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Disturbed energy metabolism after neonicotinoid exposure in relation to altered homing flight performance in honey bees

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Introduction

Neonicotinoids are suspected to be implicated in the decline of honey bee populations. As nicotinic acetylcholine receptor agonists they can disturb acetylcholine receptor signalling, leading to neurotoxicity. Several studies have shown links between neonicotinoid exposure and adverse effects on various behavioral traits, including foraging activity [1], homing flight performance [2] and reproduction [3]. Despite this large impact on sublethal traits, the molecular aspects underlying these effects are not well understood up to day. In the present pilot study, we have elucidated the link between homing flight performance and expression of selected transcripts in the brain of honey bee pollen foragers. Besides potential sub-lethal neurotoxic effects of neonicotinoids leading to disorientation which might adversely effect homing flight time and/or rate, neonicotinoids may also disturb bees`energy metabolism hence causing longer homing flight time or exhaustion. To analyze these effects, RFID experiments were conducted to monitor homing flight time and homing rate. Additionally, returned foragers were collected to analyze gene expression in their brains. We compared the expression of selected target transcripts for energy metabolism between fast returning controls and slow returning thiamethoxam of exposed bees (as a worst case scenario). Further, we analyzed the effect of feeding strategy on data scattering. Therefore, pollen foragers were exposed to thiamethoxam in single bee feeding and group feeding (10 foragers per cage) approach.

Materials and Methods

Homing flight activity (RFID)
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 using RFID system [4]. In the evening after daily bee flight activity, all returned foragers were collected and stored at -80°C until further analysis.
Homing flight times
For each returned pollen forager corresponding individual flight time was determined using specific software [4]. An overview of homing flight times is shown in Fig.1.
Gene expression 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 analyzed using quantitative PCR according to standardized protocols [5]. We analyzed the 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.
Data analysis
One-way ANOVA and Bonferroni's multiple comparison test were applied to compare means of exposed and unexposed samples. Data are shown as means ± standard error of means. Statistically significant limits were set as follow: one asterisk at 0.05 > p > 0.01, two asterisks at 0.01 > p > 0.001 and three asterisks at 0.001 > p > 0.0001. Obtained transcriptional data were imported into MEV 4.9 (Multi Experiment Viewer) software to draw heat maps.

Results and Discussion

We analyzed the 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. A comparison of all generated expression data demonstrated that data of the single bee-feeding approach scatter less than data of the ten bee-feeding approach, particularly in the control samples (Fig. 2). This finding clearly demonstrates the unequal distribution of sugar syrup (over- and under-dosing) between caged honey bees due to trophallaxis.
No significant changes were seen for all analyzed transcripts of the ten bee-feeding approach due to strong scattering of data and small sample size. In contrast, the expression of *cox 5a* and *cox 17* (Fig. 3A) was significantly altered in pollen foragers exposed to 1 ng/bee thiamethoxam in the single bee feeding approach. The expression of *cox5a* was up-regulated upon exposure to thiamethoxam whereas the expression of *cox17* was down-regulated. These expressional changes of transcripts of the oxidative phosphorylation may have adverse effects on the homing flight behavior. The homing flight time could be extended by reduced energy supply. An overview of all obtained transcriptional changes is summarized in a heat map (Fig. 3B).

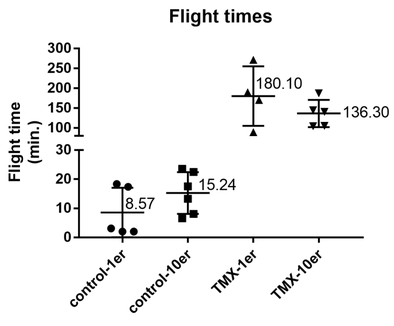

Fig. 1: Homing flight times of selected foragers. Shown are the individual flight times depicted in minutes for various treatments.

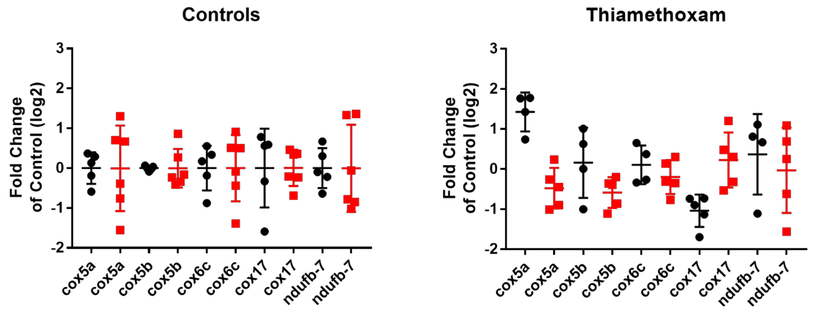

Fig. 2: Comparison of gene expression between single bee feeding and group feeding approach. The expression of *cox5a*, *cox5b*, *cox6c*, *cox17* and *ndufb-7* was analyzed in controls and thiamethoxam-exposed pollen foragers in the single bee (black circles ●) and group feeding (red squares ■) approach.

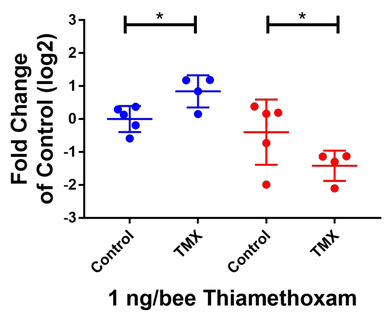

Fig. 3A: Expression of *cox5a* (blue) and *cox17* (red) in pollen foragers exposed to 1 ng/bee thiamethoxam. Significant changes are indicated by asterisk.

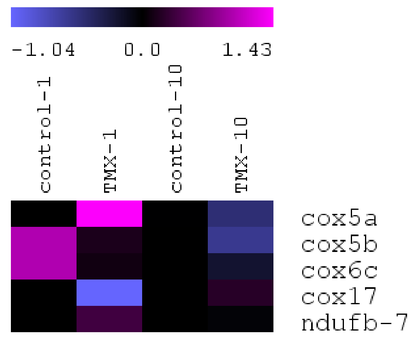

Fig.3B: Heat map summarizing all obtained expressional data. Blue indicating down-regulation and pink indicating up-regulation of the analyzed transcript

Conclusion

In summary, this small pilot study has two major findings. First, feeding strategy is very important in regard to analyzing sublethal and significant effects. Therefore, single bee feeding approach should be used in future studies as single dosing of bees is not trophallaxis dependent, hence an exact dose per bee can be determined. Second, there is a clear link between prolongation of homing flight time and energy metabolism indicated by the changed expression of transcripts of the oxidative phosphorylation, namely *cox5a* and *cox17*. Therefore, longer homing flight time may be not only due to disturbed orientation but also due to a lack of energy. As exposed and returned pollen foragers were sampled in the evening after daily bee flight, there is an delay in time between entering the hive (homing) and status of gene expression of the sampled bees. In future studies, returning pollen foragers should be sampled and stored at -80°C directly after returning to the hive to avoid this delay. As we analyzed only a few transcripts of the energy metabolism, we may have missed certain effects. Hence, global gene expression should be analyzed in future studies for example by next generation sequencing to get a complete picture about transcriptional changes due to thiamethoxam exposure in correlation to homing flight time.

References

[1] Tison L, Hahn ML, Holtz S, Rößner A, Greggers U, Bischoff G, Menzel R. 2016. Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field. Environ Sci Technol 5;50(13):7218-27.
[2] Matsumoto T. 2013. Reduction in homing flights in the honey bee Apis mellifera after a sublethal dose of neonicotinoid insecticides. Bull Insectol 66 (1): 1-9.
[3] Williams GR, Troxler A, Retschnig G, Roth K, Yañez O, Shutler D, Neumann P, Gauthier L. 2015. Neonicotinoid pesticides severely affect honey bee queens. Sci Rep. 5: 14621.
[4] Julie Fourrier . RFID ring-test protocol 2016-2019, unpublished.
[5] Christen V, Krebs J, Bünter I, Fent K. 2019. Biopesticide spinosad induces transcriptional alterations in genes associated with energy production in honey bees (Apis mellifera) at sublethal concentrations. J Hazard Mater 378:120736.

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