Neuroengineering control and regulation of behavior

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ABSTRACT

To monitor neuronal circuits involved in emotional modulation of sensory processing we proposed a plan to establish novel research techniques combining recent biological, technical and analytical discoveries. The project was granted by National Science Center and we started to build a new experimental model for studying the selected circuits of genetically marked and behaviorally activated neurons. To achieve this goal we will combine the pioneering, interdisciplinary expertise of four Polish institutions: (i) the Nencki Institute of Experimental Biology (Polish Academy of Sciences) will deliver the expertise on genetically modified mice and rats, mapping of the neuronal circuits activated by behavior, monitoring complex behaviors measured in the IntelliCage system, electrophysiological brain activity recordings by multielectrodes in behaving animals, analysis and modeling of behavioral and electrophysiological data; (ii) the AGH University of Science and Technology (Faculty of Physics and Applied Computer Sciences) will use its experience in high-throughput electronics to build multichannel systems for recording the brain activity of behaving animals; (iii) the University of Warsaw (Faculty of Physics) and (iv) the Center for Theoretical Physics (Polish Academy of Sciences) will construct optoelectronic device for remote control of opto-animals produced in the Nencki Institute based on the unique experience in laser sources, studies of light propagation and its interaction with condensed media, wireless medical robotic systems, fast readout opto-electronics with control software and micromechanics.

Keywords: Novel optoelectronic and electrophysiological tools for brain studies, neuronal basis of emotional behaviors, novel methods of analysis and modeling neurophysiological data

1. AIM OF THE STUDY

Understanding functioning of the brain in health and disease comprises one of the major challenges of contemporary science. In particular, understanding neuronal mechanisms underlying emotional modulation of sensory processing is a clinically relevant line of research. This is clearly depicted by psychiatric disorders such as addiction[19], autism[1,18] or post-traumatic stress disorders (in which natural coupling of sensory information to emotions goes awry.

The amygdala is emerging as a key component of the brain's emotional system involved in coding and updating the emotional value of information[12]. At the same time, it has been shown that sensory systems, including sensory cortices and subcortical structures, are critical for processing of emotionally valenced sensory information[13,28]. The amygdala

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consists of several cytoarchitectonically well-defined and internally distinguishable nuclei. Although precise classification of the functions of different amygdalar nuclei and their subdivisions in the context of behavior still remains a major goal of research, functional differences between them, consistent with anatomical data, have been identified[12]. Much of this knowledge was gained through immunohistochemical mapping of immediate early genes expression, and c-Fos protein expression in particular, has been one of the most commonly used techniques to deduce the contribution of particular brain region to regulation of emotional behaviors[10,12]. The actual contribution of c-Fos expressing neurons to driving such behaviors has been, however, difficult to determine due to technological limitations. In the current project, we plan to engage multidisciplinary approach in order to circumvent these problems and test the hypothesis that c-fos-expressing neuronal circuits are involved in processing and storing emotional information.

Identifying and characterizing these circuits will allow for selective modification of their activity, which would open up a possibility of regulating impaired behaviors. Specifically, we will answer the following questions:

1. How are functional connectivity of the brain structures and local cooperation between neurons related to emotional meaning of the sensory stimuli?
2. How do c-Fos-expressing neuronal circuits contribute to behaviors driven by emotionally valenced information?

To identify and characterize the neuronal circuits underlying processing of specific emotional information we need novel methods allowing to manipulate, measure, and interpret neuronal activity in a behaving animal. A major technological revolution in manipulation of specific neuronal circuits has recently been achieved by optogenetics which relies on genetic introduction of light sensitive opsins driving activation or silencing of the affected neurons[4]. Here we will take advantage of the optogenetic toolbox and create transgenic animals expressing opsins under c-fos promoter, in behaviorally activated neurons (WP 1; Fig. 1). So far, a major drawback of optogenetic approach has been its limited application to freely moving animals, as they had to be connected to the light source. In the present project, this obstacle will be surmounted by constructing a new wireless optoelectronic device (WP 2) which will be applied to freely moving animals in the IntelliCages (WP 4), where mice can be tested in close to ethological conditions. Furthermore, a novel multi-electrode systems will be created (WP 3) and used in behaving animals (WP 5) to measure the neuronal activity of many brain structures and to couple this information with ongoing behavior and emotional state. This combination of new techniques together with novel methods of data analysis and modeling (WP 6) will provide a unique setup for addressing a range of neurobiological challenges and providing an exceptional insight into the neuronal mechanisms underlying emotional modulation of sensory processing. It should be stressed that while the technologies are developed here to facilitate study of specific biological questions, their utility goes far beyond the focus of this project. Indeed, bringing the technology to the level where it can be easily applied in other contexts, with possible commercialization, is one of the major goals of this proposal. Our research will broaden the understanding of the neuronal circuits involved in coupling sensory information with emotions. In particular, we shall determine the function of active neurons, as marked by c-Fos expression, in gating emotional information and driving behaviors. Impaired processing of emotionally valenced information is observed in patients suffering from many psychiatric disorders, such as addiction, stress disorders or social symptoms of autism; the knowledge and innovations obtained in this project may open new pathways towards clinical applications aimed at treating these symptoms at the functional level.

2. CONTRIBUTION OF DIFFERENT DISCIPLINES

In the project we plan to investigate the role of c-Fos expressing neuronal circuits in processing and storing emotional information. To tackle this problem we propose: (1) to produce novel optogenetic, optoelectronic and electronic tools; (2) to use these tools in order to understand the neuronal basis of behaviors driven by emotions; (3) to use these tools in order to understand the functional connectivity of the brain structures processing and storing emotional valence of the stimuli.

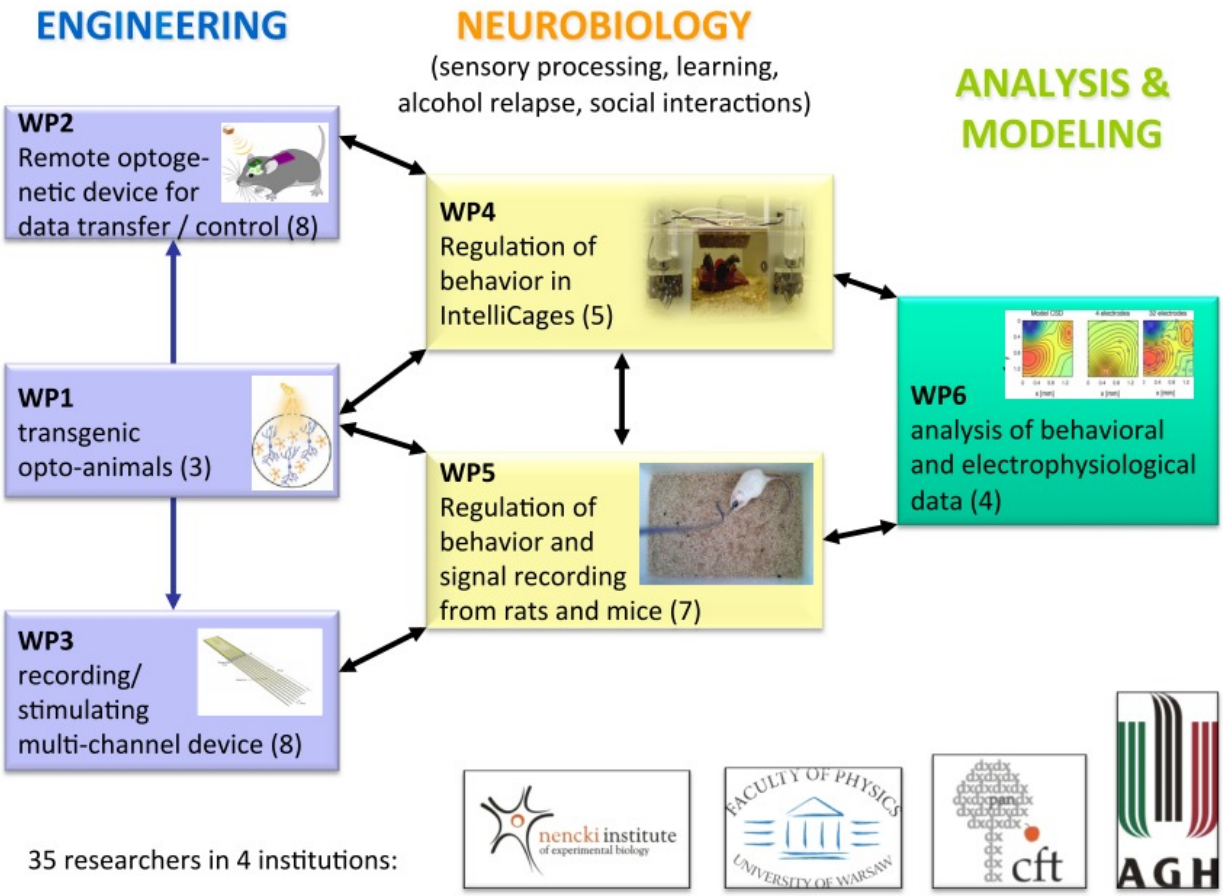

Figure 1. Specific tasks organized in work packages operating in the grant.

Novel optoelectronic and electrophysiological tools. Although the main questions driving this proposal come from neurobiology, development of novel technologies stemming from research in several fields is pivotal for the whole project. A technological breakthrough allowing to manipulate specific neuronal circuits has recently been achieved by introduction of optogenetics[4]. Optogenetics relies on genetic manipulation of neurons that introduce cell membrane proteins that respond to defined light wavelengths and facilitate or block neuronal activity in a way that mimics the endogenous function of nerve cells. It offers an unmatched tool for manipulation of single cells, cell populations and networks, to test which of those elements are involved in specific behaviors. Unfortunately, so far optogenetics has had a limited application to freely moving animals. Thus, the adjustment of optoelectronic devices for wireless use in freely moving animals is one of the major technological challenges addressed in our project. We plan to develop remotely controlled, miniaturized, fully implantable optogenetic devices which will facilitate control and regulation of animal behavior in laboratory experiments.

Despite the development of various imaging techniques, electrophysiology is still the best method that allows real-time monitoring of brain activity in parallel with ongoing behavior and with excellent time resolution. A major recent advancement in electrophysiology was the introduction of massively parallel multichannel recording systems with hundreds and thousands of channels. However, the use of such system has been limited mostly to planar multielectrode arrays (MEAs) applied to in vitro slice or cell culture preparations. To find out how the brain structures interact in different emotional states one needs to simultaneously record from multiple brain regions in unanesthetized, behaving animals. Thus here, in order to develop a multichannel recording system that will be utilized in freely moving animals, we will put stress on the miniaturization of the headsets (including optical connectors), wireless technologies, and flexibility of the system which will allow for simultaneous recording and stimulation of channels selected on the fly.

To achieve these technological advances we plan to apply expertise gained in designing electronic systems for astrophysics[8,26,29], high energy physics[2,23,27], optical systems[7,21,30], and high-throughput neuro-electronis[5,16], and provide the best available solutions. We note that similar pilot devices have already been built and successfully applied by other groups[31], providing a clear proof of principle. Combination of these novel technologies promises an exceptional insight into the neuronal mechanisms by which emotions shape behavior.

Neuronal basis of emotional behaviors. Mice and rats have become the major species employed in experimental brain research. An important factor here was their susceptibility to genetic manipulations. However, the behavioral studies on these rodents have been simplified to the point that their ethological relevance suffers appreciably. A breakthrough has been offered by the development of IntelliCages that give a unique opportunity for continuous long-term monitoring of freely moving mice in cohorts, with minimal contact with an experimenter. Thus the IntelliCages opened new possibilities for modeling complex, long-lasting and alternating in time psychiatric conditions in an environment close to natural. We have been extensively involved in producing novel IntelliCage-based behavioral tests in which emotionally-valenced information shaped animal behavior, such as in appetitive and aversive learning (learning to approach reward versus to avoid danger[11], social interactions[9,22] and alcohol addiction[25], to name just a few examples.

The next major challenge is to decipher the brain circuits governing the observed behaviors. Pivotal in this respect are the methods allowing to measure and to manipulate neuronal activity in the brains of freely moving animals. In this project, neuronal c-Fos expression activity has been selected to serve as a way to evaluate specific engagement of neuronal circuits in a variety of behaviors. Such an approach is justified by a vast body of data that has been accumulated over the last 25 years about c-Fos expression and brain mapping as well as recent experiments showing that inactivation of c-Fos expressing neurons may permanently arise in particular types of memory[14].

The combination of IntelliCage-based models with remotely controlled optogenetics (with opsins expressed under c-fos promoter) and the use of multichannel electrophysiological system to simultaneously record and manipulate multiple cortical and subcortical sensory structures will allow to create extensive maps of functional connections in emotional brain.

Novel methods of data analysis and modeling. Increasing throughput of data coming from behavioral and electrophysiological experiments calls for new approaches to data analysis. With tens of thousands of correlated mice visits and terabytes of recorded simultaneous potentials from multiple structures, one must go beyond simple averaging or Fourier methods not mentioning the mundane procedures of storing and handling the data which on this scale take a completely new dimension. We will develop and apply new methods of analysis of both types of experimental data complementing it with data modeling approaches[3,17,20]. In particular, the analysis toolbox for IntelliCage data is under-developed as this is new technology. Even simple transfer of methods from spike train analysis (treating mice visits to corners as point events) leads to new and interesting results[9]. We will extend this analogy including the physical properties of the problem (e.g., finite time of visit corresponding to the refraction time, higher variability of mice behavior than that of neurons, etc.).

In CSD analysis of multielectrode LFP data we are a leading group[24]. We have developed a way to estimate activity of individual cell groups from multielectrode LFP recordings which will be put to use in the proposed project allowing better identification of functional changes induced by differing context and emotional valence of stimuli. Further, the application of reinforcement learning models in trial-by-trial data analysis[3] to both types of data (behavioral and electrophysiological) will lend a strong grip on the dynamics of learning in the conducted experiments. The amount and quality of data provided by the IntelliCage system and the proposed multielectrode setups gives a unique opportunity to put these techniques to use to quantify learning in a much greater detail than possible up to now.

3. SIGNIFICANCE OF THE PROJECT

Brain diseases impose a heavy social and economic burden to European society. A recent analysis of the European Brain Council (Cost of Disorders of the Brain in Europe, European J. Neurol., vol 12, Supplement 1, June 2005) estimated the total cost of brain diseases in Europe to be 386 Billion Euros. The importance of research on the brain function has been noted by the Future and Emerging Technologies office of the European Commission which recently awarded substantial funding to the Human Brain Project as a Flagship Project to foster global European research in brain studies.

In the current project, which is focused on coupling emotions to sensory information, we plan to tackle the medical challenges faced by the modern society using recent technological breakthroughs in behavioral experiments. In particular, we will work on miniaturization, remote control and integration of opto-electronics and electronics in order to use optogenetics in freely moving animals, as well as to construct multielectrodes to simultaneously stimulate and record activity of multiple brain regions in freely moving animals. The cutting-edge technology will be applied in research on neuronal basis of learning, addiction and empathy, giving a promising vistas for new treatment strategies of such psychiatric and psychological conditions as post-traumatic stress disorder, alcohol addiction or autism spectrum disorder.

Optogenetics offers a great technological breakthrough allowing to manipulate specific cell types within particular brain regions and altering specific projections[15]. The function of particular neurons in the brain can be dissected now with unprecedented accuracy due to cell type-specific promoters driving expression of light-sensitive opsins. Here we plan to focus on optogenetic modifications that will allow behavioral activation of neurons by using the c-Fos as a marker, that will subsequently expressed desired opsins. Furthermore, the application of the optogenetics so far has been limited to artificial experimental conditions where animal activity was very constrained, with mice being literally on an optic lead. We plan to apply optogenetics to the freely moving animals in the IntelliCages, which will allow for research on the neurobiology of the complex behaviors in close-to-ethological conditions.

Miniaturization, remote control and integration of opto-electronics and electronics to monitor and control behavior. Advancement in recording and stimulation techniques achieved in basic research on animals will be applicable in rapidly developing field of human brain-machine-interfacing and deep brain stimulation therapies.

Sensory processing, learning and memory. A consistent number of the most costly psychiatric disorders involve altered function of the amygdala. For instance, the pivotal role of amygdalar dysfunction has been shown in major depression, bipolar disorder, schizophrenia, posttraumatic stress disorder, and addiction. Moreover, the amygdala impairments seem to be crucial for disturbed social interaction and communication, symptoms of autism spectrum disorders. The impairments affect processing of both emotional valence and arousing strength of stimuli, and the effects can be seen at the sensory level, in appetitively and aversively motivated learning, as well as in social interaction and communication. The role of discrete amygdalar, thalamic and cortical subsystems in processing of emotional information has been established[12,28], however, the dynamics of their interaction – at the level of single neurons and of neuronal circuits – is still unclear. Further characterization of these circuits will help to design targeted, effective therapies. We are now exceptionally well positioned to tackle this problem. We have designed and validated a handful of behavioral tasks and electrophysiological experimental models that allow to reliably compare the brain activity during appetitively and aversively motivated learning, as well as responses to sensory stimuli and social interactions.

Alcohol addiction. Alcoholism comprises a collection of fundamental personal and socioeconomic problems including major financial burden for the health care and welfare systems, as well as emotional one for the affected individuals and their families. The World Health Organization estimates that there are 140 million people suffering from alcoholism worldwide. Despite multiple efforts to understand the biology of alcohol addiction, little progress has been made so far to develop effective therapies[6]. One of the main reasons for the lack of successful therapy is the lack of appropriate animal models capturing all major aspects of addicted state. Towards this aim we have recently developed multidimensional tests in the IntelliCage system[25] to model in mice addiction-like behaviors that resemble criteria for alcohol addiction as they are defined by the Development of Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V). In the current project we propose to go a step further and focus on neuronal basis of the addiction-related behaviors. This is a crucial step on the way towards new pharmacological treatments of addiction.

4. WORK PLAN

To investigate mechanisms underlying processing of emotional information we will perform the following work-packages (WP, Fig. 1):
WP 1. Obtaining and validation of optogenetic tools.
WP 2. Designing, construction and testing of miniaturized optoelectronic devices combining optogenetic stimulation with basic electrophysiological recording system to remotely control and regulate animal behavior.
WP 3. Designing, construction and testing of a multielectrode system for simultaneous recording and modification (electric and optogenetic stimulation) of activity in many brain regions in anesthetized and freely moving rats and mice.
WP 4. Application of miniaturized wireless optoelectronic devices in transgenic mice tested in the IntelliCages for: (1) appetitively and aversively motivated learning, (2) alcohol relapse and (3) socially transferred emotions.
WP 5. Application of multielectrode systems to non-anesthetized animals: (1) processing of visual information associated with alcohol reward; (2) processing of sensory information related to socially transferred emotions, as well as (3) storage and retrieval of sensory information in aversively motivated learning.
WP 6. Data analysis and modeling.

The details of the work-packages are specified in the following contributions.

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