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Assessment of acute sublethal effects of clothianidin on motor function of honeybee workers using video-tracking analysis

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A B S T R A C T

Sublethal impacts of pesticides on the locomotor activity might occur to different degrees and could escape visual observation. Therefore, our objective is the utilization of video-tracking to quantify how the acute oral exposure to different doses (0.1–2 ng/bee) of the neonicotinoid "clothianidin" influences the locomotor activity of honeybees in a time course experiment. The total distance moved, resting time as well as the duration and frequency of bouts of laying upside down are measured.

Our results show that bees exposed to acute sublethal doses of clothianidin exhibit a significant increase in the total distance moved after 30 and 60 min of the treatment at the highest dose (2 ng/bee). Nevertheless, a reduction of the total distance is observed at this dose 90 min post-treatment compared to the distance of the same group after 30 min, where the treated bees show an arched abdomen and start to lose their postural control. The treated bees with 1 ng clothianidin show a significant increase in total distance moved over the experimental period. Moreover, a reduction in the resting time and increase of the duration and frequency of bouts of laying upside down at these doses are found. Furthermore, significant effects on the tested parameters are observed at the dose (0.5 ng/bee) first at 60 min post-treatment compared to untreated bees. The lowest dose (0.1 ng/bee) has non-significant effects on the motor activity of honeybees compared to untreated bees over the experimental period.

1. Introduction

The bioavailability of neonicotinoids is considered to be at a high level throughout the year depending on the respective pest control profiles in a wide range of agricultural and horticultural plants (Bonmatin et al., 2015). Neonicotinoids exhibit long persistency in soil, e.g. the half-life of clothianidin in soil is between 148 and 6900 days (Rexrode et al., 2003). Moreover, their high ability to diffuse throughout the plants due to their systemic properties allow them to spread through the xylem in growing plants. Thus, the uptake by crops and wild plants as well as the diffusion in several matrices lead to contaminated nectar, pollen (Cutler et al., 2014; Botias et al., 2015) and water (Joachimsmeier et al., 2012; Samson-Robert et al., 2014) which were collected by bee foragers and transported to the nest.

However, under field-realistic conditions, little information is known about the level of oral or contact exposure either via contaminated food (nectar, pollen, and water) or other surfaces and matrices (reviewed by Alkassab and Kirchner, 2017). Therefore, the exposure of non-target organisms, e.g. *Apis* and non-*Apis* bees, to pesticides through the residues at different concentrations is currently a vital issue in the risk assessment process (Spurgeon et al., 2016).

It has been reported that the field-relevant concentrations of these pesticides ranged between 1–10 µg/kg depending on the most frequently detected residues in pollen and nectar in the seed-treated crops (Cresswell, 2011; Botias et al., 2015; Kunz et al., 2015). Recently, Rundlöf et al. (2015) reported that the detected residues of clothianidin in the pollen or nectar of seed-treated canola ranged between 6.7–16 µg/L in nectar and 6.6–23 µg/kg in pollen. Subsequently, the exposure of pollinators to neonicotinoids at sublethal concentrations is not excluded. Nowadays, increasing attention is paid to sublethal effects due to their subsequent impacts on the development of the insect pollinators (Schneider et al., 2012; Arce et al., 2016). Among them, *Apis* and non-*Apis* bees are considered as the most important pollinators worldwide, as they play a key role in the maintenance of biodiversity and food production (Kleijn et al., 2015; Potts et al., 2016).

Moreover, the concerns about adverse effects of neonicotinoids on insect pollinators have led to two-year restrictions on the use of three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) as seed treatment in bee-attractive crops in the European Union to evaluate their potential environmental impacts (European Commission, 2013).

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The neonicotinoids' mode of action is known as acetylcholine mimics acting as agonists of nicotinic acetylcholine receptors (nAChRs), which in turn activate persistently the cholinergic receptors leading to hyperexcitation and eventually death (Jeschke and Nauen, 2008). Moreover, Palmer et al. (2013) reported that the exposure to neonicotinoids causes a depolarization-block of neuronal firing and inhibition in the nicotinic responses.

Although imidacloprid and clothianidin were classified in the same group "N-nitroguanidines," clothianidin acts as a super agonist compared to imidacloprid serving as a partial agonist (Brown et al., 2006). However, imidacloprid-related effects on honeybees have been well investigated by many researchers (e.g. Decourtye et al., 2004; Eiri and Nieh, 2012; Blanken et al., 2015; Wegener et al., 2016), whereas few studies were carried out to assess clothianidin-related effects on honeybees (e.g. Cutler et al., 2014; Rundlöf et al., 2015)

Recently, most ecotoxicological studies have been carried out to test the effects of sublethal exposure to pesticides on various endpoints, especially the behavioral endpoints, due to the sensitivity and effectiveness of these endpoints in the ecological risk assessment (see review Alkassab and Kirchner, 2017). Motion activities play an important role for *Apis* and non-*Apis* bees, since they are involved in different behavioral aspects, e.g. foraging and communication. Some studies looked at the influence of xenobiotics on bees' mobility by investigating the locomotion modifications and foraging activity (Williamson et al., 2014; Schneider et al., 2012). Special attention was given to assessing sublethal effects on foraging behavior, which plays a key role in the development and fitness of the colony (Sherman and Visscher, 2002). In relation to the effects on motor function, imidacloprid was reported to reduce the foraging activity of honeybees as well as bumblebees (Decourtye et al., 2004; Feltham et al., 2014; Arce et al., 2016), delay a forager's return visit to the feeder (Yang et al., 2008; Schneider et al., 2012), impair navigation and homing flights (Schneider et al., 2012; Fischer et al., 2014), and lead to fewer waggle dance circuits (Eiri and Nieh, 2012). Moreover, bees treated with imidacloprid exhibit trembling or may decrease the frequency of waggle dancing upon their return to the nest (Kirchner, 1999).

Few studies have been conducted to investigate the related effects of these pesticides on the motion activities of honeybees because there are sometimes limitations to determining and quantifying the effects using an efficient tool. A preliminary visual observation was performed, showing an increased motor activity of bees contact-treated with imidacloprid at 1.25 ng/bee even after 15 min of the treatment, whereas impairment of the movement was observed at doses ≥ 5 ng/bee (Lambin et al., 2001). On the other hand, bees treated orally with clothianidin exhibited no changes in walking, sitting and flying but spent more time laying on their backs (upside down) after 24 h at the dose 0.34 ng/bee (Williamson et al., 2014).

Nevertheless, sublethal impacts on the locomotor activity might occur at different degrees and could escape visual observation. However, if the foragers are foraging permanently from a monoculture of crops seed-treated with neonicotinoids, they might be exposed to sublethal doses from the contaminated nectar during their foraging trips. Thus, our objective is to use the video-tracking method to quantify how the acute oral exposure to clothianidin influences the motor activity of honeybees in the time course experiment. The total distance, resting time and the period of laying upside down are measured by analysis of the video-recordings. To our knowledge, this study provides the first detailed data about clothianidin-related effects on the locomotor activity of honeybees, where low sublethal doses were also tested to correspond to the realistic field exposure levels.

2. Materials and methods

2.1. Pesticide

Clothianidin was obtained in dry powder (99% purity) from Bayer Crop Science, Germany. The solubility of clothianidin in water is high (327 mg/L), but due to the difficulty in dissolving the crystals in water, a stock solution was pre-dissolved in acetone with a concentration of 200 mg/L. Then, the previous solution was mixed with distilled water, thereby gaining a solution of 1 mg/L. For acute oral treatment, dilution series were implemented to obtain concentrations in a 2 M sucrose solution of 5, 25, 50, 100 µg a.i./kg syrup which are equivalent to dosages per 20 mg syrup of 0.1, 0.5, 1 and 2 ng/bee. Untreated bees were fed a 2 M sucrose solution, in addition to an equal amount of solvent 0.0025–0.05%. Fresh solutions were prepared weekly from frozen aliquots of the stock solution. The sucrose solution was prepared with distilled water and kept in the refrigerator at 2–4 °C.

2.2. Bees

Winter workers of the honeybee *Apis mellifera* were collected from a single healthy colony form an apiary at the Ruhr University, Germany. A treatment against *Varroa destructor* was performed in the late summer with 80% formic acid. The colony comprised about 7000 workers and a fertile 1-year-old queen.

2.3. Experimental protocols

For each dose, 24 individual winter bees were randomly collected from the colony, then placed in the arena (a 9 cm Petri dish with filter paper and four small holes on the side for ventilation), transferred to the laboratory and left to acclimatize for 30 min under red light. Since the bees need only a short time to find the food, we offered the sugar solution (20 mg in the cap of a 1.5 ml centrifuge tube) after 25 min of acclimatization for the three pairs of bees, i.e. six Petri dishes, and observed them; when two bees (one treatment and on control) ate the total amount at the same time (± 1 min) within the 5 min, the caps were removed, and the time of the experiment started. Within the next 30 min, the next three pairs were collected and left to acclimatize. All treatments were recorded at the same time of day, 10:00–15:00 o′clock.

The time course experiment was conducted to analyze the locomotor activity of bees, where pairwise (treated and untreated bees) video recording (Canon camera; Powershot SX500 IS; 30 photo/s) was carried out for 10 min during 30–40, 60–70, 90–100 min post-treatment.

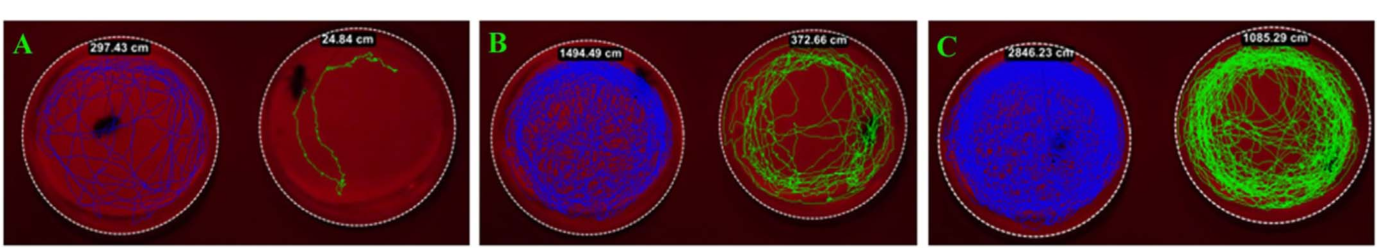

Fig. 1. Example of the tracking process and the distance moved inside the tested arena. Treated honeybee (blue track) with 2 ng after 30 min of the treatment compared with untreated bee (green track) throughout the 10 min of video recording. (A) total distance moved after one minute, (B) total distance moved after five minutes, (C) total distance moved after ten minutes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In total, 1440 min (12 pairwise bees × 3 periods after treatment × 4 doses) of video recording of 96 bees were assessed. The total distance moved (cm) throughout the arena (Fig. 1a–c), resting time (total stopped period throughout the 10 min of video recording) of twenty-four bees per dose (twelve control and twelve treatment) were analyzed using an automatic video tracking program (Kinovea, experimental version; 0.8.23). The program provides ca. 18,000 values, one for each image and cumulative total distance at the end. The total duration of laying upside down and the frequency of bouts were measured visually from playbacks of the recorded video using the Kenova stopwatch.

2.4. Statistical analysis

The One-way ANOVA was used to assess the effect of treatments on the total distance moved compared to the control, since the data was normally distributed. *Post hoc* pairwise comparisons were corrected with a Bonferroni correction.

Furthermore, the Kruskal-Wallis test with the Bonferroni procedure was performed to examine the differences between the control and the treatments for resting time and total duration of laying upside down and frequency of bouts, where the data was not normally distributed.

All statistical analyses were performed using SPSS.24 software (SPSS, Chicago, IL, USA). The level of significance in all tests was set to $p < 0.05$.

3. Results

3.1. Effect on total distance moved

Within the experimental period, i.e. 120 min (30 min acclimatization and 90 min after treatments), no bees died, therefore all tested doses are considered as sublethal for the bees.

The exposure to acute sublethal doses of clothianidin except for the lowest dose (0.1 ng/bee) yields a stimulatory effect, where the distances moved by the treated bees increase in the tested arena over the time of the experiment compared to untreated bees (Fig. 2). No significant differences occur between the distances moved by untreated bees over the time of the experiment, where the average distances moved are (mean ± SE) 1386.23 ± 78.95 cm after 30 min, 1428 ± 90.22 cm after 60 min and 1229.58 ± 84.15 cm after 90 min (One-way ANOVA, $p > 0.05$, $n = 48$).

Bees exposed to acute sublethal doses of clothianidin exhibit a significant increase in the total distance moved directly after 30 min of the treatment at 1 ng/bee (mean ± SE; 2268.52 ± 214.43 cm) and 2 ng/bee (mean ± SE; 2170.55 ± 119.49 cm; One-way ANOVA, $p < 0.01$, $n = 12$ for treatment and 48 for control).

A delayed effect of clothianidin on locomotor activity at 0.5 ng/bee is observed, where the total distance moved increased significantly compared to untreated bees first after 60 min (2177.71 ± 190.72 cm) and 90 min (1964.21 ± 151.30; One-way ANOVA, $p < 0.01$, $n = 12$ for treatment and 48 for control).

On the other hand, a reduction of the total distance moved is observed in bees treated with the highest dose (2 ng/bee), presenting a shorter distance moved 90 min post-treatment (1669.49 ± 165.83) compared to the distance of the same group after 30 min (2170.55 ± 119.49 cm; One-way ANOVA, $p = 0.02$, $n = 12$). At this time point, the treated bees show an arched abdomen and start to lose their postural control.

The lowest dose (0.1 ng/bee) has no effects on the distance moved compared to untreated bees, where the average distances moved in the tested arena were 1317.61 ± 261.57 cm after 30 min, 1269.87 ± 214.92 cm after 60 min, and 1203.27 ± 245.29 cm after 90 min.

3.2. Effect on resting time

The resting time of treated bees is affected by all tested doses except 0.1 ng/bee, where significant reductions in the resting time of treated bees compared to untreated bees are observed (Kruskal-Wallis test, $p < 0.05$, $n = 12$ for treatment and 48 for control; Fig. 3). There are non-significant differences in the resting time of the untreated bees, where the median of resting time is 164.05 s (interquartile range IQR; 143.69) after 30 min, 102.72 s (IQR; 168.54) after 60 min and 140.76 s (IQR;181.03) after 90 min (Kruskal-Wallis test, $p > 0.05$, $n = 48$). Bees treated with 0.5 ng show significantly shorter resting times compared to untreated bees first 60 min after treatment (median (IQR); 15.15 s

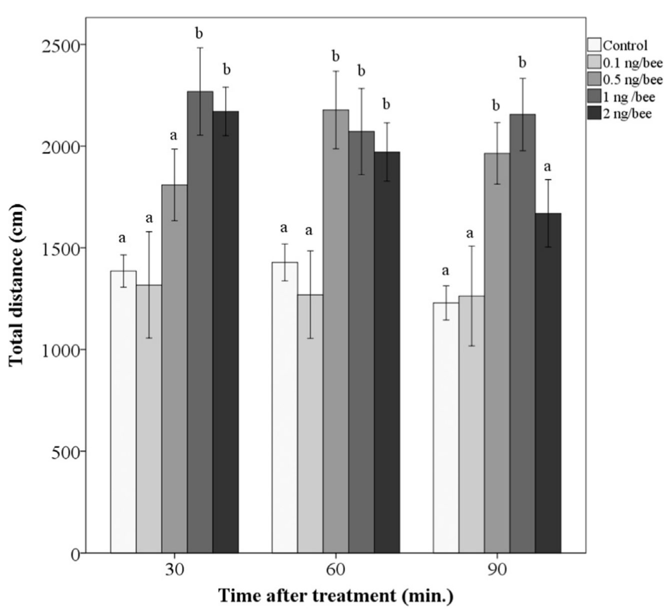

Fig. 2. Total distance moved by honeybee workers throughout the 10 min of video recording after the time course experiment ($n = 48$ for control and $n = 12$ for each treatment). The bars represent mean ± SE. Different letters indicate the significant differences between the control group and treatments (One-way ANOVA, $p < 0.05$).

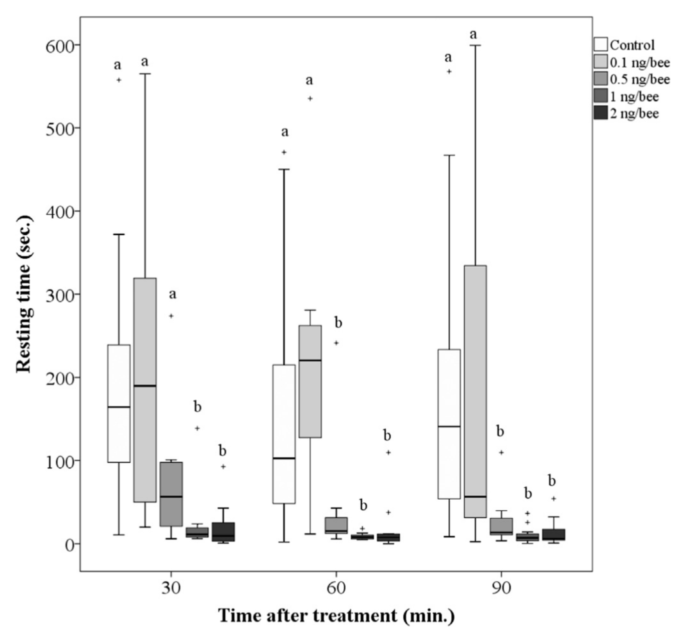

Fig. 3. Resting time (total stopped period) of honeybee workers throughout the 10 min of video recording after the time course experiment ($n = 48$ for control and $n = 12$ for each treatment). Treatments are shown as boxplots with the median, the edges of the box indicate the 25th and 75th percentiles. Outliers are shown as crosses. Different letters indicate the significant differences between the control group and the treatments (Kruskal-Wallis test, $p < 0.05$).

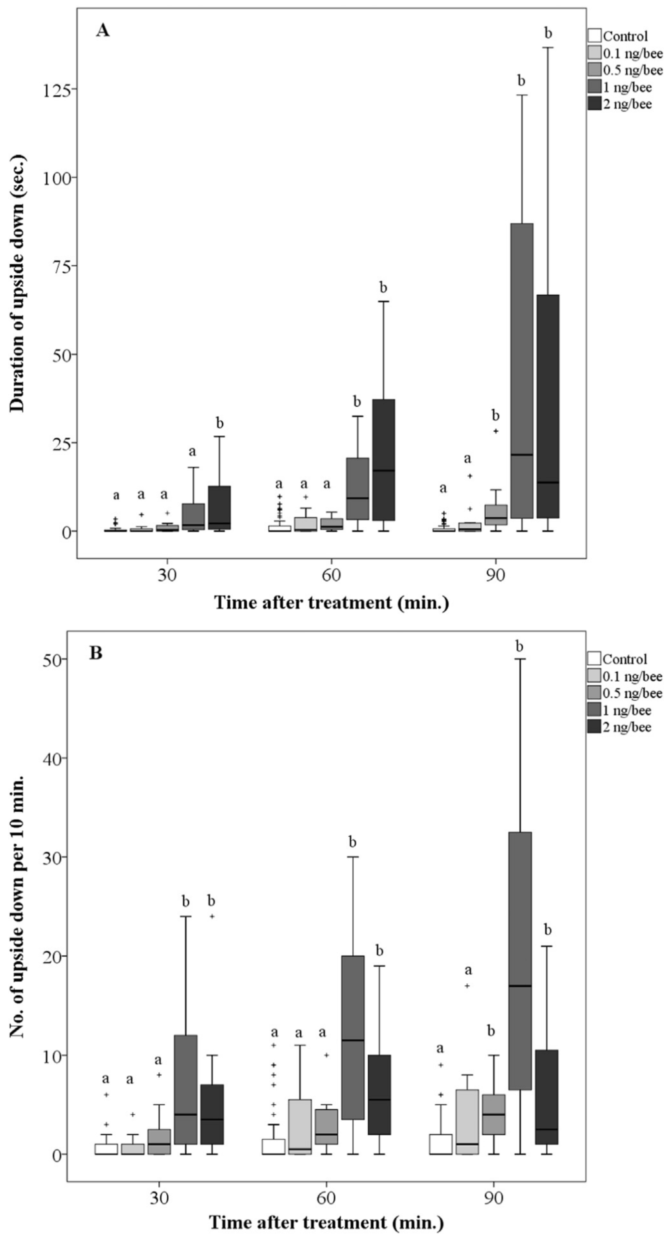

Fig. 4. (A) Duration of upside down period, (B) the number of upside down bouts during the 10 min of video recording after the time course experiment (N = 48 for control and N = 12 for each treatment). Treatments are shown as boxplots with the median, the edges of the box indicate the 25th and 75th percentiles. Outliers are shown as crosses. Different letters indicate the significant differences between the control group and the treatments (Kruskal-Wallis test, $p < 0.05$).

(21.83); Kruskal-Wallis test, $p < 0.05$, n = 48 for control and 12 for treatment). The bees treated with 1 and 2 ng have very short resting times over the experimental period, where the median resting time is 11.13 s (IQR; 13.52). for the treatment with 1 ng and 9.36 s (IQR; 23.68) for the treatment with 2 ng after 30 min (Kruskal-Wallis test, $p < 0.05$, n = 48 for control and 12 for treatment). Regarding behavior, these clearly reflect the hyperactivity effects of clothianidin on the exposed bees even after a short ingestion time.

3.3. Effect on postural control

The oral exposure to clothianidin at sublethal doses (≥ 0.5 ng/bee) causes a significant increase of the total duration of upside down and the frequency of bouts compared to the control group (Fig. 4a and b).

A delayed effect of clothianidin on postural control is observed at 0.5 ng/bee, where treated bees spent significantly more time laying upside down (median (IQR)); 3.67 s (IQR; 7.67) compared to untreated bees only after 90 min of the treatment (Kruskal-Wallis test, $p < 0.05$; n = 48 for control and 12 for treatment).

The time spent upside down is significantly longer for the bees treated with 1 ng over the experimental period (median (IQR); 1.72 (7.74), 9.33 (20.08) and 21.58 (89.08) s. after 30, 60 and 90 min of the treatment, respectively) compared to the control group (Kruskal-Wallis test, $p < 0.05$; n = 48 for control and 12 for treatment). Likewise, bees treated with 2 ng show longer upside down periods, where the median time spent upside down is 2.17 (IQR; 16.37), 17.15 (IQR; 34.63), and 13.75 (IQR; 76.84) s. after 30, 60 and 90 min of the treatment, respectively compared to untreated bees (Kruskal-Wallis test, $p < 0.05$; n = 48 for control and 12 for treatment).

On the other hand, bees treated with 0.5 ng/bee exhibit significantly more frequent bouts of laying upside down only 90 min post-treatment compared to untreated bees, where the median is 4.0 times during the 10 min of video recording (Kruskal-Wallis test, $p < 0.05$; n = 48 for control and 12 for treatment). Bees treated with 1 and 2 ng/bee spent significantly more time laying upside down compared to untreated bees over the experimental period, where the median ranged between 4.0 times after 30 min to 17.0 times after 90 min (Kruskal-Wallis test, $p < 0.05$; n = 48 for control and 12 for treatment).

The lowest dose (0.1 ng/bee) had no effect on the postural control compared to untreated bees over the time of the experiment (Kruskal-Wallis test, $p > 0.05$; n = 48 for control and 12 for treatment).

4. Discussion

In our time course experiment, we provide the first detailed information on how a sublethal dose of clothianidin can alter the locomotor activity of honeybees, where different phases of honeybees' intoxication are observed. Using the video tracking software, we could supply more accurate measurements of the total distance moved compared to visual observation, since any changes in the distance and velocity are easy to identify with the software. The analysis of the bees' responses within a short period after exposure provides useful information in terms of the duration of a single foraging trip.

Since the oral exposure through pollen and nectar is considered to be the most frequent exposure pathway, it is important in the ecotoxicological studies to investigate the effects of field-relevant doses on the tested organism. EFSA (2012) reported that the amount of a contaminant that is consumed by the bee, i.e. the effective dose, of a forager bee depends on: (1) energy demand for flying per time unit, i.e. 8–12 mg sugar/hour considering 50% sugar content (Balderrama et al., 1992) (2) the sugar concentration of the collected nectar which could range between 15–84% among crops (Knopper et al., 2016). In the case of oilseed rape, the sugar concentration in the flower's nectar ranged between 14.6–40.6% (Alkassab et al., 2016), which means that the bees will consume 13.6–17.6 mg sugar/hour at 15% sugar content and 9.6–13.6 mg sugar/hour at 40% sugar content. Winston (1987) reported the flight duration of a trip (between 30 and 80 min). Therefore, we estimate that the exposure doses during a foraging trip could range between 0.02–0.2 ng/bee, depending on the most frequently detected residues in the nectar of clothianidin seed-treated crops (1–10 µg/kg). Nevertheless, the exposure level will be much higher during multiple foraging trips, where a forager bee can perform up to 10 trips per day (Winston, 1987). In our experiment, we chose a range of doses (0.1–2 ng/bee) to cover the field-relevant dose.

As the results show, the lowest dose of clothianidin (0.1 ng/bee), which could be considered comparable to a field-realistic dose, had no recognizable effects under laboratory conditions on the tested motor functions of honeybees. Nevertheless, we show here that acute exposure to clothianidin at sublethal doses > 0.1 ng/bee lead to changes in the motion behavior of the treated bees in a time- and dose-dependent way.

Therefore, if residues in flowers were to be higher than 10 μg/kg especially in the case of summer oilseed rape (Rundlöf et al., 2015); adverse effects on the bee's behavior are not excluded.

The significant increase in total distance moved and the decrease of resting time directly after 30 min of the treatment with ≥ 1 ng/bee showed the strong effect and fast diffusion of clothianidin in the bodies of treated bees to achieve the hyperactivity phase within a short period. This hyperactivity is an initial result of the clothianidin effect on the nervous system, caused by an activation of cholinergic receptors leading to an impairment of normal neuronal excitability (Palmer et al., 2013). A similar effect was reported after acute topical exposure of honeybees to imidacloprid at 1.25 ng/bee, where a stimulatory effect on locomotor activity after 15 min of administration was observed (Lambin et al., 2001). On the other hand, imidacloprid-treated bees at higher applied doses (≥ 5 ng/bee) exhibited a significant reduction of movement and an increase of the immobility phase 30 min post-treatment (Lambin et al., 2001). An explanation for these contradictory effects is that the low doses induce an activation of cholinergic receptors leading to a stimulatory effect on locomotor activity, whereas high doses induce a non-specific toxic impact in the whole body including hypoactivity and paralysis.

Another study showed that the topical treatment of young honeybees with a high dose (3.8 ng/bee) of thiamethoxam induced locomotor deficits 48 h after exposure (Charreton et al., 2015). Williamson et al. (2014) reported that the oral exposure to imidacloprid at a high sublethal dose (3.7 ng/bee over 24 h) also caused a reduction of movement in the tested adult honeybees. Nevertheless, the bees were treated in our experiment with a single sublethal dose, whereas Williamson et al. exposed the bees to pesticides through an ad libitum feeding approach over 24 h, i.e. the bees ingested the pesticides gradually over the 24 h and could metabolize part of the taken amount, wherefore their dose remained too low to induce changes in the walking, flying, or standing still behavior.

In our time course experiment, we observed that the bees treated with 2 ng/bee began to exhibit symptoms of locomotor deficits, where the total distance moved 90 min post-treatment decreased in comparison with the distance of the same group after 30 min. This result indicates that the exposure time also plays a key role in the intoxication: A short-term exposure will have a stimulatory effect on locomotor activity, whereas long-term exposure will generate impairment effects, i.e. a reduction in the foraging activity (Feltham et al., 2014; Tosi et al., 2017).

Nevertheless, a delayed effect of clothianidin on locomotor activity at 0.5 ng was observed, where the total distance moved increased non-significantly after 30 min but significantly compared to untreated bees after 60 min and 90 min. This delayed effect of the low dose indicates that a longer time is required until the excitability of receptors will be achieved and thus enhance the toxic effects (Palmer et al., 2013). Schneider et al. (2012) also reported delayed effects on the activity of bees treated with a low dose (0.05 ng/bee), where treated bees on the next day showed less time for a foraging trip as well as spending shorter periods of time at the feeder. However, Williamson et al. (2014) reported that the exposure to imidacloprid, thiamethoxam and clothianidin at sublethal doses (ca. 0.4–0.5 ng/bee over 24 h) did not significantly alter the walking, flying, or standing still behavior of the tested honeybees. This could be due to the different exposure way.

Furthermore, impairment of the righting reflex after turning upside down was observed, where the bees treated with ≥ 0.5 ng/bee exhibited an increase of the number and duration of upside down bouts compared to the control group. Moreover, bees treated with high doses ≥ 1 ng/bee after 60 min showed an abnormal walking performance with spastic gait drags and an arched abdomen. Likewise, Schneider et al. (2012) observed an abnormal activity of the bees treated with 1 ng and 2 ng clothianidin. They reported that treated bees displayed movement with an arched abdomen, then falling over and lying on their back. Another study conducted by Williamson et al. (2014) also showed that bees exposed to imidacloprid, thiamethoxam and clothianidin at sublethal doses (ca. 0.4–0.5 ng/bee over 24 h) lost their ability to right themselves after falling over. This abnormality in the motion performance is due to a lack of motor coordination. However, neonicotinoids as neurotoxins are described to act as agonists on nicotinic acetylcholine receptors (nAChRs) (Thany, 2009); their effects in relation to the insects' nAChRs are very complex, including various behavioral and physiological aspects (Salgado and Saar, 2004). Neuronal nAChRs are present in various regions in the insect nervous tissue (Bai et al., 1991). In honeybees, the distribution of nAChRs in the ventral unpaired median (VUM) neurons has yet to be studied, but it has been considered that VUM-neurons are homologous to dorsal unpaired median (DUM) neurons (Schröter et al., 2007). Thany (2009) reported at least three distinct nAChR subtypes on cockroach DUM-neurons which are sensitive to clothianidin. These neurons play an important role in different aspects of the motor function of several insect species, e.g. cockroaches (Benzidane et al., 2010) and locusts (Duch et al., 1999) and could be correlated with the lack of motor coordination.

Thus, it could also be that different subtypes of nAChRs are abundant in various motoneurons and therefore could serve as targets for the effects of neonicotinoids to modify the motor performance of honeybees. However, further studies are required on this topic. Moreover, it is possible that the modifications in the motor performance of neonicotinoid-treated bees correlate with other impacts on foraging activity including the impairment of navigation and homing flights (Fischer et al., 2014), a decreased frequency of waggle dancing (Kirchner, 1999; Eiri and Nieh, 2012), a reduction of the visit rate to the feeder (Yang et al., 2008; Schneider et al., 2012) and a reduction of the foraging activity reported in honeybees as well as bumblebees (Decourtye et al., 2004; Feltham et al., 2014). Blanken et al. (2015) reported that chronic exposure to imidacloprid reduces the flight capacity of the tested bees, which could correlate with the effects of neonicotinoids on the motoneurons and muscle system as we show here. Most recently, Tosi et al. (2017) found that the acute exposure to thiamethoxam increased flight duration and distance, whereas the chronic exposure reduced the flight duration and distance.

In conclusion, our results strongly suggest that the usage of a video-tracking method is an accurate method to investigate sublethal effects of pesticides and measure any locomotive changes in honeybees.

Moreover, since the acute sublethal impacts of the clothianidin on the motor functions of honey bees have not previously been investigated, the results presented here provide the first detailed information in that regard. Nevertheless, it would be useful for future studies to investigate the chronic sublethal effects throughout long-term exposure, since the exposure time could have a key role in the toxicity profile of the pesticides (Feltham et al., 2014; Alkassab and Kirchner, 2016; Arce et al., 2016). Moreover, a comparative study of sublethal effects of pesticides and the sensitivity of other bee species such as bumblebees and solitary bees should be conducted. Additionally, more information about the distribution of neonicotinoid-sensitive receptors in motoneurons and neuromuscular junctions is required.

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