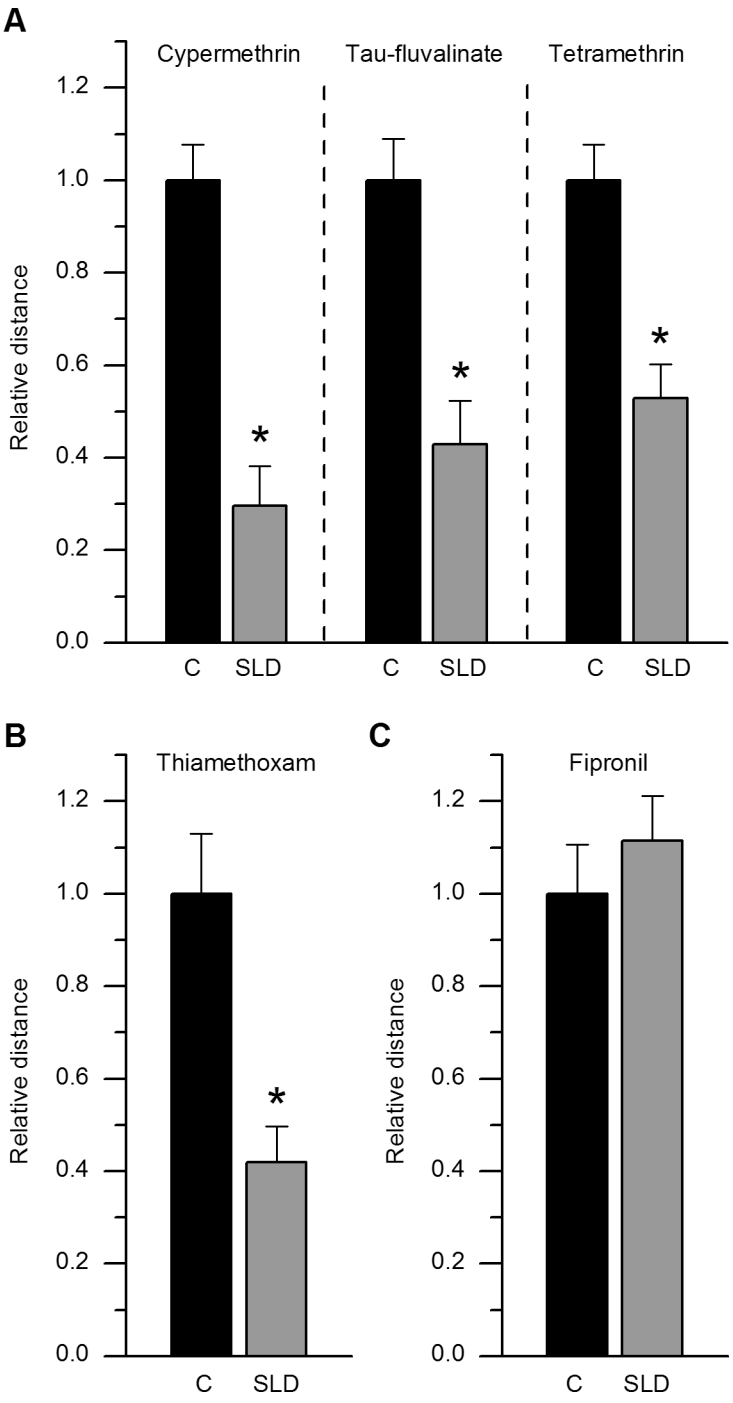

Fig 3. Evidence for locomotor deficits after exposure to a sublethal dose (SLD_{48h}) of a pyrethroid or a neonicotinoid but not a phenylpyrazole. A, The average (± S.E.M) relative distance covered by young bees is significantly decreased 6±2h after exposure to either a SLD_{48h} of cypermethrin (2.5 ng/bee), tau-fluvalinate (33 ng/bee) or tetramethrin (70 ng/bee). **B**, A significant decrease in distance is observed after exposure to a SLD_{48h} of thiamethoxam (3.8 ng/bee) as well. **C**, The relative distance covered by bees after exposure to a SLD_{48h} of fipronil (0.5 ng/bee) is similar to the distance covered by control bees. In the case of fipronil, whereas early deleterious effects cannot be evidenced by the locomotion assay, an increased mortality is observed five days after exposure. For cypermethrin, n = 19 control and n = 20 exposed bees respectively. For tau-fluvalinate, n = 12 control and n = 19 exposed bees respectively. For tetramethrin, n = 20 control and n = 20 exposed bees respectively. For thiamethoxam, n = 19 control and n = 19 exposed bees respectively. For fipronil, n = 19 control and n = 20 exposed bees respectively.

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Fig). Given the experimental stability of control groups, we could readily assess the five treatments as a part of a single LMM model. The distance covered by individuals was significantly lower in all treated groups compared to control, except for the fipronil trial (S2 Table, S2 Fig). In bees exposed to an SLD_{48h} of cypermethrin, the mean covered distance was significantly decreased by 71 ± 9% relative to control bees (Fig 3, S1 Fig, S2 Table). Tau-fluvalinate was also very potent at the SLD_{48h}, and the covered distance was significantly diminished by 58 ± 10% (Fig 3, S1 Fig, S2 Table), while tetramethrin (70 ng/bee) significantly decreased the distance covered by 48 ± 7% (Fig 3, S1 Fig, S2 Table). At the chosen SLD_{48h}, all 3 pyrethroids appear qualitatively equally potent. Exposure to an SLD_{48h} of the neonicotinoid thiamethoxam (3.8 ng/bee) resulted in a similar significant decrease by 58 ± 8% of the distance covered (Fig 3, S1 Fig, S2 Table). Exposure to an SLD_{48h} of fipronil (0.5 ng/bee) did not produce any significant effect on locomotion (Fig 3, S1 Fig, S2 Table).

Mortality several days after exposure to fipronil in the absence of early locomotor deficits

In parallel with locomotion assays, long-term survival was measured after exposure to the insecticide SLD_{48h}, to explore whether the locomotor deficits that we have measured could induce any mortality several days after exposure in laboratory conditions. The current legislation imposes that acute contact mortality tests have to be routinely performed for 48h [26]. However, if mortality increases by more than 10% between 24 and 48 h, the assay should be extended up to 96 h. Here, for all pyrethroids and the neonicotinoid tested, mortality rates were stable between 24 and 48 h. We found that the SLD_{48h} was sublethal at 120 h as well, indicating that the early locomotor deficit observed does not compromise survival five days after exposure, at least in a controlled laboratory environment. At 120 h, the SLD_{48h} of cypermethrin, tetramethrin, tau-fluvalinate or thiamethoxam did not induce mortality more than their respective controls (Fisher exact tests, $P>0.14$). Interestingly, whereas fipronil was the only modality in which no locomotor deficits were detected, with a mortality rate stable between 24 and 48 h (1 and 2% respectively, $P = 0.6515$), the SLD_{48h} of this insecticide started to produce an increased mortality at 72 h (14%, $P<0.0001$ as compared to 48 h) and a high mortality rate at 120 h after exposure (78%, n = 180 bees) as compared to control bees (1.5%, n = 180 bees, $P<0.0001$). It is noteworthy to mention that the survival of control bees was stable between 48h and 120h (99.5% and 98.5% respectively, n = 180, $P = 0.6229$).

Discussion

For pollinators, sublethal effects of insecticides increase toxicological risks and thus should be taken more into account in the methods of risk evaluation [32]. For the first time, we analyzed the sublethal effects of pesticides from three major insecticide classes on young bees (day 1 after emergence) in a standardized honeybee walking locomotion test. Emphasis has been put on pyrethroids that were much less studied than the two other insecticide classes despite 1) their high toxicity towards insects, 2) their pervasive use in agriculture and 3) their prevalence in hives. Specific experimentally-determined sublethal doses were selected for each insecticide (see Methods). In our study, assuming a mean individual bee weight of 0.1 g [1], the SLD_{48h} of pyrethroids were 25 ppb for cypermethrin, 330 ppb for tau-fluvalinate and 700 ppb for tetramethrin. By comparison, quantitatively similar values of 13 pyrethroid residues have been detected in North American hives [1]. For instance, in foundation wax, cypermethrin and fluvalinate were found in 23.8 and 100% of samples at maximal levels of 131 and 10120 ppb respectively (average levels 51.6 and 2006 ppb respectively). Knowing that multiple pyrethroids can be found in the same hive, preimaginal bees and newly emerged bees were thus potentially