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)

Acknowledgments

We thank Michel Pélissier for his technical help with the locomotion arena and the laboratory beekeepers who provided domestic honeybees. We thank Dr Cynthia Mc Donnell for carefully reading the manuscript.

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.

References

1. Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, Vanengelsdorp D, et al. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS One. 2010; 5(3):e9754. Epub 2010/03/25. doi: 10.1371/journal.pone.0009754 PMID: 20333298; PubMed Central PMCID: PMC2841636.
2. Chauzat MP, Faucon JP. Pesticide residues in beeswax samples collected from honey bee colonies (Apis mellifera L.) in France. Pest Management Science. 2007; 63(11):1100–6. PMID: 17879980
3. Chauzat MP, Cauquil L, Roy L, Franco S, Hendrikx P, Ribiere-Chabert M. Demographics of the European apicultural industry. PLoS One. 2013; 8(11):e79018. Epub 2013/11/16. doi: 10.1371/journal.pone.0079018 PONE-D-13-02274 [pii]. PMID: 24236084; PubMed Central PMCID: PMC3827320.
4. VanEngelsdorp D, Speybroeck N, Evans JD, Nguyen BK, Mullin C, Frazier M, et al. Weighing risk factors associated with bee colony collapse disorder by classification and regression tree analysis. J Econ Entomol. 2010; 103(5):1517–23. Epub 2010/11/11. PMID: 21061948.

5. Vanengelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, et al. Colony collapse disorder: a descriptive study. PLoS One. 2009; 4(8):e6481. Epub 2009/08/04. doi: 10.1371/journal.pone.0006481 PMID: 19649264; PubMed Central PMCID: PMC2715894.
6. Pisa LW, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs CA, Goulson D, et al. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ Sci Pollut Res Int. 2015; 22(1):68–102. Epub 2014/09/17. doi: 10.1007/s11356-014-3471-x PMID: 25223353; PubMed Central PMCID: PMC4284392.
7. Henry M, Beguin M, Requier F, Rollin O, Odoux JF, Aupinel P, et al. A common pesticide decreases foraging success and survival in honey bees. Science. 2012; 336(6079):348–50. Epub 2012/03/31. doi: 10.1126/science.1215039 [pii]. PMID: 22461498.
8. Whitehorn PR, O'Connor S, Wackers FL, Goulson D. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science. 2012; 336(6079):351–2. Epub 2012/03/31. doi: 10.1126/science.1215025 [pii]. PMID: 22461500.
9. Eisenstein M. Pesticides: Seeking answers amid a toxic debate. Nature. 2015; 521(7552):S52–5. Epub 2015/05/21. doi: 10.1038/521S52a [pii]. PMID: 25992672.
10. El Hassani AK, Dacher M, Gauthier M, Armengaud C. Effects of sublethal doses of fipronil on the behavior of the honeybee (Apis mellifera). Pharmacol Biochem Behav. 2005; 82(1):30–9. Epub 2005/08/17. doi: S0091-3057(05)00241-8 [pii] doi: 10.1016/j.pbb.2005.07.008 PMID: 16102801.
11. Vidau C, Diogon M, Aufauvre J, Fontbonne R, Vigues B, Brunet JL, et al. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by Nosema ceranae. PLoS One. 2011; 6(6):e21550. Epub 2011/07/09. doi: 10.1371/journal.pone.0021550 PONE-D-11-04998 [pii]. PMID: 21738706; PubMed Central PMCID: PMC3125288.
12. Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, et al. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ Sci Pollut Res Int. 2015; 22(1):5–34. Epub 2014/09/23. doi: 10.1007/s11356-014-3470-y PMID: 25233913; PubMed Central PMCID: PMC4284386.
13. Khambay BPS, Jewess PJ. Pyrethroids. In: Gilbert LI, editor. Comprehensive Molecular Insect Science. Amsterdam: Elsevier; 2005. p. 1–29.
14. Hardstone MC, Scott JG. Is Apis mellifera more sensitive to insecticides than other insects? Pest Manag Sci. 2010; 66(11):1171–80. Epub 2010/07/31. doi: 10.1002/ps.2001 PMID: 20672339.
15. Decourtye A, Devillers J, Cluzeau S, Charreton M, Pham-Delegue MH. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicol Environ Saf. 2004; 57(3):410–9. PMID: 15041263.
16. Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S, et al. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee Apis mellifera. Arch Environ Contam Toxicol. 2005; 48(2):242–50. PMID: 15750780.
17. Decourtye A, Devillers J, Aupinel P, Brun F, Bagnis C, Fourrier J, et al. Honeybee tracking with microchips: a new methodology to measure the effects of pesticides. Ecotoxicology. 2011; 20(2):429–37. Epub 2011/01/27. doi: 10.1007/s10646-011-0594-4 PMID: 21267650.
18. van Dame R, Meled M, Colin M-E, Belzunces LP. Alteration of the homing-flight in the honey bee Apis mellifera L. Exposed to sublethal dose of deltamethrin. Environmental Toxicology and Chemistry. 1995; 14(5):855–60. doi: 10.1002/etc.5620140517
19. Winston ML. The biology of the honey bee: harvard university press; 1987. 281 p.
20. Carlson FD, Wilkie DR. Muscle Physiology: Prentice-Hall; 1974. 170 p.
21. Lambin M, Armengaud C, Raymond S, Gauthier M. Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. Arch Insect Biochem Physiol. 2001; 48(3):129–34. Epub 2001/10/24. doi: 10.1002/arch.1065 [pii]. PMID: 11673842.
22. El Hassani A, Dacher M, Gary V, Lambin M, Gauthier M, Armengaud C. Effects of Sublethal Doses of Acetamiprid and Thiamethoxam on the Behavior of the Honeybee (Apis mellifera). Arch Environ Contam Toxicol. 2008; 54(4):653–61. doi: 10.1007/s00244-007-9071-8 PMID: 18026773
23. Williamson SM, Willis SJ, Wright GA. Exposure to neonicotinoids influences the motor function of adult worker honeybees. Ecotoxicology. 2014; 23(8):1409–18. Epub 2014/07/12. doi: 10.1007/s10646-014-1283-x PMID: 25011924; PubMed Central PMCID: PMC4165879.
24. Eiri DM, Nieh JC. A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing. J Exp Biol. 2012; 215(Pt 12):2022–9. Epub 2012/05/25. doi: 10.1242/jeb.068718 215/12/2022 [pii]. PMID: 22623190.
25. Teeters BS, Johnson RM, Ellis MD, Siegfried BD. Using video-tracking to assess sublethal effects of pesticides on honey bees (Apis mellifera L.). Environ Toxicol Chem. 2012; 31(6):1349–54. Epub 2012/04/11. doi: 10.1002/etc.1830 PMID: 22488825.