

NEONICOTINOIDS

Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation

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Neonicotinoid pesticides can negatively affect bee colonies, but the behavioral mechanisms by which these compounds impair colony growth remain unclear. Here, we investigate imidacloprid's effects on bumblebee worker behavior within the nest, using an automated, robotic platform for continuous, multicolony monitoring of uniquely identified workers. We find that exposure to field-realistic levels of imidacloprid impairs nursing and alters social and spatial dynamics within nests, but that these effects vary substantially with time of day. In the field, imidacloprid impairs colony thermoregulation, including the construction of an insulating wax canopy. Our results show that neonicotinoids induce widespread disruption of within-nest worker behavior that may contribute to impaired growth, highlighting the potential of automated techniques for characterizing the multifaceted, dynamic impacts of stressors on behavior in bee colonies.

Animal pollinators support biodiversity and agricultural yields (1, 2), and there is growing concern over the causes and consequences of declining bee populations (3, 4). Mounting evidence indicates that neonicotinoid pesticides can negatively affect both commercial honey bee (5, 6) and wild bee (7) populations. Neonicotinoids are agonists of nicotinic acetylcholine receptors and therefore disrupt cholinergic signaling in the insect central nervous system. Neonicotinoids are believed to reduce growth of developing brood (7–9) by impairing foraging behavior [including navigation (5, 10) and floral learning (11, 12)], leading to reduced colony resource intake (13, 14). Recent work, however, shows that neonicotinoid exposure can impair colony growth without altering foraging (15) [and vice versa (16)].

In addition to foraging, workers in social insect colonies perform critical tasks within the nest (e.g., larval incubation and feeding, cleaning, and nest construction) that are vital for colony development. Although nest workers are

exposed to neonicotinoid residues (7, 17) that may affect behavior [including physiology (18, 19) and locomotion (20–22)], neonicotinoids' effects on within-nest behaviors are poorly understood.

To investigate imidacloprid's effects on bumblebee (*Bombus impatiens*) nest behavior, we combined a system for automated behavioral tracking of uniquely identified workers [BEEtag (23, 24)] with a robotic observation platform (Fig. 1, A to C), allowing long-term (12-day, Fig. 1 and figs. S1 and S2) tracking of uniquely identified bumblebee workers and queens. Colonies were given ad libitum access to either pure nectar (control, $n = 9$), or nectar containing field-realistic concentrations of imidacloprid, a globally prevalent neonicotinoid [$n = 9$, 6 parts per billion (ppb)].

Chronic imidacloprid exposure impairs a suite of worker behaviors within the nest. Workers in imidacloprid-exposed colonies spent significantly less time active (Fig. 1D and fig. S2). Imidacloprid exposure also reduced rates of nursing among workers (Fig. 1E and fig. S2) and shifted spatial occupancy toward the nest periphery (Fig. 1F and fig. S2).

Behavioral effects of imidacloprid differed markedly between night and day [14:10 light:dark (L:D) cycle] within the colony (Fig. 1); reductions in activity were stronger at night (Fig. 1D and fig. S2) than during the day (but were significant during both night and day, see fig. S2) and effects on daytime activity declined over time (with effects undetectable by the end of the exposure period, Fig. 1D). Reductions in nursing and distance from the nest center were significant at night, but not during the day (Fig. 1, E and F, and fig. S2). Imidacloprid reduced social network density compared to controls, consistent with effects of thiacloprid in honey bees

(25), although this effect was also only significant at night (Fig. 1G; network density, night: bootstrap $p = 0.0042$; network density, day: bootstrap $p = 0.71$). Imidacloprid increased movement speed in workers, although this effect appears delayed and is only significant during the day (fig. S2). Whereas previous work has shown that imidacloprid exposure can lead to either hyperactivity or immobility depending on dose (26), these results show that both effects can occur at the same concentration. Imidacloprid also reduced activity and nursing in queens (fig. S2), consistent with results in honey bees (27) and bumblebees (28, 29). Body concentrations of imidacloprid measured after the experiment were independent of foraging activity, confirming that even nonforaging nest workers are exposed to imidacloprid (fig. S3, mean concentration = 2.25 fmol imidacloprid per milligram of body mass).

To confirm that imidacloprid induces direct and rapid changes in nest behavior after exposure, we recorded behavior of workers in four additional *B. impatiens* colonies on the days immediately before and after individually administered, acute consumption of 0 (control), 0.1, or 1.0 ng of imidacloprid (Fig. 2; see supplementary text for justification of doses).

Acute imidacloprid exposure altered nest behavior within 24 hours, with effects qualitatively similar to those of chronic exposure (Fig. 2 and tables S1 to S4). Bees fed 1.0 ng of imidacloprid had reduced activity and nursing, were located further from the nest center, and had reduced social interactions compared to controls (Fig. 2 and table S2). Bees fed 1.0 ng of imidacloprid showed reduced foraging, driven by a reduction in nonforagers initiating foraging after treatment—rather than a decrease in foraging among foragers (tables S2 to S4). Bees fed 0.1 ng of imidacloprid showed no significant differences in behavior compared to controls (fig. 2 and tables S1 to S4).

To gain insight into the mechanisms underlying the multiple behavioral outcomes of imidacloprid exposure, we developed a spatially explicit, agent-based model of worker nest movements (supplementary text and figs. S4 to S6). Bees were modeled as either active (moving) or inactive (not moving) at each time point and Markovian transitions were used to switch between these states (fig. S4). The transition rates were modulated by contact with nestmates and the bee's location on or off the nest and were directly estimated from experiments (fig. S5). When bees were active, they moved with a random walk biased toward the nest center (fig. S4; parameters also fit from experiments).

We then used this model to disentangle the effects of imidacloprid on activity, space use, and social interactions and found evidence that these multiple outcomes of imidacloprid exposure are functionally linked; simulations isolating imidacloprid's direct effects on activity (both spontaneously in isolated workers and when activity is modulated by social contact; fig. S5) resulted in shifts in spatial occupancy and interaction rate within the nest (fig. S6). These effects are

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compounded by imidacloprid's reduction of attraction to the nest center (fig. S6 and supplementary text).

To test neonicotinoids' effects on nest behavior under realistic conditions, we quantified nest thermoregulation performance in free-foraging

B. impatiens colonies (Fig. 3, A and B). Colonies were given ad libitum access to nectar containing imidacloprid (6 ppb, $n = 9$) or pesticide-free nectar ($n = 9$) within the nest, but foraged outdoors to gather pollen. For each colony, we measured the surface temperature of the brood

and air temperature within the nest chamber, in addition to outdoor air temperature (Fig. 3, A and B). Imidacloprid impaired thermoregulation of the developing brood (Fig. 3, C and D, and fig. S7, permutation test, $p = 0.005$, tables S5 and S6) and nest air temperature (Fisher's

Fig. 1. Chronic exposure to imidacloprid alters nest behavior and social interactions in bumblebee colonies.

(A and B) Schematic diagrams of (A) robotic platform for multicolony (in a 4 by 3 array) behavioral tracking and (B) a single colony chamber. (C) Example tracking of nest workers, with unique identification numbers shown in green. Orange dotted line shows the nest structure. (D) Colony mean percentage of time active over 7 consecutive days (with time indicating hours after exposure). Filled circles represent mean activity levels for a single colony (averaged across all individual workers) for a single 5-min trial, and solid lines show mean values for treatment groups (control colonies, $n = 9$, in green; imidacloprid-exposed colonies, $n = 9$, in red). Gray blocks and Sun/Moon symbols show the 14:10 hour L:D cycle in the tracking arena. (E) Percentage of time engaged in nursing. (F) Mean distance to the nest center and (G) social network density [proportion of possible pairwise interactions between workers that actually occur, during a single 5-min trial] by treatment group and time of day. * $p < 0.05$, ** $p < 0.005$, based on 10,000 hierarchical bootstrap replicates. Solid markers in (E) to (G) show group means, and black bars indicate 95% bootstrap confidence intervals, with control and imidacloprid-exposed colonies shown in green and red, respectively. n.s., not significant.

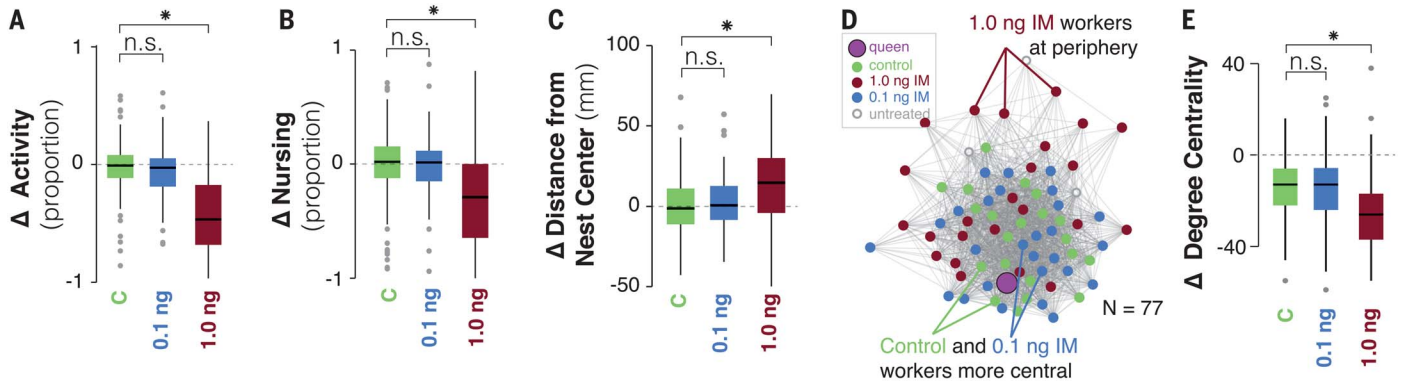
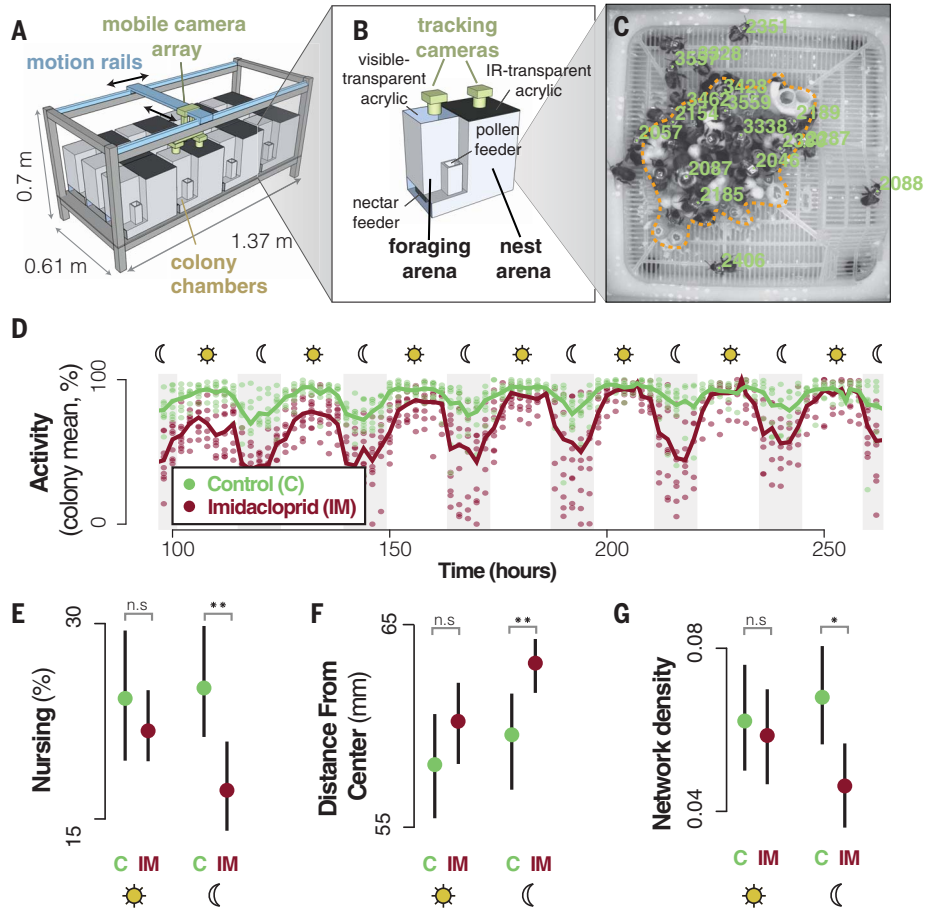


Fig. 2. Effects of imidacloprid on nest behavior occur rapidly after acute exposure. Changes in (A) activity, (B) nursing, and (C) distance from nest center of workers after exposure to different imidacloprid treatments. (D) Social network diagram of a representative colony, with nodes positioned by a force-directed algorithm. Circles represent individual bees, with gray lines drawn between bees that interacted during a 1-hour trial. The queen is shown as a purple circle, and untreated workers are shown as open gray circles.

(E) Change in degree centrality (i.e., number of unique social interactions) after exposure by treatment. Boxplots show median (thick black lines), interquartile range (solid box), and range (thin lines, 75th and 25th percentile $\pm 1.5 \times$ IQR), with outliers shown in gray. Behavioral changes were calculated as the difference in individual behavior 24 hours after versus 24 hours before exposure. In all panels, workers exposed to 1.0, 0.1, or 0 ng of imidacloprid are shown in red, blue, and green, respectively. * $p < 0.001$. n.s., not significant.

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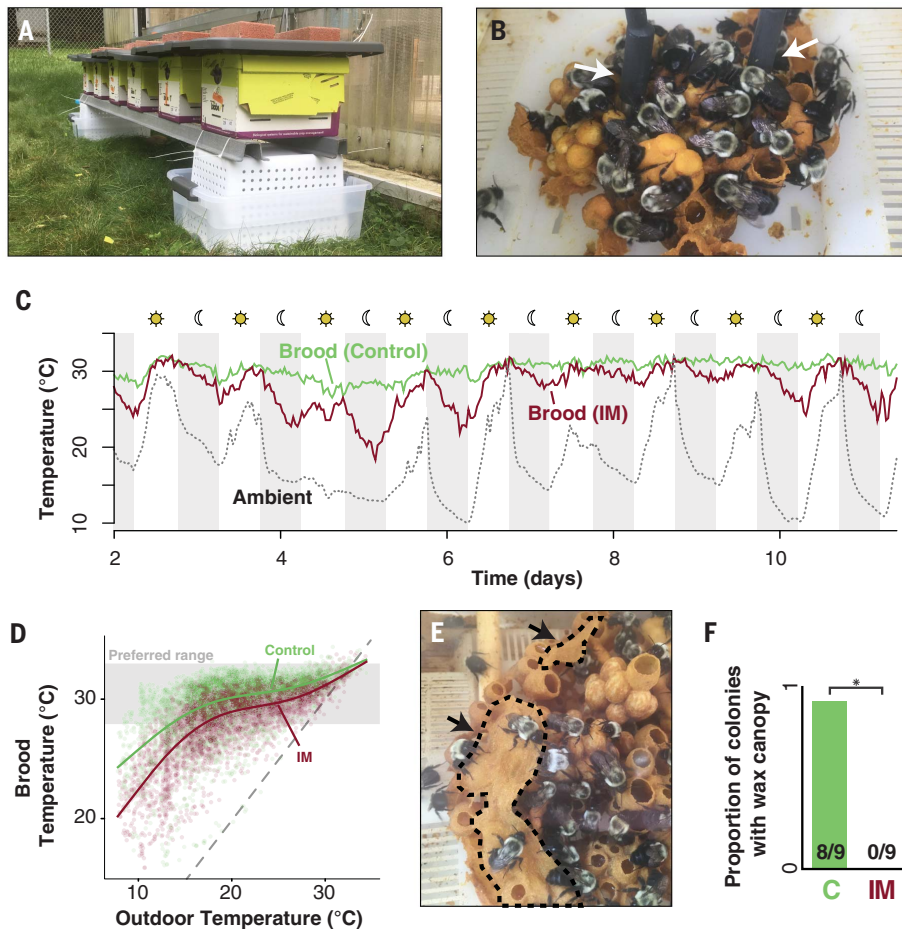


Fig. 3. Chronic imidacloprid exposure disrupts brood thermoregulation in *Bombus impatiens*. (A) Outdoor *B. impatiens* colonies with digital temperature sensors (B) (white arrows). (C) Example brood (solid lines) temperatures from one imidacloprid-exposed colony (red, IM) and one control (green, C) colony. Dotted line shows outdoor air temperature. (D) Brood versus outdoor temperatures for control (C, green) and treated (IM, red). Transparent markers show individual measurements across all colonies, and solid lines show LOESS-smoothed trends by treatment. Dashed line: brood temperature = outdoor temperature. (E) Example of a partially constructed insulating wax canopy (black arrows and dashed lines) covering brood cells. (F) Proportion of colonies that had a partially or completely constructed wax canopy by the end of the experiment, by treatment. Asterisk indicates significant difference between groups ($p = 0.0005$, permutation test).

exact test, $p = 0.009$, tables S5 and S6), with stronger effects occurring at lower temperatures (Fig. 3D). This result confirms that neonicotinoids' effects on thermogenesis in individual, isolated honey bees (18) and bumblebees (19) extend to colony temperature regulation under field conditions. We found a significant interaction between exposure and the direction of temperature change, suggesting that the effect of imidacloprid on thermoregulation may be stronger when air temperature is rising (fig. S7 and tables S5 and S6). Imidacloprid-treated colonies were also less likely to construct an insulating wax canopy around the developing brood, an important behavioral adaptation to cold (30) (Fig. 3, E and F, permutation test, $p = 0.0005$).

Large-scale field studies have revealed that the impacts of neonicotinoids on bee colonies can vary substantially depending on environmental context (6, 7), highlighting the need for improved understanding of the mechanisms by which neonicotinoids affect workers and colonies. Our results suggest that reduced brood growth in neonicotinoid-exposed colonies (7) could result from impaired nursing behavior and temperature control by nest workers, in addition to reduced colony resource intake (13, 14). These results support previous findings that neonicotinoids impair worker hygienic be-

havior in honey bees at higher concentrations [e.g., 50 ppb or higher (27)] and over extended exposure periods [e.g., 12 weeks (31)].

Our results highlight the multifaceted behavioral impacts of neonicotinoid exposure; imidacloprid's effects on nest behavior vary substantially with time of day (Fig. 1 and fig. S2), exposure affects both mobility and sensory decision-making (Figs. 1 and 2, fig. S5, and supplementary text), and the impacts of imidacloprid on brood thermoregulation are nonlinear (Fig. 3 and tables S5 and S6) and dynamic (fig. S7 and tables S5 and S6). These results illustrate the potential of high-throughput, automated analysis for improving our understanding of the context-specific effects of neonicotinoids, as well as efficiently screening agrochemicals more generally for sublethal impacts on pollinators.

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Author contributions: J.D.C., C.M.S., N.E.P., S.A.C., and B.L.dB. designed experiments. J.D.C., R.L.O., and C.M.S. performed acute exposure experiments. J.D.C., C.G., and B.L.dB. designed and constructed the robotic tracking arenas and platform. J.D.C., R.L.O., C.M.S., A.B., and M.E. collected data and performed experiments. J.D.C. performed automated tracking, behavioral analysis, and prepared solutions for chronic behavior and field experiments. J.D.C., A.N.F.V, and B.D. designed and implemented the agent-based model. C.M.S. mixed the solutions of imidacloprid for acute experiments and performed statistical analysis for acute experiments and thermoregulation experiments. C.M.S. and J.D.C. performed statistical

analysis for chronic imidacloprid experiments. All authors wrote the manuscript. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** Associated data and custom scripts are deposited at Zenodo (32).

SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S12
Tables S1 to S6
References (33–54)
Movies S1 to S3

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Trouble at the hive

Neonicotinoid pesticides cause mortality and decline in insect pollinators. One repeatedly noted effect is a reduction in bee colony size. However, the mechanism behind this reduction is unclear. Crall *et al.* performed complex real-time monitoring of bumblebee behavior within their nests (see the Perspective by Raine). Neonicotinoid exposure reduced nurse and caretaking behaviors, which affected productivity and harmed colony thermoregulation. These changes in behavior acted together to decrease colony viability, even when exposure was nonlethal.

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