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Conditioning your bee in one, two, three! – Effect of a neonicotinoid on learning performance in honey bees

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Introduction

It has been shown that pesticides, especially neonicotinoids, impair honey bee learning, memory and locomotion even at sub-lethal doses (for an overview, see *Blacquière et al. 2012*). Assessment of these effects required either Proboscis Extension Response (PER)-conditioning (*Williamson & Wright 2013*), tagging bees with RFID-chips (*Schneider et al. 2012*) or video-analysis (*Teeters et al. 2012*). All these methods have limitations, e.g. excessive manual labor, costs or a lack of standardization. To overcome these restrictions, we developed the semi-automatic conditioning device APIS (**A**utomatic **P**erformance **I**ndex **S**ystem, *Kirkerud et al. 2013*). It allows to conveniently quantify effects of chemicals such as pesticides by introducing bees into a walking chamber where they learn to associate odours with mild electric shocks. The bees' movement is continuously sampled by infrared photosensors, from which learning, memory and locomotion is automatically assessed. The advantage of our device is the integration of all controlling elements in a single, easy to use device, operated through a customised program. Here we present data on the impact of the neonicotinoid Thiamethoxam upon the bees learning and memory in APIS.

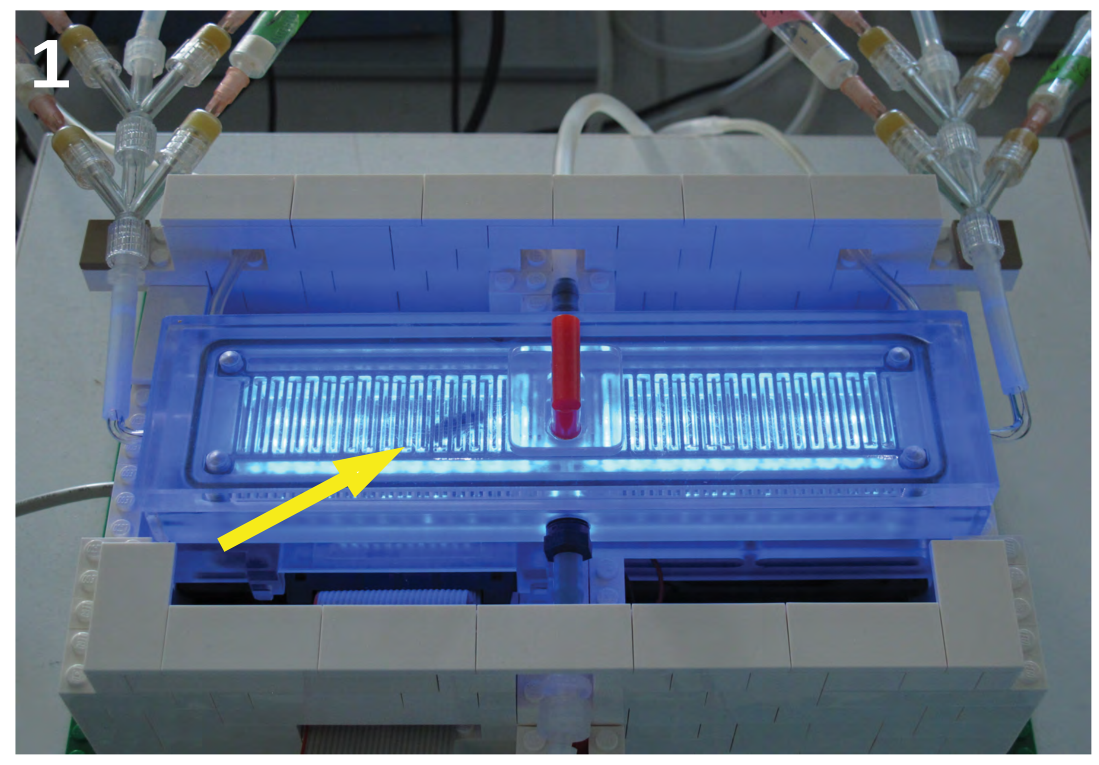

Figure 1: APIS is 148 mm long, 20 mm wide and 6 mm deep, enabling unhindered walking on floor or ceiling of a honey bee (yellow arrow). The interior is covered with an electrifiable metallic grid, and infrared-sensors constantly record the bees' position and behaviour. Odours were injected from the distal ends of the chamber via computer-controlled valves. Bees had to learn that one odour predicted electric shocks and the other odour not, and that the shocks could be avoided by escaping to the opposite side of the injected odour.

Conclusion

We use APIS (Automatic Performance Index System) to investigate effects of pesticides on learning and memory of honey bees.

Treatment with sub-lethal doses of the neonicotinoid Thiamethoxam impairs odour discrimination (Figure 3 & 4).

However, bees compensate the effect within 24 hrs (Figure 5) - further investigations are needed for better understanding of this compensation.

Methods

Treatment & conditioning

Individual bees were caught and kept for 24 hrs in custom-made containers where they could feed on:
- 70µl of sucrose solution (control group)
- 0.03 ng Thiamethoxam in 70µl of sucrose solution (low dose)
- 0.3 ng Thiamethoxam in 70µl of sucrose solution (medium dose)
- 3 ng Thiamethoxam in 70µl of sucrose solution (high dose)

3 ng equals 60% of the reported oral LD_{50} of Thiamethoxam (*AFSSA 2009*). After 24 hrs, bees were conditioned in a eight-trial differential conditioning paradigm: they received 4 times one odour (CS+) paired with shock pulses (10V, 8.5mA, 2Hz) and four times another odour (CS-) not paired with shocks (see *Figure 2*). The shocks stopped when the bee moved to the side opposite of the odour injection and resumed on its return during odour stimulation.
3 minutes after end of conditioning, a short-term memory test was performed by giving the two odours without shock. After that, bees were transferred to their custom-built containers where they spent the night until their memory was tested again 24 hours after conditioning.

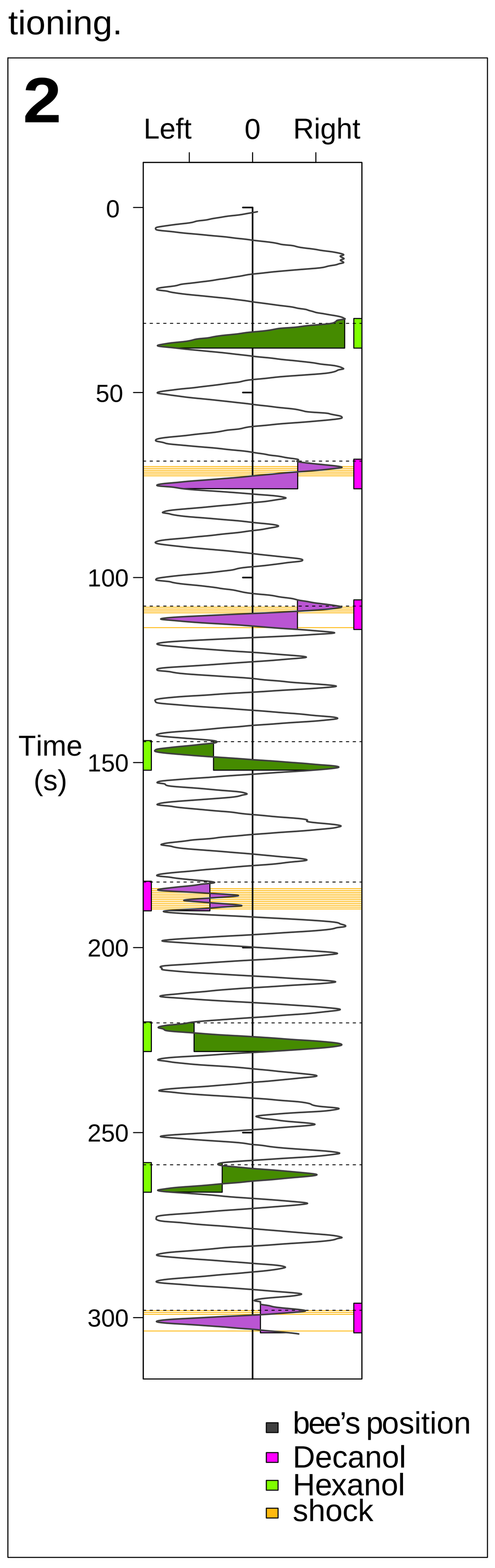

Figure 2: Example trace of a bee during conditioning.

Data analysis

A customised script was written in R to analyse the acquired tracking data of each individual bee (see *Figure 2* for an example trace).
Avoidance was assessed by calculating the integral of the position trace with respect to the bees' starting position for each odour stimulus period (highlighted as coloured polygons in *Figure 2*). Only the period between odour onset and shock onset (2s) was used for analysis. This served as a general descriptor of the bees' response.
The bees' avoidance response was fitted by a linear mixed model, with stimulus (shocked odour or neutral odour, respectively), trial and treatment as predictors and bee identity as random effect to account for repeated measurements.
Fitted means and 95% credible intervals of the quantified variables were estimated using the "fixef" function and the "sim" function from the R-packages "lme4" and "arm", respectively.
Hypothesis tests were executed by calculating the probabilities that the fitted mean of one group recided within the credible interval of another.

Thiamethoxam-bees show impaired learning and memory

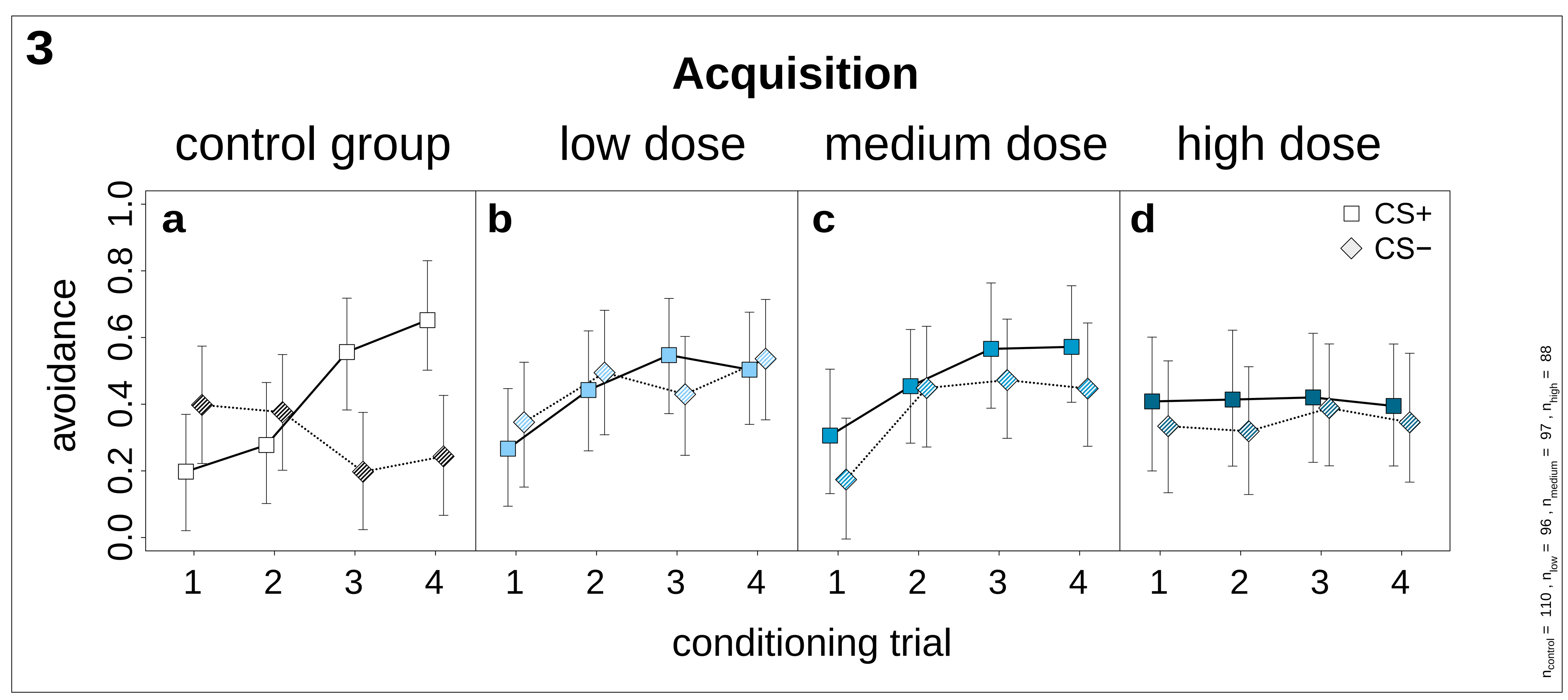

Figure 3: Avoidance to shocked odour (CS+) and safe odour (CS-) during conditioning phase. Control bees (panel a) successfully avoided the shocked odour to a greater extent than the safe odour after the second trial, reflected in the divergence of the response curves. The bees treated with either low or medium doses of Thiamethoxam, however, showed an increased avoidance response to both odours over the 4 trials, and no clear differentiation (panel b and c). Bees treated with high dose of Thiamethoxam showed neither an increase in avoidance response over training trials nor differentiation between CS+ and CS- (panel d).

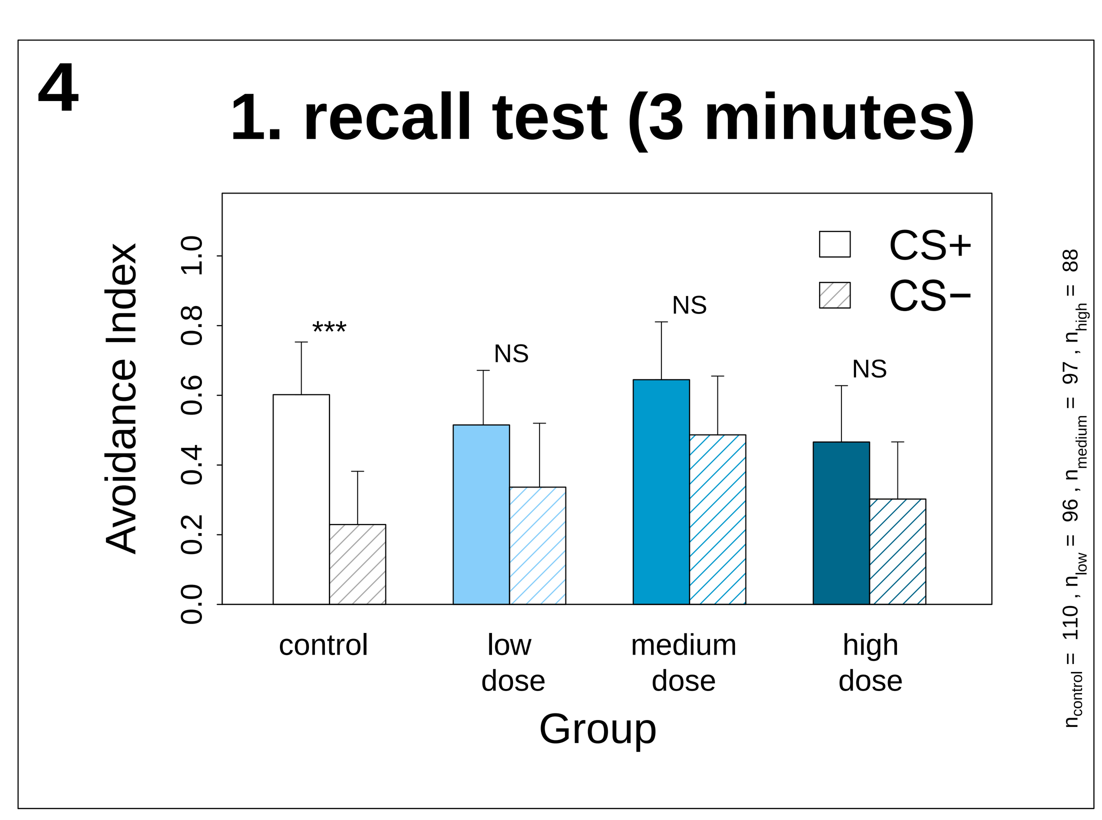

Figure 4: During a memory test 3 minutes after end of conditioning, the control bees avoided the CS+ more than the CS-, whereas bees from all three pesticide treatment groups did not differentiate in their avoidance.

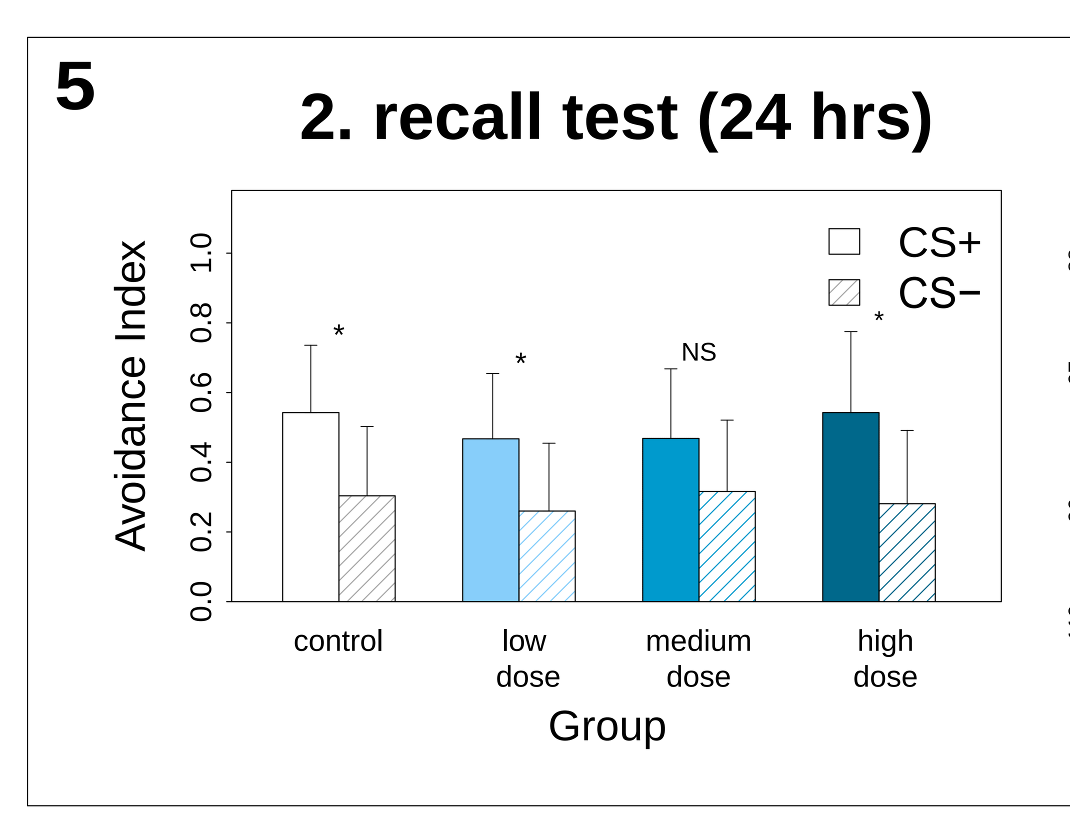

Figure 5: However, the bees that received low and high Thiamethoxam treatment showed similar performance as the control bees during the long-term memory test 24 hours later. This suggests possible compensatory mechanisms and that the pesticide-treated bees might have learned the classical association (even though they did not show a differentiation during conditioning and the 1. recall test).

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