

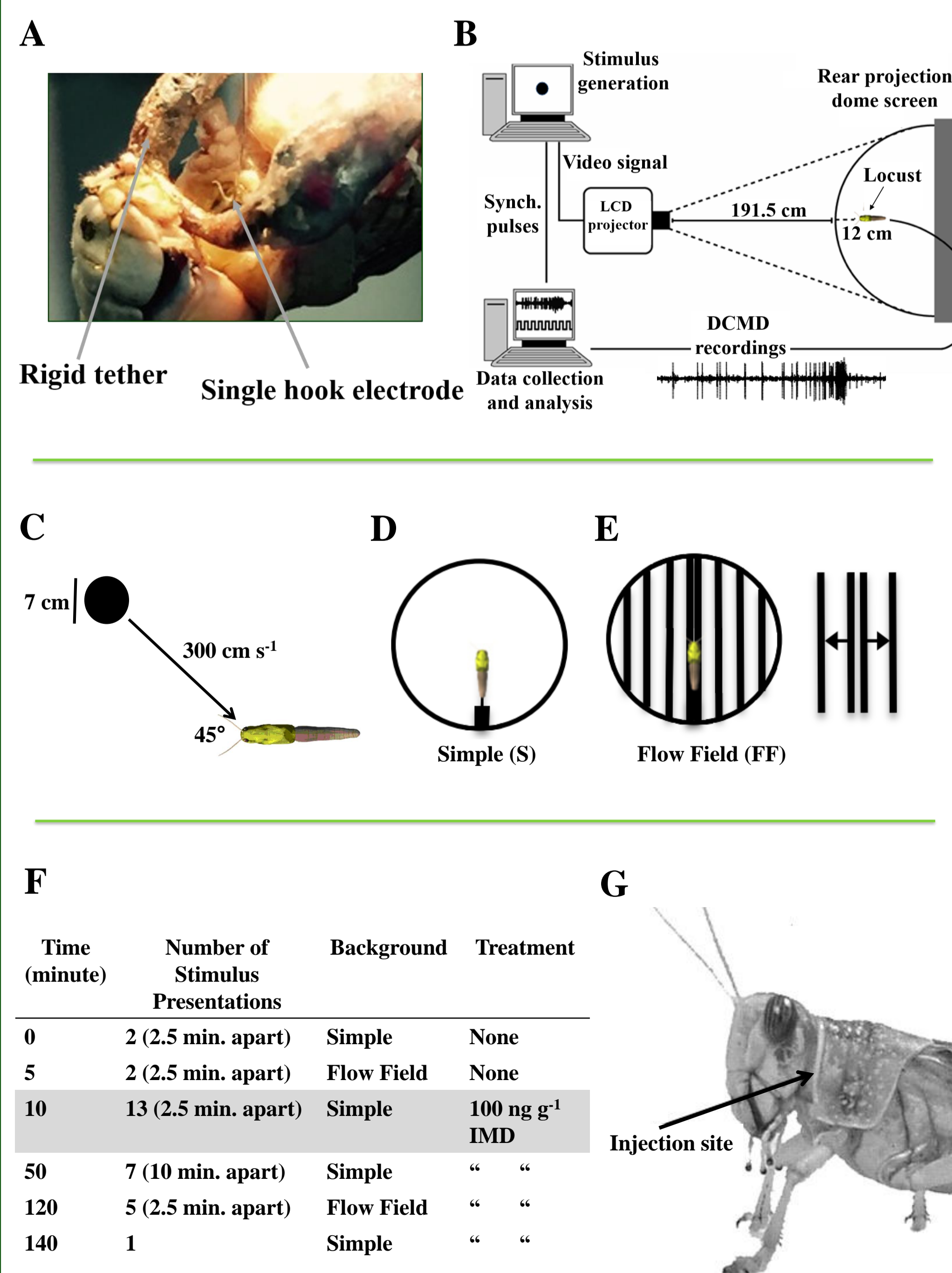
1. INTRODUCTION

Imidacloprid (IMD), a nicotinic acetylcholine receptor (nAChR) agonist, is a neonicotinoid pesticide used widely in agriculture. We tested sublethal effects of IMD on a behaviourally-relevant neural pathway in locusts (*Locusta migratoria*), which are important agricultural pests. The Descending Contralateral Movement Detector (DCMD) is a motion sensitive neuron in the locust brain involved in collision avoidance and escape from predators. It produces a well characterized, stereotypical response to a looming stimulus, that is dependent on the size of the looming object and its speed. Different response profiles are generated when displaying the stimulus over a simple versus flow field background, and this is related to upstream processing of visual information. By testing the effects of IMD on the firing profile of the DCMD, we can learn more about the role of the nAChR in this pathway.

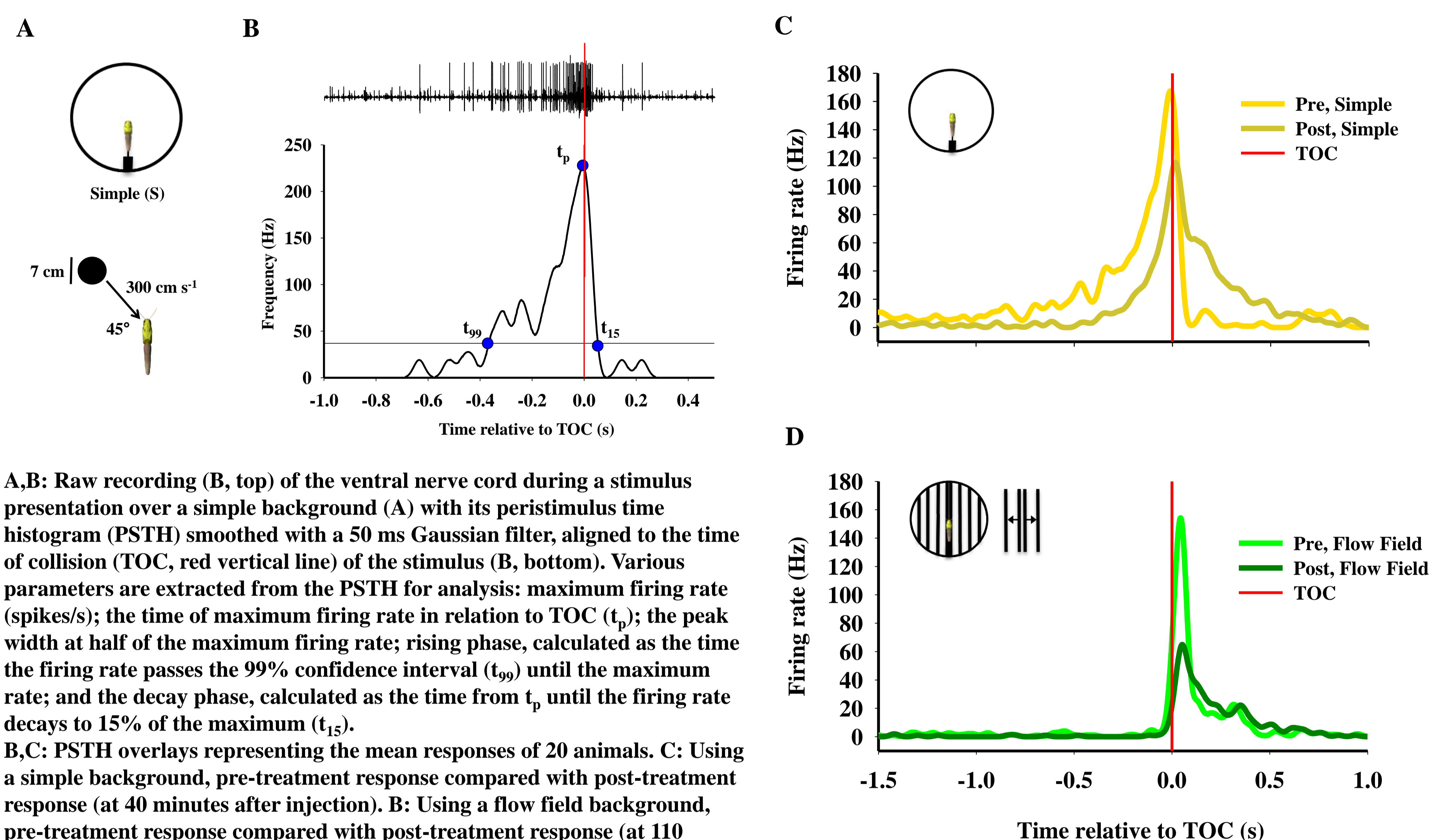
Peristimulus time histograms (PSTH) of the response of the DCMD to a looming stimulus over a blank (top) and flow field (middle) background, and the change in subtense angle of the stimulus (bottom) time aligned to the time of collision (TOC). The response of the DCMD to a looming stimulus over a flow field background contrasts that of the simple background in that it has a shorter rising phase (t_{99} to t_p), a longer decay phase (t_p to t_{15}), and a lower maximum firing rate. Figure adapted from McMillan et al. (2015).

2. METHODS

Locusts ($n=20$) were dissected ventrally (A) and mounted in the set-up facing the apex of the dome screen (B). A single stimulus (C) was presented over two background types (D, E). Temporal controls preceded treatment with IMD (F), which was injected (G). Experiments were replicated with a Vehicle control containing acetone and saline ($n=5$). Raw neuronal data was spike sorted (Offline Sorter) and spike times were aligned to time of collision of the stimulus (TOC). Peristimulus time histograms were generated with a 50 ms Gaussian smoothed filter (Neuroexplorer).

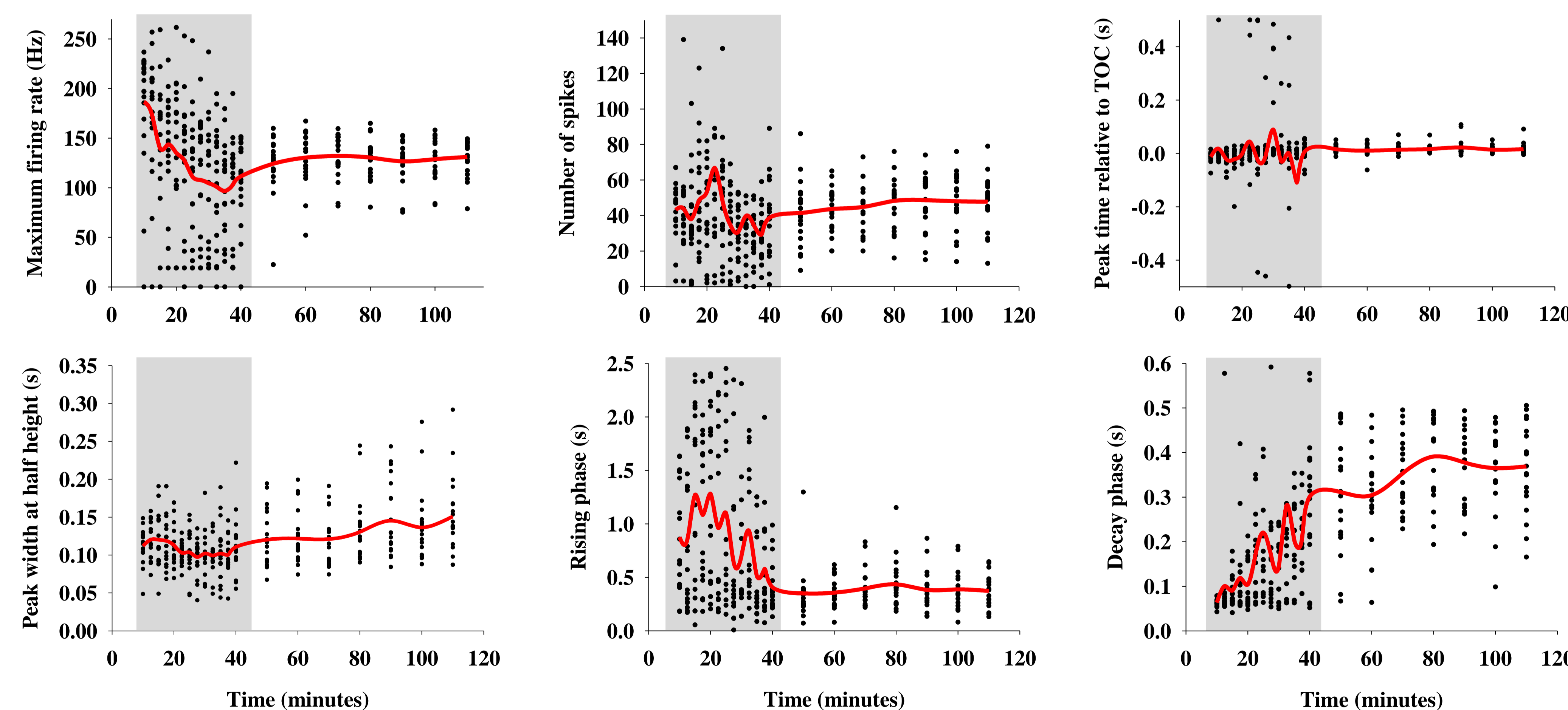


3. RAW DATA AND PERISTIMULUS TIME HISTOGRAMS



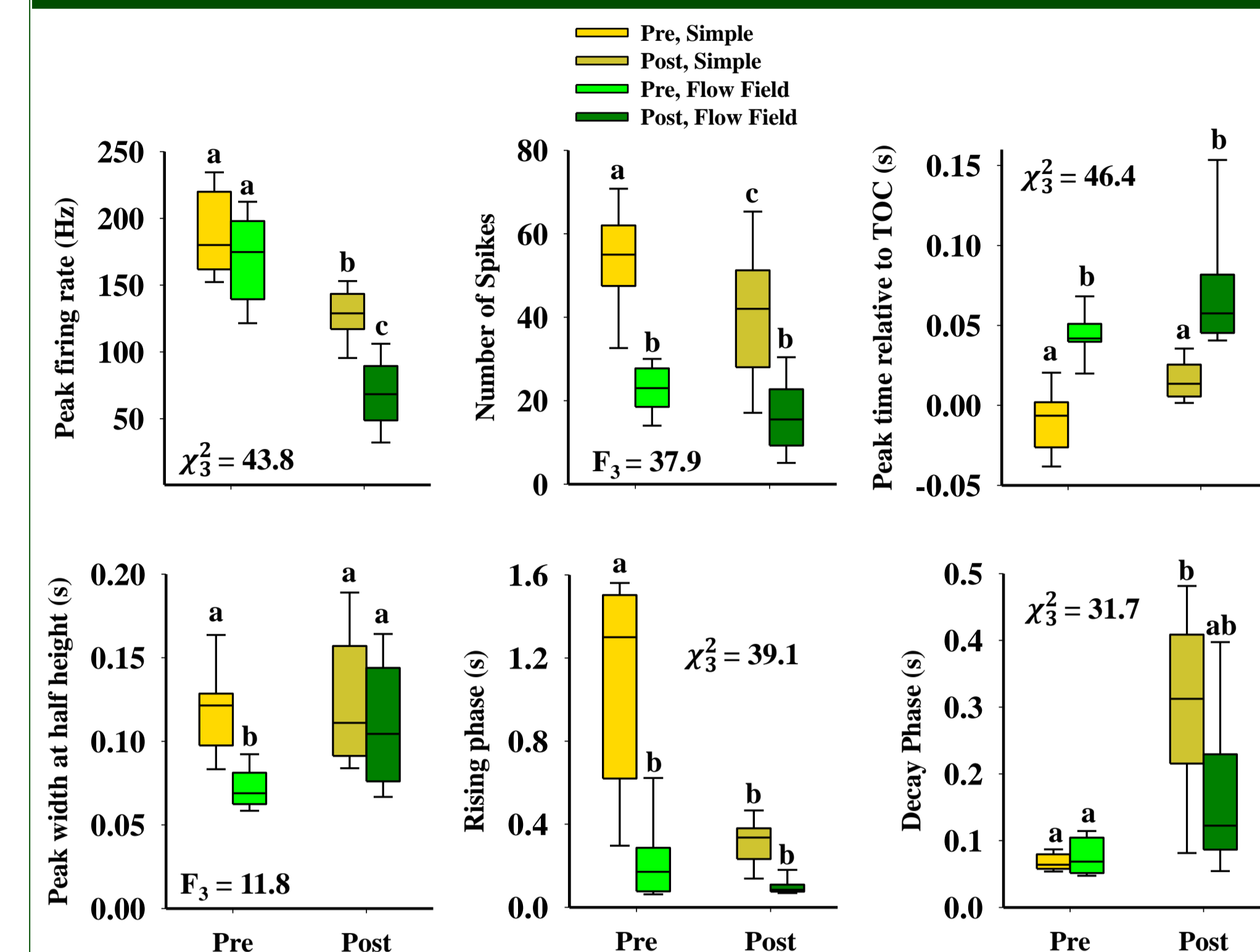
A,B: Raw recording (B, top) of the ventral nerve cord during a stimulus presentation over a simple background (A) with its peristimulus time histogram (PSTH) smoothed with a 50 ms Gaussian filter, aligned to the time of collision (TOC, red vertical line) of the stimulus (B, bottom). Various parameters are extracted from the PSTH for analysis: maximum firing rate (spikes/s); the time of maximum firing rate in relation to TOC (t_p); the peak width at half of the maximum firing rate; rising phase, calculated as the time the firing rate passes the 99% confidence interval (t_{99}) until the maximum rate; and the decay phase, calculated as the time from t_p until the firing rate decays to 15% of the maximum (t_{15}). B,C: PSTH overlays representing the mean responses of 20 animals. C: Using a simple background, pre-treatment response compared with post-treatment response (at 40 minutes after injection). B: Using a flow field background, pre-treatment response compared with post-treatment response (at 110 minutes after injection).

4. IMD EFFECT OVER TIME



Effect of IMD on PSTH properties over 100 minutes directly after injection with IMD. The stimulus was presented over a simple background every 2.5 minutes until minute 40 (light grey shading), and then 10 minutes apart until minute 110. Each column of data points represents the responses of all 20 animals. Mean is drawn in red. From injection until after minute 40 the responses are highly variable with no apparent trend. Presentations at minutes 50 through 110 have stabilized, and there is no significant difference between the responses at minute 50 and any of the later responses.

5. RESULTS



Comparison of pre-treatment parameters with simple and flow field backgrounds, and post-treatment parameters. Effect of post-treatment parameters were measured at 40 minutes after injection (simple) and 110 minutes after injection (flow field), as the effect was stable after 40 minutes. Significance shown with alternate letter. Tested with Friedman Repeated Measures ANOVA on Ranks (F statistic = normality passed, χ^2 statistic = normality failed).

6. SUMMARY

- A sublethal dose of IMD significantly effected the ability of this neural pathway to detect object motion
- Effects highly variable up to 40 minutes post treatment
- Effects stable after 40 minutes post treatment

Summary Table of Results. ↓ - significant decrease, ↑ - significant increase, ns – not significant.

Parameter	Post-treatment, simple	Post-treatment, flow field
Peak firing rate	↓	↓
Number of spikes	↓	ns
Peak time	ns	ns
Peak width half height	ns	↑
Rising phase	↓	ns
Decay phase	↑	ns

7. FUNDING AND REFERENCES

Funding provided by the Natural Sciences and Engineering Research Council of Canada, the Canada Foundation for Innovation, the University of Saskatchewan and the Margaret MacKay Scholarship

- Judge, S. and Rind, F. (1997). The locust DCMD, a movement-detecting neurone tightly tuned to collision trajectories. *J. Exp. Biol.* 200, 2209–2216.
- Silva, A. C., McMillan, G. A., Santos, C. P. and Gray, J. R. (2015). Background complexity affects response of a looming-sensitive neuron to object motion. *J. Neurophysiol.* 113, 218–231.
- Tomizawa, M. and Casida, J. E. (2003). Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annu. Rev. Entomol.* 48, 339–64.