Multi-omics for ecotoxicology

Honey bees show a biphasic stress response when exposed to Imidacloprid

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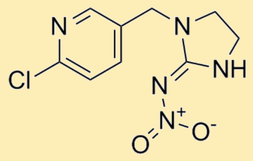
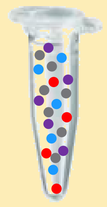
Understanding and managing the sublethal effects of chemical contaminants is a key challenge in ecotoxicology. Multi-omics can be used to identify biomarkers and causal mechanisms underlying stress responses. Here, a honey bee model is used to demonstrate the utility of multi-omics to uncover time- and concentration-dependent responses to a sublethal pesticide exposure.

4-day old honey bee larvae (from 3 hives) n = 192

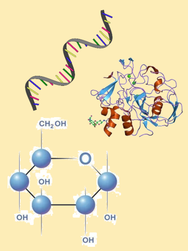


Exposed *in vitro* to three sublethal concentrations of Imidacloprid (IMI):
IMI1 = 0.834 mg/L (Low)
IMI2 = 1.67 mg/L (Medium)
IMI3 = 3.34 mg/L (High)

Larvae sampled: 1, 24, 48 and 72 hours post-exposure



Multi-omics extractions from single larvae samples



Transcriptomics (RNA-seq), proteomics and metabolomics (LC-MS) data generated.

Results

A strong initial acute response was detected in treated larvae at 1 h (transcriptome) to 24 h (proteome and metabolome) post-exposure (Fig 1). This was characterised by an increase in stress response pathways, including DNA damage response and oxidative stress, and a decrease in Ca^{2+}/Calmodulin, development, (e.g., Notch, Wnt, TGFβ, Hippo, Hedgehog, FoxO) and neurotransmission (e.g., phototransduction and circadian rhythm) signaling pathways (Figure 2). The magnitude of these early impacts increased in a concentration-dependent manner, particularly in the transcriptome (Fig 1 and 2).

After a period of recovery at 48 h, a delayed response was detected (in all omics) at 72 h (Fig 1). Development and immune pathways were downregulated in this late response. There was also evidence of perturbed energy metabolism and upregulation of amino acid and lipid catabolism (Fig 2). These findings suggest IMI causes a long-term energy cost at the expense of development and immune processes. Unlike the early response, these delayed effects were not concentration dependent. While this later response was not a strong as the initial acute stress phase, it highlights the potential for long-term adverse effects in honey bees exposed to IMI as larvae.

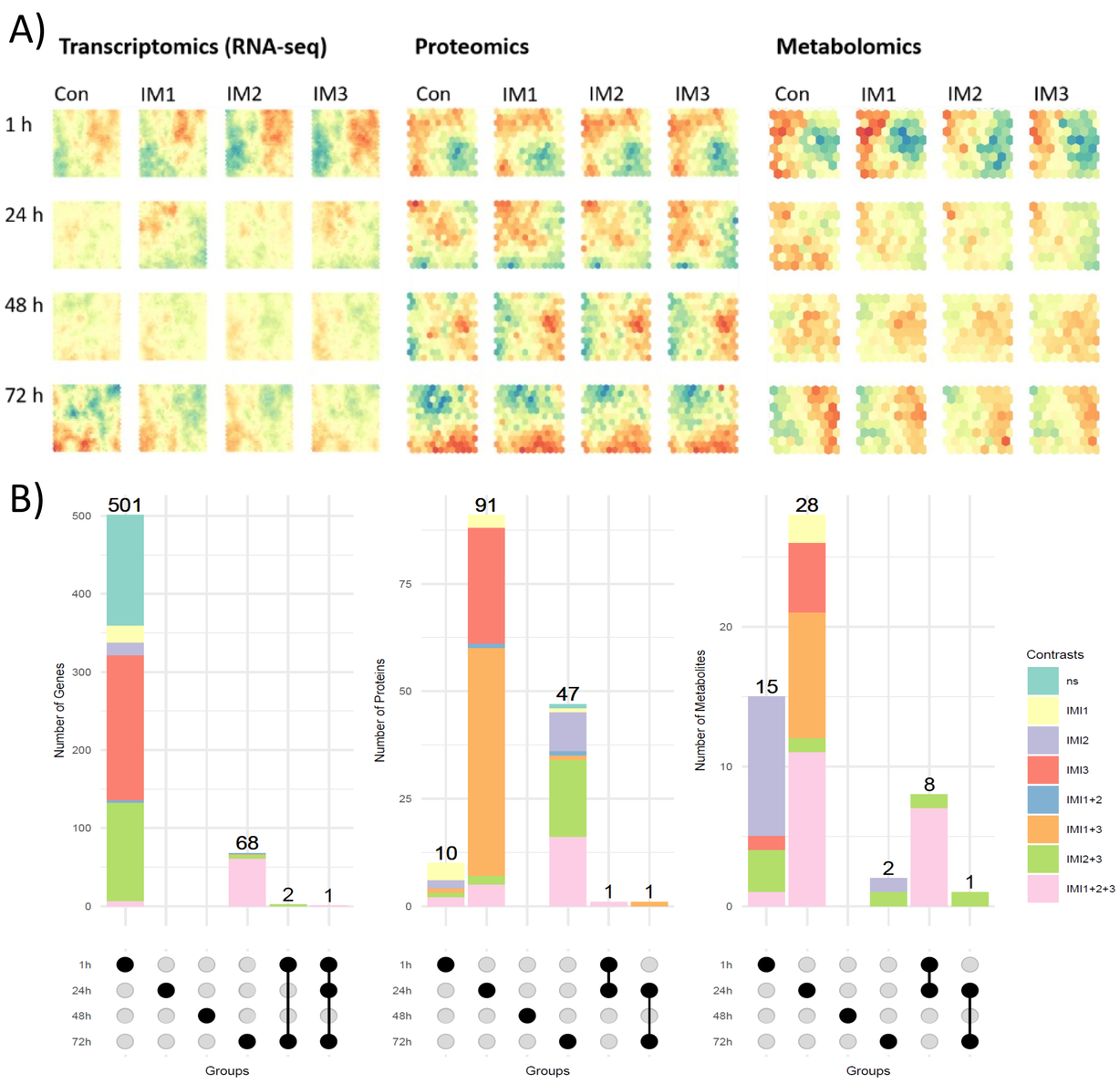


Figure 1. A) Self organizing maps (SOMs) showing aggregation of all features based on mean expression (RNA) or abundance (proteins and metabolites) by time and concentration. All topographies within each omics data set are comparable as the positions of the cells are fixed in the maps; this enables simultaneous visual inspection of expression/abundance changes across concentrations and over time by colour (blue = down and red = up, relative to average normalised expression/abundance value). **B)** Upset plots showing the numbers and intersections of differentially expressed genes and differentially abundant (DA) proteins and metabolites, in treated larvae relative to controls, at each time point for each concentration of IMI. ns = DA features which are not significant in any individual contrast.

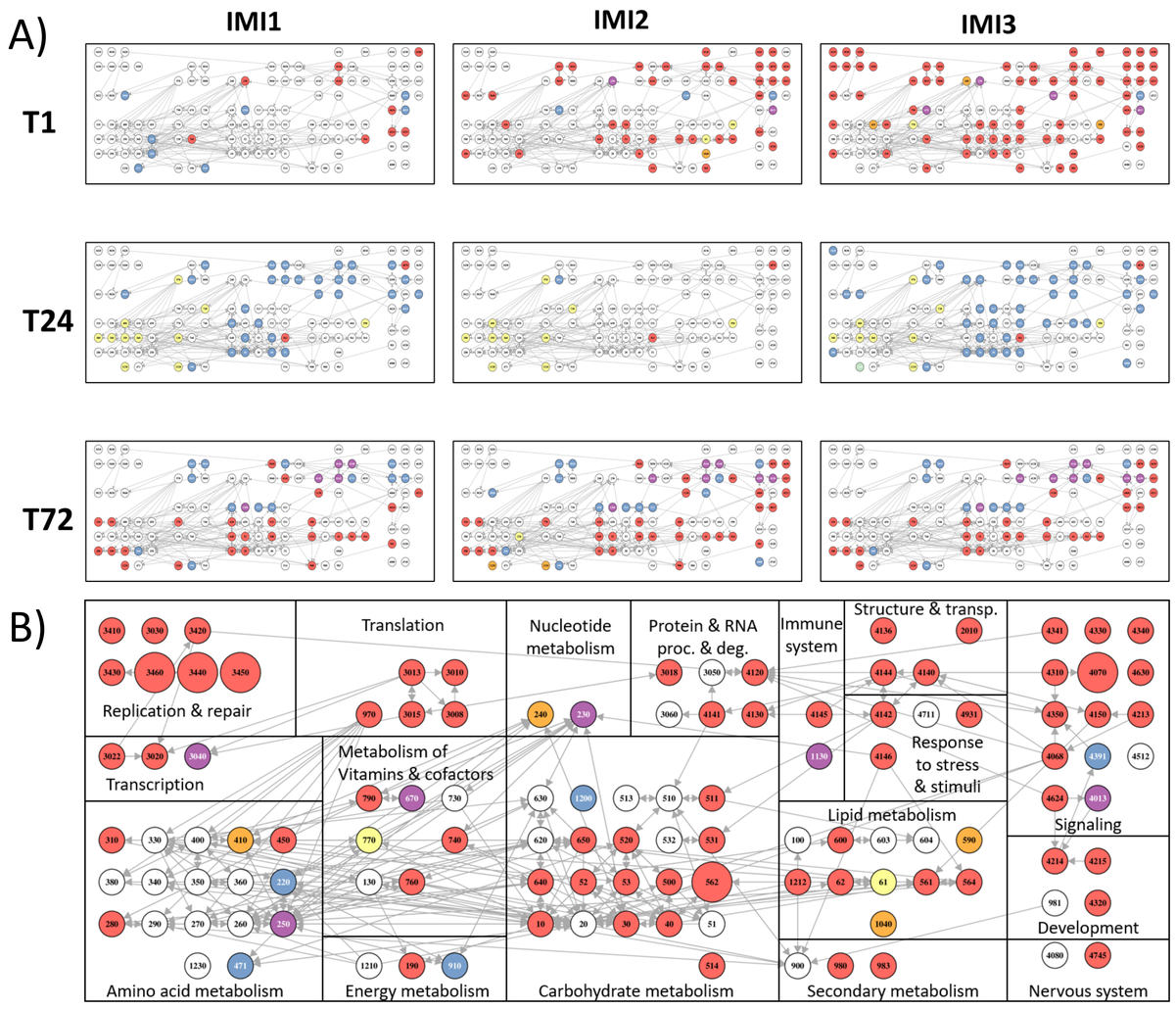
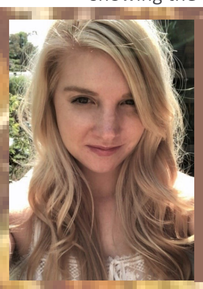


Figure 2. A) Network maps of KEGG pathways containing significantly impacted features (red = genes, blue = proteins, yellow = metabolites, purple = genes and proteins, orange = genes and metabolites, green = proteins and metabolites) for each time point and each concentration of IMI, relative to controls. **B)** Key of the network map (using T1 combined concentrations) showing the functional grouping of impacted KEGG pathways.

Conclusions

This study demonstrates the utility of multi-omics to explore complex time and concentration relationships in chemical stress studies, and to characterise underlying causal mechanisms.

The use of multi-omics enabled detection of different functional and temporal responses that would not have been seen by a single omics technology alone. Initial acute stress responses to IMI were detected that increased in a concentration-dependent manner while delayed effects on development and energy metabolism were similar irrespective of dose. These findings highlight the potential for honey bees to have long-term adverse outcomes from early life exposure to even very sublethal concentrations of IMI.



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