Light-inducible transcriptomic and epigenomic changes underlying brain plasticity in honey bees

Nils Becker[1], Robert Kucharski[2], Sylvain Foret[2], Ryszard Maleszka[2], Wolfgang Rössler[1]
[1]University of Würzburg, Biozentrum, Department of Behavioral Physiology & Sociobiology, Am Hubland, D-97074 Würzburg, Germany
[2] The Australian National University, Research School of Biology, Acton, ACT 0200, Australia

Introduction

A worker honey bee performs different tasks throughout its life span. The shift from one task to another, especially from in-hive tasks to outside tasks requires partly drastic adaptations to a different environment. One major difference between the in-hive environment and the outside is exposure to light as bees leave the dark hive (Fig. 1).

Light plays an essential role in the life of the forager bees in terms of visual navigation and spotting of food sources. It is apparent that for these important tasks foragers need to be optimally prepared by adaptive changes in the neuronal circuitry. Indeed, the transition from in-hive tasks to foraging is associated with remarkable changes in brain structure, and with synaptic plasticity[1]. Even the exposure of adult worker bees to artificial light is sufficient to induce structural synaptic plasticity in visual subcompartments of the mushroom bodies[2].

To investigate how synaptic plasticity in visual centers of the brain is controlled at the level of genes, we compared gene expression levels in the optic lobes of light exposed and dark kept honey bees via whole transcriptome sequencing (RNAseq) and quantitativ real time PCR (qPCR).

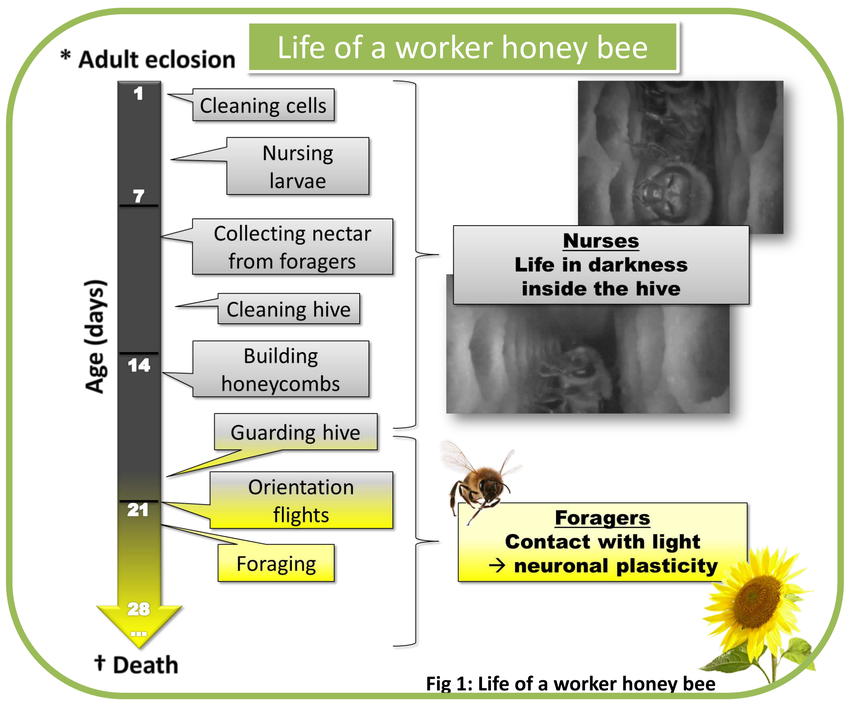


Fig 1: Life of a worker honey bee

Methods

Procedure

- Bees were collected directly after eclosion and were kept in cages in total darkness.
- At the age of **1** or **7** days the bees were exposed to 5 x 45 min pulses of artificial full-spectrum day-light whereas an age-matched control group was kept in darkness (Fig. 2).
- The bees were snap frozen directly after the last pulse of the light program (Fig. 2).
- Optic lobes of 5 or 3 brains were dissected and pooled for RNAseq (n=2) and qPCR (n=8) analysis (Fig. 3).

Light program, sampling point

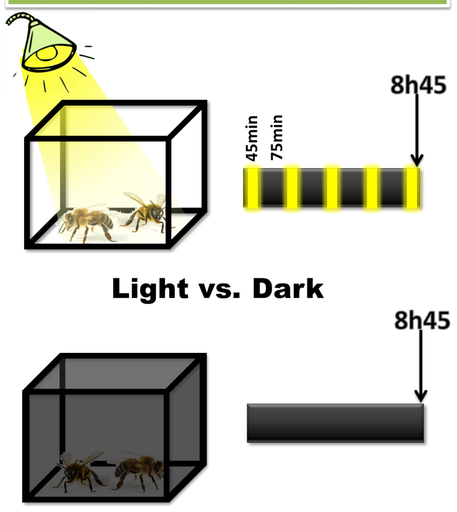


Fig 2: Light regime and sampling points

Brain dissection and pooling

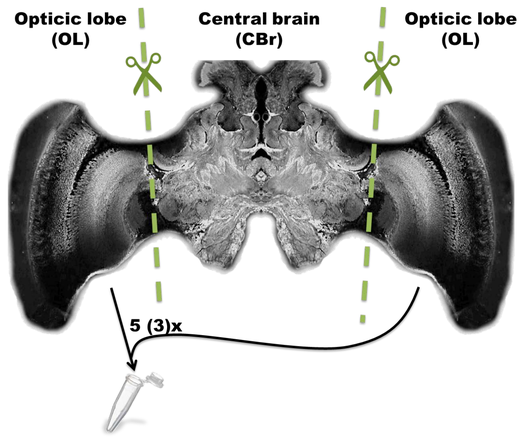


Fig 3: Brain dissection and pooling

RNAseq with 1 day old bees

→ Identification of differentially expressed genes in the optic lobes. See top 52 in Tab 1.

Validation with qPCR

→ Of so far 7 tested genes 3 could be confirmed for **1** and **7** day old bees by qPCR: *CNPY-1-like* (GB50831), *Ip3k1* (GB41220), *Trim71* (GB48462).

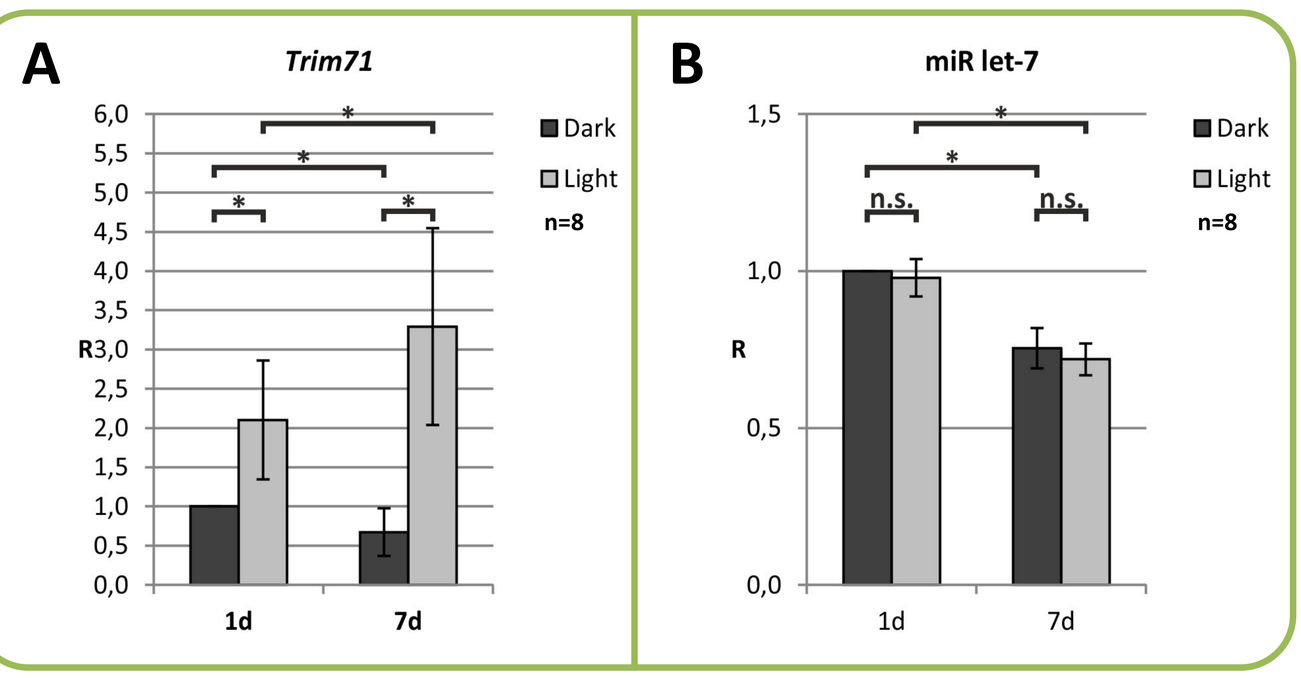
→ *Trim71* is regulated by the micro RNA let-7[3].

Results

Gene (BeeBase)	Light induced expression	Reproducibility in experiment 1 and 2	General function
GB55613*	Up	YES	Unknown
GB54595*	Down	YES	histone H3-K27 demethylase
GB45148	Up	YES	Vitamin A related
GB45147*	UP	YES	Vitamin A related
GB45024	UP	YES	Vitamin A related
GB45023	Up	YES	Vitamin A related
GB41220*	Up	YES	IP3 kinase
GB42985	Up	Weaker induction in 2	pyruvate lyase
GB41002	Up	YES	timeless
GB43805	Up	YES	metallo-endopeptidase
GB46312	Up	Weaker induction in 2	cuticular protein
GB55396	Up	YES	Unknown
GB50831*	Up	YES	neurite outgrowth enhancer
GB48462	Up	YES	E3 ubiquitin protein ligase
GB43732	Up	YES	serine/threonine-protein kinase
GB44871	Up	YES	glycine N-methyltransferase
GB47279	Up	YES	cytochrome P450
GB43514	Up	YES	lipase, member H
GB49843	Up	YES	neuronal PAS domain protein
GB54962	Up	YES	Unknown
GB42197	Up	YES	Unknown
GB47484	Up	YES	Histone-like?
GB47382	Up	YES	Histone H4
GB41720	Up	YES	Pleckstrin-like
GB48492*	UP	YES	Take-out
GB42467	UP	YES	phototransduction
GB42673	UP	Weaker induction in 1	RDH10/retinol dehydrogenase
GB43649	UP	YES	chloride channel
GB55043	UP	YES	kainate glutamate receptor
GB43823*	UP	YES	chemosensory protein CSP1
GB41593*	UP	YES	cell migration regulator
GB40046	UP	YES	neuronal mt transport protein
GB55050	UP	YES	transmembrane transporter
GB41277*	UP	YES	light-induced ubiquitylation
GB45365	UP	YES	transmembrane transporter
GB47948	UP	YES	myosin light chain kinase
GB41720	UP	YES	Plekstrin-like
GB51220	UP	YES	cytochrome b-561
GB40552	UP	YES	Unknown
GB45910	UP	YES	Crystallin
GB45906	UP	YES	Crystallin 2
GB46514 GB46515*	UP	YES	Acetylcholinesterase (both loci)
GB44095	UP	YES	Cation channel
GB42227	UP	Weaker induction in 1	Homeobox related
GB51580*	UP	YES	acyl-CoA synthetase
GB41339	UP	Weaker induction in 2	acid phosphatase
GB52448	UP	YES	Unknown
GB53210	UP	YES	Unknown
GB47697 GB47697	UP	YES	Unknown (both loci)
GB41709	UP	YES	Unknown

Table 1: Top 52 differentially expressed genes in the optic lobes identified by RNAseq
These 52 genes show the biggest difference in their expression level between 1 day old light exposed-, and 1 day old dark kept bees. Genes with an epigenetic function are highlighted in green; *methylated genes;

Fig 4: Relative gene expression ratios (R) by qPCR analysis
Relative gene expression ratios (R) were calibrated against 1 day old dark kept bees (1d Dark). **(A)** shows R for *Trim71* (GB48462), and **(B)** shows R for its negative regulator micro RNA let-7. 1d: 1day old bees; 7d: 7 day old bees;



Conclusion and Outlook

- RNAseq revealed genes which show a difference in their expression levels in the honey bees' optic lobes between the two treatment groups (Tab. 1).
- Several of these genes (i.e. *CNPY-1-like* (GB50831), *Ip3k1* (GB41220)) have been associated with neuronal plasticity and therefore are good candidates to play a role in light induced synaptic plasticity in the honey bee brain[4,5].
- Some genes like *Trim71* (GB48462) and *histone demethylase UTY-like* (GB54595) could rather contribute to an adaptation to a changed environment by epigenetic mechanisms which influence the transcription of a broad number of genes simultaneously.
- qPCR studies could confirm some of the tested genes found with RNAseq, but failed to confirm others. This might be due to the partly different bee handling and different honey bee races used in both applications as RNAseq was performed in Canberra, Australia with *A.m. var. ligustica*, whereas qPCR experiments took place in Würzburg, Germany with *A.m. var. carnica*.
- Future studies will aim at finding a causality between expression of the candidate genes, synaptic plasticity and subsequent behavior. For this issue pharmacological or siRNA knockdown experiments are planned.

References: [1]Groh et al. 2012, J Comp Neurol; [2]Christina Scholl et al. 2014, Developmental Neurobiology; [3]Lin et al. 2007, Mol Biol Evol; [4]Windhort et al. 2012, Cellular Signalling; [5]Bornhauser et al. 2003, J Biological Chemistry