: a magnetic stimulation study. *J. Neurophysiology*, Bethesda, 1995, 73(6): 2608.
- 9) Rizzolatti G., Fadiga L., Gallese V., Fogassi L. Premotor cortex and the recognition of motor actions, *Cogn. Brain Res.*, Amsterdam, 1996, 3:131-141.
- 10) Gallese V., Fadiga L., Fogassi L., Rizzolatti G. Action recognition in the premotor cortex, *Brain*, Oxford, 1996, 119:593-609.
- 11) Fogassi L., Gallese V., Fadiga L., Luppino G., Matelli M., Rizzolatti G. Coding of peripersonal space in inferior premotor cortex (area F4), *J. Neurophysiol.*, Bethesda, 1996, 76:141-157.
- 12) Rizzolatti G., Fadiga L., Matelli M., Bettinardi V., Paulesu E., Perani D., Fazio F. Localization of grasp representations in human by PET: 1. Observation versus execution. *Exp. Brain Res.*, New York, 1996, 111:246-252.
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Ministero della Salute

Dipartimento per la Sanità Pubblica Veterinaria, la
Nutrizione e la Sicurezza degli Alimenti
Direzione Generale della Sanità Animale e del Farmaco
Veterinario
Ufficio VI

prot. 25805/11 del 06/06/2011

OGGETTO:

D.lgs. 116/92 in materia di protezione degli animali
utilizzati ai fini sperimentali e scientifici.
Trasmissione autorizzazioni ai sensi dell'art. 9.
- Decreto n° 91/2011-B

Ministero della Salute
DGSA

0009320-P-20/05/2011
1.5.1.q.3/2011/10



Università degli Studi di Ferrara
c.a. Dott.ssa Silvia GANDINI
e-mail: gndslv@unife.it

Stabilimento Utilizzatore
Dipartimento di Scienze Biomediche
e Terapie Avanzate
c.a. Dr. Luciano FADIGA
e-mail: fdl@unife.it

Stabilimento Utilizzatore
Fondazione Istituto Italiano di Tecnologia
c.a. Dr. Umberto CARDELLINO
e-mail: umberto.cardellino@iit.it

E p.c.

Regione Emilia Romagna
Servizio Veterinario
ABovo@Regione.Emilia-Romagna.it

ASL Ferrara
Distretto Veterinario n° 1
c.a. Dr.ssa FAGGIOLI
p.faggioli@ausl.fe.it

A.S.L. 3 Genovese
Dipartimento di Prevenzione
Struttura Complessa Sanità Animale
sanitaanimale@asl3.liguria.it

Prefettura di Ferrara
protocollo.prefe@pec.interno.it

Ufficio Veterinario per gli
Adempimenti Comunitari
sanvet-pr@postacert.sanita.it

Si trasmette il decreto ministeriale n° 91/2011-B rilasciato in data 18/05/2011 che reca
l'autorizzazione di cui all'oggetto.

Copia conforme della predetta autorizzazione è altresì trasmessa, per quanto di competenza,
agli altri Enti in indirizzo.

IL DIRETTORE DELL'UFFICIO VI



Ministero della Salute

DIPARTIMENTO PER LA SANITÀ PUBBLICA VETERINARIA, LA NUTRIZIONE
E LA SICUREZZA DEGLI ALIMENTI
DIREZIONE GENERALE DELLA SANITÀ ANIMALE E DEL FARMACO VETERINARIO
UFFICIO VI

DECRETO N° 91 /2011- B

IL DIRETTORE GENERALE

Visto l'articolo 9 del decreto legislativo 27 gennaio 1992 n°116, che demanda al Ministro della Sanità l'autorizzazione in deroga all'art. 4, comma 3 dello stesso decreto legislativo per gli esperimenti da effettuarsi senza anestesia;

Visti gli articoli 4 e 16 del Decreto Legislativo 30 marzo 2001, n° 165, che demanda ai Dirigenti l'adozione degli atti che impegnano l'amministrazione verso l'esterno;

Visto l'articolo 7 secondo comma del decreto legislativo 27 gennaio 1992, n°116;

Vista la domanda del Prof. Luciano FADIGA del Dipartimento di Scienze Biomediche e Terapie Avanzate - Sezione di Fisiologia Umana dell'Università degli Studi di Ferrara, sede legale in Ferrara, Via Savonarola, 9, intesa ad ottenere l'autorizzazione in deroga ai sensi dell'articolo 9 del decreto legislativo 27 gennaio 1992, n°116, concernente gli studi che saranno effettuati presso lo stabilimento utilizzatore del Dipartimento di Scienze Biomediche e Terapie Avanzate - Sezione di Fisiologia Umana dell'Università degli Studi di Ferrara, sito in Ferrara, Via Fossato di Mortara, 17/19 e presso lo stabilimento utilizzatore della Fondazione Istituto Italiano di Tecnologia, sito in Genova, Via Morego, 30, sotto la diretta responsabilità del Prof. Luciano FADIGA, laureato in Medicina e Chirurgia;

Visto il progetto di ricerca allegato alla domanda;

Visto il parere favorevole n. 22548 del 13/05/2011 espresso dall'Istituto Superiore di Sanità;

Atteso che le finalità del progetto sono comprese tra quelle di cui all'articolo 3, comma 1;

Ritenuto che l'anestesia è incompatibile con il fine degli esperimenti stessi;

Considerato che:

a) lo stabulario del Dipartimento di Scienze Biomediche e Terapie Avanzate - Sezione di Fisiologia Umana dell'Università degli Studi di Ferrara è regolarmente autorizzato come stabilimento utilizzatore ai sensi dell'art. 12 del D.lgs. 27 Gennaio 1992, n° 116, con decreto n° 38/2009 - A del 20/02/2009 e che il Prof. Luciano FADIGA, laureato in Medicina e Chirurgia, è responsabile dell'assistenza agli animali e del funzionamento delle attrezzature;

b) lo stabulario della Fondazione Istituto Italiano di Tecnologia, è regolarmente autorizzato come stabilimento utilizzatore ai sensi dell'art. 12 del D.lgs 27 Gennaio 1992, n°116 con decreto n°29/2011-A del 16/02/2011 e che il Dott. Umberto CARDELLINO, laureato in Medicina Veterinaria è responsabile dell'assistenza agli animali e del funzionamento delle attrezzature;

Preso atto che il Medico Veterinario responsabile del controllo della buona esecuzione delle procedure d'esperimento, ai sensi dell'art. 6, comma 4 sono il Dr. Paolo SQUARZONI e il Dr. Umberto CARDELLINO;

DECRETA



Il Prof. Luciano FADIGA del Dipartimento di Scienze Biomediche e Terapie Avanzate – Sezione di Fisiologia Umana dell’Università degli Studi di Ferrara, è autorizzato per la durata massima di dodici mesi, a eseguire l’esperimento di seguito riportato, ai sensi del primo comma dell’art. 9 del D.lgs. 27 Gennaio 1992, n°116:

“Studio di Matrici di Multielettrodi per la derivazione dell’attività unitaria neuronale della corteccia sensorimotoria del ratto”

L’esperimento sarà eseguito sotto la diretta responsabilità del Prof. Luciano FADIGA, laureato in Medicina e Chirurgia;

L’esperimento sarà eseguito presso lo stabulario del Dipartimento di Scienze Biomediche e Terapie Avanzate – Sezione di Fisiologia Umana dell’Università degli Studi di Ferrara, regolarmente autorizzato come stabilimento utilizzatore ai sensi dell’art. 12 del D.lgs. 27 Gennaio 1992, n°116 con decreto n°38/2009 – A del 20/02/2009 e presso lo stabulario della Fondazione Istituto Italiano di Tecnologia, regolarmente autorizzato come stabilimento utilizzatore ai sensi dell’art. 12 del D.lgs 27 Gennaio 1992, n°116 con decreto n°29/2011-A del 16/02/2011.

IL DIRETTORE GENERALE

C. Cerretti

18 MAG. 2011



Ministero della Salute

Dipartimento per la Sanità Pubblica Veterinaria, la
Nutrizione e la Sicurezza degli Alimenti
Direzione Generale della Sanità Animale e del Farmaco
Veterinario
Ufficio VI

Ministero della Salute

DGSA

0002987-P-17/02/2011

I. S. i. q. 3/2009/332



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OGGETTO:

D.lgs. 116/92 in materia di protezione degli animali
utilizzati ai fini sperimentali e scientifici.
Trasmissione autorizzazioni ai sensi degli art. 12..
- Decreto n° 29/2011- A

Si trasmette il decreto ministeriale n° 29/2011- A rilasciato in data 16/02/2011 che reca
l' autorizzazione di cui all' oggetto.

Copia conforme della predetta autorizzazione è altresì trasmessa, per quanto di
competenza, agli altri Enti in indirizzo.

p. IL DIRETTORE DELL'UFFICIO VI

Prot 7049/u
del 18/02/2011

Fondazione Istituto Italiano
di Tecnologia - GENOVA
c.a. Dr. Umberto CARDELLINO
e-mail: umberto.cardellino@iit.it

E p.c.

Regione Liguria
Assessorato Sanità - Servizi Veterinari
alimenti.veterinaria@regione.liguria.it
Monica.Reali@regione.liguria.it

A.S.L. 3 Genovese
Dipartimento di Prevenzione
Struttura Complessa Sanità Animale
sanitaanimale@asl3.liguria.it

PREFETTURA DI GENOVA
prefettura.prefge@pec.interno.it

Ufficio Veterinario per gli
Adempimenti Comunitari
sanvet-ge@postacert.sanita.it



Ministero della Salute

DIPARTIMENTO PER LA SANITÀ PUBBLICA VETERINARIA, LA NUTRIZIONE
E LA SICUREZZA DEGLI ALIMENTI
DIREZIONE GENERALE DELLA SANITÀ ANIMALE E DEL FARMACO VETERINARIO
UFFICIO VI

DECRETO N° 23 /2011 - A

IL DIRETTORE GENERALE

Visto l'articolo 12 del **decreto legislativo 27 gennaio 1992 n°116**, che demanda al Ministero della Sanità la concessione all'autorizzazione di **stabilimento utilizzatore** di animali a fini sperimentali o ad altri fini scientifici;

Visto l'articolo 3 che stabilisce i fini per i quali è consentita l'utilizzazione degli animali negli esperimenti;

Visti gli artt. 5 e 6 che fissano i requisiti generali del benessere animale cui devono corrispondere le strutture e la conduzione delle stesse;

Vista la nota della **Fondazione Istituto Italiano di Tecnologia (IIT)**, con sede legale in **Genova, via Morego, n. 30, codice fiscale n° 97329350587**, con la quale comunica come da planimetria dello stabilimento già autorizzato la rinuncia dei locali collocati al sesto piano dell'edificio di cui al decreto ministeriale 67/2008-A del 19.05.2008, mentre rimangono in essere i locali collocati al piano -1 dell'edificio e di cui al decreto ministeriale n°154/2009-A del 07.08.2009 e nel contempo variazione del medico veterinario e responsabile dello stabilimento utilizzatore;

Visti i Decreti n°67/2008-A del 19.05.2008 e 154/2009-A del 07.08.2009;

Vista la planimetria allegata alla domanda dove vengono definiti i locali che verranno utilizzati per fini sperimentali;

Visto il verbale di sopralluogo effettuato dall'ispettore veterinario del Ministero della Salute presso lo stabilimento utilizzatore sito in Genova, via Morego, n. 30, in data 02 Luglio 2009;

Visto il successivo parere favorevole espresso dall'Azienda Sanitaria Locale di Genova/3, in data 27 Luglio 2009;

Considerato che nulla osta alla richiesta di cui sopra;

Visti gli articoli 4 e 16 del decreto legislativo 30 marzo 2001, n°165, che demanda ai dirigenti l'adozione degli atti che impegnano l'amministrazione verso l'esterno;

Preso atto che il responsabile dell'assistenza agli animali, del funzionamento delle attrezzature, della gestione delle strutture e del personale ad esse adibito, ai sensi dell'articolo 12 del D.Lgs. 116/92, è il **Dott. Umberto CARDELLINO**, laureato in Medicina Veterinaria;

Preso atto che il Medico Veterinario responsabile, dell'assistenza veterinaria e della consulenza sul benessere animale ai sensi dell'art. 12 del D.Lgs. 116/92, comma 2, punto 5, nonché della buona esecuzione della procedura degli esperimenti, ai sensi del D.Lgs. 116/92, art. 6, comma 4, è il **Dott. Umberto CARDELLINO**;



DECRETA

La **Fondazione Istituto Italiano di Tecnologia (IIT)**, con sede legale in Genova, via Morego, n. 30, codice fiscale n° 97329350587, è autorizzato allo stabilimento utilizzatore nel quale sono utilizzati fini sperimentali o altri fini scientifici a norma dell'articolo 12 del decreto legislativo 27 gennaio 1992 n°116, le seguenti specie animali:

- a) **Topo** (*Mus musculus*)
- b) **Ratto** (*Rattus norvegicus*)
- c) **Cavia** (*Cavia Porcellus*)

Il **Dott. Umberto CARDELLINO**, laureato in Medicina Veterinaria, è responsabile dell'assistenza agli animali, del funzionamento delle attrezzature, della gestione delle strutture e del personale ad esse adibito, ai sensi dell'articolo 12 del D.lgs 116/92;

Il Dott Umberto CARDELLINO è responsabile della corretta tenuta del registro di utilizzazione degli animali e conformemente all'articolo 15 del D.lgs 116/92, trasmette al Ministero della Salute, entro il 31 marzo di ogni anno, le tabelle statistiche contenenti i dati degli animali utilizzati;

Il **Dott. Umberto CARDELLINO**, laureato in medicina veterinaria, è responsabile della consulenza, dell'assistenza veterinaria e della consulenza sul benessere animale ai sensi dell'art. 12 del D.lgs 116/92, comma 2, punto 5, nonché della buona esecuzione della procedura degli esperimenti ai sensi del D.lgs 116/92, art. 6, comma 4. Per questa ultima competenza il predetto professionista assicura la propria presenza durante l'esecuzione delle procedure sperimentali, tranne nel caso di prove di tipo routinario e sistematico non implicanti tecniche sperimentali a rischio per la tutela del benessere animale, la cui esecuzione è realizzata secondo prassi consolidate di laboratorio.

Il presente decreto annulla e sostituisce i decreti n°67/2008-A del 19.05.2008 e 154/2009-A del 07.08.2009.

IL DIRETTORE GENERALE

Cetana Fi

16 FEB. 2011