The Ant Odometer: Stepping on Stilts and Stumps

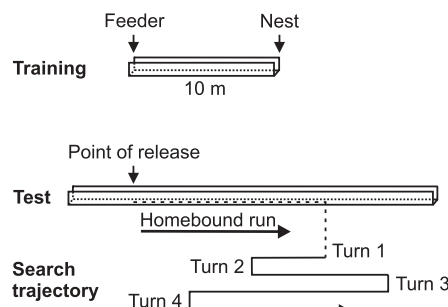Matthias Wittlinger,[1]* Rüdiger Wehner,[2] Harald Wolf[1]

Desert ants, *Cataglyphis*, navigate in their vast desert habitat by path integration. They continuously integrate directions steered (as determined by their celestial compass) and distances traveled, gauged by as-yet-unknown mechanisms. Here we test the hypothesis that navigating ants measure distances traveled by using some kind of step integrator, or "step counter." We manipulated the lengths of the legs and, hence, the stride lengths, in freely walking ants. Animals with elongated ("stilts") or shortened legs ("stumps") take larger or shorter strides, respectively, and concomitantly misgauge travel distance. Travel distance is overestimated by experimental animals walking on stilts and underestimated by animals walking on stumps.

Foraging Saharan desert ants, *Cataglyphis fortis*, use a mode of dead reckoning known as path integration (1, 2) to monitor their current position relative to the nest and to find their way home (3). This enables them to return on a direct route, rather than retracing the tortuous outbound journey performed when searching for food items in their flat desert habitat, which is often completely devoid of landmarks. The path integrator requires two kinds of input information: about directions steered, as obtained via the ant's celestial compass (4), and about distance traveled, as gauged by the ant's odometer.

The cues by which ants measure travel distance during locomotion have not yet been discovered. However, there are several promising hypotheses. The "energy hypothesis" posits that the (surplus) energy required for locomotion (as opposed to rest) is used to calculate travel distance. This hypothesis is of long standing in arthropod research (5), but is not applicable to the problem of the ant odometer, because ants assess their walking distances with great accuracy, irrespective of the load they carry (6). The "optic flow hypothesis" has been proven in honeybees, which integrate visual flow-field cues during their foraging flights to gauge flight distance (7, 8). In *Cataglyphis* ants, this mechanism plays a minor role, if it has any effect (9) (for further discussion of the optic flow hypothesis, see SOM). Even in complete darkness, on featureless platforms, or with the ventral halves of their eyes covered, the animals are still able to assess traveling distance correctly during

their homing runs (9, 10). Also, lateral optic flow does not have any influence at all on distance estimation (11). Considering the relatively constant locomotor speed of desert ants, a time-lapse integrator might function to measure walking distance—although this possibility has been refuted in slightly different experimental contexts (12). Thus, ants appear to rely primarily on idiothetic cues, most probably derived from the movements of their legs (13). Although this step integrator or "pedometer" hypothesis was initially proposed as early as 1904 (14), it has remained untested. Here, we examine whether or not ants with manipulated leg lengths, walking on stilts or on stumps, exhibit changes in their stride lengths and, consequently, misgauge their travel distance during homebound runs.
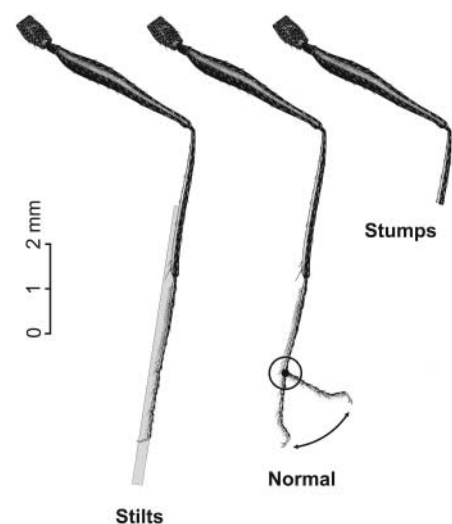
The ants were trained to walk from their nest entrance to a feeder, over a distance of 10 m and in a linear alloy channel (Fig. 1, top). After at least 1 day of training, the animals were caught at the feeding site and transferred to a test channel, aligned parallel to the training channel (Fig. 1, bottom). Once transferred into this test channel, the ants performed their homebound

runs, and we recorded the point at which the ants switched from their straight and steady return path to their characteristic nest-searching behavior. This point is marked by a 180° U-turn (15), followed by a run pacing back and forth around the anticipated location of the nest entrance. Details of the experimental procedure are given in (16).

Ants that had reached the feeder on a foraging trip through the training channel were caught and subjected to experimental manipulation. To increase stride length on the animals' homebound runs, their legs were splinted and extended with pig bristles glued to the tibia and tarsus, as illustrated in Fig. 2 (stilts). To decrease stride length, the legs were shortened by severing the tarsomeres in the middle of the tibia segment (Fig. 2, stumps). Operated animals were supplied with a food item and transferred to the test channel, with the food item in their mandibles ("test 1"). These ants started determined homeward runs, and when they had covered the assumed distance to the nest, they switched to the nest-searching behavior outlined above. The ants walking back homeward on stilts clearly overshot [15.30 m, interquartile range (IQR) = 3.24 m, $n = 25$], whereas ants with shortened legs undershot (5.75 m, IQR = 1.81 m, $n = 25$) with regard to their normal homing distance (10.20 m, IQR = 2.40 m, $n = 25$) (Fig. 3A). There are statistically significant

Fig. 1. Schematic diagram of channel layout, as well as training and testing procedures applied in *Cataglyphis* foragers (not drawn to scale).

Fig. 2. Manipulation of ant legs as performed in the present study. In stilts, attached pig bristles elongated the legs; second, normal unmodified legs, with approximate range of tarsus movement indicated; third, shortened legs (stumps). The right hind leg is shown from anterior [see figure 1 in (18)].

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differences ($P \leq 0.001$, Kruskal-Wallis test) among these groups.

The modified and tested ants were put back into their nest and were tested again when they turned up at the feeder during one of the following days ("test 2"). Having already performed their outbound runs to the feeder on stilts or stumps, these animals exhibited homing runs almost identical to those of normal, unmodified ants (Fig. 3B). Ants walking on stilts (stilts, $n = 25$) searched for the nest at 10.55 m (IQR = 1.45 m) distance from the release point and ants with shortened legs at 10.25 m (IQR = 1.76 m) (stumps, $n = 25$). No significant differences were observed between these groups or with regard to unmodified animals (above, 10.20 m).

These results support the hypothesis that desert ants use a pedometer for distance measurement, or a step integrator [loosely speaking, a step counter, although the ants most probably do not literally count (17)]. According to the pedometer hypothesis, ants that have traveled to the feeder on normal legs and have had their leg length modified at the feeder should cover a different distance on their homebound journey. This is because the same number of strides made during the outbound travel, as registered by the step integrator, should presumably carry them over longer (stilts) or shorter (stumps) distances, respectively. And later, on reemerging from the nest with their legs still modified, the number of strides will be the same again for outbound and inbound travel (although different from the situation with normal legs in both cases). No

such straightforward interpretation is possible for any of the competing hypotheses.

This interpretation rests on the assumption, however, that stride length is indeed altered by the manipulation of leg length, roughly in proportion to the imposed length changes. Thus, a quantitative assessment of the (manipulated) ants' stride lengths and their relation to walking distances is also necessary. It was impossible to count the ants' strides during experiments (for instance, by filming the complete outbound and homebound journeys on high-speed video). Instead, stride lengths were determined in a different set of animals, both normal and manipulated, in a small pen erected on the desert floor. As in most animals, stride length in normal *Cataglyphis* depends on at least two parameters. The first is leg length and, thus, body size, as body morphology is isomorphic in *Cataglyphis fortis* workers (18). That is, larger ants take correspondingly longer strides. The second is walking speed. The faster an ant runs, the larger are the strides it takes (19–21). Normalization of homebound travel distances with regard to both body size and walking speed is thus required for a quantitative interpretation of the above experiments. High-speed video recordings of running ants were analyzed to determine the actual changes in stride lengths caused by the stilts and stumps manipulations described above. Walking speeds were determined in the above experimental animals by the time required for uninterrupted straight segments of homebound travel (and confirmed on the high-speed movies in a different set of animals).

To derive a quantitative prediction of how far the manipulated ants would have to run on their homebound journey, based on the pedometer hypothesis, the experimental data were first normalized with regard to body size. That is, relative stride lengths with regard to body dimensions were calculated to eliminate effects of size variation. Second, the actual stride lengths were determined in normal and manipulated ants. Ants walking on stilts took significantly longer strides ($P < 0.01$, t test; 14.8 mm, SD = ± 2.5 mm, or +13.9%) than did normal animals (13.0 mm, SD = ± 1.98 mm), and ants on stumps made significantly shorter strides ($P < 0.001$; 8.6 mm, SD = ± 1.73 mm, or –33.2%). These values were then used to derive a prediction for the respective undershoot or overshoot of manipulated ants, based on the assumption that distance is measured by a step integrator, that is, that the ants took the same number of strides during their outbound journey to the feeder and when returning to the nest on manipulated legs (hatched boxes in Fig. 3A). The predicted values show that there is general agreement between the predicted and observed homebound travel distances in manipulated ants, further corroborating the step integrator hypothesis.

However, there are some differences (significant in stilts, $P = 0.015$; not significant in stumps, $P = 0.125$; Mann-Whitney rank sum test) between the observed and the predicted homebound travel distances. Impairment of the ants by the preparation procedures cannot account for these effects. Any serious impairment would tend to shorten the ants' homebound runs. The animals walking on stilts clearly traveled for much longer distances than the normal controls, however, and they did so with apparently normal vigor. Indeed, experimentally modified ants were observed to stilt or stump through their habitat on successful foraging trips several times a day and for many days thereafter (see movie S1 in SOM).

The differences between observed and predicted homing distances may be attributable, though, to altered walking speeds in the manipulated ants. In fact, normal ants traveled at an average 0.31 m/s, and ants walking on stumps averaged 0.14 m/s, a value in good agreement with their shortened legs and stride lengths (see similarity of predicted and experimentally determined homing distances in Fig. 3A). Ants on stilts however, rather than walking at increased speeds, were also slightly slower than normal animals, walking at an average 0.29 m/s. This was presumably due to the added load of glue and pig bristles on their legs. As noted above, changed walking speeds are associated with correspondingly altered stride lengths, which would appear to explain the unexpectedly short prediction value in Fig. 3A (top hatched box). We thus corrected this prediction with the established relations between walking speed and stride length (19, 20). We assumed as a first approximation (and conservatively, when con-
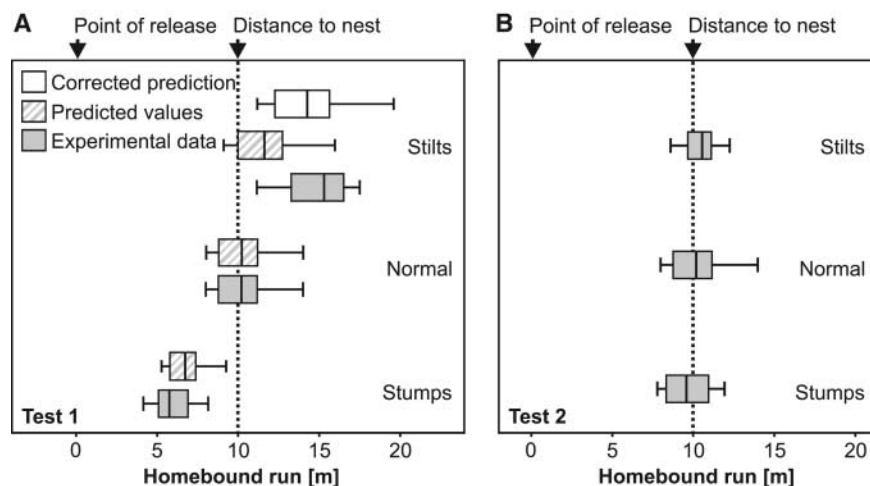
Fig. 3. Homing distances of experimental ants, tested immediately after the lengths of their legs had been modified at the feeding site. (**A**) Leg lengths were normal during the outbound journey but manipulated during the homebound run, resulting in different homing distances. (**B**) Ants tested after reemerging from the nest after previous manipulation. In this situation, leg lengths were equal, although manipulated, during outbound and homebound runs. Box plots show median values of the homing distances recorded in $n = 25$ ants per experiment (as well as IQRs, box margins, and 5th and 95th percentiles, whiskers). Median values of the initial six turning points of an ant's nest-search behavior were considered as the centers of search, indicating homing distance. The hatched box plots in (**A**) illustrate the centers of search as predicted from the high-speed video analyses of stride lengths in normal and manipulated animals. The open box represents the prediction corrected for slow walking speed. Details in text.

sidering the actually imposed changes in leg length) that ants might run by as much faster on stilts as they ran slower on stumps (0.48 m/s, a value regularly observed in highly motivated normal ants and almost reached by the fastest ants on stilts). This procedure indeed yields a value that is not significantly different from the observed homing distances in ants on stilts (open box in Fig. 3, A; 14.25 m, IQR = 3.35 m), thus confirming the consistency of our data with the step integrator hypothesis.

The slower speeds of the ants walking on stilts further rule out the only alternative explanation of our homing distance data (Fig. 3A, solid boxes). In principle, a step integrator and a time-lapse integrator would both yield the same homing distances, even in ants with manipulated leg and stride lengths, if only the ants kept their stride frequencies constant [or in normal ants, walking speed—which in fact they almost do under normal conditions (*19, 20*)]. Constant stride frequency would result in a change in walking speed in proportion to altered stride length and a resulting difference in homing distance during a set (outbound) travel time. This assumption is evidently not correct, though, given the walking speeds of the experimental animals.

Future studies will have to address the mechanism of the proposed step integrator, for example, whether it actually registers steps by means of proprioreceptors, or whether it integrates activity of a walking pattern generator, and to what extent sensory feedback regarding stride length and walking performance is considered.

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Social Modulation of Pain as Evidence for Empathy in Mice

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Empathy is thought to be unique to higher primates, possibly to humans alone. We report the modulation of pain sensitivity in mice produced solely by exposure to their cagemates, but not to strangers, in pain. Mice tested in dyads and given an identical noxious stimulus displayed increased pain behaviors with statistically greater co-occurrence, effects dependent on visual observation. When familiar mice were given noxious stimuli of different intensities, their pain behavior was influenced by their neighbor's status bidirectionally. Finally, observation of a cagemate in pain altered pain sensitivity of an entirely different modality, suggesting that nociceptive mechanisms in general are sensitized.

Although most consider true empathy to be an exclusive ability of higher primates, empathy may be a phylogenetically continuous phenomenon with subclasses such as "emotional contagion" well within the reach of all mammals (*1*). However, there is little evidence for adult-adult empathy outside of primates. In rats (*2*) and pigeons (*3*), the pain-related distress of a conspecific can serve as a conditioning stimulus. Rats produced operant responses to terminate the distress of a conspecific (*4*), but this might be better explained by arousal than altruism (*5*). One theory of human empathy postulates "physiological linkage" between empathizing individuals (*6*).

In one study, empathic accuracy for negative emotion was highest in those dyads featuring high levels of time synchrony of autonomic measures (*7*). We hypothesized that if empathy does indeed exist in mice, the real-time observation of pain in one mouse might affect the responses of its conspecifics to painful stimuli.

We first used a sensitive nociceptive assay, the reflexive 0.9% acetic acid abdominal constriction ("writhing") test. We placed mice singly within transparent Plexiglas cylinders to observe writhing behavior. For comparison, we placed two same-sex mice within each cylinder and injected either one or both mice. In the "both writhing" (BW) condition, each mouse observed the other in pain; in the "one writhing" (OW) condition, the injected mouse observed an uninjected counterpart. BW mice displayed significantly more pain behavior than isolated mice, but only when their counterparts were cagemates (Fig. 1A). The hyperalgesia was marginally enhanced in same-sex siblings living together, but a separate experiment confirmed that close genetic relatedness was not required (fig. S1). Writhing behavior in BW dyads co-occurred in time at levels significantly exceeding those expected by chance (Fig. 1B) and significantly more so in cagemate pairs than stranger pairs. The hyperalgesia and behavior co-occurrence developed over 14 to 21 days of being housed together (Fig. 1, C and D). In general, observed behaviors other than writhing were similar across all conditions (figs. S2 and S3), although evidence suggested higher levels of anxiety or stress produced by the noxious stimulus in stranger pairs relative to cagemates (fig. S4). Because the observed effects on pain behavior were higher in cagemates, stress is not a likely mediator.

When strangers were tested in dyads, a significant decrease in writhing behavior was observed in the OW condition compared to that observed in isolation (Fig. 1A). The inhibition was entirely specific to males (fig. S5) and is likely due to distraction or social stress–induced analgesia.

These findings imply the communication of pain from one mouse to another. To determine the transmitting sensory modality, we blocked sensory inputs individually, by placing physical barriers to sight and/or touch or by rendering mice anosmic or deaf (*8*). The only manipulation that significantly abolished the BW/OW hyperalgesia was a visual blockade using an opaque Plexiglas barrier (Fig. 2A). [Despite their albinism, the CD-1 mice used in these studies display no deficits in visually dependent behavioral tasks (*9*).] The opaque barrier also

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