

Precise tactile stimulation of worker ants by a robotic manipulator reveals that individual responses are density- and context-dependent

Matthias Rüegg^{1,2} | Alba Motes-Rodrigo¹  | Alexandre Tuleu¹ | Nathalie Stroeymeyt^{1,3}  | Thomas O. Richardson^{1,3}  | Mahmut Selman Sakar²  | Laurent Keller⁴

¹Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

²Institutes of Mechanical Engineering and Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

³School of Biological Sciences, University of Bristol, Bristol, UK

⁴Social Evolution Unit, Chesières, Switzerland

Correspondence

Alba Motes-Rodrigo
Email: alba.motesrodrigo@unil.ch

Laurent Keller
Email: laurentkeller01@gmail.com

Funding information

H2020 European Research Council, Grant/Award Number: 741491 and 802628; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 310030_200437

Handling Editor: Huijie Qiao

Abstract

1. Ant workers are often specialized in specific tasks, and it is well-established that the main task an ant performs in the colony can be used to predict its sensitivity and responses to task-associated stimuli. An often-overlooked aspect of ants' task specialization is that individuals often switch tasks throughout the day and are not always engaged in functional tasks. Furthermore, the tasks individuals engage in are often correlated with other context-specific factors, such as worker density, which can independently influence individuals' behaviour. Given this intra-individual variation in task engagement and its correlation with density, it is currently unknown how these two factors interact to modulate ants' sensitivity and responses to stimuli.
2. To address this question, we built a robotic manipulation system that allowed us to teleoperate a dummy inside ant colonies and to provide simulated antennations to ants when performing different tasks in areas with different worker densities. We coupled this manipulation system with a custom-built automated tracking system (FORT) that allowed us to track individual identities and locations as well as to record the ants' responses to the dummy stimulation.
3. We found independent effects of task and worker density on ants' responsiveness and alarm towards the dummy. Ants were less likely to respond and be alarmed by the dummy when stimulated in areas with high worker density. Responsiveness but not alarm was further influenced by the task being performed, with ants doing broodcare being the least responsive. Our results suggest that ants' behaviour is density-dependent and that ants experience a process of habituation to tactile stimulation. Additionally, ants' responsiveness is modulated by the task they are performing at a given time, showing that sensitivity to stimuli is context-dependent.

Matthias Rüegg and Alba Motes-Rodrigo contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2023 The Authors. *Methods in Ecology and Evolution* published by John Wiley & Sons Ltd on behalf of British Ecological Society.

4. Our robotic set-up constitutes a valuable tool to systematically investigate social insect behaviour under unprecedented experimental control to unravel the individual-level behavioural rules that underpin the organization of social insect colonies. The integrated system presented here opens new research avenues to empirically investigate the effects of more complex stimuli on social insect behaviour and has the potential to significantly further our understanding of decentralized collective systems.

KEYWORDS

ant behaviour, automated behavioural tracking, robotic manipulator, tactile stimulation, task, worker density

1 | INTRODUCTION

Ants are one of the most successful taxa on the planet as they are found in almost every ecosystem on earth. This ecological success is partly due to an efficient division of labour, where different individuals in the colony specialize on particular tasks such as brood care, foraging, defence or cleaning (Hölldobler & Wilson, 1990). Previous studies have shown that the main task a worker performs in the colony is related to its sensitivity and responsiveness to task-specific stimuli (Robinson & Page, 1989) and that individuals specialized on different tasks present consistent differences in behaviour (e.g. probability of alarm) towards stimuli across time and contexts (Jandt et al., 2014). For instance, major and minor workers of the carpenter ant *Camponotus mus* differ in their appetitive responses towards sugar solutions (Josens et al., 2018), and minor workers of the leaf-cutter ant *Atta capiguara* are disproportionately more likely to be alarmed by dead conspecifics than major workers (Hughes & Goulson, 2001).

Although ant workers spend most of their time performing the task they are specialized in, individuals can switch tasks throughout the day (Robinson, 1992) and are not always performing clearly functional tasks. For instance, over a 10-h window, individual workers of *Temnothorax allardycei* spend a large proportion of their time (32%) in undifferentiated activities such as moving through the nest and an even larger proportion of their time (55%) quiescent (Cole, 1986). This intra-individual variation in task participation remains largely unstudied, and it is currently unknown whether workers' sensitivity to task-associated stimuli varies only according to the main task ants perform in the colony or whether the specific task being carried out at a particular point in time also has an effect. Empirically testing how the task an ant is performing influences its responses towards stimuli is challenging for several reasons.

Generally, it is difficult to conduct controlled behavioural experiments with social insects because removing them from their social environment strongly affects their behaviour, increasing for instance their locomotor activity (Koto et al., 2015). Conducting behavioural experiments *within* social insect colonies without disturbing all individuals and altering their behaviour is also very complicated and has only been possible in recent years thanks to technological advances

in the fields of robotics and imaging (Franks et al., 2022; Landgraf et al., 2008). Evaluating the effects of the task being performed on responsiveness to stimuli in ants poses the additional complication that task engagement is strongly correlated with worker density, which can also modulate stimuli responsiveness independently. Ants can estimate conspecific density based on the frequency of interactions they experience (Gordon, 2020), and worker density has been shown to influence individual interaction rates in the jet ant *Lasius fuliginosus* (Gordon et al., 1993) and aggression levels towards non-nestmates in the Argentine ant *Linepithema humile* (Buczowski & Silverman, 2005). Furthermore, workers display strong spatial fidelity, and individuals performing a specific task tend to cluster in certain areas of the nest, which increases worker density in certain locations (Dornhaus & Chittka, 2005; Franks & Tofts, 1994; Mersch, 2016; Richardson et al., 2022). For example, nurses frequently stay close to the brood, whereas foragers rarely interact with nurses because they stay near the nest entrance (Mersch et al., 2013). Worker density and task are further interconnected insofar as the number of ants performing a given task can increase or reduce the likelihood that other workers will engage in the same task. For instance, in the red harvester ant, noncleaning workers are more likely to begin to clean when they encounter other workers engaged in cleaning (Gordon & Mehdiabadi, 1999). In summary, the task an ant is performing at a given point in time and the density of workers around its location are highly related and both contribute to the decentralized organization of social insect colonies. Despite their importance for regulating colony activity, whether and how these two factors interact to modulate ants' sensitivity and responsiveness to task-associated stimuli remains unknown.

The aim of this study was therefore to evaluate how the behavioural responses of workers are simultaneously influenced by the task being performed and the density of workers at the location where a stimulus is encountered. Given that traditional behavioural assays do not allow individuals to be tested without altering their social context and the task they are performing, we developed a robotic manipulation platform that allows us to teleoperate a magnetically tethered dummy inside ant colonies and to provide standardized tactile stimuli to specific ants in specific nest locations. Interactive robots and teleoperated dummies have rapidly gained

popularity in the last two decades as tools to better understand biological systems (reviewed by Krause et al., 2011). Robotic set-ups have been used to study attraction and aggression in fish (Bierbach et al., 2018) and lizards (Martins et al., 2005) as well as song development in finches (Simon et al., 2023), among others. In social insect research, robots have been used to investigate the function of the honeybee waggle dance (Landgraf et al., 2008; Michelsen et al., 1992), decision-making in cockroaches (Halloy et al., 2007) and tandem running in ants (Franks et al., 2022). Robotic manipulation presents the advantage over traditional behavioural tests of providing a standardized signal to all tested individuals. In addition, dummies can be teleoperated within animals' natural group composition and allow to select the timing and location of the stimulus presentation, solving the experimental difficulties highlighted above (Gartland et al., 2022; Krause et al., 2011).

In our experiments, we coupled a robotic manipulation system with a custom-built automated tracking system that recorded the spatial coordinates of each individual multiple times per second. We used this integrated system to investigate how workers of the ant *Leptothorax acervorum* respond to a tactile stimulus simulating antennation depending on the task they are performing and the density of workers at the time and location of the stimulus presentation. To assess the roles of the task being performed and worker density on behaviour while controlling for individual and stimuli characteristics, we presented the same tactile stimuli to the same workers in different areas of the nest and while the workers were engaged in different tasks. We then measured their overall responsiveness and alarm behaviours towards the stimuli.

2 | METHODS

2.1 | Robotic manipulation platform

The robotic manipulation platform consisted of a dummy magnetically coupled with an external control system that moved the dummy among the ants and generated a precise tactile stimulus (Figure 1 and Figures S1 and S2). Each dummy was made of a rectangular magnet 2.5 mm long, 1.5 mm wide and 0.5 mm tall (HKCM) and two artificial antennae fabricated from 2-mm-long synthetic paint brush fibres (LR 15060, Lascaux). The magnets were coated with gold to present an inert surface and maintain a smooth motion above the floor of the nest throughout week-long experiments. The artificial antennae were glued to the magnet using quick-drying glue (Pattex ultra). After fabrication, all dummies were cleaned with ethanol and placed in an incubator at 80°C for 4 h to allow any residual odours associated with the glue to evaporate. A different dummy was used for every colony. The external control system operated under the floor of the nest and consisted of two cylindrical permanent magnets (2 mm in diameter and 10 mm in height, HKCM) for navigation and two electromagnetic coils (RFS1317-824KL, Coilcraft) for oscillating the dummy in place and generating the tactile stimulus. The magnets and coils were mounted on nested cartesian (VT-80, Physik

Instrumente) and rotary (DT-34, Physik Instrumente) stages. Both the stage controller (C-884, Physik Instrumente) and the signal generator that produced the tactile stimulus (HMF2525, Rohde&Schwarz) were remotely controlled via the 'keyboardAntbot' program, a custom program that allows to teleoperate the manipulator system based on visual feedback from the live video stream (see below). Both the position of the dummy in space and the start time of each tactile stimulus were manually controlled by the experimenter.

2.2 | Automated tracking system

The automated tracking system used, named the FORmicidae Tracker (FORT, Figure 1, Figures S1 and S2), is an updated and improved version of the fiducial tracking system presented by Mersch et al. (2013). The FORT tracking system consists of a 48 megapixel and 35 mm monochromatic camera (Flare 48MP, IO Industries) equipped with an enlarging lens (apo-rodagon-n 50, Rodenstock) and a near infrared long-pass filter (lp850, MidOpt). Given that behavioural tracking took place both at day and night, we paired the monochromatic camera with a custom-built infrared illumination system, consisting of 48 high-power Infrared LEDs (SFH 4235, Osram). To reduce unwanted heating of the ant nests as well as to minimize the motion blur of fast-moving ants, these LEDs operated in pulses of 1 to 3 milliseconds that were electronically synchronized with the camera's frame grabber (Coaxpress G3 Quad DL, Euresys). Images were acquired at an eight images per second rate, and all vision processing was performed online on a high-end consumer grade PC (Intel 9700K CPU, 16Gb DDR4-3200 memory). The choices of exclusively conducting online vision processing and to only store a low-resolution compressed video stream were motivated by the need to keep the amount of stored data to a minimum in order be able to conduct experiments over long periods of time (up to 4 weeks). The custom-designed climate control system that allowed us to regulate temperature and humidity throughout the experiments consisted of a piezoelectric vaporizer combined with an exhaust fan and heating resistors (up to 45W). All blueprints, firmware and code of the FORT are published under various open-source licences at <https://formicidae-tracker.github.io>.

2.3 | Experimental set-up

Three *Leptothorax acervorum* colonies consisting of a queen and approximately 100 workers were collected on the 13th of August 2020 in Anzeindaz, Switzerland. No specific permits were required to collect or test these colonies as the ants were native to Switzerland. The colonies were housed in closed plastic test boxes with a Teflon carpet, which allowed the dummy to smoothly slide over the floor during the experiments. The walls of the boxes were covered with Fluon to prevent escapes. The boxes were equipped with a water tube and a 75 × 52 × 2 mm³ cardboard nest covered with an infrared-passing glass filter that was

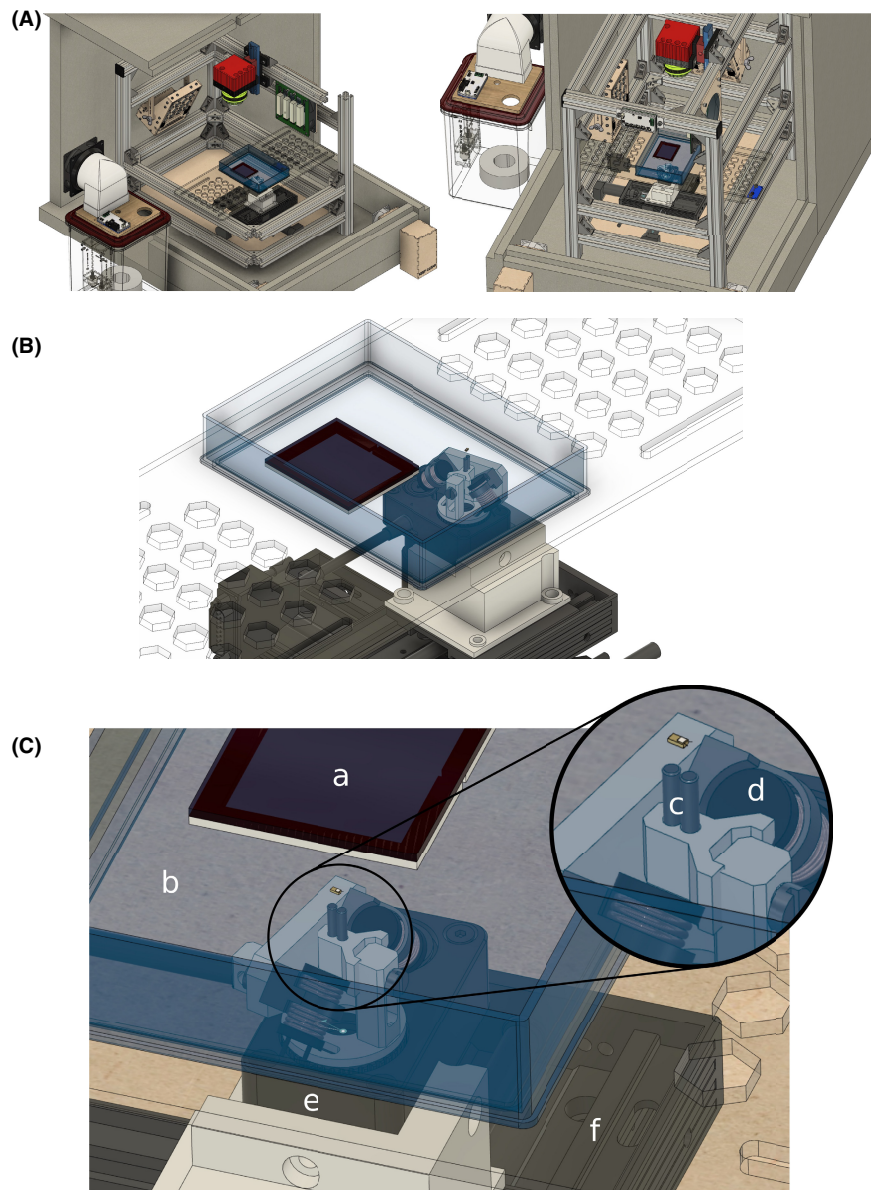


FIGURE 1 (A) Partial (left) and complete (right) schematic representations of the automated tracking system inside an open climate-controlled box paired with the robotic manipulator. The monochrome camera is depicted in red/yellow. The climate-control system is depicted on the left of the tracking system. (B) Close-up top view of the robotic manipulator, the test box in blue and the nest covered by a red-tainted glass. The robotic manipulator operates under the test box and the dummy can be seen above the two guidance magnets. (C) Close-up of the robotic manipulator and the tagged dummy (golden rectangle in close-up) placed inside the test box. The test box includes a cardboard nest (A) covered with a red glass to simulate darkness and a Teflon covered base (B). The robotic magnetic control system is placed below the test box and consists of two guidance magnets (C) and two electromagnetic coils (D) mounted on one rotational stage (E) and two translational (F) stages.

opaque for visible light. The ants were fed once a week with diluted sugar water, *Drosophila* flies and artificial ant diet (Bhatkar & Whitcomb, 1970). At the time of the experiments, the colonies had been in the laboratory for approximately 6 months. Ten days before the experimental manipulation, all the workers, the queen and the dummy were tagged using unique fiducial markers (ARTag, Apriltag) of 0.7×0.7 mm (Figure 2; Mersch et al., 2013; Richardson et al., 2022) and moved to a clean nest together with the brood. All brood items of all developmental stages were included in the

experiment to keep the colonies' composition as natural as possible. The number of brood items in the tested colonies was counted every 3 h during the experimental period (24 values per colony; mean number of brood items, colony 209: 253; colony 207: 254; colony 201: 334). The tags were glued onto the ants' thorax using fast-drying glue (Pattex ultra). Ants were tracked using the above-mentioned FORT system, which in addition to recording individual ant trajectories over extended periods of time, generates a live video stream and real-time data on individual identities that allow

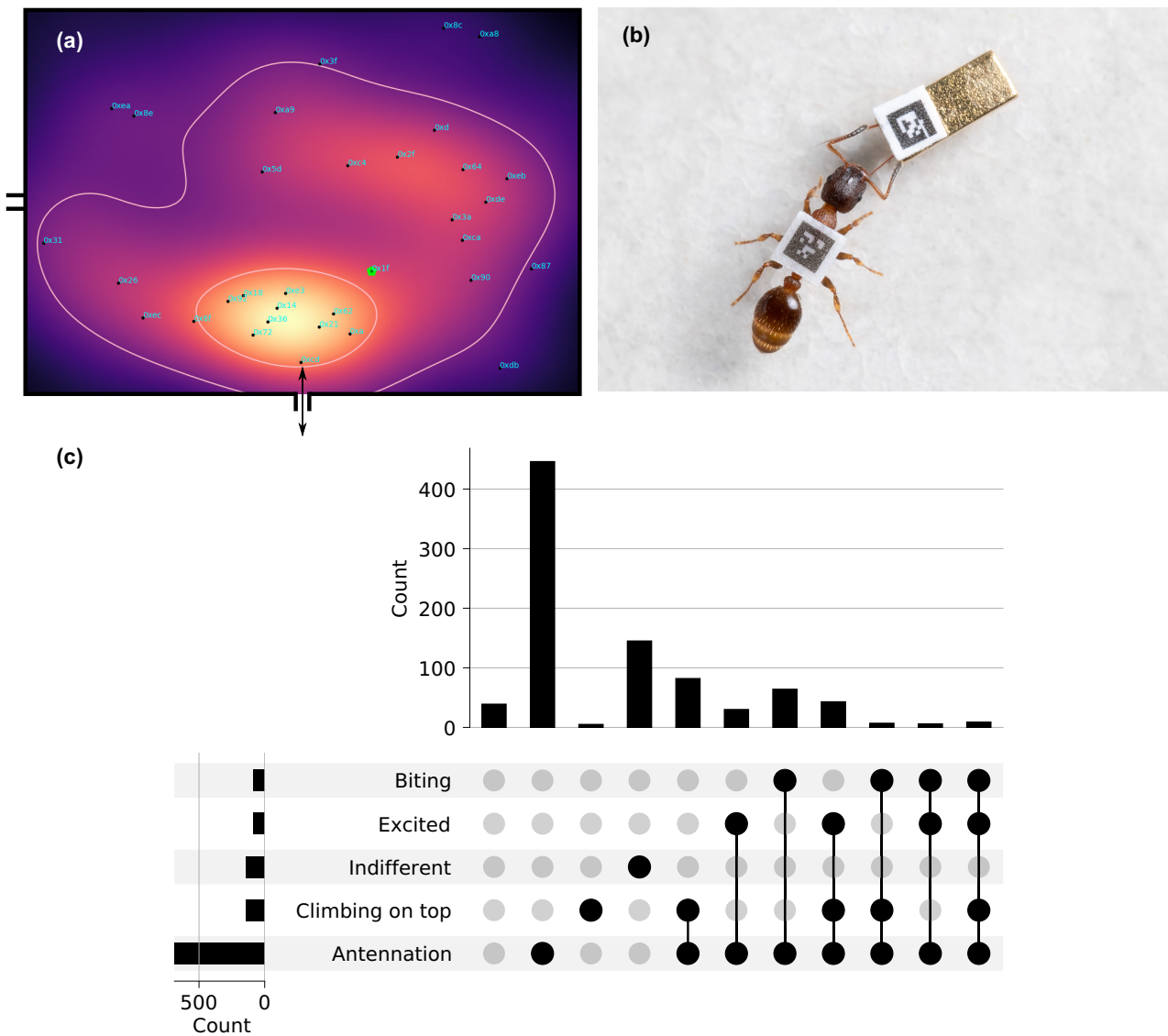


FIGURE 2 (A) Live worker density mapping for a single colony. The contours delimit the high-, medium- and low-density zones. Warmer colours represent higher-densities and colder colours represent lower densities. Each number in blue represents an ant and the green symbol marks the position of the dummy. The double arrow marks the nest entrance and the double line on the left of the figure represents the secret entrance through which the dummy was introduced into the nest. (B) A tagged ant antennating the tagged dummy. (C) Frequencies of the different behavioural responses observed (left) and the different behavioural combinations observed (top). Black circles represent the presence of a behaviour, grey circles represent absence of a behaviour and black circles linked by lines represent observed combinations of behaviours.

the experimenter to operate the manipulation system and guide the dummy inside the ant colony. The ants were kept under a daily climate cycle of 12-h daylight–12-h darkness and a temperature of 17°C at 70% relative humidity throughout the experiment.

2.4 | Experimental design

For each colony, the experiment was divided into a seven-day acclimation phase, followed by a six-day experimental phase. The experimental phase consisted of two 4-h sessions per day on six consecutive days. Every session consisted of four, 1-h periods,

alternating periods when targeted stimulations were conducted (moving periods) and periods when no targeted stimulations were conducted and the dummy remained immobile (static periods, Table S1). We randomly selected half of the sessions to start with a moving period and the other half to start with a static period. During static periods, all interactions between the dummy and the ants were initiated by the ants themselves and therefore outside the experimenters' control. By contrast, during the moving periods, a stimulation took place when the dummy entered into physical contact with the targeted ant. Each stimulation consisted of a simulated antennation event where the dummy was directed towards an ant and upon establishing physical contact, performed a sinusoidal wiggling

signal. The experimenter controlled the start time of the stimulation but once the stimulation started, the oscillation was preprogrammed to have a given frequency and duration. For these experiments, the oscillations were set to last 15 s and have a constant frequency of 1 Hz. This oscillation frequency was chosen based on pilot testing as the highest frequency that did not displace or harm the ants. Stimulations were directed exclusively towards adult workers and not brood items (even when ants were doing broodcare).

Although the same ants were occasionally tested multiple times in the same period, the same ant was never tested multiple times in a row, and an attempt was made to test as many different ants as possible in a given density zone (see below) at least once per 1-h period. Across moving periods, we performed an average of 19 stimulations per period (range 10–37 per period, corresponding to one stimulation every 3 to 10 min), targeted at an average of 14 different ants (range 8–24). This resulted in a total of 702 stimulations across all sessions and colonies. In some cases ($N=51$), stimulation events were excluded from the analysis as they included technical problems. These instances involved the loss of the magnetic capture of the dummy, debris jamming under the magnet and forceful encounters with ants after the dummy was stuck or the operator made a mistake.

To investigate the effect of worker density on behaviour, each 4-h session took place in one of three densities zones. To define density zones in real-time, the trajectory of every ant was retrieved from the FORT tracking system and plotted every 10 s in an equidistant density contour plot overlaid on the live feed. This plot contained three delimited zones calculated using Gaussian kernel density estimation, which provided visual feedback for the experimenter to direct the dummy towards an ant in the desired density zone ('gaussian_kde' function of 'scipy' python package using Scott's rule for bandwidth estimation and uniform weights, Figure 2A). The density zone where the interactions of a given period took place (i.e. low, middle or high) was pseudo-randomized a priori for each colony so that each zone was targeted in four sessions during the experiment.

To investigate whether the dummy could potentially acquire a chemical profile that influenced the ants' behaviour, the first six experimental sessions were performed with a dummy placed inside the nest 7 days before the start of the first session. The remaining six sessions were each conducted with a freshly fabricated dummy (not acclimatized) that was introduced into the colony less than 1 h before the start of the experiments. Dummy acclimatization (acclimatized or not), density zone (low, middle or high) and period type (moving or static) were fully crossed so that each colony was subjected to each combination four times during the experiment. To avoid disturbing the colony, each dummy was first introduced into the nest through a secondary nest opening that remained closed otherwise rather than via the main nest entrance.

2.5 | Data collection and coding

Interactions between ants and the dummy were detected with the FORT-myrmidon postprocessing library (<https://formicidae-tracker.github.io>). Two ellipses were defined for each ant, one comprising the head and antenna region and the other comprising the body. The dummy was defined by a single ellipse. Interactions were detected when the head ellipse of a given individual collided with the head or body ellipses of another individual or the dummy.

One hundred and fifty interactions between ants and the dummy during static periods were randomly selected to evaluate differences in worker behaviour towards moving and static dummies. In a preliminary inspection of the video recordings from the periods when the dummy was teleoperated (moving periods), five distinct behavioural responses towards the dummy were identified (Table 1). For each stimulation during moving periods, we computed the normalized worker density around the stimulated ant from the density map (i.e., a value corresponding to the colour of the pixel of the target ant in Figure 2A). To evaluate how other spatially structured aspects of the nest such as brood distribution influenced ant

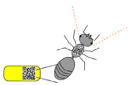
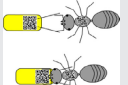
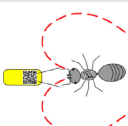

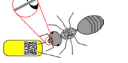
Depiction	Description
	Indifferent: The ant is touched by the wiggling dummy but shows no response (i.e. the head does not orient towards the dummy or the ant just walks/runs away without interacting with the dummy)
	Antennation: The ant touches the dummy with the antennae
	Excited runs: After antennating with the dummy, the ant quickly moves away and proceeds to interact in quick succession with ants nearby or to check on eggs. Occasionally the ant will return to interact again with the dummy
	Climbing on top: The ant climbs the dummy placing its head and thorax on top of it for more than 1 s
	Biting: The ant bites the dummy's rods

TABLE 1 Five behavioural responses of ants towards the dummy.

behaviour, we extracted the coordinates of each visible brood item from a video frame every 3h from the video recordings collected during the experiments. Next, we built brood-density maps using the same method that we employed to build worker density maps and assigned normalized brood densities to each stimulated ant based on the map closest in time to the stimulation.

From video recordings, we manually coded whether the ants responded to the dummy or not after a stimulation, how the ant responded (Table 1) and the task or action that the focal ant was performing right before the stimulation. We defined alarm as any response that included strong excitement (e.g. excited runs) and/or aggression (e.g. biting; Wilson & Regnier, 1971 see Table 1 for definitions). Climbing on top of the dummy was not classified as alarm because it was not obviously related with behaviours associated with anxiety or agitation. In addition, for each stimulation during moving periods and interaction with the dummy during static periods, we recorded the ant ID, the colony ID and whether the dummy was acclimatized or freshly made (see Table S2 for model structures).

3 | RESULTS

3.1 | General description of the behavioural responses to tactile stimulation

In total, we analysed 801 interactions between dummies and 208 different ants. Of these, 651 were interactions where the dummy was mobile (i.e. stimulations where the dummy was teleoperated to interact with an ant). Most ants (77%) were targeted only once during the experiments. We observed five different types of responses towards the dummy after stimulation: indifference, antennation, excited runs, climbing on top and biting (Table 1). If the ants exhibited any behaviour towards the dummy other than indifference, we considered that they had responded to the dummy (value of 1 in response variable of Model 3, see Supplementary Methods). Ants usually exhibited a single-behaviour response (569 out of 801 events), but occasionally multiple behaviours were performed sequentially as a response to the dummy stimulation (232 events). Excited runs, biting or any sequence containing either or both response types was classified as alarm (value of 1 in response variable of Models 2 and 4, see Supplementary Methods). The frequencies of each response as well as the observed combinations of response types are shown in Figure 2C.

Antennation was the most frequent behaviour observed both as a single-behaviour response and as part of response combinations. The behaviours involved in the alarm response (biting and excited runs) only occurred as part of combinations (Figure 2C). Ants were less likely to antennate the dummy when it was moving than when it was static (GLMM 1: $\chi^2=35.01$, $df=1$, $p<0.001$, estimate \pm SD = -2.72 ± 0.63 , Table S2). However, ants were significantly more likely to display alarm towards the dummy when it was moving than when it was static (GLMM 2: $\chi^2=60.31$, $df=1$, $p<0.001$, estimate \pm SD = 9.33 ± 1.73 , Table S2). These results indicate that ants

perceive the dummy as a bigger threat when is moving compared to when it is static.

3.2 | Factors influencing the probability of responding to the dummy

In Model 3 (Table S2), we evaluated how the probability of ants to respond to the moving dummy was influenced by the interaction between the density of workers and the task the ant was performing just before the stimulation took place (Figure S3), the interaction between the brood density and the task, and the acclimatization of the dummy (i.e., whether or not it had been in the colony during the 7 days prior to the experiment). Given that the interactions between worker or brood density and task were not significant (full-reduced model comparison lacking interactions: $\chi^2=14.14$, $df=8$, $p=0.078$), we proceeded to evaluate the individual fixed effects of the predictors from a model lacking interaction terms (full-null model comparison: $\chi^2=44.22$, $df=7$, $p<0.001$).

Worker density had a significant effect on the probability to respond to the dummy (GLMM Likelihood ratio test = 17.42, $df=1$, $p<0.001$; Figure 3B). Ants were less likely to respond the higher the density of conspecifics around them at the time of stimulation (estimate \pm SE = -0.51 ± 0.12). A possible explanation for these results could be that areas with different worker densities are populated by distinct subsets of workers with intrinsic differences in response thresholds. To exclude this possibility, we compared the probability to respond to the dummy of individuals which had been tested in two of the discrete density areas generated during the data collection process that significantly differ in response probability (i.e. we compared the probability of response of ants stimulated at least once in both the high- and middle-density zones, $N=53$; and the high- and low-density zones, $N_{ants}=53$; Figure S4). These comparisons revealed that the probability of the same ant responding to the dummy was significantly lower when it was in the high-density zone, compared to when it was in the middle- or low-density zones but did not differ between the middle- and low-density zones (Table S3). The same results were obtained when only a single, randomly selected value per ant and density zone was considered (Table S3). These comparisons between responses of single individuals at different locations in the nest indicate that the association between local density and response probability was not a side effect of differences in the average behavioural responses between worker task groups occupying different parts of the nest, but instead represent dynamical changes in the individual responses to tactile stimuli as a function of the immediate local conspecific density.

Brood density did not influence the response probabilities of workers (GLMM Likelihood ratio test = 0.10, $df=1$, $p=0.75$, Figure 3A). However, because continuous brood density and continuous worker density were weakly but significantly correlated (Pearson's r [$df=649$] = 0.09, $p<0.01$), ant density could have been masking an effect of brood density in Model 3. To exclude this possibility, we re-evaluated the effect of brood density on ants'

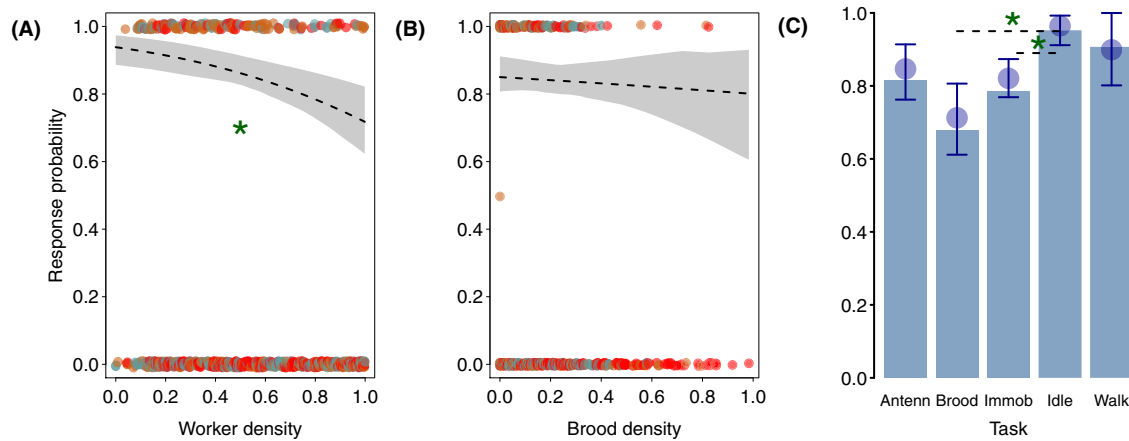


FIGURE 3 Overall responsiveness to tactile stimulation. (A) The probability of responding to the dummy as a function of normalized worker density at the area where the stimulation took place. (B) The probability of responding to the dummy as a function of normalized brood density at the area where the stimulation took place. (C) The probability of responding to the dummy depending on the task the ants were performing at the time of stimulation. 'Antenn' stands for antennation, 'Brood' stands for brood care and 'Immob' stands for immobile. In panels (A and B), each dot is an individual coloured according to colony. The dashed line is the fitted model and margins of the shaded area represent the 95% confidence intervals of the model. In panel (C), blue circles are the fitted values calculated by the model and vertical lines represent the 95% confidence intervals of the model estimates calculated via bootstrapping. Bar heights represent observed mean response probabilities per task. Asterisks denote significant differences between groups.

probability of response by refitting Model 3 excluding worker density as a predictor. The results of this model confirmed that brood density had no significant effect on workers' probability of response (GLMM estimate \pm SE = -0.06 ± 0.13 , $p = 0.62$).

The probability of response to the dummy was also influenced by the task that the ants were performing just before receiving the stimulation (GLMM Likelihood ratio test = 20.01, $df = 4$, $p < 0.001$; Figure 3C). Ants tending to brood and immobile ants were significantly less likely to respond to the dummy than idle ants. To exclude the possibility that the differences in probability of response towards the dummy according to task were due to different tasks being performed by different subsets of workers with intrinsic differences in response thresholds, we re-evaluated the pairwise differences between tasks revealed by Model 3, considering only those individuals that had been tested when performing both tasks in the pair. Both previously significant pairwise comparisons remained significant (Figure 3, Table S4).

Finally, the probability of response to the dummy was not significantly affected by the acclimatization of the dummy (GLMM Likelihood ratio test = 0.87, $df = 1$, $p = 0.35$) as ants responded similarly to acclimatized and freshly made dummies.

To evaluate whether physical contact between the stimulated worker and the brood influenced the probability of the worker responding to the dummy, we compared the probability of response of ants that had been tested both when they were in physical contact with brood and when they were not ($N = 55$). When ants were in contact with brood they were less likely to respond to the dummy than when they were not (McNemar chi-square = 72.75, $p < 0.001$, odds ratio = 6.15, 95% CI [3.99, 11.57]), and this difference remained significant when only one randomly selected response per

ant in each condition (presence or absence of brood) was considered (McNemar chi-square = 19.59, $p < 0.001$, odds ratio = 3.92, 95% CI [2.18, 9.85]).

3.3 | Factors influencing the probability of alarm

In Model 4 (Table S2), we tested whether the probability of being alarmed by the moving dummy was influenced by the interactions between the density of workers and the task being performed just before the dummy stimulation, the interaction between brood density and the task, and the acclimatization of the dummy. Given that the models with and without interactions did not differ ($\chi^2 = 8.69$, $df = 8$, $p = 0.37$), we proceeded to evaluate the individual fixed effects of the predictors in a model lacking interactions (full-null model comparison: $\chi^2 = 31.61$, $df = 7$, $p < 0.001$).

The density of workers around the stimulated ant had a significant effect on the probability that ants were alarmed by the dummy (GLMM Likelihood ratio test = 14.78, $df = 1$, $p < 0.001$, estimate \pm SE = -0.46 ± 0.12 ; Figure 4A). Ants were significantly more alarmed the lower the density of workers in the area where the stimulation took place. Similarly, brood density had a significant effect on the ants' alarm probability (GLMM Likelihood ratio test = 5.20, $df = 1$, $p = 0.02$, estimate \pm SE = -0.36 ± 0.17 ; Figure 4A), with ants being more alarmed at lower brood densities.

The probability of ants to be alarmed by the dummy was not significantly affected by the task the ants were performing at the time of the stimulation (GLMM Likelihood ratio test = 4.50, $df = 4$, $p = 0.34$, Figure 4B) nor by the acclimatization of the dummy (GLMM Likelihood ratio test = 1.84, $df = 1$, $p = 0.17$).

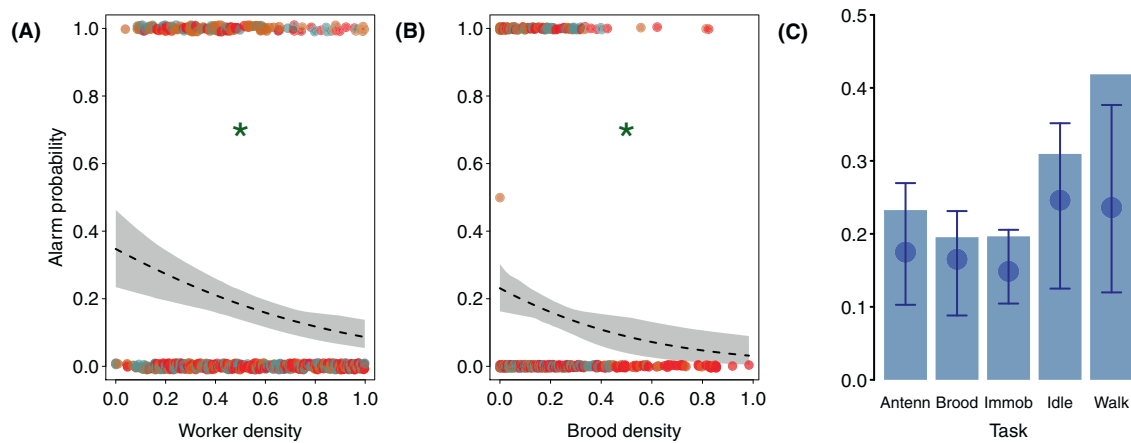


FIGURE 4 Alarm to tactile stimulation. (A) The probability of being alarmed by the dummy as a function of normalized worker density at the area where the stimulation took place. (B) The probability of being alarmed by the dummy as a function of normalized brood density at the area where the stimulation took place. (C) The probability of being alarmed by the dummy depending on the task the ants were performing at the time of stimulation. ‘Antenn’ stands for antennation, ‘Brood’ stands for brood care and ‘Immob’ stands for immobile. In panels (A and B), each dot is an individual coloured according to colony. The dashed line is the fitted model and the margins of the shaded area represent the 95% confidence intervals of the model. In panel (C), blue circles are the fitted values calculated by the model and vertical lines represent 95% confidence intervals of the model estimates calculated via bootstrapping. Bar heights represent observed mean alarm probabilities per task.

As for Model 3, we conducted additional analyses focusing on ants that had been tested both in the high- and middle-worker density zones or in the high- and low-worker density zones used during data collection to exclude effects caused by intrinsic differences in response thresholds between ants located in the different density zones. We focused on these pairwise comparisons because the probability of ants to be alarmed differed between these density zones when worker density was considered as a discrete variable (Figure S4). These pairwise tests confirmed the significant effect of worker density both when all observations per ant and zones were considered as well as when a single, randomly selected observation per ant and zone was considered (Table S5). Similarly, we performed equivalent analyses evaluating whether the probability of alarm of individual ants differed when they were tested in different brood density areas by binning brood density into three discrete categories like those used for worker density. Contrary to the effects of worker density on alarm probability, ants did not differ in their probability of alarm depending on the brood density area where they were stimulated (full-reduced model comparison lacking brood density area: $\chi^2=4.75$, $df=2$, $p=0.09$).

3.4 | Testing the habituation hypothesis

The association between the probability of response and local worker density could stem from a habituation process, whereby ants in a high-density area receive more interactions and so become less responsive towards stimuli. To test this hypothesis, we first evaluated whether worker density at the location where a stimulation took place was correlated with the number of interactions in the

previous 1, 2 and 5 min. In all cases, worker density and number of interactions were significantly and positively correlated (Pearson's correlation 1 min: r ($df=649$)=0.59, $p<0.001$; Pearson's correlation 2 min: r ($df=649$)=0.59, $p<0.001$; Pearson's correlation 5 min: r ($df=649$)=0.54, $p<0.001$, Figure S5).

Next, we evaluated whether the number of previous interactions with nestmates had an effect on the probabilities of responding and being alarmed by the dummy when controlling for worker density and task being performed at the stimulation (Models 5 and 6). The number of nestmate interactions that an ant received during the minute before the stimulation had a negative effect on the probability of responding to the dummy (GLMM estimate \pm SE = -0.45 ± 0.14 , $\chi^2=10.3$, $df=1$, $p=0.001$, Model 5). This effect was still significant when the time window considered was expanded to 2 min (GLMM estimate \pm SE = -0.40 ± 0.14 , $\chi^2=8.05$, $df=1$, $p=0.004$) and 5 min (GLMM estimate \pm SE = -0.40 ± 0.14 , $\chi^2=8.31$, $df=1$, $p=0.003$). By contrast, the number of nestmate interactions that an ant received during the minute before the stimulation by the dummy did not have a significant effect on the probability of being alarmed by the dummy in any of the time windows (Model 6 1 min: estimate \pm SE = -0.15 ± 0.15 , $\chi^2=1.12$, $df=1$, $p=0.29$; 2 min: estimate \pm SE = 0.05 ± 0.14 , $\chi^2=0.14$, $df=1$, $p=0.71$; 5 min: estimate \pm SE = 0.15 ± 0.14 , $\chi^2=1.22$, $df=1$, $p=0.27$) before the interaction.

To rule out the possibility that these differences may stem from intrinsic behavioural differences associated with the overall level of activity of different ants, we investigated whether for a given ant, the probabilities of response were influenced by the ant's current interaction rate. We classified the stimulation by the dummy as having occurred during either a high- or a low- interactivity period. High- and low-interactivity periods were those where the number

Window length	Dependent variable	McNemar chi-square	Odds ratio	95% CI	p
1 min	Prob. Response	93.94	5.69	3.94, 9.34	<0.001
2 min	Prob. Response	92.92	4.67	3.49, 7.47	<0.001
5 min	Prob. Response	117.04	6.71	4.66, 11.12	<0.001

TABLE 2 Comparisons of ants' probabilities of responding to the dummy (Prob. Response) during high and low-sociality windows.

of nestmate interactions experienced by the target ant was higher or lower (respectively) than its average number of nestmate interactions measured during the 12h pre- and the 12h poststimulation. These analyses showed that ants were less likely to respond to the dummy when the dummy stimulation took place during a high-activity period compared to when the ant was in a low-activity period. This result was not influenced by the length of the time window considered (Table 2).

4 | DISCUSSION

We developed a new robotic manipulation platform consisting of an electromagnetic control system and a magnetically actuated ant-sized mobile dummy that can be used to stimulate individuals at specific nest locations. After validating the dummy as an effective stimulus, we used this system to conduct a behavioural experiment in ants employing simulated antennation as a tactile stimulus in situ, without extricating the individual workers from their social environment or interrupting the task they were conducting. In our experiment, we assessed how the specific task that the individuals were performing at a given point in time as well as the density of workers and brood around those individuals influenced their probability to respond and their probability of being alarmed by a mobile tactile stimulus.

The probability of an ant responding to the dummy varied depending on the task they were performing at the time of the interaction, with ants doing brood care being the least likely to respond. By contrast, ants that were inactive (idle or immobile; Charbonneau et al., 2017) when the interaction took place had a higher probability of responding to the dummy. In isolation, these results could be explained by the existence of different average response thresholds in different groups of ants (inactive vs broodcaring ants; Charbonneau et al., 2017). However, individual-level analysis revealed that the different response probabilities observed were not the result of intrinsic variation in response thresholds among workers but rather represented a plastic response mediated by the task being performed. The higher responsiveness of idle ants compared with ants performing brood care could be explained if there was an individual cost associated with task switching (Goldsby et al., 2012). Individuals performing a task that switch to a new task might delay the performance of the new and/or the old task due to the time required for cognitive retrieval of the motor patterns involved in the tasks (Chittka et al., 1997). For instance, *Temnothorax rugulatus* workers have longer inter-task intervals when they switch tasks than

when they stop and then retake the same task they were previously performing, suggesting that there is a temporal cost in task switching (Leighton et al., 2017). In line with our finding that idle ants have higher probabilities to respond to the dummy than ants performing brood care, previous studies found that inactive ants pay smaller task switching costs than ants performing brood care (Leighton et al., 2017). This could be because inactive ants do not really switch task when they start interacting with the dummy but merely 'activate', whereas brood caring ants must truly switch task. Alternatively, the differences in responsiveness between idle ants compared with brood-caring ants could be due to a decreased sensitivity to stimuli given their longer intervals of inactivity preceding the dummy stimulation. Previous studies have shown that the length of the inactivity interval preceding an interaction with another ant predicts the delay between interaction and response following a negative exponential curve (Cole, 1991). Given that the length of the inactivity period of brood-caring ants would be close to 0, this could explain why they would require a longer activation period that went beyond the 15 s of the stimulation than idle ants with longer inactive intervals. Contrary to the overall probability of response, we did not find that the specific task the ants were performing at the time of the interaction influenced their probability of alarm.

The density of workers in the area where the dummy stimulation took place negatively influenced the ants' probability of response. There are two possible explanations for this pattern. The first is that ants with high response thresholds are more likely to stay in denser regions of the nest. Alternatively, ants may modulate their response behaviour depending on the worker density at their spatial location. To disentangle between these two explanations, we compared the response of workers that interacted with the dummy in at least two discrete zones with different worker densities. These analyses revealed that workers' sensitivity to tactile stimulation is context-dependent as they exhibited higher response probabilities when stimulated in less dense zones. Worker density also influenced the probability that ants were alarmed by the dummy. As was the case with overall responsiveness, individual-level analysis revealed that these results were not due to ants with different alarm thresholds preferentially staying in areas with specific densities. Instead, ants probability of alarm was lowered in areas with higher worker density.

Our results on density-dependent responsiveness and the negative effects of previous number of interactions on probability of response suggest that habituation, a simple form of associative learning that involves a reduction in responsiveness to a repeated stimulus (Wilson, 1998) plays a key role in modulating

responsiveness via physical contact in ant colonies. Habituation to odorant stimuli has been previously described in ants. In *Pheidole* desert ants, the mean level of aggression between workers from different colonies was significantly lower if the ants had interacted before (Langen et al., 2000). Similarly, in the Argentine ant, repeated exposure to the alarm pheromone of nestmates reduced alarmed responses until after four or five exposures, there was no behavioural response at all (Maccaro et al., 2020). Although tactile habituation has rarely been investigated in insects, Hölldobler and Wilson (1990) proposed that the variation in antennal postures exhibited by ants during trophallaxis could be explained by a necessity to avoid habituation to the tactile stimuli. Support for the tactile habituation hypothesis in our study comes from our results showing that the number of previous contacts was negatively related to ants' probabilities of response, meaning that ants become habituated to tactile stimuli in higher density zones because they are exposed to more frequent contacts with conspecifics. Similarly, individual level analysis revealed that the probability of response was lower when the interaction with the dummy took place in a time window where workers had experienced above-average numbers of social contacts compared to windows where ants experience below-average numbers of contacts. By contrast, worker density effects on ants' alarm probabilities could not be explained by the habituation hypothesis as the number of previous interactions did not predict alarm probability when conspecific density and task was controlled for. Instead, the reduction of ants' probability of alarm in denser areas could be explained if higher worker density leads to higher or more diverse volatile compound concentrations (pheromones or cuticular hydrocarbons) that would elevate ants' alarm thresholds. Higher concentrations and/or diversity of volatile compounds could perhaps convey 'safety in numbers' information or reduce aggression, as being aggressive in areas of higher conspecific density is likely to increase the risk of injury (Hughes & Banks, 2016).

Brood density did not influence ants' probability of response, but had a significant effect on ants' probability of alarm. Individual-level pairwise tests comparing ants' probability of alarm when stimulated in multiple discrete brood density areas revealed that the observed significant effect of brood density on the alarm probability likely stem from intrinsic differences in alarm thresholds between ants located in areas of different brood density. This could be due to an age effect as younger ants tend to stay closer to brood (Mirenda & Vinson, 1981; Sendova-Franks & Franks, 1995) and in some species have been shown to display lower levels of aggression than older ants (Amador-Vargas, 2012).

The robotic platform presented here allowed us to independently evaluate how the local worker density and the task an ant is performing independently influence its behavioural responses by applying controlled, standardized tactile stimuli that simulate antennation to targeted ants inside the nest. Using this platform, we were able to target individuals performing various tasks inside areas with different densities without disturbing overall colony activity. This study shows a successful application

of a robotic manipulation to investigate the individual-level behavioural rules that underpin the organization of ant colonies. These experiments validate the use of teleoperated mobile dummies to investigate social insect behaviour in situ and open new research avenues to explore the effects of more complex stimuli, such as the combination of tactile and chemical signals, on ant behaviour.

In future studies, the robotic platform could be programmed to operate in an automated fashion, to further reduce the role of human bias and increase the throughput of behavioural experiments. Furthermore, given that the dummy is generic in its shape, composition, and colour (a gold-coated magnet) its design is not specific to a single species of social insect and can be easily scaled to match the size of larger organisms such as bees and termites by using larger magnets and stages. Another potential extension of this system would be to 3D print different ant shapes that can be fitted on the dummy to explore responses to parasitic species (Fischer et al., 2020). In addition, future studies could incorporate variables such as age and (if appropriate) caste in the experimental design to study their effects on inter-individual behavioural variation as well as explore how different species with varied social organization and parasitic pressures differ on worker behaviour. Focusing on a subset of individuals over longer periods of time would also allow to evaluate interesting questions beyond the scope of this paper such as individual consistency of behavioural responses over time. Overall, the system employed here can be scaled and adapted to address a variety of questions in different model organisms to better understand the behavioural commonalities among these taxa and thus further our knowledge of decentralized collective systems.

AUTHOR CONTRIBUTIONS

Matthias Rüegg, Alba Motes-Rodrigo, Nathalie Stroeymeyt, Thomas O. Richardson, Mahmut Selman Sakar and Laurent Keller conceived the experiments and designed the methodology; Matthias Rüegg and Mahmut Selman Sakar developed the robotic manipulation platform and Alexandre Tuleu developed the automated tracking system; Matthias Rüegg collected the data; Alba Motes-Rodrigo analysed the data; Alba Motes-Rodrigo and Laurent Keller led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

N.S. and T.O.R. acknowledge funding by an ERC Starting Grant ('DISEASE', no. 802628, to N.S.). L.K. acknowledges funding from an ERC Consolidator Grant ('resiliANT', no. 741491) and from an FNS Grant ('Molecular evolution and genome symbiosis in double-clonal ants', no. 310030_200437).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.14244>.

DATA AVAILABILITY STATEMENT

All information about the tracking system employed in this study can be found in the system's linked GitHub repository (Tuleu, 2023). Video examples as well as raw data and code used for statistical analysis and plotting can be found in the OSF project folder (Motes-Rodrigo & Rüegg, 2023).

ORCID

Alba Motes-Rodrigo  <https://orcid.org/0000-0002-4444-7723>
 Nathalie Stroeymeyt  <http://orcid.org/0000-0001-8047-449X>
 Thomas O. Richardson  <http://orcid.org/0000-0002-0220-4897>
 Mahmut Selman Sakar  <https://orcid.org/0000-0002-7226-3382>

REFERENCES

- Amador-Vargas, S. (2012). Behavioral responses of acacia ants correlate with age and location on the host plant. *Insectes Sociaux*, 59(3), 341–350. <https://doi.org/10.1007/s00040-012-0226-x>
- Bhatkar, A., & Whitcomb, W. H. (1970). Artificial diet for rearing various species of ants. *The Florida Entomologist*, 53(4), 229. <https://doi.org/10.2307/3493193>
- Bierbach, D., Landgraf, T., Romanczuk, P., Lukas, J., Nguyen, H., Wolf, M., & Krause, J. (2018). Using a robotic fish to investigate individual differences in social responsiveness in the guppy. *Royal Society Open Science*, 5(8), 181026. <https://doi.org/10.1098/rsos.181026>
- Buczowski, G., & Silverman, J. (2005). Context-dependent nestmate discrimination and the effect of action thresholds on exogenous cue recognition in the Argentine ant. *Animal Behaviour*, 69(3), 741–749. <https://doi.org/10.1016/j.anbehav.2004.06.027>
- Charbonneau, D., Sasaki, T., & Dornhaus, A. (2017). Who needs 'lazy' workers? Inactive workers act as a 'reserve' labor force replacing active workers, but inactive workers are not replaced when they are removed. *PLoS One*, 12(9), e0184074. <https://doi.org/10.1371/journal.pone.0184074>
- Chittka, L., Gumbert, A., & Kunze, J. (1997). Foraging dynamics of bumble bees: Correlates of movements within and between plant species. *Behavioral Ecology*, 8(3), 239–249. <https://doi.org/10.1093/beheco/8.3.239>
- Cole, B. J. (1986). The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): Time budgets and the evolution of worker reproduction. *Behavioral Ecology and Sociobiology*, 18(3), 165–173. <https://doi.org/10.1007/BF00290820>
- Cole, B. J. (1991). Short-term activity cycles in ants: A phase-response curve and phase resetting in worker activity. *Journal of Insect Behavior*, 4(2), 129–137. <https://doi.org/10.1007/BF01054607>
- Dornhaus, A., & Chittka, L. (2005). Bumble bees (*Bombus terrestris*) store both food and information in honeypots. *Behavioral Ecology*, 16(3), 661–666. <https://doi.org/10.1093/beheco/ari040>
- Fischer, G., Friedman, N. R., Huang, J.-P., Narula, N., Knowles, L. L., Fisher, B. L., Mikheyev, A. S., & Economo, E. P. (2020). Socially parasitic ants evolve a mosaic of host-matching and parasitic morphological traits. *Current Biology*, 30(18), 3639–3646.e4. <https://doi.org/10.1016/j.cub.2020.06.078>
- Franks, N. R., Podesta, J. A., Jarvis, E. C., Worley, A., & Sendova-Franks, A. B. (2022). Robotic communication with ants. *Journal of Experimental Biology*, 225(15), jeb244106. <https://doi.org/10.1242/jeb.244106>
- Franks, N. R., & Tofts, C. (1994). Foraging for work: How tasks allocate workers. *Animal Behaviour*, 48(2), 470–472. <https://doi.org/10.1006/anbe.1994.1261>
- Gartland, L. A., Firth, J. A., Laskowski, K. L., Jeanson, R., & Ioannou, C. C. (2022). Sociability as a personality trait in animals: Methods, causes and consequences. *Biological Reviews*, 97(2), 802–816. <https://doi.org/10.1111/brv.12823>
- Goldsby, H. J., Dornhaus, A., Kerr, B., & Ofria, C. (2012). Task-switching costs promote the evolution of division of labor and shifts in individuality. *Proceedings of the National Academy of Sciences of the United States of America*, 109(34), 13686–13691. <https://doi.org/10.1073/pnas.1202233109>
- Gordon, D. M. (2020). Movement, encounter rate, and collective behavior in ant colonies. *Annals of the Entomological Society of America*, 114(5), 541–546. <https://doi.org/10.1093/aesa/saaa036>
- Gordon, D. M., & Mehdiabadi, N. J. (1999). Encounter rate and task allocation in harvester ants. *Behavioral Ecology and Sociobiology*, 45(5), 370–377. <https://doi.org/10.1007/s002650050573>
- Gordon, D. M., Paul, R. E., & Thorpe, K. (1993). What is the function of encounter patterns in ant colonies? *Animal Behaviour*, 45(6), 1083–1100. <https://doi.org/10.1006/anbe.1993.1134>
- Halloy, J., Sempo, G., Caprari, G., Rivault, C., Asadpour, M., Tache, F., Said, I., Durier, V., Canonge, S., Ame, J. M., Detrain, C., Correll, N., Martinoli, A., Mondada, F., Siegart, R., & Deneubourg, J. L. (2007). Social integration of robots into groups of cockroaches to control self-organized choices. *Science*, 318(5853), 1155–1158. <https://doi.org/10.1126/science.1144259>
- Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Springer Berlin Heidelberg. <https://doi.org/10.1007/978-3-662-10306-7>
- Hughes, N. K., & Banks, P. B. (2016). Olfactory contacts mediate plasticity in male aggression with variable male density. *Journal of Mammalogy*, 97(2), 444–454. <https://doi.org/10.1093/jmammal/gyv188>
- Hughes, W. O. H., & Goulson, D. (2001). Polyethism and the importance of context in the alarm reaction of the grass-cutting ant, *Atta capiguara*. *Behavioral Ecology and Sociobiology*, 49(6), 503–508. <https://doi.org/10.1007/s002650100321>
- Jandt, J. M., Bengston, S., Pinter-Wollman, N., Pruitt, J. N., Raine, N. E., Dornhaus, A., & Sih, A. (2014). Behavioural syndromes and social insects: Personality at multiple levels: Behavioural syndromes and social insects. *Biological Reviews*, 89(1), 48–67. <https://doi.org/10.1111/brv.12042>
- Josens, R., Lopez, M. A., Jofré, N., & Giurfa, M. (2018). Individual size as determinant of sugar responsiveness in ants. *Behavioral Ecology and Sociobiology*, 72(10), 162. <https://doi.org/10.1007/s00265-018-2581-8>
- Koto, A., Mersch, D., Hollis, B., & Keller, L. (2015). Social isolation causes mortality by disrupting energy homeostasis in ants. *Behavioral Ecology and Sociobiology*, 69(4), 583–591. <https://doi.org/10.1007/s00265-014-1869-6>
- Krause, J., Winfield, A. F. T., & Deneubourg, J.-L. (2011). Interactive robots in experimental biology. *Trends in Ecology & Evolution*, 26(7), 369–375. <https://doi.org/10.1016/j.tree.2011.03.015>
- Landgraf, T., Moballeghe, H., & Rojas, R. (2008). Design and development of a robotic bee for the analysis of honeybee dance communication. *Applied Bionics and Biomechanics*, 5(3), 157–164. <https://doi.org/10.1080/11762320802617552>
- Langen, T. A., Triplet, F., & Nonacs, P. (2000). The red and the black: Habituation and the dear-enemy phenomenon in two desert Pheidole ants. *Behavioral Ecology and Sociobiology*, 48(4), 285–292. <https://doi.org/10.1007/s002650000223>
- Leighton, G. M., Charbonneau, D., & Dornhaus, A. (2017). Task switching is associated with temporal delays in *Temnothorax rugatulus* ants. *Behavioral Ecology*, 28(1), 319–327. <https://doi.org/10.1093/beheco/arw162>

- Maccaro, J. J., Whyte, B. A., & Tsutsui, N. D. (2020). The ant who cried wolf? Short-term repeated exposure to alarm pheromone reduces behavioral response in Argentine ants. *Insects*, 11(12), Article 12. <https://doi.org/10.3390/insects11120871>
- Martins, E. P., Ord, T. J., & Davenport, S. W. (2005). Combining motions into complex displays: Playbacks with a robotic lizard. *Behavioral Ecology and Sociobiology*, 58(4), 351–360. <https://doi.org/10.1007/s00265-005-0954-2>
- Mersch, D. P. (2016). The social mirror for division of labor: What network topology and dynamics can teach us about organization of work in insect societies. *Behavioral Ecology and Sociobiology*, 70(7), 1087–1099. <https://doi.org/10.1007/s00265-016-2104-4>
- Mersch, D. P., Crespi, A., & Keller, L. (2013). Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science*, 340(6136), 1090–1093. <https://doi.org/10.1126/science.1234316>
- Michelsen, A., Andersen, B. B., Storm, J., Kirchner, W. H., & Lindauer, M. (1992). How honeybees perceive communication dances, studied by means of a mechanical model. *Behavioral Ecology and Sociobiology*, 30(3–4), 143–150. <https://doi.org/10.1007/BF00166696>
- Mirenda, J. T., & Vinson, S. B. (1981). Division of labour and specification of castes in the red imported fire ant *Solenopsis invicta* Buren. *Animal Behaviour*, 29(2), 410–420. [https://doi.org/10.1016/S0003-3472\(81\)80100-5](https://doi.org/10.1016/S0003-3472(81)80100-5)
- Motes-Rodrigo, A., & Rüegg, M. (2023). *Ant-dummy project documentation and code*. <https://doi.org/10.17605/OSF.IO/4HT26>
- Richardson, T. O., Stroeymeyt, N., Crespi, A., & Keller, L. (2022). Two simple movement mechanisms for spatial division of labour in social insects. *Nature Communications*, 13(1), 6985. <https://doi.org/10.1038/s41467-022-34706-7>
- Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annual Review of Entomology*, 37(1), 637–665. <https://doi.org/10.1146/annurev.en.37.010192.003225>
- Robinson, G. E., & Page, R. E. (1989). Genetic basis for division of labour in an insect society. In *The genetics of social evolution*. CRC Press.
- Sendova-Franks, A. B., & Franks, N. R. (1995). Spatial relationships within nests of the ant *Leptothorax unifasciatus* (Latr.) and their implications for the division of labour. *Animal Behaviour*, 50(1), 121–136. <https://doi.org/10.1006/anbe.1995.0226>
- Simon, R., Varkevisser, J., Mendoza, E., Hochradel, K., Elsinga, R., Wiersma, P. G., Middelburg, E., Zoeter, E., Scharff, C., Riebel, K., & Halfwerk, W. (2023). RoboFinch: A versatile audio-visual synchronised robotic bird model for laboratory and field research on songbirds. *Methods in Ecology and Evolution*, 14, 1092–1103. <https://doi.org/10.1111/2041-210X.14063>
- Tuleu, A. (2023). *FORMicidae tracker (FORT): Open source online tracker*. <https://doi.org/10.5281/ZENODO.10018588>
- Wilson, D. A. (1998). Habituation of odor responses in the rat anterior piriform cortex. *Journal of Neurophysiology*, 79(3), 1425–1440. <https://doi.org/10.1152/jn.1998.79.3.1425>
- Wilson, E. O., & Regnier, F. E. (1971). The evolution of the alarm-defense system in the formicine ants. *The American Naturalist*, 105(943), 279–289. <https://doi.org/10.1086/282724>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Top view of the manipulator (grey) operating under the testing box (blue). The nest is illustrated by the reddish square inside the testing box.

Figure S2. Sideview of the manipulator operating under the testing box (blue).

Figure S3. Distribution of tasks being performed by the target ants in each density zone at the time of stimulation.

Figure S4. Differences in response probability (A) and alarm probability (B) of ants depending on the discrete worker density zone where they were stimulated.

Figure S5. Correlations between normalized worker density at the location where each stimulation took place and the number of interactions each stimulated ant experienced in the previous minute ($r=0.57$, $p<0.001$), two minutes ($r=0.59$, $p<0.001$) or five minutes ($r=0.54$, $p<0.001$).

Table S1. Example of a testing schedule for one of the colonies investigated.

Table S2. Summary of model structures.

Table S3. Pairwise comparisons of the probability to respond towards the dummy of ants tested in two density zones which were significantly different in Model 3 when density was modeled as a discrete variable containing three levels.

Table S4. Pairwise comparisons of the probability to respond towards the dummy of ants tested when doing multiple tasks which were significantly different in Model 3.

Table S5. Pairwise comparisons of the probability to be alarmed of ants tested in two density zones which were significantly different in Model 4 when density was modeled as a discrete variable containing three levels.

How to cite this article: Rüegg, M., Motes-Rodrigo, A., Tuleu, A., Stroeymeyt, N., Richardson, T. O., Sakar, M. S., & Keller, L. (2024). Precise tactile stimulation of worker ants by a robotic manipulator reveals that individual responses are density- and context-dependent. *Methods in Ecology and Evolution*, 15, 117–129. <https://doi.org/10.1111/2041-210X.14244>