

## Supplementary methods.

### (a) Measurements of brain biogenic amine levels

Flies and bees were flash-frozen in liquid nitrogen and brains were extracted from partially lyophilized heads as previously described [1]. Fly brains were pooled, ten per sample, after dissection, while bee brains were analysed individually. Brains were centrifuged at 15000 G for 5 min at 4°C, and homogenized by ultra-sonication in 0.2 M perchloric acid containing 10 pg  $\mu\text{l}^{-1}$  of the HPLC standard dihydroxybenzylamine (15  $\mu\text{l}$  for flies, 60  $\mu\text{l}$  for bees). Samples were incubated on ice in the dark for 20 min prior to being centrifuged at 15000 G and 4°C for 15 minutes. Biogenic amine levels from 10  $\mu\text{l}$  of the supernatant of each sample was analysed using an Agilent 1200 Series HPLC system. Samples were separated across an HR-80 column (ESA Biosciences, inc., Chelmsford, MA, USA), and biogenic amine content was quantified using an ESA Coulochem III electrochemical detector with an ESA 5011A analytical cell (Agilent Technologies, Santa Clara, CA). Amounts of octopamine, dopamine, and serotonin were quantified relative to known amounts of each of these chemicals. All chemicals were supplied by Sigma-Aldrich (St. Louis, MO, USA).

### (b) RFID measurements of honey bee foraging

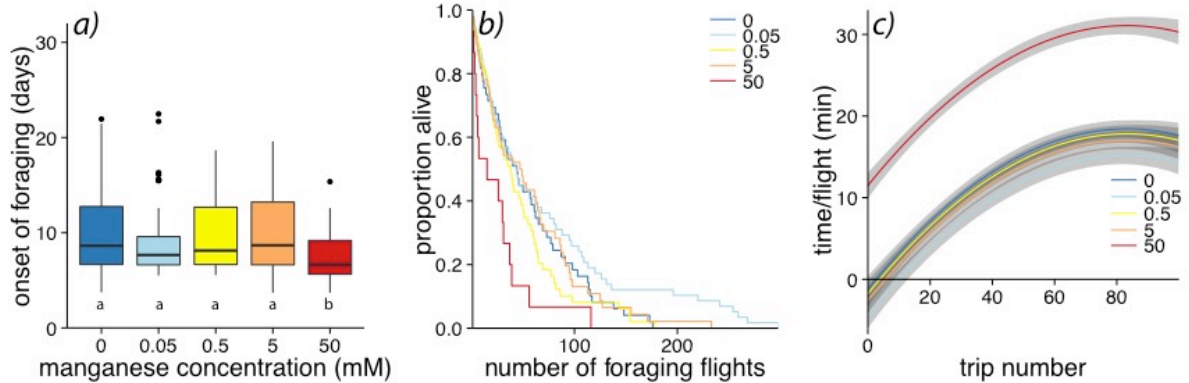
We tracked honey bee behaviour using an RFID system (Invengo Information Technology Co. Ltd., Shenzhen, China). This system allowed us to track the foraging activity of individual honey bees throughout their lifespan [2]. Small, 1 mg, transponder tags were glued to the back of each bee. Each tag transmitted a unique digital signal whenever it passed across antennae at the hive entrance, recording the activities of individual bees. We conducted two independent replicates with eighty tagged honeybees in each treatment group.

### (c) Statistical analysis

Levels of octopamine, dopamine, and serotonin in honey bees were measured in the brains of individual bees, which were treated as the dependent variable. Each amine was analyzed independently. Data were analyzed with Linear mixed regression models. The concentration of  $\text{Mn}^{2+}$  was treated as a fixed parameter in the model. 100 individual brains (20 per group) were run through the HPLC system in batches of 25. 'Batch' was treated as a random factor. Biogenic amine levels in flies were analysed using Kruskal-Wallis tests in the same manner as for RFID experiments. The brain levels of each biogenic amine was analysed by a separate linear mixed model, fitting the amine levels against diet  $\text{Mn}^{2+}$  concentration and controlling for the HPLC analysis batch. Age at onset of foraging RFID data were analysed by a Kruskal-Wallis test followed by *post hoc* tests. Onset of foraging was defined as the time-point when a honey bee first left the hive for a period greater than five minutes and successfully returned [3]. Kaplan-Meier log-rank tests were used to compare survival curves for lifetime foraging trips between treatments. To compare how foraging flight time changed with experience, we utilized a random slope polynomial regression model. All statistical analyses were conducted in R 3.0.0 [4].

1. Søvik, E., Cornish, J. L. & Barron, A. B. 2013 Cocaine tolerance in honey bees. *PLoS One* **8**, e64920. (doi:10.1371/journal.pone.0064920)
2. He, X., Wang, W., Qin, Q., Zeng, Z., Zhang, S. & Barron, A. B. 2013 Assessment of flight activity and homing ability in Asian and European honey bee species, *Apis cerana* and *Apis mellifera*, measured with radio frequency tags. *Apidologie* **44**, 38–51. (doi:10.1007/s13592-012-0156-7)

3. Capaldi, E. A. et al. 2000 Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature* **403**, 537–540. (doi:10.1038/35000564)
4. R Development Core Team 2013 *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.



**Fig S1. Effects of Mn<sup>2+</sup> on honey bee foraging – trial 2.** (A) Age of onset of foraging for bees treated with 0 – 50 mM Mn<sup>2+</sup>. Box-plots show median and inter-quartile ranges of the first day of foraging ( $\chi^2 = 10.4718$ ,  $df = 4$ ,  $p = 0.0332$ ). Comparisons between groups were analyzed with Dunn's *post hoc* test. Groups that did not differ are denoted by the same lower-case letter. (B) Trips completed by bees treated with 0 – 50 mM Mn<sup>2+</sup> during foraging period. The Kaplan-Meier survival curves represent the number of trips completed by bees in each treatment group. The end point for each individual was taken as the number of trips they completed before leaving the colonies for the last time ( $\chi^2 = 3.1$ ,  $df = 1$ ,  $p < 0.0001$ ). (C) Relationship between time spent on each foraging trip and foraging experience ( $F_{6,393} = 59.43$   $p < 0.0000001$ ,  $R^2 = 0.42$ ).