1.3 Disturbed energy metabolism after neonicotinoid exposure as cause of altered homing flight activity of honey bees

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Abstract

Neonicotinoids are implicated in the decline of honey bee populations. As nicotinic acetylcholine receptor agonists they disturb acetylcholine receptor signalling, leading to neurotoxicity. Several behavioural studies have shown links between neonicotinoid exposure and adverse effects on foraging activity, homing flight performance and reproduction but the molecular aspects underlying these effects are not well understood. We have elucidated the link between homing flight performance and expression of selected transcripts in the brain of honey bees. Besides possible neurotoxic effects of neonicotinoids leading to disturbed orientation and therefore prolongation of homing flight time, neonicotinoids may also disturb energy metabolism, also causing longer homing flight time. To test the second hypothesis, pollen foragers were fitted with RFID chips, exposed to 1 ng/bee thiamethoxam in single bee feeding and 10 bee-feeding settings and released 1km from the hive. The homing flight time was monitored. In the evening, all returned foragers were collected and stored at -80°C until further analysis. After homing flight data analysis, brain RNA of fast returning controls and slow returning exposed foragers of both feeding strategies was isolated and energy metabolism transcript expression was analysed using quantitative PCR. We analysed expression of *cox 5a*, *cox 5b*, *cox 6c* and *cox 17*, all transcripts of complex IV and *ndufb-7*, part of complex I of the oxidative phosphorylation. Comparing all generated expression data demonstrated that data of the 1 bee-feeding approach scatter less than data of the 10 bee-feeding approach. This finding clearly shows the unequal distribution of sugar syrup between caged honey bees due to trophallaxis. In addition, no significant changes were seen for all analysed transcripts of the 10 bee-feeding approach due to strong scattering of data and small sample size. In contrast, the expression of *cox 5a* and *cox 17* was significantly altered in foragers exposed to 1 ng/bee thiamethoxam in the single bee feeding approach and there was a strong correlation between the down-regulation of *cox 17* and the prolongation of homing flight time. In summary, this small study has two major findings. First, feeding strategy is very important as regards significant effects and single bee feeding approach should be used in future studies. Second, there is a clear link between prolongation of homing flight time and energy metabolism. Therefore, longer homing flight time may be not only due to disturbed orientation but also due to a lack of energy. Further studies are needed to analyse this point in more detail.

1.4 Gene expreslsion analysis in honey bees as novel tool for assessing effects of plant protection products

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Abstract

To date, molecular approaches are not well established in bee research. This holds in particular for investigation into molecular adverse effects of plant protection products (PPPs). Furthermore, molecular tools in standardized, replicable experimental setups are not yet incorporated in standard protocols within the framework of OECD guidelines or other test guidelines for assessing effects and risks of PPPs. In the last few years, we applied gene expression analysis techniques, such as RT-qPCR and RNA-sequencing, to evaluate effects of a series of important PPPs, including insecticides, fungicides or PPPs used in organic farming. We performed short-term laboratory exposures of honey bee workers for 24 to 72 hours and assessed molecular responses in the brain. Our analyses demonstrate that environmental concentrations of PPPs cause significant alteration in gene expression of target genes that are associated with alteration of important physiological pathways. The