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Multi-modal cue integration in the black garden ant

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Abstract

In a constantly changing environment, it is advantageous for animals to encode a location (such as a food source) relying on more than one single cue. A certain position might, in fact, be signalled by the presence of information acquired through different sensory modalities which may be integrated into cohesive memories. Here, we aimed to investigate multi-sensory learning capabilities and multi-modal information integration in *Lasius niger* ants. Individual ants were placed in a Y-maze where odour information always led to a food reward; moreover, arm and wall colour were also predictive but only when co-occurring with odour in a specific combination. At test, the odour cue was made uninformative (it was present in both arms). Ants were still able to correctly locate the reward by integrating odour with the right colour and side combination. In a second experiment, we tested whether multi-modal cue integration can take place in a single trial. To this end, ants were exposed to a rewarded odour in a single-arm maze and could experience the Y-maze (with all available cues) only once. At test (which was identical to that of Experiment 1), ants showed a slight preference for the correct colour-side combination, although not significantly different from chance level. Our results showed the capability of black garden ants to code apparently redundant contextual information and to create and rely on conditional relationships between the information available. We argue that future studies should deepen the inquiry on the timing and progression of multi-modal cue learning.

Keywords Episodic memory · Ant · Cognition · Route learning · Navigation · Cue integration

Introduction

In ants, food foraging and consequently colony survival strictly depend on spatial navigation, which not surprisingly constitutes a sophisticated and extraordinary behavioural adaptation in ant species. Once a food source has been located, the successful forager has to memorise the food source and pinpoint the nest to allow for multiple visits. To

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this end, ants can acquire information from multiple cues of different modalities (Knaden and Graham 2016; Knaden 2019). One such cue is of proprioceptive nature: ants can track their own movement and direction to calculate a home vector which guides them back to the vicinities of the nest. However, to accurately pinpoint the nest or food sources, ants often additionally memorise visual or olfactory cues during learning walks (Collett and Collett 2000; Wehner et al. 2004; Kohler and Wehner 2005; Wittlinger et al. 2006; Collett et al. 2013; Knaden and Graham 2016; Collett and Zeil 2018; Hoinville and Wehner 2018; Freas et al. 2019).

The use of visual cues in navigation attained much scientific attention, predominantly in ant species emphasising the visual domain such as desert ants (Knaden 2019). To reach a goal, ants use a mechanism called view-based matching (Knaden and Graham 2016; Collett and Zeil 2018; Hoinville and Wehner 2018), where they compare the current view to memorised views of the surrounding landscape from previous visits. The discrepancies between the current and the previous views allow the ant to orient itself in space. The wide-field surrounding (panorama) strongly influences

situation-dependent recall in ants (Graham and Cheng 2009; Wystrach et al. 2011). Relying on panoramas instead of distinct visual landmarks is also compatible with the generally low spatial resolution of ants' eyes (Wystrach et al. 2011). In other words, ants tend to memorise the overall visual context rather than specific landmarks in the environment. Apart from the panorama, ants are also able to perceive colours and associate these with rewards (Yilmaz et al. 2017; Fernandes et al. 2018; Online Resource 1).

Olfactory cues are heavily used not only in ant navigation, often in the form of trail pheromone (Beckers et al. 1992; Czaczkes et al. 2015), but also in species that navigate without trail pheromones, such as desert ants (Wolf 2005; Steck et al. 2011; Steck 2012; Buehlmann et al. 2013, 2015). Distinctive olfactory landmarks help ants to locate food or the nest, and odours can induce recall of previously visited food sites (Czaczkes et al. 2014) or active searches for sites exhibiting that odour (Provecho and Josens 2009). Moreover, ants are able to learn different odours (Wolf 2005; Steck et al. 2011; Steck 2012; Czaczkes et al. 2014) and to form odour–reward associations even after a single exposure (Oberhauser et al. 2019).

Ants are not just able to register multiple information at once. It is crucial to point out that the multi-modal nature of insect navigation can provide more precision than a single-cue system (Steck et al. 2011; Collett 2012; Knaden and Graham 2016; Hoinville and Wehner 2018). Ants can, in fact, extract and learn bimodal cues (visual and olfactory) simultaneously, and their combined presence enhances each other's conspicuousness, thus favouring the learning process (Steck et al. 2011). Such synergism is indicative of a neural integration system of information from different modalities (Knaden 2019). It has been proposed that multiple cues are registered and weighted according to their expected predictive power of the presence of a reward. These are then compared and combined to accurately pinpoint the nest or food sources, averaging the different cues into a cohesive decision (Kohler and Wehner 2005; Collett et al. 2013; Wystrach et al. 2016; Collett and Zeil 2018; Hoinville and Wehner 2018).

In the proposed mechanism, the integration of multi-modal cues is of additive nature, in which each information is contributing to the decision in a weighed manner, but does so independently of the other cues. However, ants might face tasks where the predictive power of a cue is dependent on the presence or absence of another in a conditional manner: an olfactory cue may only be predictive of a reward if another contextual cue is concurrently present. In such a situation, it will not be sufficient to sum up the predictive power of all cues, but instead they will be integrated in a composite, single memory.

It has been suggested that the conditional use of multi-modal information may be one of the fundamental mechanisms of navigation, both for vertebrates and invertebrates (Cheng 1995, 2000, 2009). Bees were shown to be able to use cues conditionally (Cheng 2005; Pahl et al. 2007; Mota et al. 2011). For example, in the experiment by Mota et al. (2011), honeybees were trained to associate a particular scent to a reward, but only when presented concurrently with a specific coloured light. The subjects, then tested in a proboscis extension reflex paradigm, were successful in learning the conditional association. Moreover, other studies have demonstrated that the memory valence of different sensory cues can be linked or even transferred when presented together (Reinhard et al. 2004, 2006; Guo and Guo 2005; Leonard and Masek 2014). This strengthens the idea that insects may possess a neural integration system for multi-modal cues. Yet, evidence of conditional learning in ants is sparse.

To discover whether ants are able to use multi-sensory cues to form composite memories, we tested *L. niger* ants in a Y-maze in which they had to conditionally integrate olfactory, visual and spatial cues across 12 training trials to obtain a reward.

Ants are surprisingly fast learners. It has already been demonstrated that they can acquire information through very few or sometimes even single exposures. Studies demonstrated that ~70% of *L. niger* ants can learn a feeder location after only one visit (Grüter et al. 2011; Czaczkes et al. 2013; Oberhauser et al. 2018). However, these studies focused on a single predictor, which is a much less demanding task with respect to multi-modal conditional learning. To test whether ants were able to solve the task as fast as they can do in single-cue situations, we presented them with visual and spatial cues during a single trial after having learned olfactory cues.

Methods

Subjects

We used 4 queenless *L. niger* colony fragments collected from different colonies on the University of Regensburg campus consisting of ~ 1000 workers each. Queenless colonies behave normally and are often used in foraging experiments (Dussutour 2005; Mailleux et al. 2011). Each fragment was kept in a plastic box ($30 \times 20 \times 40$ cm) with a floor of plaster and a circular plaster nest (14 cm in diameter and 2 cm thick). Temperature (21–25 °C) and humidity (45–55%) were kept constant, and colonies were kept in a 12:12 light:dark cycle. Each colony was fed 0.5 M sucrose solution ad libitum and was deprived of food 4 days prior to each test. Water was provided ad libitum.

Experiment 1—information integration

The aim of this experiment was to discover if ants are able to learn a visual context, a side and a scent simultaneously. While the scent was always predictive of the location of a reward, side and context were predictive only when considered together, not by themselves. To this end, ants were trained on a Y-maze to associate a scent (either lemon or rosemary) presented on the maze arm with a 1.0 M sucrose (Merck KGaA, Darmstadt, Germany) solution drop. The side of the reward was alternated (left or right) consistently with the colour of the background (e.g. when the Y-maze had a blue background, reward was on the left, when the background was yellow, reward was on the right). During the test phase, the rewarded scent, which represented the only reliable information per se, was applied on both arms and thus became uninformative. To locate the reward, ants, therefore, had to integrate background colour with side. A schematic representation of the procedure is available in Fig. 1.

Training

In the training phase, ants were allowed on a 15-cm-long, 1-cm-wide runway (referred to as entering runway) that led to a Y-maze (arm length 10 cm, bifurcation angle 120°). Both the stem of the Y-maze and the entering runway were covered with unscented paper overlays. The two arms were covered with paper overlays of a different scent each. The scented runways were prepared by placing the paper overlays in an enclosed box containing 100 μl of either rosemary or lemon essential oil. Ants have been shown to not have any innate preferences for either (Oberhauser and Czaczkes 2018). The paper overlays were left in the box for at least 2 h before being used. The maze was tapered at the bifurcation to ensure that ants perceived both scented arms at the same time (following Czaczkes 2018). One of the two arms led to a drop of 1.0 M sucrose solution, corresponding to a high-value reward for *L. niger* (Detrain and Prieur 2014). The other arm led to a drop of water, visually similar but bearing no reward. Around the Y-maze, a 5-cm-tall wall was placed. The wall surface could either be blue or yellow. In a pilot experiment, we demonstrated that ants can clearly distinguish between these two colours (see Online Resource 1). The first ant that reached the sucrose drop and started drinking was marked with a dot of paint and allowed to drink until satiation while all other ants were put back into the nest. After drinking fully, it was allowed back to the nest to unload the sucrose to nest mates via trophallaxis (mouth-to-mouth feeding, Hölldobler and Wilson 2009).

After unloading, only the marked ant was selectively allowed onto the setup using a movable bridge once all the paper overlays of the Y-maze had been replaced with
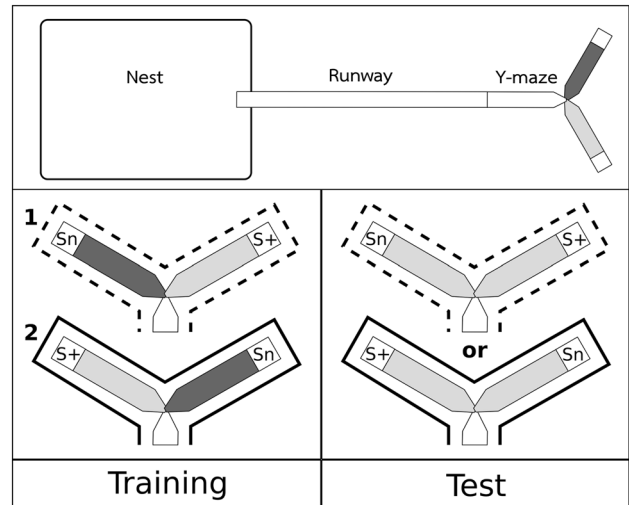

Fig. 1 On top, aerial view of the full experimental apparatus. Ants could access from the nest to the runway that led to the Y-maze. On the bottom, schematic representation of the Y-mazes, as set up for experiment 1. During training, the ants were let onto a Y-maze. During a first visit (number 1 in the picture) one of the arms of the Y-maze was coated with lemon scented paper (light grey) and led to a 1.0 M sucrose solution (S+). The other arm was coated with rosemary scented paper (dark grey) and led to a water drop (Sn). The walls around the Y-maze could either be yellow or blue (dashed or solid line around the maze). The colour of the walls and the position of the rewarded scent (left or right) were always associated (e.g. blue walls, lemon on right, yellow walls, lemon on left). After the first visit, the ant was let back to the nest, the position of the scent was switched as well as the wall colour (as number 2 in the picture). Across the total of 12 training trials that were performed wall colour and consequently the position of the lemon scent were alternated. In the testing phase, we removed the scented paper predicting Sn (rosemary) and left the rewarded scent on both arms. At this point, the scent information became uninformative. To still be able to locate the reward, the ants needed to remember that when the walls are blue, reward could have been found on the left, and vice versa with the yellow walls, effectively demonstrating the ability to integrate side and colour information. Note that no reward was presented in the testing phase (in the figure, S+ indicates the arm considered rewarding if the ants did learn the task). Rewarded scent (lemon or rosemary) and colour–side association were balanced across individuals

new ones. This was done for 11 more times, resulting in a total of 12 training visits. On each visit, the position of the rewarded scent was changed, so that both sides were rewarded in alternation across visits. The wall colour was changed accordingly, to always have the same colour associated with the same side. For each visit, we recorded (1) pheromone depositions on the way towards the drop and on the way back to the nest only on the scented part of the setup (pheromone deposition is a stereotyped behaviour in *L. niger* and can be quantified by eye, Beckers et al. 1992); (2) the ant's initial decision, scored when the ant crossed a decision line 2 cm inwards of a Y-maze arm; (3) the final decision, scored when the ant crossed a decision line 8 cm inwards of an arm. For each ant, the rewarded scent was kept constant,

but we randomized the rewarded scent, background colour at start, rewarded side at start and colour-side associations across ants.

Test phase

Ants were tested on their 13th visit to the Y-maze. No sucrose was present in the test visit. The background colour was either blue or yellow. The rewarded scent, however, was now placed on both arms of the maze and thus made uninformative. Now, ants were only able to choose the "correct" arm (consistent with the colour-side association) if they concurrently learned the association between background colour and side during training. After the tested ant reached the end of either arm, it was immediately allowed on a piece of paper and gently placed back to the Y-maze stem, to repeat the test. This way, each ant made five decisions during the test phase, providing an estimate of choice reliability and drop-out probability. In this test phase, there was no time interval between each one trial and the next one; therefore, paper inlays could not be replaced during these five choices.

Experiment 2—information integration in an episode

Experiment 1 demonstrated that ants learn the association between background colour and side despite the presence of scent as sufficient predictor for reward during training (see "Results"). Next, we tested if ants were still able to succeed in the testing phase after only one exposure. A schematic representation of the procedure is available in Fig. 2.

Training

In this experiment, the training setup was a 10-cm-long and 1-cm-wide runway instead of a Y-maze. This runway was scented and a drop of either water or 1.0 M sucrose solution was placed at its end. The scent of the runway was consistent with the drop quality, to let the ant form an association between the scent and the reward. As before, multiple ants were allowed on the setup; the first ant that started drinking on the drop was marked and the others were moved back to the nest. Only the marked ant, thereon, was allowed onto the setup after having replaced the paper overlays for five further training visits, resulting in a total of six visits (including the first). Each visit alternated between sucrose solution (visits 1,3,5) or a drop of water (visits 2, 4, 6) and the overlay scent was alternated accordingly, so that one scent always predicted a reward while the other always predicted a water drop. The rewarded scent was balanced between ants. For
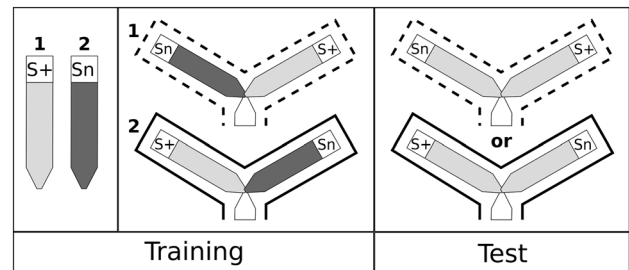

Fig. 2 Schematic representation of the procedure in experiment 2. For an aerial view of the full apparatus see Fig. 1. In the training phase the ant was initially let onto a straight runway. This could have been covered with lemon scented paper (light grey) and leading to a drop of 1.0 M sucrose solution (S+), or with rosemary scented paper (dark grey) and leading to a drop of water (Sn). In this experiment, the runway was surrounded by white walls. Across the six trials, we alternated the two scents and consequently the reward with the neutral. After the first 6 training trials, the ant was let onto a Y-maze, with the exact same setup of the training phase in the first experiment. The Y-maze training was repeated for only two trials, to let the ant experience each combination (e.g. blue walls, lemon on the left, yellow walls, lemon on the right) once. After these last two training trials, the ants were tested with the same procedure used for experiment 1. Here, to still predict the reward position, the ants not only needed to integrate all the available information during training, but also had to be able to do so remembering only a single event. Note that no reward was presented in the testing phase (in the figure, S+ indicates the arm considered rewarding if the ants did learn the task)

each visit, we recorded pheromone deposited both on the way to the drop and on the way back on the 10-cm-long scented overlay.

On the 7th visit, ants were confronted with a Y-maze identical to the training setup in the first experiment. The two arms presented the two scents (lemon and rosemary), and the walls were either blue or yellow. At the end of one arm, 1.0 M sucrose solution was placed and a water drop was placed on the other, according to the scent–reward association established in visits 1–6. The 8th visit was identical to the 7th, but both the rewarded side and the colour of the wall were switched. For both of those visits, we recorded the number of pheromone depositions on the scented portion (on the way to the drop and back) and the side choice, as described for the first experiment. The latter in particular was used to assess whether the ants had learned the scent–reward association. These two visits were used to make the ants experience the association between spatial (side) and contextual (wall colour) information, other than the conditioned stimulus (the scent). In both visit 7 and 8, the ants could have integrated together all the information to form a cohesive memory of an episode. We needed to present an event for each colour and side to prevent the ant from exclusively relying on either the side or the background colour as predictor for food presence.

Test phase

The test phase was identical to experiment 1: the rewarded scent was presented on both arms of the Y-maze and thus made uninformative. No water or sucrose solution was presented on either side. The wall colour was either blue or yellow. If ants remembered an episode in which they experienced the rewarded scent being on one particular side with one particular coloured background (visit 7 and 8), they should be able to choose the "correct" arm. Colour of background, colour–side association and correct scent were balanced between ants, as well as background colour order in visits 7 and 8. During the test, we recorded the initial and final decision of ants, as described in experiment 1. After the tested ant reached the end of one arm, it was allowed onto a piece of paper and was gently placed back on the Y-maze stem to repeat the test. As in experiment 1, each ant was tested five times to assess choice reliability as well as dropout probability. Again, due to the tight scheduling of trials, the paper overlays could not be replaced between visits.

Statistical analysis

Statistical analyses were carried out in R 3.3.3 (R Core Team 2017). Following Forstmeier and Schielzeth (2011), we only added factors in the models for which we had a priori reasons for including, namely correct scent (lemon or rosemary), correct side (left or right) and wall colour (blue or yellow). Our primary dependent variable was the binomial arm choice of the ants. We also include analysis on the pheromone deposition in Online Resource 3 (we decided to not include it in the main paper as pheromone is often interpreted as a measure of relative preference. Since in all our tests the ants had to choose between either a reward or nothing, the relativeness was less crucial). As we found no difference between initial and final decisions, only the initial decision was used in the analysis (see Online Resource 3 for the supporting analysis).

To see in which of the trials the ants learned the association between the scent and reward, we looked at ant choice during training visits. Given the fact that we had multiple observations of each individual, and that some individuals were from the same colony, we employed generalized linear mixed effect models using the package lme4 (Bates et al. 2015), with ants nested in colonies as a random intercept effect. Y-maze choice data were coded as binomial data (1 for choosing rewarded and 0 for choosing unrewarded scent) and so were modelled using a binomial distribution with a logit link function. We then carried out a post hoc analysis with Bonferroni correction using the package emmeans (Lenth 2018) to test each visit probability against chance level.

Subsequently, we analysed the ants' choice during the test phase. We only included the first testing trial of each ant (see Online Resource 3 for the full analysis regarding testing repeated measures), and accordingly added colony as a random intercept effect. We then used the package car (Fox and Weisberg 2011) to test which factors of the model had a significant effect on the dependent variable.

We tested model fit using the DHARMa package (Hartig 2018). When needed, we used a zero-inflated model with the pscl package (Zeileis et al. 2008; Jackman 2017). Plots were generated using the packages ggplot2 (Wickham 2009) and cowplot (Wilke 2017).

Results

Only the main results are reported below. The full analysis is available in Online Resource 3, while the raw data for the experiment are available in Online Resource 2.

Experiment 1—information integration

During training, in the second visit, 62.5% (20/32) of the ants choose the correct scent (GLMM post hoc with estimated means, probability $= 0.683$, SE $= 0.102$, $z = 1.627$, $p = 1$). Already in the third visit, the percentage rose to 75% (24/32) (GLMM post hoc with estimated means, probability $= 0.831$, SE $= 0.077$, $z = 2.915$, $p = 0.039$), plateauing in the fourth visit at 90% (29/32) (GLMM post hoc with estimated means, probability $= 0.977$, SE $= 0.025$, $z = 3.443$, $p = 0.002$) and remaining stable across all other visits. In the test trial, 87.5% (28/32) of the ants correctly chose the side that was associated with the background colour (GLMM post hoc with estimated means, probability $= 0.875$, SE $= 0.058$, z $= 3.64$, p $= 0.0003$) (Fig. 3a). We found no effect of any of the modelled predictors.

Experiment 2—information integration in an episode

In both trial 7 and 8, 96.9% (31/32) ants choose the correct scent (GLMM post hoc with estimated means, probability $= 0.969$, SE $= 0.031$, $z = 3.38$, $p = 0.001$). However, in the test phase, the percentage of ants correctly choosing the side associated with the presented background colour dropped to 65.6% (21/32) (GLMM post hoc with estimated means, probability $= 0.656$, SE $= 0.084$, $z = 1.737$, $p = 0.0823$) (Fig. 3b). We found no effect of any of the modelled predictors.
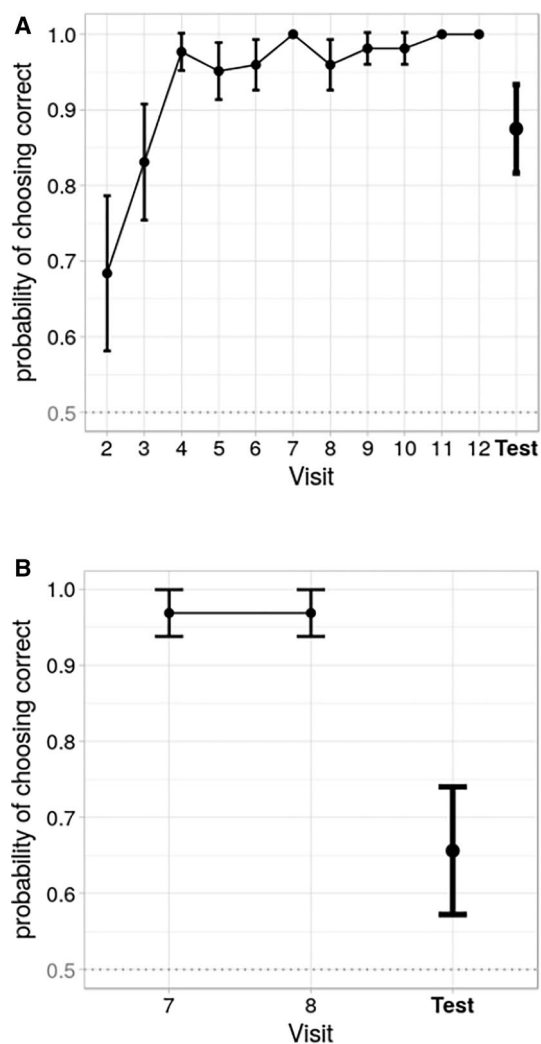
correct arm of the maze by integrating side and colour cues. These results clearly demonstrate that foraging ants not only learn contextual cues in addition to the most predictive cue, but also integrate them conditionally to find a food source.

Recent research suggests that insects can combine cues weighed by their uncertainty in the current context (Wystrach et al. 2015; Wehner et al. 2016; Huber and Knaden 2017; Hoinville and Wehner 2018; Strube-Bloss and Rössler 2018) rather than creating information hierarchies in which one cue reliably dominates over the others. Accordingly, hierarchical-like decisions, in which it appears as if animals only learned one cue in the environment could, in fact, be based on a very strong weight on one cue, but still involve processing of additional cues. While, for instance, *Myrmica* foragers were found to rely predominantly on visual cues in bright light, they switched readily to olfactory cues when light intensity decreased (Cammaerts Tricot 2012; Jones et al. 2018). Such behaviour does not imply an exclusive reliance on learning of either cue. In our study, ants clearly did not use information in a strictly hierarchical order; if ants exclusively relied on odour cues, they would have performed at chance level in the test. Conversely, if they had just relied on contextual cues, we would not have observed a rise in correct choices in the second training trial (even though still not significantly different from chance level, see below). In the second training trial, only the odour information is reliable, as the colour–side combination is completely novel.

However, weighing of cues alone would have not led to success in our experiment, unless the cues were conditionally integrated. In fact, while such weighing can create a better prediction as a result of an additive integration process, the different cues do not influence each other, nor does the weight of one change the other. In our experiment, scents predicted the presence of the reward with 100% certainty, while colour and side had only a 50% certainty by themselves, as each colour and each side were rewarded equally. The predictive power of colour with side becomes 100% only when considered conditionally: if colour A then side B, thus switching from an additive weighed process to a conditional one. Such learning of additional, seemingly redundant, information about the environment might initially demand higher costs, but can greatly decrease susceptibility to environmental perturbations and risk of disorientation when foraging (Bregy et al. 2008; Steck et al. 2011; Schultheiss et al. 2016; Wehner et al. 2016), and, thus, is worthy of energetic investment by the animal.

During training in the first experiment, 62.5% of ants chose the correct odour on the second trial, 75% of them made a significant choice towards the correct side at the third visit and 90% correct choices was reached on the fourth visit. Even though the percentage of correct choices in the second trial seems consistent with previous evidence of single-trial

Fig. 3 **a** Probability of ants choosing the correct side during training visits and during test for experiment 1 according to the GLMM model. Dots represent average probability, error bars are SE, dotted line is chance level. $N = 32$. Already in the third training trial performance was above chance level. In the test, the ants chose correctly even in the absence of odour cues (GLMM post-hoc with estimated means, probability = 0.875, SE = 0.058, $z = 3.64$, $p = 0.0003$). **b** Probability of ants choosing the correct side during training visits with colour background and during test for experiment 2 according to the GLMM model. Dots represent mean probability, error bars are SE, dotted line is chance level. $N = 32$. For both visit 7 and 8, performance is at 96%. In the testing phase, however, the probability of choosing correctly is 65.6%, not different from chance level (GLMM post hoc with estimated means, probability = 0.656, SE = 0.084, $z = 1.737$, $p = 0.0823$)

Discussion

In the first experiment, *L. niger* ants were provided with an olfactory cue that fully predicted the location of a reward in a Y-maze along with contextual cues (maze wall colour and arm side). Yet, once the olfactory cue was made uninformative, 87.5% of ants were still able to successfully locate the

learning (Grüter et al. 2011; Czaczkes et al. 2013; Oberhauser et al. 2018), our sample size was not sufficient to find significance. We are, however, aware that changing the sample size after having collected and analysed the data can often lead to type I errors, so we decided to discuss and present the data as they are.

Intriguingly, the number of correct decisions on trial 2 of experiment 1 and at the test of experiment 2 were very similar (62.5% vs 65.6%), suggesting a similar single-trial learning effect, even though the percentage is still not significantly different from chance level. Nonetheless, trial 2 of experiment 1 and the test of experiment 2 differ fundamentally in their levels of complexity; even though all three components are presented in both cases, in experiment 1 the scent alone had 100% predictive power. One could hypothesize that ants can learn faster, or prioritize learning, only for some sensory modalities. However, different from the second trial of experiment 1, to locate the reward in the test of experiment 2, scent alone was not sufficient. To this end, both colour and side combined were needed. The fact that the same percentage of ants chose correctly in a situation where one cue would have sufficed (scent cue, experiment 1) and in a situation where stimuli had to be combined (colour and side, experiment 2), suggests that the animals attend to all sensory modalities combined, and start forming conditional relationships between them even before knowing whether they will be predictive of a reward. Future studies are needed to confirm this hypothesis, as our results were not conclusive. More exhaustive evidence on the ants' learning curves for single and multi-modal cues would be of interest.

It is worth discussing how in visit 7 of experiment 2, the first presenting all three information sources, ants were confronted with two novel cues, not predictive in themselves, while a perfectly reliable odour cue was present at once. In an associative learning context, we should have expected an effect of blocking (Kamin 1967), where the presence of a predictor previously associated with a reward prevents learning of subsequent cues. However, in our experiment, this seemingly did not take place. This effect has been amply observed not only in bees (Smith and Cobey 1994; Couvillon et al. 1997, 2001; Hosler and Smith 2000; Cheng and Spetch 2001; Blaser et al. 2004, 2006; Guerrieri et al. 2005), but also other invertebrates (Sahley et al. 1981; Acebes et al. 2009). However, trial 7 of experiment 2 did not only introduce new predictors, but also fundamentally changed the training context. This could explain why blocking was not observed. In fact, a novel training context may trigger an increase in attention, and the relative salience of the previously acquired predictors changes consequently. It has been observed that the phenomenon of blocking can be highly dependent on the salience of the predictors (Funayama et al. 1995; Couvillon et al. 2001). It has also been shown that in some circumstances the presence of a previously

conditioned stimulus can facilitate learning of subsequently presented stimuli (Bouton et al. 1986; Batsell et al. 2001) Future studies should be designed to disentangle these possible explanations.

To conclude, the results of our experiments show that *L. niger* ants extract and readily combine contextual cues while foraging to locate a food source. This ability may rely on weighed cue integration with the addition of a sophisticated conditional integration process on top of an additive one. Moreover, we found a striking similarity in performance among the test phase of the second experiment and the second training trial of the first experiment. Such similarity suggests that information load had no effect on performance, as ants may immediately focus on multi-modal cues (Bregy et al. 2008; Steck et al. 2011; Schultheiss et al. 2016; Wehner et al. 2016). Ants also seemed to be able to integrate those cues upon encountering them for the first time, and even before having assessed their predictive power.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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