Visual motion detection and collision avoidance behaviours are disrupted by a neonicotinoid insecticide and its metabolites

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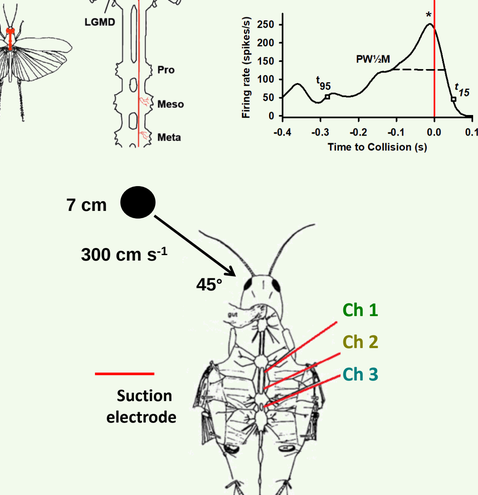
INTRODUCTION

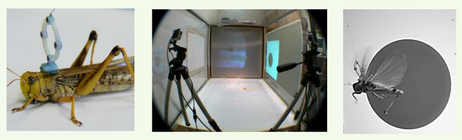
Neonicotinoid insecticides (NIs) affect many aspects of insect physiology and behaviour, and are having significant detrimental effects on many non-target species, including wild and domestic bees. We previously showed that sublethal doses the NI imidacloprid (IMD) significantly reduces collision avoidance behaviours and visual motion detection of a looming sensitive neuron in the locust, the Descending Contralateral Movement Detector (DCMD)[1]. These effects were present 2 and 24 hours after treatment. While NIs are nAChR partial agonists, increased spontaneous activity of the DCMD was only observed in the first 20 to 30 minutes after treatment. IMD is metabolized to several secondary compounds that can be more persistent and bioavailable and it is unknown whether these compounds affect visual processing[2].

Here, we tested the sublethal effects of IMD and two of its metabolites, 5-hydroxy-imidacloprid (5OH) and imidacloprid-olefin (OLE) on collision avoidance behaviour, visual processing, and action potential propagation. We hypothesized that if previously observed effects on DCMD firing were caused primarily by an IMD metabolite the effects would be more pronounced when the metabolite was applied directly.

MATERIALS AND METHODS



Imidacloprid (IMD) and two metabolites, 5-hydroxy-imidacloprid (5OH) and imidacloprid-olefin (OLE) were used at sublethal quantities (10 ng/locust).



We recorded behavioural responses of loosely tethered locusts in a wind tunnel presented with looming visual stimuli before and after treatment with IMD, OLE or 5OH.

We tested the effects of IMD, OLE, and 5OH on DCMD responses to a looming disk. Dorsal dissections exposed the ventral nerve cords and ganglia to allow for three simultaneous recordings. DCMD spike times were used to construct peristimulus time histograms (PSTHs) from which response parameters were measured, including peak time and peak frequency (*) rise phase (t_{95} to *), decay phase (* to t_{15}), and peak width at half maximum (PW½M). Conduction velocity was calculated as the reciprocal of the delay between DCMD spikes in adjacent channels (CV = 1/b-a), and normalized within each animal before and after treatment as a proportion of the first spike in the pre-treatment recording.

IMD AND METABOLITES IMPAIR AVOIDANCE BEHAVIOUR

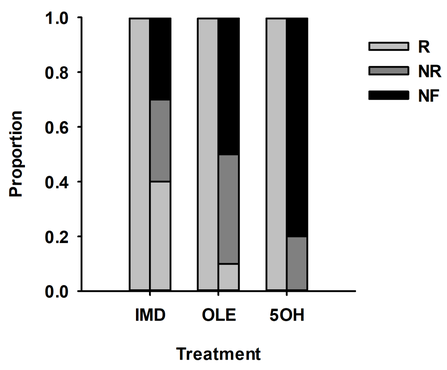


Figure 1: Flight steering and escape behaviours were affected by imidacloprid and its metabolites. While all animals responded to the looming stimulus with an escape manoeuvre (R, responding: stop, turn, or glide) before treatment (left columns), these behaviours decreased or attenuated 1 hour after injection of a single, sublethal (10ng) dose of IMD, OLE, or 5OH (right columns). Treatment with IMD, OLE and 5OH reduced or abolished behavioural responses, resulting in locusts that either flew without responding (NR) or did not fly (NF).

IMD & METABOLITES DECREASE VISUAL MOTION DETECTION

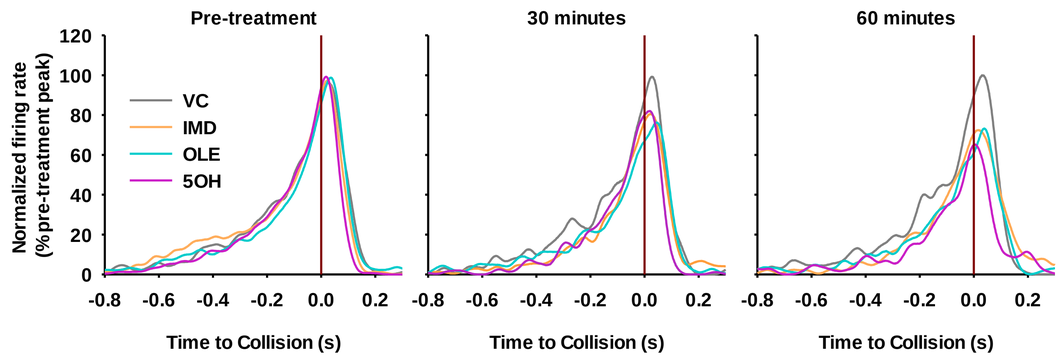


Figure 2: Mean peristimulus time histograms (PSTHs) from all animals in each treatment group normalized as a percent of the peak firing rate before treatment. IMD, OLE and 5OH caused a decrease in peak firing rate relative to the vehicle control (VC) and altered the DCMD response profile, and the effects were most pronounced 60 minutes after treatment. Vertical line at 0 s represents the projected time of collision of the looming stimulus.

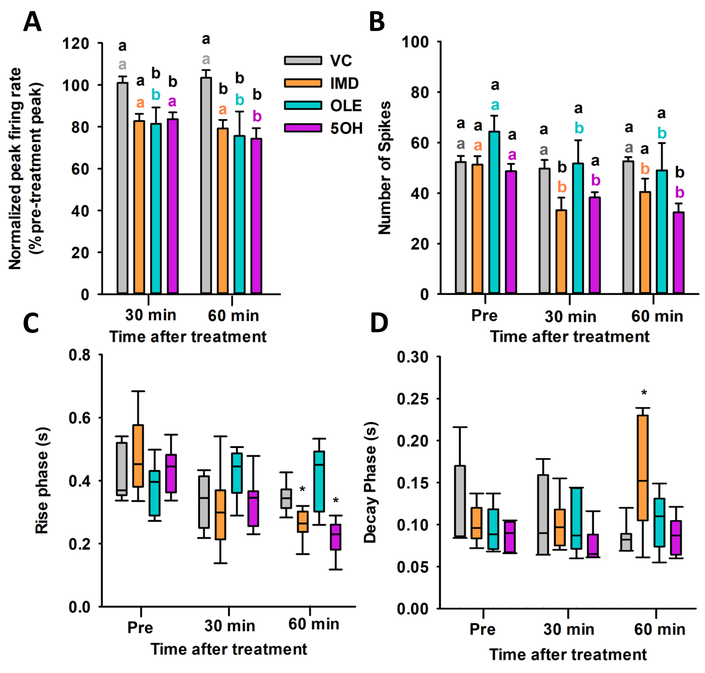


Figure 3: Comparison of response parameters measured from PSTHs, including the normalized peak firing rate (A), the total number of spikes per stimulus presentation (B), and the rise (C), and decay (D) phases of the histograms. Letters above bars represent significance of Two-Way Repeated Measures ANOVA: black letters compare treatments to VC within each timepoint, and coloured letters compare within treatment over time. Asterisks above box plots denote significance of treatment compared to VC within timepoint from One Way ANOVA tests. IMD, OLE and 5OH had most significant effects on peak firing rate and total number of spikes at both time points.

IMD & METABOLITES REDUCE CONDUCTION VELOCITY

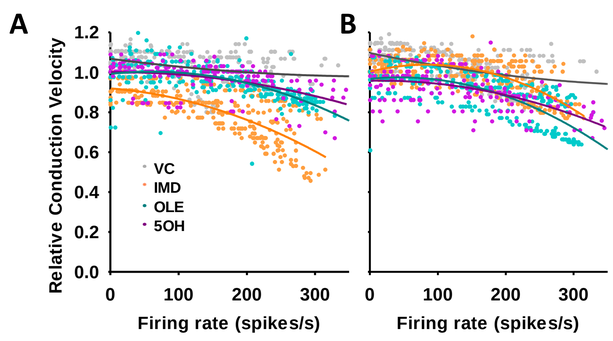


Figure 4: Relative DCMD conduction velocity (CV) versus firing rate along the ventral connective (A) and across the mesothoracic ganglion (B) at 60 minutes after treatment. CV decreased after treatment with IMD, OLE or 5OH, which was enhanced at higher firing rates.

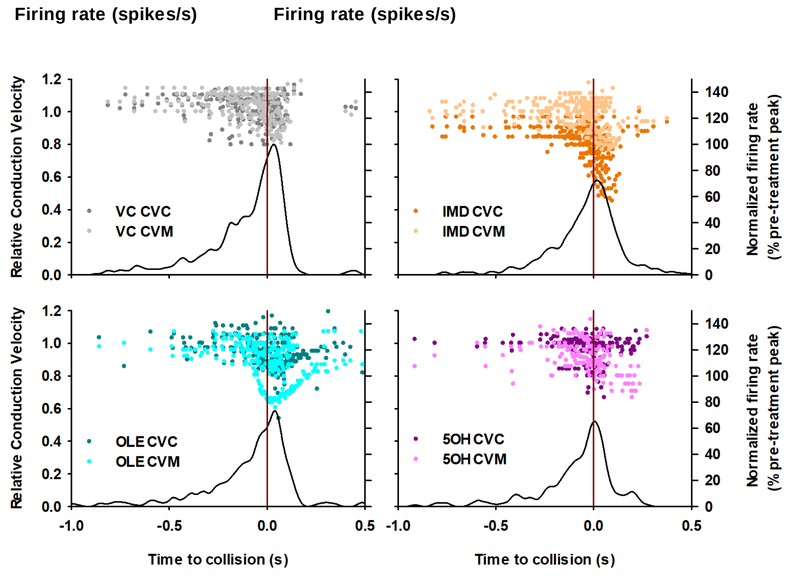


Figure 5: CV versus time of collision along connective (CVC) or across the mesothoracic ganglion (CVM) for all animals (top plots) and corresponding mean PSTHs (bottom plots) at 60 minutes after treatment. While lower CV values were associated with higher firing rates, the effect was enhanced following treatment of IMD, OLE or 5OH despite lower peak firing rates.

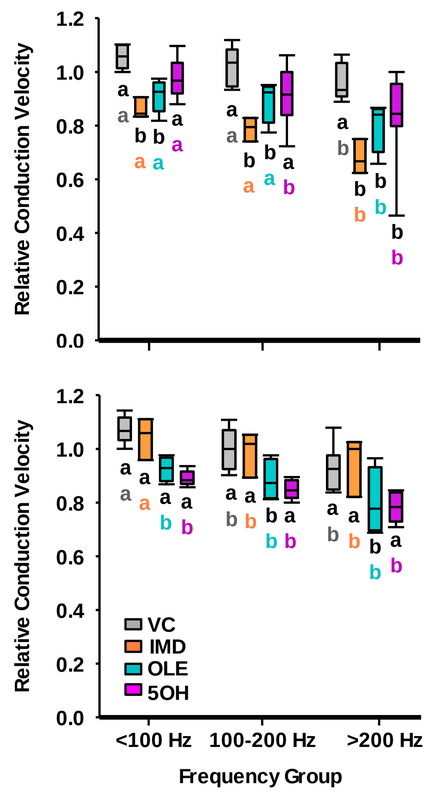


Figure 6: Boxplots illustrating the effects of dose and firing rate on DCMD CV along the connective (A) and across the mesothoracic ganglion (B). Letters below boxes denote results of Two Way RM ANOVA with black letters comparing treatments to VC within each frequency group and coloured letters comparing within treatment across frequency groups. CV decreased at high frequencies across the ganglion but not along connective normally, but was found to decrease with increasing frequency after treatment with IMD, OLE or 5OH along the connective. OLE caused a significant reduction in CV across the ganglion compared to VC.

SUMMARY & CONCLUSIONS

Behaviour
- IMD and metabolites reduce flight and escape behaviours.
- 5OH has the most pronounced effect (80% of animals unable to fly).

Visual motion detection
- Treatment reduced DCMD peak firing rate, with greatest effect from 5OH.

Conduction velocity
- IMD significantly reduced CV, which was most pronounced at higher firing frequencies.
- CV was most affected along the connective rather than across the mesothoracic ganglion

Conclusions
- Behaviour was most affected by 5OH, corresponds with a lower peak DCMD firing rate.
- Similar effects of IMD and metabolites on DCMD firing suggest a common binding site and affinity for nAChRs located on its dendrites and presynaptic neurons
- These novel results show that, in addition to the effects of neonicotinoids on nAChRs on the dendrites, NIs also affect action potential propagation suggesting possible secondary effects on voltage-gated channels along the axon[3]

REFERENCES & ACKNOWLEDGEMENTS

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1. Parkinson R.H. et al (2017) A sublethal dose of a neonicotinoid insecticide disrupts visual processing and collision avoidance behaviour in *Locusta migratoria. Sci Rep 7:936*

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3. Cross & Robertson (2016) Ionic mechanisms maintaining action potential conduction velocity at high firing frequencies in an unmyelinated axon. *Physiol Rep* 4:10