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## Research

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## Animal behaviour

## Negative impact of manganese on honeybee foraging

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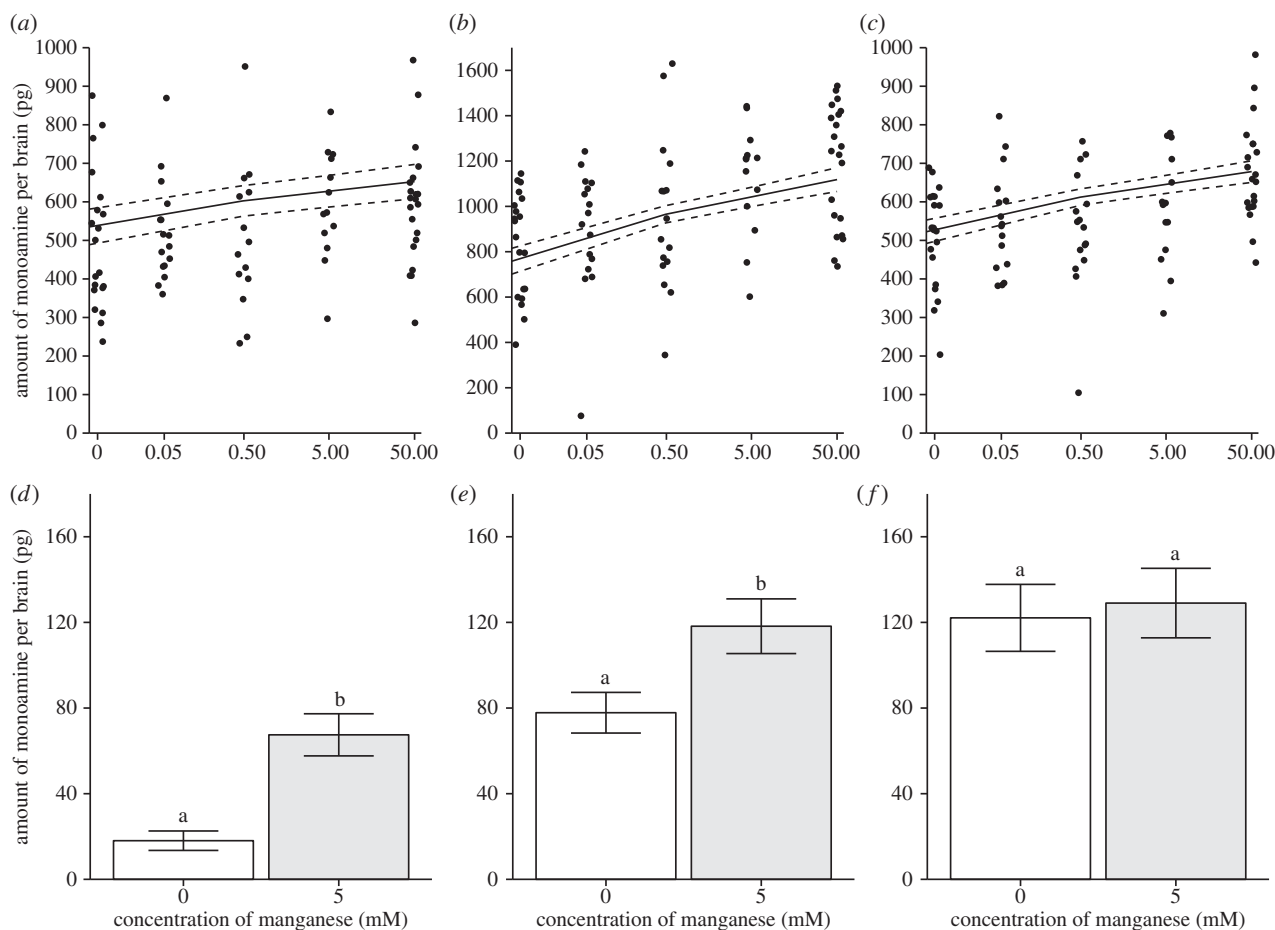
Anthropogenic accumulation of metals such as manganese is a well-established health risk factor for vertebrates. By contrast, the long-term impact of these contaminants on invertebrates is mostly unknown. Here, we demonstrate that manganese ingestion alters brain biogenic amine levels in honeybees and fruit flies. Furthermore, we show that manganese exposure negatively affects foraging behaviour in the honeybee, an economically important pollinator. Our findings indicate that in addition to its direct impact on human health, the common industrial contaminant manganese might also have indirect environmental and economical impacts via the modulation of neuronal and behavioural functions in economically important insects.

## 1. Introduction

The possible impact of environmental contaminants on human health is often measured only in terms of its direct impact on human biology [1]. By contrast, their possible indirect impact on human health via negative effects on other organisms is often ignored. Consequently, some environmental pollutants could have a significant effect on human health by affecting, for example, pollinators of important food crops even when present at levels that are not considered toxic.

Understanding the possible negative impact of metals such as manganese on insects could be important when considering the alarming reports on the continual loss of insect pollinators [2], which include the honeybee [3]. As honeybees bring nectar and pollen back to the nest where it is concentrated before being consumed [4,5], this can lead to the accumulation of contaminants such as metals in both honey and bee tissues [5,6]. Previous studies have shown that some metals can affect the responsiveness of honeybees to sucrose [7] without an impact on their visitation rates of contaminated flowers [8,9]. These data suggest that in areas where metals are present in nectars, bees are likely to carry them back to their hive. For example, elements such as selenium, aluminium and nickel can have an impact on behaviours of honeybees, bumblebees and other pollinators [7–10]. By contrast, the impacts of common anthropogenic metal pollutants such as manganese on bee health are not as well understood, despite their well-known effects on the physiology of plants [11] and vertebrates [12].

Previous work indicated that exposure to  $Mn^{2+}$  affects feeding behaviour of bees and flies [13,14] and is associated with changes in their brain transcriptome [15]. In addition, the concentration coefficient for  $Mn^{2+}$ , defined as tissue accumulation relative to amounts consumed, is higher for honeybees than that of all other metals studied to date [16]. Because excessive  $Mn^{2+}$  levels have been found in commercial honeys, and its levels in honeys reflect the levels seen in the immediate environment [16,17], relatively small increases in environmental levels of  $Mn^{2+}$  could lead to significantly higher accumulation of this metal in honeybee tissues relative to other metal ions. As exposure to excessive



**Figure 1.** Relationship between  $Mn^{2+}$  treatments and biogenic amine levels. Linear mixed regression estimates (95% CIs) and data for (a) octopamine, (b) dopamine and (c) serotonin in honeybee brains as a function of  $Mn^{2+}$  levels (see table 1 for statistics). Levels of (d) octopamine ( $t_{34} = -4.5631$ ,  $p = 0.00006$ ,  $n = 18$ ), (e) dopamine ( $t_{34} = -2.5393$ ,  $p = 0.0159$ ,  $n = 18$ ) and (f) serotonin ( $t_{31} = -0.3063$ ,  $p = 0.7614$ ,  $n = 18$ ) from fly brains. Data are presented as mean  $\pm$  s.e. Different letters above bars denote statistical difference.

**Table 1.** Linear-mixed regression models for the effect of  $Mn^{2+}$  exposure on biogenic amine levels in honeybees.

fixed effect	$Mn^{2+}$ concentration				
dependent variable	d.f.	estimate	s.e.	F	p-value
octopamine	1	33.04	11.48	8.238	0.00525
dopamine	1	97.16	20.61	21.904	0.00001
serotonin	1	43.11	10.42	16.894	0.00009

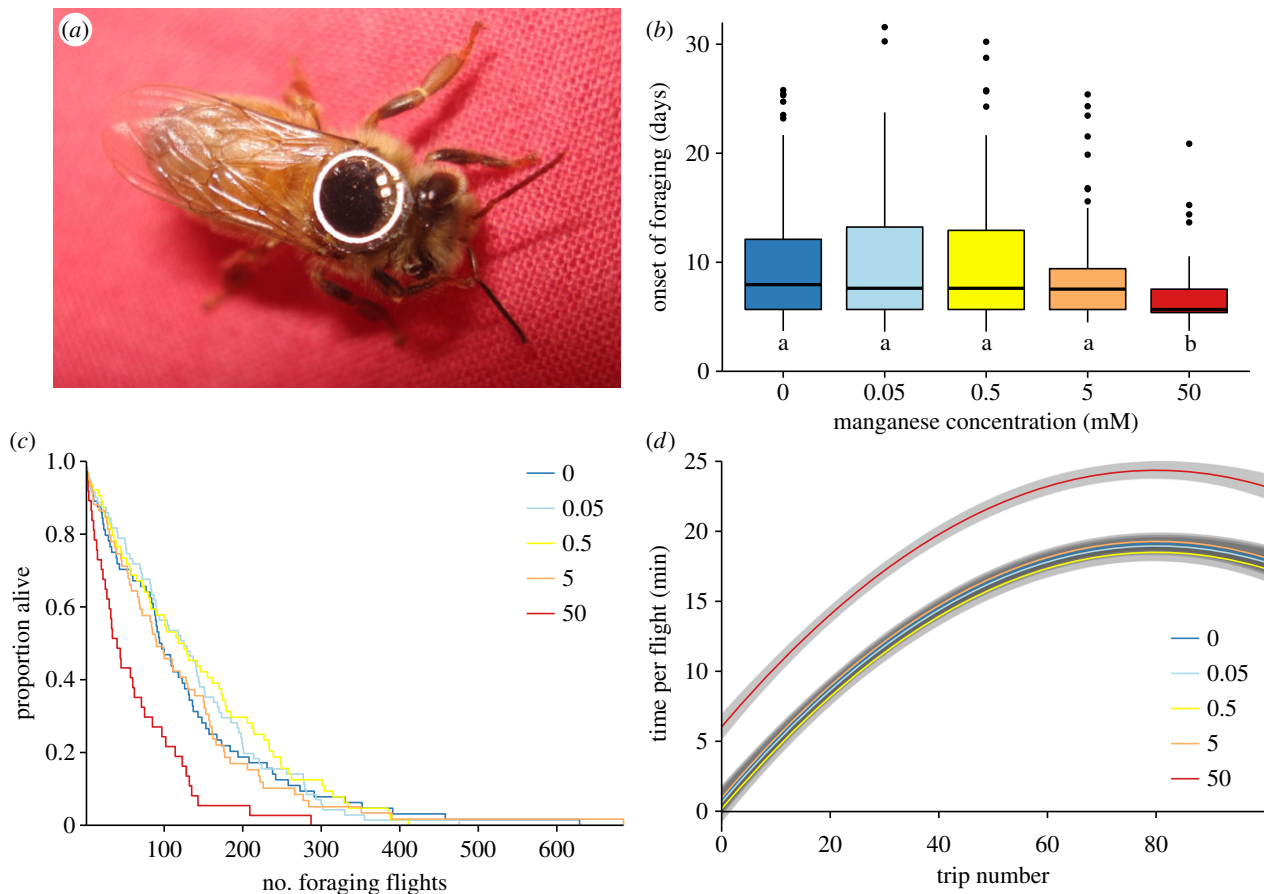
$Mn^{2+}$  levels affect biogenic amine signalling in the mammalian brain [18], and biogenic amines are key modulators of honeybee foraging [19], we investigated the possible impact of dietary  $Mn^{2+}$  on brain aminergic signalling pathways and foraging behaviour in honeybees.

## 2. Material and methods

We quantified levels of octopamine, dopamine and serotonin from the brains of honeybees (*Apis mellifera*) and fruit flies (*Drosophila melanogaster*) fed differing levels of  $Mn^{2+}$  using high-pressure liquid chromatography as described elsewhere [20].  $Mn^{2+}$  was supplied in either 1.5 M sucrose (bees) or standard *Drosophila* medium (flies) over a period of 4 days. We tracked the individual bees treated with  $Mn^{2+}$  using an RFID system that allowed us to track foraging activity throughout the lifespan. See electronic supplement material for additional details.

## 3. Results and discussion

We found that consumption of  $Mn^{2+}$  by honeybees leads to a dose-dependent increase in brain levels of octopamine, dopamine and serotonin (figure 1a–c and table 1). These findings disagree with previous reports in mammalian models and the fruit fly, which showed  $Mn^{2+}$  caused dopaminergic neurotoxicity and reduced levels of dopamine in the brain [21–23]. To confirm that our current observations were not unique to the honeybee, we treated fruit flies with sub-toxic levels of  $Mn^{2+}$  and examined its impact on biogenic amine levels. As in the honeybee, we found that ingestion of 5 mM  $Mn^{2+}$  by *Drosophila* caused an increase in brain levels of octopamine and dopamine, but not serotonin (figure 1a–f). Together, these results indicate that exposure to  $Mn^{2+}$  at levels that are considered safe for humans can still affect insect behaviour.



**Figure 2.** Effects of  $\text{Mn}^{2+}$  on honeybee foraging. (a) Honeybee forager tagged with an RFID transponder. (b) Boxplots show age at onset of foraging for bees treated with 0–50 mM  $\text{Mn}^{2+}$  ( $\chi^2 = 25.4634$ , d.f. = 4,  $p < 0.0001$ ). Different lower case letters below bars denote statistically different groups. (c) Kaplan–Meier survival curves showing the number of foraging trips completed by honeybees treated with 0–50 mM  $\text{Mn}^{2+}$  between onset of foraging and death ( $\chi^2 = 17.6$ , d.f. = 1,  $p < 0.0001$ ). (d) Polynomial regression of the relationship between time spent outside the hive per foraging trip and number of foraging trips taken ( $F_{6,393} = 85.55$ ,  $p < 0.0001$ ,  $R^2 = 0.51$ ).

As increased biogenic amines in the honeybee brain are associated with precocious foraging [24,25], we next used the tracking of individual bees to study the effects of  $\text{Mn}^{2+}$  treatment on the ontogeny of bee foraging (figure 2a). Similar to our previous report [14], here we found that honeybees treated with 50 mM  $\text{Mn}^{2+}$  showed a precocious transition from in-hive behaviours to foraging ( $\chi^2 = 25.4636$ , d.f. = 4,  $p < 0.0001$ ; figure 2b; electronic supplementary material, figure S1A). Surprisingly, precocious foragers completed significantly fewer foraging trips over their lifetime ( $\chi^2 = 17.6$ , d.f. = 1,  $p < 0.0001$ ; figure 2c; electronic supplementary material, figure S1B), which suggests that long-term exposure of beehives to  $\text{Mn}^{2+}$  could negatively affect colony fitness. Furthermore, although all treatment groups increased the length of their foraging trips over time, the initial trips of 50 mM  $\text{Mn}^{2+}$ -treated bees were significantly longer ( $F_{6,393} = 85.55$ ,  $p < 0.000001$ ,  $R^2 = 0.51$ , figure 2d; electronic supplementary material, figure S1C).

Our studies support a model in which  $\text{Mn}^{2+}$  treatment leads to an early transition to foraging by increasing brain aminergic signalling, which is in agreement with previous studies of biogenic amines in honeybees [19]. However, our findings are also in contrast to previous studies in mammals [18] and *Drosophila* [21], which indicated that  $\text{Mn}^{2+}$  exposure leads to dopaminergic neuronal loss, and overall reduced levels of dopamine in the brain. We do not yet understand the main reason for the differences between our current findings and previous reports. Noteworthy, the  $\text{Mn}^{2+}$  doses we

have used in our studies were far below previously reported neurotoxic levels [21]. As a result, these previously published data together with our current findings suggest that the interaction of  $\text{Mn}^{2+}$  with biogenic amine signalling and behaviour comprises two phases: exposure to low  $\text{Mn}^{2+}$  levels leads to an increase in biogenic amine synthesis but, once above the neurotoxic threshold, it leads to a reduction in biogenic amine levels.

Our finding that  $\text{Mn}^{2+}$  treatment leads to extended initial foraging trips suggest that  $\text{Mn}^{2+}$ -induced precocious foraging might be associated with decreased navigational abilities or lower physical fitness. As increase in time spent on individual foraging flights has previously been linked to declining health and decreased navigational abilities of foragers [26], our findings further support the hypothesis that exposure to even low levels of  $\text{Mn}^{2+}$  could affect the long-term health of bees.

As  $\text{Mn}^{2+}$  induces precocious foraging and the foraging performance of precocious foragers is significantly lower than typical-age foragers [27], our data indicate that in addition to the increased environmental pressures from parasites, pathogens, insecticides and modern agricultural practices on the health of pollinators [2], it is important to consider other potential anthropogenic factors such as metal pollution as possible risk factors. Consequently, better understanding of these factors would lead to improved risk assessment, and improved management practices of pollinators and other beneficial invertebrates.

**Data accessibility.** All data has been deposited in Dryad (doi:10.5061/dryad.9j0j8).

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**Author contributions.** E.S., Y.B.-S. and A.B.B. designed experiments, analysed data and wrote the paper. E.S., C.J.P. and A.L.M. conducted experiments.

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**Conflict of interests.** We have no competing interests.

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