To our knowledge, the identification of a strong walking deficit in young bees after contact exposure to an SLD_{48h} of thiamethoxam has never been reported before. At 1 ng/bee, no walking deficit was detected [22], whereas the SLD_{48h} used in the present study (3.8 ng/bee) clearly impairs locomotion. At an oral SLD of 1.3 ng/bee (i.e. 25–33 fold lower than the LD_{50}, [34, 50]), a fraction of bees also fail to perform their normal homing flight [7]. The neonicotinoids primary mode of action has been studied in honeybee neurons and is compatible with neurotoxic actions on the nervous cholinergic system [51–54]. Several nicotinic receptor subtypes are involved in complex behaviors and memory processes, and may be differentially altered by sublethal doses of neonicotinoids [55]. Fipronil did not affect locomotion at the SLD_{48h} (see also [10]), but surprisingly induced significant mortality at 5 days after exposure. We did not observe this phenomenon with other insecticides (see also [34] for cypermethrin and thiamethoxam), strongly suggesting that it is insecticide-specific. Whereas SLD_{48h} of all three pyrethroids and the neonicotinoid clearly impair the distance covered by bees, analysis of more subtle behaviors could resolve undetected fipronil-induced deficits. For instance, longer recording durations could reveal subtle alterations in inter-individual interactions, grooming behaviors and time spent near a food source [25, 29, 56].

In conclusion, the locomotion test allowed the identification of important deficits in young bees. It revealed that these effects are insecticide-specific and cannot be simply extrapolated from LD_{50} values. This assay could thus be used as a preliminary analysis before implementing more sophisticated homing-flight experiments or more subtle memory or orientation tests [7, 18, 57]. It is worth noting that such a laboratory locomotion test is formalized, standardized and displays the least sensitivity to seasonal, phenologic, weather and landscape variations [58]. The recent temporary ban of neonicotinoids in Europe, due to their high toxicity towards the honeybee, calls for alternative methods of pest control, which thus become a priority for modern agriculture, but also a societal issue. Pyrethroids, that already represent one fifth of the global pesticides market [59], have already been used as an alternative solution to restricted or banned pesticides. Their toxicity identified using a simple locomotion test suggests that pyrethroids can be as toxic as a neonicotinoid towards bees, and therefore implies that the molecules to be used would need to be carefully selected.

Supporting Information

S1 Fig. Individual distances covered by bees in each group. Individual distances (in meters) covered by control bees and exposed bees are plotted as white and grey dots respectively, for each insecticide. Average distances (± S.E.M) are shown for each modality. Mean distances in control groups were similar (3.14 ± 0.24 m, 3.26 ± 0.29 m, 3.50 ± 0.27 m, 3.22 ± 0.42 m, 3.37 ± 0.35 m for cypermethrin, tau-fluvalinate, tetramethrin, thiamethoxam and fipronil respectively, see S2 Table for statistics). Mean distances after exposure to a SLD_{48h} were 0.93 ± 0.27 m, 1.40 ± 0.31 m, 1.85 ± 0.26 m, 1.35 ± 0.25 m, 3.22 ± 0.33 m for cypermethrin, tau-fluvalinate, tetramethrin, thiamethoxam and fipronil respectively (see Fig 3 for numbers of bees in each group).
(TIF)

S2 Fig. Effect size estimates for variations of distance covered by individuals (a) among control groups of the five trials and (b) between treatment and control groups. Horizontal bars stand for the 95% confidence intervals returned by the post-hoc multiple pairwise comparisons. The vertical dashed line indicates the no-effect level.
(TIF)

S3 Fig. Observed average instantaneous speed during the 3-min recording time as compared to the 95% confidence limits (shaded area) for an expected steady-state average speed. The instantaneous speed during the 3-min recording time was measured in a pilot study performed on 80 non-exposed individual bees. The mean instantaneous speed (mm.s-1) was averaged per 10-s slots among the 80 individual bees. We compared the observed averages with the 95% confidence interval (CI) range expected under the hypothesis of steady-state average instantaneous speed. The 95% CI range was obtained from a bootstrapping procedure whereby the speed data were randomly shuffled along the temporal axis. We recomputed 200 random rearrangements of the raw database and then extracted the average speed values at the 2.5% and 97.5% ranks for each 10s step to delineate the 95% CI. Average speed tended to decrease as time lapses, with observed values being closer to (or slightly above) the upper CI boundary during the first minute of recording, and closer to the lower CI boundary during the third minute of recording. At the very last 10s recording slot, average speed fell below the expected steady-state confidence limits. We therefore considered that the 3-min standard recording duration was appropriate to cover statistically steady-state locomotion samples in our control-*vs*.-treated experiments.
(TIF)

S1 Table. Mortality tests for the determination of sublethal doses.
(XLSX)

S2 Table. Statistical outputs of LM and LMM models comparing distances covered by individuals (m) among control groups of the five trials, and between treatments. The post-hoc pairwise comparisons indicate that only the fipronil treatment did not significantly affect distances. See S2 Fig for effect size estimates.
(DOCX)

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Author Contributions

Conceived and designed the experiments: MC AD MH CC. Performed the experiments: MC CC. Analyzed the data: MC AD MH JCS PC CC. Contributed reagents/materials/analysis tools: MC AD MH GR CC. Wrote the paper: MC AD MH GR JCS PC CC.

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