exposed to cumulated doses [1] that are compatible with SLD_{48h} used in the present study. However, the gaps in the current knowledge on pesticides toxicokinetics (e.g. on the transfer rate of pesticides from hive matrices to the body of a young bee) precludes comparing the level of exposure resulting from contact with contaminated waxes and the level of exposure resulting from a laboratory procedure in which a droplet of contaminated solution is applied on the thorax. Currently, a model that would link these two modes of exposure is unfortunately lacking for a risk evaluation to be accurately performed. For fipronil, our SLD_{48h} was 5 ppb. By comparison, 1.4% of wax samples contained a maximum of 35.9 ppb of fipronil, 0.3% of pollen samples contained a maximum of 28.5 ppb [1]. For thiamethoxam, our SLD_{48h} was 38 ppb. By comparison, 0.3% of pollen samples contained a maximum of 53.3 ppb of thiamethoxam and it was not detected in wax [1].

An SLD_{48h} of all the insecticides tested (except fipronil) triggered serious locomotor deficits. According to available $LD_{50\ at\ 48h}$ values for cypermethrin, that vary from 25 to 121 ng/bee, [33, 34], an SLD_{48h} of cypermethrin 10 to 48 fold lower than the LD_{50} seriously impairs locomotion. The SLD_{48h} for tau-fluvalinate is between 75 and 600 fold lower than published LD_{50} values (2.5 μg—20 μg/bee, [34, 35]). In the case of tetramethrin, the effective SLD_{48h} was only two times lower than the available LD_{50} [36]. Very different maximal ratios between LD_{50} and SLD_{48h} values for the three tested pyrethroids (2, 48 and 600 for tetramethrin, cypermethrin and tau-fluvalinate, respectively) suggest that within a chemical family, deleterious effects of individual insecticides have to be evaluated separately. A similar locomotor deficit was observed with a SLD_{48h} of tau-fluvalinate and a 13 fold lower SLD_{48h} of cypermethrin. This result is consistent with a 20 fold lower LD_{50} obtained with cypermethrin than with tau-fluvalinate [34]. Tau-fluvalinate is commonly used against the bee parasite *Varroa destructor*. The locomotor deficits observed here after exposure to low doses of tau-fluvalinate surely challenge the widespread concept that it can safely be used in hives.

Since honeybees' skeletal muscles do not express functional voltage-gated Na^+ channels, locomotion deficits cannot be explained by a direct action of pyrethroids on such channels in muscle [37, 38]. Locomotor deficits can then be potentially attributed to non-mutually exclusive explanations. First, the locomotor deficits observed in our study could be a consequence of pyrethroid effects on sodium channels located in the central nervous system (in brain and other ganglia) impairing information processing and motor command [39]. Alternatively, impaired electrical activity of sensory neurons housed in the antennae, that are more pyrethroid-sensitive than central neurons, may affect sensory perception and thus impede locomotion [39, 40]. Structural differences between tau-fluvalinate, cypermethrin and tetramethrin in the acidic and alcohol moieties (Fig 1) may produce the different sets of interactions within the channel pore revealed by molecular modeling [41–43], thus giving molecular support for drug-specific modifications in the honeybee sodium channel kinetic parameters [39]. The cloning and expression of the honeybee voltage-dependent sodium channel, $AmNa_v1$ [43] together with the analysis of the changes induced by the different pyrethroids using numerical simulation bring a set of important tools that will be useful to fully characterize and understand the binding differences between these pyrethroids and hence their differential toxicity. Deficits could also be related to pyrethroid potency on secondary targets such as voltage-gated calcium channels [44, 45] that are broadly distributed in honeybee tissues [46, 47]. Calcium channels underlie action potentials in muscles of the honeybee or other insects, [37, 38, 48, 49]. Effects on muscle calcium channels would thus not only impair locomotion but thermoregulation or hemolymph circulation as well. A direct effect on bee muscle cells has actually been shown *in vitro* [37]. Our recent cloning and expression of *Apis mellifera* calcium channels ($AmCa_v$) will allow for more systematic testing of pyrethroids [46, 47].

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)