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Report on training stay funded by NENS stipend

"Optogenetics as a tool for studying the serotonergic system"

Home laboratory: Prof. Dr. M. Bader, Max-Delbrueck-Center for Molecular Medicine, Berlin-Buch, Germany
Host laboratory: Prof. Dr. S. Kasparov, University of Bristol, UK
Duration of stay: 10-14 February; 11 June- 23 July 2013

The training stay in the laboratory of Dr. Kasparov was very fruitful. Before starting the actual work I had to complete Home Office course in order to obtain personal license to be able to perform experiments *in vivo* with mice.

The main technique I learned during the stay was optogenetics. Optogenetic technology allows modulation of the activity of certain neuronal populations with the light. To this end a light sensitive protein, for example channelrhodopsin-2 (ChR2) should be expressed in a defined cell and integrated in its plasma membrane. After activation by blue light, it forms a cation permeable channel, thereby depolarizes the cell and elicits action potentials.

I was interested in applying this technology to study the serotonergic neurons. For this purpose, I used two mouse lines which were transported to the University of Bristol prior my arrival to the host laboratory. The first model, TpH2-mhChR2-YFP BAC transgenic mouse, expresses an improved ChR2/EYFP fusion protein (mhChR2::YFP) directed to serotonergic neuronal populations by the *Tph2* promoter and allows direct activation of 5-HT neurons by blue light. The second mouse model, C57BL/6N-Tg(Tph2-iCre/ERT2)6Gloss/J, expresses Cre recombinase under the control of the mouse *Tph2* promoter. Local injections of viruses, expressing ChR2 upon Cre-recombinase mediated removal of a stop cassette, into the brain of this mouse line allows subregion-specific expression of CHR2.

Using these two models, I trained in:

- Stereotactical intracranial injections of virus
- Optrode implantation
- Light modulation

In particular, I performed acute optrode (15 pc x 400 µm core, 7 mm needle part, Figure 1A) implantation in the dorsolateral part of dorsal raphe (0,3mm lateral, -1mm caudal, 3mm ventral from lambda, Figure 1B) in four Tph2-ChR2 and one control mouse . Prior operation animals were anesthetized by intraperitoneal (i.p.)

injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and were followed and supplied if necessary with the additional dose of anesthetics during the whole procedures. Mouse was implanted with the EMG electrodes in the left hind paw. Recording of the EMG signal as a response to a coarse pressure (total force of 2,5 N, 10 sec) was performed in three conditions with 5 min interval: prior light stimulation, during light stimulation (blue light, 1/30 sec frequency, in total 20 sec, the last 10 sec pressure), post light stimulation (5 min after the light stimulation). I could observe that prior light stimulation both control and Tph2-ChR2 mice had similar EMG response to applied pressure (Figure 1C, D). However, during the light stimulation I could observe a nociceptive effect of stimulation of serotonergic neurons in Tph2-ChR2 but not in control mice (Figure 1C, D).

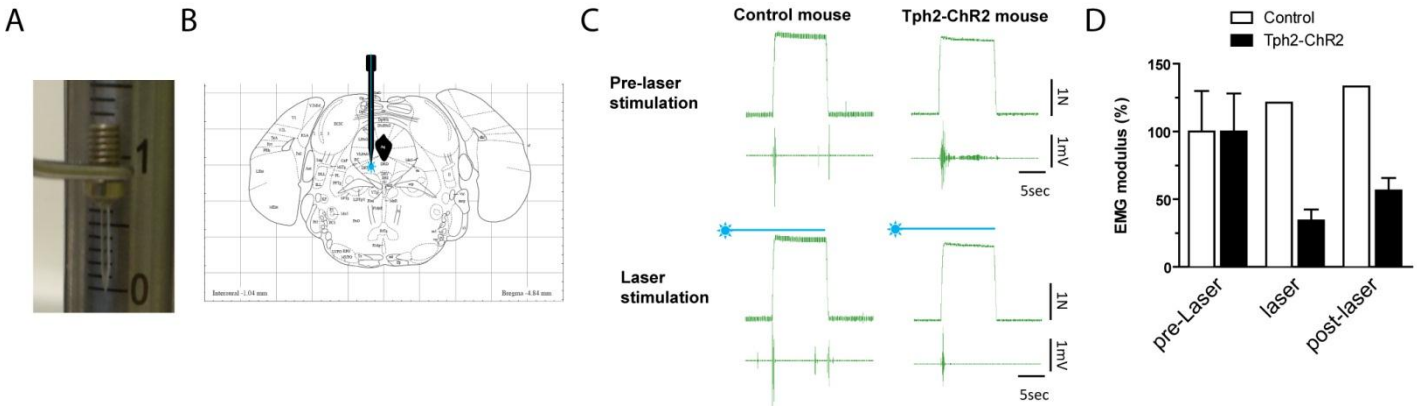

Figure 1. Nociceptive response in Tph2-ChR2 mice upon light activation of serotonergic neurons. **A**: Representative picture of used optrodes (15 pc x 400 µm core, 7 mm needle part). **B**: Schematic picture of the optrode implantation (dorsolateral dorsal raphe; 0,3mm lateral, -1mm caudal from lambda). **C**: representative pictures of applied stimulus (upper panel, 2,5 N of total force) and EMG recorded from left hind paw (lower panel) prior and during light stimulation in control and Tph2-Chr2 mice. **D**: EMG response during laser stimulation.

Next in order to be able to modulate activity of serotonergic neurons in Tph2-iCre mice I injected AAV (titre 10^{12}) carrying either ChR2 (experimental mice) or just GFP (control mice) in the dorsolateral part of dorsal raphe (0,3mm lateral, -1mm caudal from lambda) 0,5 µl at 2,8 mm and 3 mm ventral from lambda. Following four days of recovery tamoxifen was injected i.p. in concentration 120 mg/kg (in sesame oil) three times, one day apart. Two weeks after the first tamoxifen injection I tested anxiety-like phenotype of mice prior and after activation of DL part of dorsal raphe. During 20 min of marble burying test (MBT) the mice showed the tendency to burry less marbles (Figure 2A) under the light activation (pulse: 10 msec ON, 20 msec OFF, 20mV intensity; 30 sec ON, 60 sec break). During 5 min of open field test under light stimulation (as for MBT) mice had reduced track length (Figure 2B, C) as well as decreased average velocity (Figure 2D).

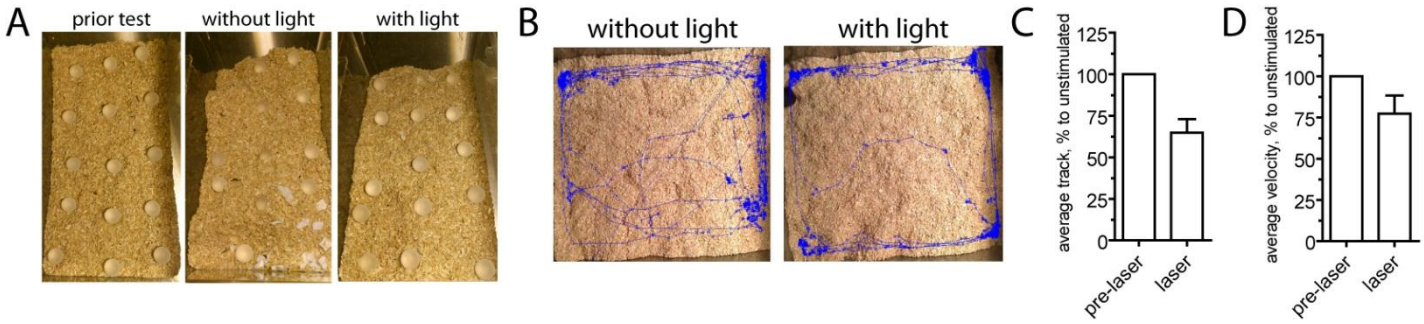

Figure 1. Anxiety-like behavior upon light activation of serotonergic neurons. **A**: Representative pictures of the arena in which marble burying test was performed. **B**: Schematic picture of the track from an Tph2-iCre mouse injected with AAV carrying ChR2 without and with light activation during 5 min of open field. **C** Average track length during 5 min of open field test of Tph2-iCre mouse injected with AAV carrying ChR2 without and with light activation. Calculated as % to the same mouse without light activation. **D**: Average velocity during 5 min of open field test of Tph2-iCre mouse injected with AAV carrying ChR2 without and with light activation. Calculated as % to the same mouse without light activation.