OPTOGENETIC PACEMAKING TO REWIRE NEURONAL CIRCUITS

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1. Summary of the project

Acquired traumatic injuries to the central nervous system cause irreversible damage, leading to a dramatic decrease in the quality of life of affected individuals. The impact of these lesions can be extremely marked if key layers of information processing are injured, even though a great portion of affected neuronal circuits remain intact. The aim of the project was to lay the foundations of a novel pharmacological approach by defining a proof-of-principle based on optogenetics and photopharmacology (light-regulated drugs). The goal was to artificially maintain the activity of an injured neuronal circuit by selectively illuminating non-damaged neurons expressing ontogenetic molecules such as channelrhodopsin-2, or applying drugs, such as photoswitchable tethered ligands (PTLs). Upon photon absorption, these molecules change membrane potential and act as pacemakers of neuronal activity, thus artificially emulating the action of damaged synapses and keeping circuits operational.

We hypothesized that these conditions favoured restoration of nervous functions and tests were applied in vivo in *Xenopus tropicalis*. This species is advantageous: small size, fast experimental possibilities, a sequenced genome showing many genes conserved in humans, genetically modifiable, and well-established regenerative features in its CNS. Three specific goals were planned: i) establishment of new PTLs, ii) generation of transgenic lines of *X. tropicalis* expressing optogenetic switches in the CNS, and iii) effect on nervous regeneration of optically pacemaking non-damaged neurons, either by optogenetics or PTLs.

As a result, the project has: i) generated novel PTLs capable of controlling cellular/neuronal activity, ii) used transgenic lines of *X. tropicalis* to visualize the time course of reformation of a nervous pathway in vivo, iii) identified factors promoting the correct rewiring of nervous connectivity after injury, and iv) successfully applied PTLs to control neuronal functions. Preliminary results suggest a beneficial contribution of keeping a damaged circuit active through the use of PTLs.

In summary, our data supports the applicability of PTLs to the nervous system, by providing an exquisite control of drug activity. *X. tropicalis* has proved to be an excellent screening platform, acting as high-throughput component of mammalian animal models. The research groups led by Dr Artur Llobet (coordinator) at the Bellvitge Biomedical Research Institute (IDIBELL) and by Dr Pau Gorostiza at the Institute for Bioengineering of Catalonia (IBEC) ran the project.

2. Results

Coordinated efforts of both research groups participating in the project have made it possible to obtain a wide range of results, ranging from those that can be considered essentially methodological to findings with a clear translational component. The first part of the project was mainly methodological because it required the development of technical approaches, which five years ago were a complete challenge. The second half of the project illustrates the applicability of methodologies developed. Results obtained laid the foundations of the research that is currently taking place in our research groups.

We want to highlight that the project aimed at combining optogenetics and work with *Xenopus tropicalis,* which is an emerging model organism. There have been dramatic changes in both fields during the period 2010-2015 and we believe that the work performed has significantly contributed to the development of these fields. We have evidenced for the first time the applicability of a new range of molecules regulated by light to control neuronal activity and behaviour in living animals.

During the first half of the project, the efforts were centred on establishing methods. Dr. Gorostiza's group worked on the development of novel photoswitchable tethered ligands (PTLs). These compounds display a pharmacological activity that can be modulated by light. For example, upon shining blue light a drug can be inactive and in the presence of green light, active. In this way, it is possible to switch the activity of a certain drug on and off. The approach was developed for: i) peptides that interfere with endocytosis, which

were termed traffic-light peptides, and ii) an allosteric modulator of the glutamate receptor mGluR5, termed alloswitch-1.

In the same period, Dr. Llobet's group worked on the establishment of transgenic lines of *X. tropicalis*. The goal was to identify promoters capable of labelling neuronal subpopulations in the olfactory pathway through the use of genetically encoded fluorescent reporters (e.g. GFP). Olfactory neurons are dorsally located and are very accessible to all kinds of in vivo experiments. The transparency of tadpoles allows easy observation of neurons with very little invasiveness. Through a 10 Kb promoter of lhx2 gene it was possible to label a group of olfactory sensory neurons and M/T cells, which are responsible for conveying and receiving information in the olfactory bulb, respectively. The next step was to place an ontogenetic molecule such as ChR2 under the lhx2 promoter. ChR2 is a membrane ion channel that opens in the presence of blue light. As a result, neurons become active in the presence of blue light.

But, by the middle of the project, PTLs were providing very promising results in vitro. Since the time required to establish a transgenic line of *X. tropicalis* is 8-12 months we decided to push experiments involving the generated PTLs in wild-type animals rather than focusing on optogenetics. We thus moved all our efforts to the work on optopharmacology, which involves direct application of compounds without requiring genetic manipulation. We continued with the genetic modification of *X. tropicalis* but it was mostly used to generate animals expressing fluorescent reporters in neuronal populations. In this way, the project was centred on: i) the use of PTLs to control neuronal activity, and ii) the use of tadpoles expressing fluorescent reporters to assess the regeneration of neuronal connections.

We achieved great success when we were able to control the movement of tadpoles using alloswitch-1. Upon exposure to the drug, swimming activity became light dependent. In the presence of green light, tadpoles stopped moving but under blue light swimming reappeared. This was the first demonstration that a given behaviour can be externally switched on and off when the activity of the metabotropic glutamate receptor, mGluR5, is modulated through alloswitch-1. This experiment was a proof-of-principle of the main

hypothesis of the proposal. The next step was to use a similar approach to maintain the activity of a damaged circuit to evaluate if recovery from injury was improved.

To this aim we worked with NBT-GFP tadpoles. This transgenic line expresses GFP in neuronal processes. Since nerves show a strong fluorescence, it is possible to damage them and follow their re-formation in living animals. We observed that tadpoles reform an injured olfactory nerve in 4 days. Interestingly, reformation is usually correct but aberrant nerves form in ~10% of cases. Considering that aberrant connectivity is responsible for spasticity, loss of the control of reflexes, pain and other complications, we have an excellent model to investigate the factors that promote correct rewiring vs wrong rewiring of nervous connections after injury.

The next step was to apply a specific PTL in the olfactory bulb, termed TSP. This compound is active on AMPA receptors, which mediate most of the excitatory neurotransmission in the brain. We illuminated tadpoles with different light patterns over 4 days and analysed whether nerves improved their re-formation. Preliminary data shows that nerves re-form in two phases: reconnection and consolidation. Apparently, nerve reconnection is positively influenced by an enhanced activity of the olfactory bulb. The complexity of the experiments has precluded obtaining conclusive data but preliminary results are promising. Current laboratory efforts are in this direction.

We want to highlight that the research lines established have been very important to complement laboratory activities in the research groups of Drs. Llobet and Gorostiza. For example, work with PTLs has been key to gaining understanding of the molecular mechanisms of clathrin-mediated endocytosis of synaptic vesicles and *X. tropicalis* tadpoles have been extremely useful for in vivo studies of synapse elimination. Work initiated with this grant from the Fundació La Marató de TV3 continues and research groups maintain regular collaboration and meetings.

Finally, the outcome of the project could be summarized by the publication of 2 papers in high-impact journals: Nature Chemical Biology and Angewandte Chemie - International Edition, and 1 methodological paper in Journal of Biological Methods. Some of the results

obtained have been presented to international meetings, such as the European meeting of the Federation of Neurosciences (FENS) in the editions of 2012 and 2014.

3. Relevance and possible implications

We are very satisfied with the possible applicability of results obtained:

1) We have described that alloswitch-1 is capable of controlling animal motor behaviour with light. This finding is a proof-of-principle, showing the possibilities of photopharmacology. It is thus possible to control neuronal networks with the application of light to exogenous compounds (Pittolo et al., Nature Chemical Biology, 10 (10): 813-815 (2014)).

2) We have used *X. tropicalis* tadpoles to show the role of p4.2, a 20 amino acid derived from SPARC, which is a protein secreted by glia, in eliminating synapses. Synapse elimination plays a fundamental role in development, traumatic brain injuries and neurodegeneration. Our results open the possibility of using the peptide to perform a light-dependent, targeted elimination of aberrant connections established after brain injuries (López-Murcia et al., PNAS, 2014). At the beginning of the grant we showed a method to modify peptides of the clathrin pathway with light (Nevola et al., Angewandte Chemie - International Edition, 52 (30): 7704-7708 (2013)). In future experiments we could modify peptide p4.2 to make it light sensitive and use it to selectively eliminate undesired synaptic contacts.

3) Our methods show that we can do an in vivo pacemaking of neurons with PTLs. We are currently using TSP, a compound interacting with AMPA receptors. We are still evaluating the effects of TSP by changing light patterns, incubation time, etc. on synapse reformation in the olfactory bulb after injury. Our results already validate the feasibility of the approach. The pathway is open. Now is time to find the best possible molecules and study how to achieve maximum benefit from optopharmacological drugs.

4. References

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