Imidacloprid impairs visually-based collision avoidance

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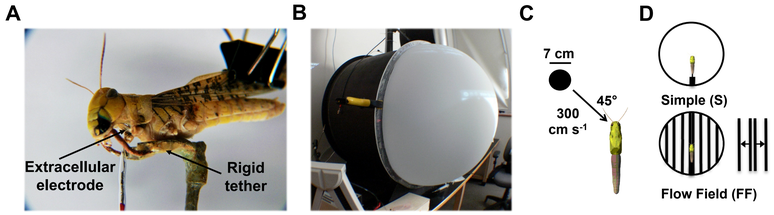
ABSTRACT

Neonicotinoid insecticide (NI) use is widespread and preclusive, despite demonstrated risk to pollinators and other invertebrates[1,2]. We defined sublethal effects of imidacloprid (IMD), a NI, on a well described and tractable motion detection neuron in the locust, the Descending Contralateral Movement Detector (DCMD). The DCMD receives coded input from the compound eyes via its pre-synaptic partner, the Lobula Giant Movement Detector (LGMD), and has monosynaptic excitatory inputs on motorneurons for flight and jumping[3,4]. Bursting of the DCMD may cue escape behaviours by surpassing a rate threshold in active motorneurons[5,6]. The 48h injected LD50 for IMD was estimated to 2,500 ppb in male locusts. We show sublethal IMD doses (10 and 100 ppb) affect DCMD responses to looming stimuli. While DCMD firing within the first hour of treatment was highly variable between animals and involved periods of sporadic firing and/or neural silence, the response stabilized in the second hour but does not return to pre-treatment patterns. Twenty-four hours after treatment with 10 or 100 ppb IMD, the peak firing rate within DCMD bursts remained significantly reduced. Observed effects on DCMD firing are translated to deficits in collision avoidance behaviours: exposure to 10 ppb IMD attenuated escape manoeuvers, while the ability to fly and walk was attenuated by 100 ppb IMD. These effects are sustained 2 and 24 hours after treatment. We show that IMD causes significant and lasting damage to an important pathway involved with visual sensory coding and escape behaviours at doses well below the LD50.

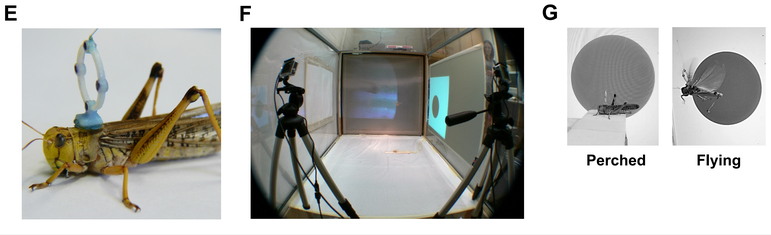
METHODS

LD50 experiments. Male (n=160) and female (n=66) locusts were injected with doses of IMD between 1 and 10,000 ppb. Mortality was calculated at 48 hours after treatment, and normalized to vehicle control groups on each day.

Electrophysiology. Male locusts were dissected ventrally to record extracellularly from the ventral nerve cord (**A**) and mounted in the flight simulator (**B**). Animals were presented with a looming stimulus (**C**) against simple and flow field backgrounds (**D**). Recordings were made over 2 hours after treatment with 0.1, 1, 10 (n=5 each), or 100 ppb IMD (n=20) or the vehicle (n=5), and at 24 hours after treatment with 10 or 100 ppb IMD (n=9 each) or the vehicle (n=7).



Behavioural assays. Tethers were affixed to the dorsal pronotum (**E**) and locusts were positioned in a wind tunnel (**F**). Locusts were presented with looming stimuli while perched and flying (**G**), and collision avoidance behaviours were scored. Animals were treated with 10 or 100 ppb IMD (n=10 each) or the vehicle (n=15), and behavioural assays were repeated 2 and 24 hours after treatment.



Males are more sensitive than females to IMD

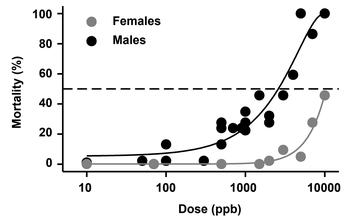


Figure 1: The 48 hour injected LD50 of imidacloprid is estimated to 2,500 ppb for male locusts. Female locusts displayed a greater resistance, and 50% mortality was not achieved over the range of doses used. More studies are required to determine the cause of this disparity. For this reason, only male locusts were used in subsequent experimentation.

IMD decreases neural responses to object motion

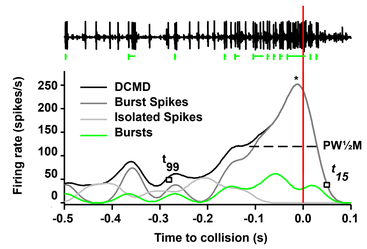


Figure 2: Control DCMD response to a looming stimulus (top) and corresponding peristimulus time histograms (PSTHs; bottom) aligned to the time of collision of the looming stimulus (0s). DCMD Burst initiation and burst duration are highlighted with vertical and horizontal green lines, respectively. Four overlaid PSTHs represent the full DCMD response, spikes within bursts, isolated spikes and bursts. Parameters were measured from the four PSTHs to define each DCMD response profile: peak firing rate and peak time (y and x at *); rise phase (t_{99} to *); decay phase (* to t_{15}); and peak width at half maximum (PW½M).

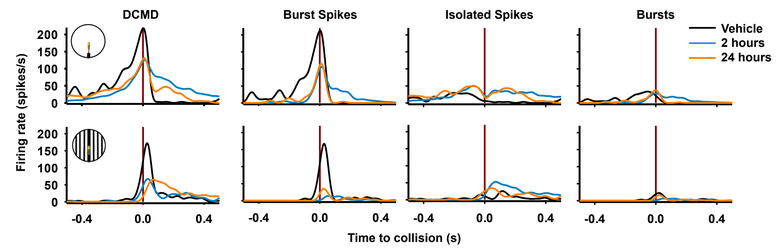


Figure 3: PSTH overlays of DCMD responses to looming stimuli over simple (top row) and flow field (bottom row) backgrounds, divided into the four rate codes. Overlays show the DCMD response profile at 2 (blue) and 24 (orange) hours after treatment with 100 ppb IMD, compared with the vehicle (black). 100 ppb IMD results in a reduced peak firing rate at both 2 and 24 hours, while other response parameters were altered differently between the time points.

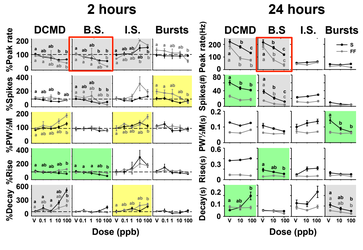


Figure 4: Comparison of DCMD response variables (mean±SEM) at 2 and 24 hours after an acute treatment with imidacloprid or the vehicle. At 2 hours after treatment, response variables are displayed as a percent of pre-treatment values, while at 24 hours after treatment actual values are plotted. Grey backgrounds represent parameters that were significant for both simple and flow field backgrounds at one or more imidacloprid doses. Yellow backgrounds highlight parameters that were significant for stimuli presented over a flow field background only, and green backgrounds highlight parameters that were significant for simple background only. The only response parameter that was significantly affected by 10 and 100 ppb imidacloprid at both 2 and 24 hours after treatment, with both simple and flow field backgrounds was the peak firing rate within bursts (red boxes). This parameter was used to describe the inhibitory effect of imidacloprid (figure 5).

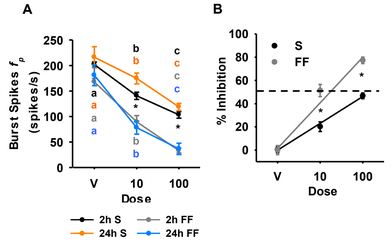


Figure 5: Effect of imidacloprid on DCMD peak firing rate within bursts (mean±SEM). Data from 2 and 24 hours after an acute treatment with imidacloprid (A) show no significant difference between timepoints within dose and background, while there are significant differences between doses and backgrounds. 2 and 24 hour data is combined to visualize the percent inhibition of each dose (B). 10 ppb imidacloprid results in 50% inhibition of the peak firing rate within bursts for stimuli presented over a flow field background, while similar inhibition is achieved with 100 ppb imidacloprid with stimuli presented over a simple background.

IMD decreases behavioural responses to object motion

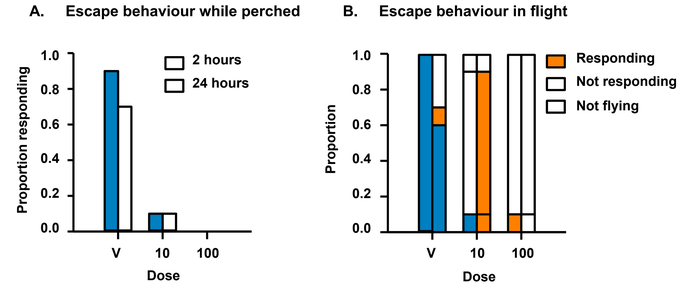


Figure 6: Escape behaviours at 2 (left-hand columns) and 24 (right-hand columns) hours after treatment with a single dose of imidacloprid (10 or 100 ppb) or the vehicle. Animals were presented with a looming visual stimulus while perched (A) or in flight (B). 10 ppb IMD inhibited escape behaviours while perched and in flight, while the ability to fly or walk was unaffected. With 100 ppb IMD, escape behaviours were inhibited, and animals were also unable to fly or walk.

Summary & Conclusions

LD50
- Locusts displayed low sensitivity to imidacloprid compared to other insects
- Females can support doses up to four times higher than males.

Electrophysiology
- 0.1 and 1.0 ppb imidacloprid did not significantly affect DCMD firing
- 100 ppb imidacloprid resulted in significant effects on many DCMD firing parameters
- Effects are more pronounced at 2 hours than 24 hours after treatment
- Peak firing rate within bursts: only parameter significantly affected with 10 and 100 ppb imidacloprid at 2 and 24 hours after treatment for stimuli presented over simple and flow field background
  - Firing rate threshold to elicit downstream motoneuron spikes[5,7]
  - Burst timing is correlated with the generation of escape manoeuvers[6]
- 50% inhibition achieved with 10 ppb imidacloprid with flow field:
  - Flow field emulates optic flow and mimics "natural" visual feedback in flight
  - Suggests freely behaving animals affected by lower doses

Behaviour:
- Effects of imidacloprid sustained 2 and 24 hours after treatment
- 10 ppb IMD inhibits escape manoeuvers
  - Visual processing disrupted
- 100 ppb IMD inhibits locomotion (walking and flying)
  - Motor control of wing and leg muscles disrupted

Our data suggest a strong correlation between reduced peak DMCD firing rate within bursts and an inability to perform escape manoeuvers, caused by an acute sublethal dose (0.4% LD50) of imidacloprid.

ACKNOWLEDGEMENTS & REFERENCES

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