

Fig 2. Video tracking of bees using a vertical arena. **A**, Examples of paths followed during 3 minutes by four individual young bees (day 1 after emergence). **B**, Superimposed paths followed by eighty individual bees. Overall, arena sides were more frequently visited. **C**, Locomotor ability measured at day 1, 2 and 6 after emergence (bees kept in an incubator). Mean distance (\pm S.E.M) covered by bees slightly increased from 3.2 to 3.8 meters between day 1 and 2 ($p < 0.01$, $n = 138$ and 63 respectively) and did not significantly further increase as shown at 6 days after emergence ($n = 38$).

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assay displayed variable trajectories, as illustrated for four individuals (first day after emergence) monitored for 3 minutes at a frequency of 1 Hz (Fig 2A). During 3 minutes, each bee can explore only a fraction of the arena. However, overall, bees visited all parts of the arena, although sides were visited more often, possibly owing to a positive thigmotaxis phenomenon (Fig 2B, superimposed trajectories of 80 control bees). The total distance covered was chosen as a proxy for the bee's locomotor ability. In laboratory conditions, a bee's locomotor ability only slightly increased with age as shown by distances measured from bees kept in a cage for 6 days (Fig 2C). Mean distance covered by bees increased from 3.2 ± 1.0 to 3.8 ± 1.6 m between day 1 and 2 (Mann-Whitney $U = 3119$, $n_1 = 138$, $n_2 = 63$, $p < 0.01$) and did not significantly further increase at day 6 after emergence (4.0 ± 2.5 m, $n = 38$). Bees already showed good locomotion skills at day one after emergence, making this age suitable for the following locomotion assays on insecticide-treated bees.

Locomotion in bees exposed to a SLD_{48h}

Average distances covered by young bees (day 1 after emergence) were measured after exposure to an SLD_{48h} of one of the three pyrethroids: cypermethrin (2.5 ng/bee), tau-fluvalinate (33 ng/bee) and tetramethrin (70 ng/bee). For ease of comparison, distances covered by exposed bees were standardized to the average distance covered by corresponding control bees, set at 1 (Fig 3, relative control distances in black). S1 Fig also reports individual actual distances in meters (S1 Fig). All the five control groups delivered statistically identical locomotion properties, with no significant distance variation in any pairwise combination of trial (S2 Table, S2

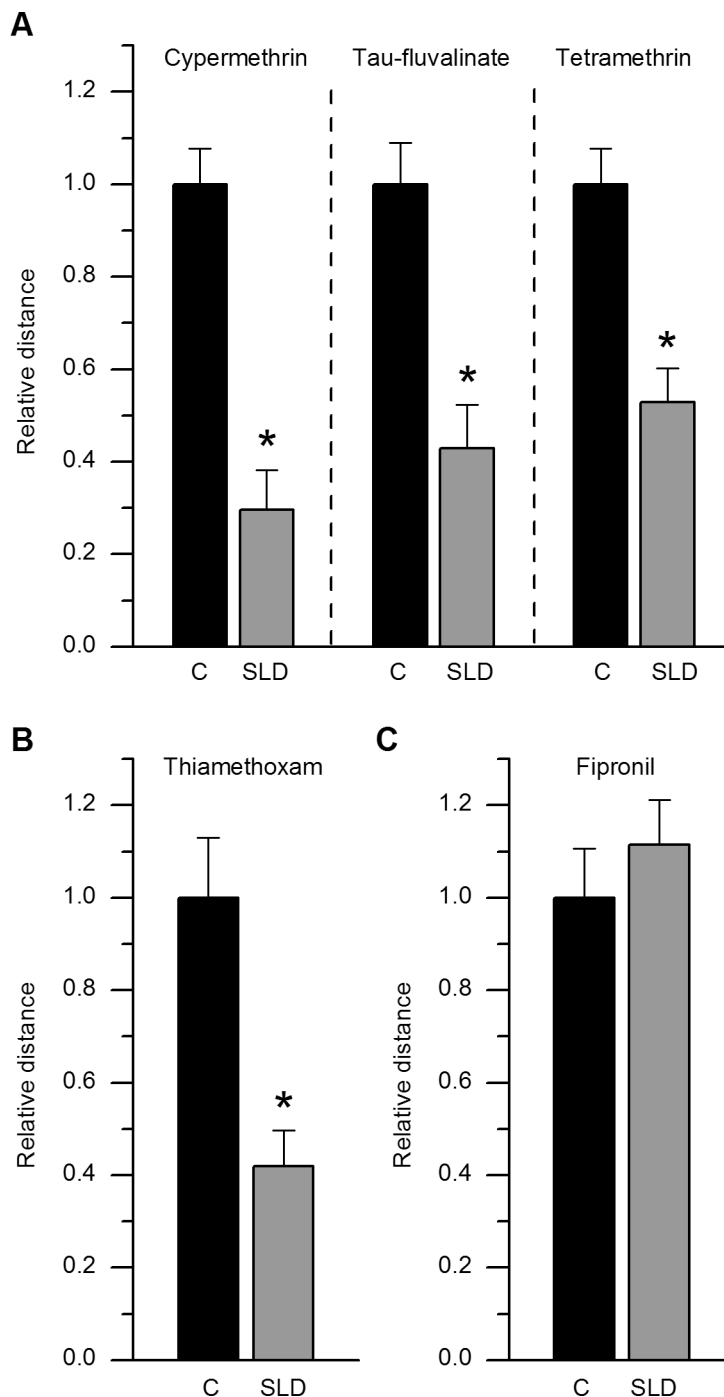


Fig 3. Evidence for locomotor deficits after exposure to a sublethal dose (SLD_{48h}) of a pyrethroid or a neonicotinoid but not a phenylpyrazole. **A**, The average (\pm S.E.M) relative distance covered by young bees is significantly decreased 6 \pm 2h after exposure to either a SLD_{48h} of cypermethrin (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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