Pyrethroids inhibit K_{2P} channels and activate sensory neurons: basis of insecticide-induced paraesthesias

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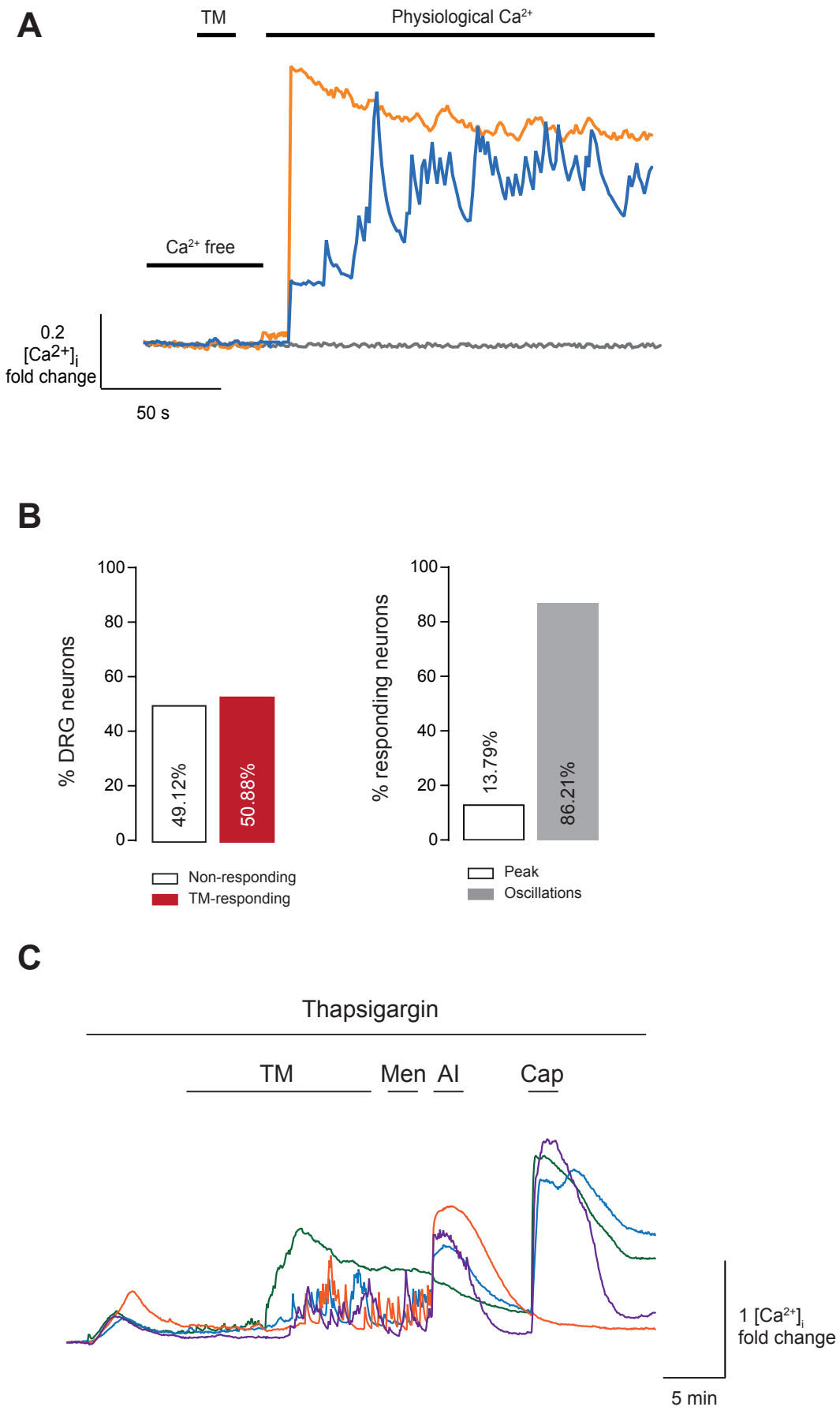
Abbreviated title: Insecticides inhibit K_{2P}s to activate nociceptors

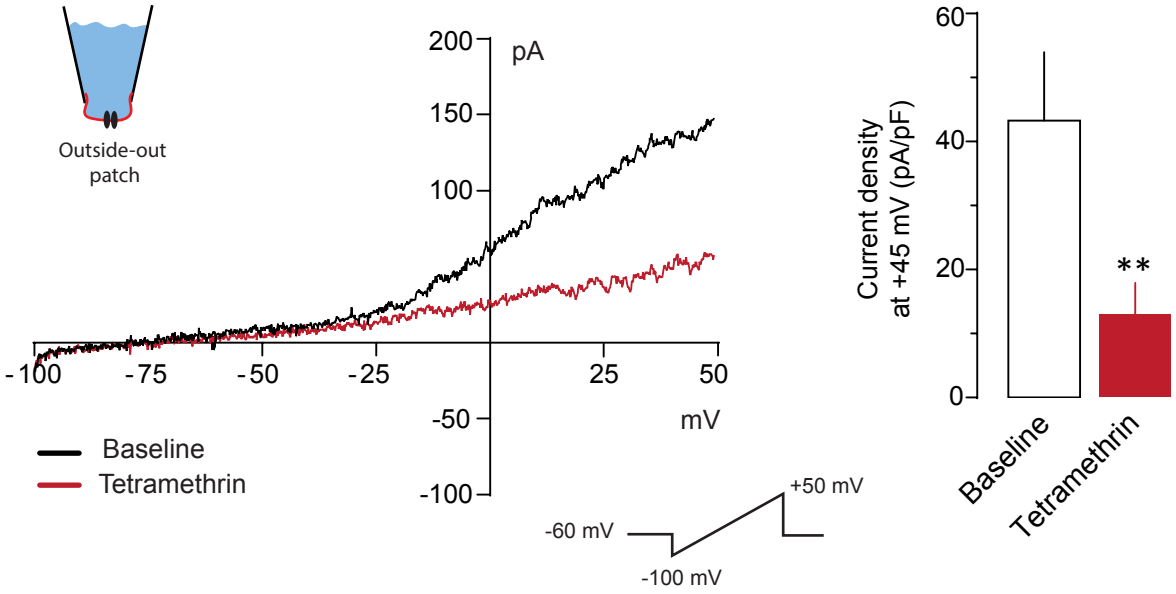
Supplementary Figure 1: Tetramethrin-mediated Ca^{2+} increase is due to extracellular Ca^{2+} entry and it is independent of Ca^{2+} release from intracellular stores

A. Examples of wild-type mouse DRG sensory neurons stimulated with Tetramethrin (TM, 20 μM) in nominally Ca^{2+}-free conditions and after reintroduction of Ca^{2+} into the extracellular solution. None of the neurons studied in Ca^{2+}-free conditions elicited a response (0 out of 126; 0%). Reintroduction of extracellular Ca^{2+} produced an increase in cytosolic Ca^{2+} in 70 out of 126 neurons (55.6%). Examples of two neurons activated by TM upon Ca^{2+} reintroduction are shown in blue (with an oscillatory pattern) and orange (single Ca^{2+} peak). A TM non-responding neuron is shown in gray. Intracellular calcium was measured by Fura-2 ratiometric imaging as described in methods. **B.** DRG wild-type neurons stimulated with tetramethrin (20 μM) in the presence of thapsigargin (1 μM) to empty intracellular calcium stores. Neurons were challenged with thapsigargin which was kept in the bath for the whole duration of the experiment. Left: Quantification of the percentage of neurons responding to tetramethrin from a total of 57 cells. Right: Percentage of tetramethrin-responding cells showing an oscillatory calcium pattern or responding with a single calcium peak. **C.** Representative traces of wild-type mouse DRG sensory neurons treated with thapsigargin. Neurons were exposed to tetramethrin (20 μM), menthol (Men, 100 μM), AITC (100 μM), capsaicin (Cap, 1 μM) and KCl (not shown) and intracellular calcium was measured by Fura-2 ratiometric imaging.


A
TM
Physiological Ca2+
Ca2+ free
0.2
[Ca2+]i
fold change
50 s
B
% DRG neurons
100
80
60
40
20
0
49.12%
50.88%
Non-responding
TM-responding
% responding neurons
13.79%
86.21%
Peak
Oscillations
C
Thapsigargin
TM
Men
AI
Cap
1 [Ca2+]i
fold change
5 min

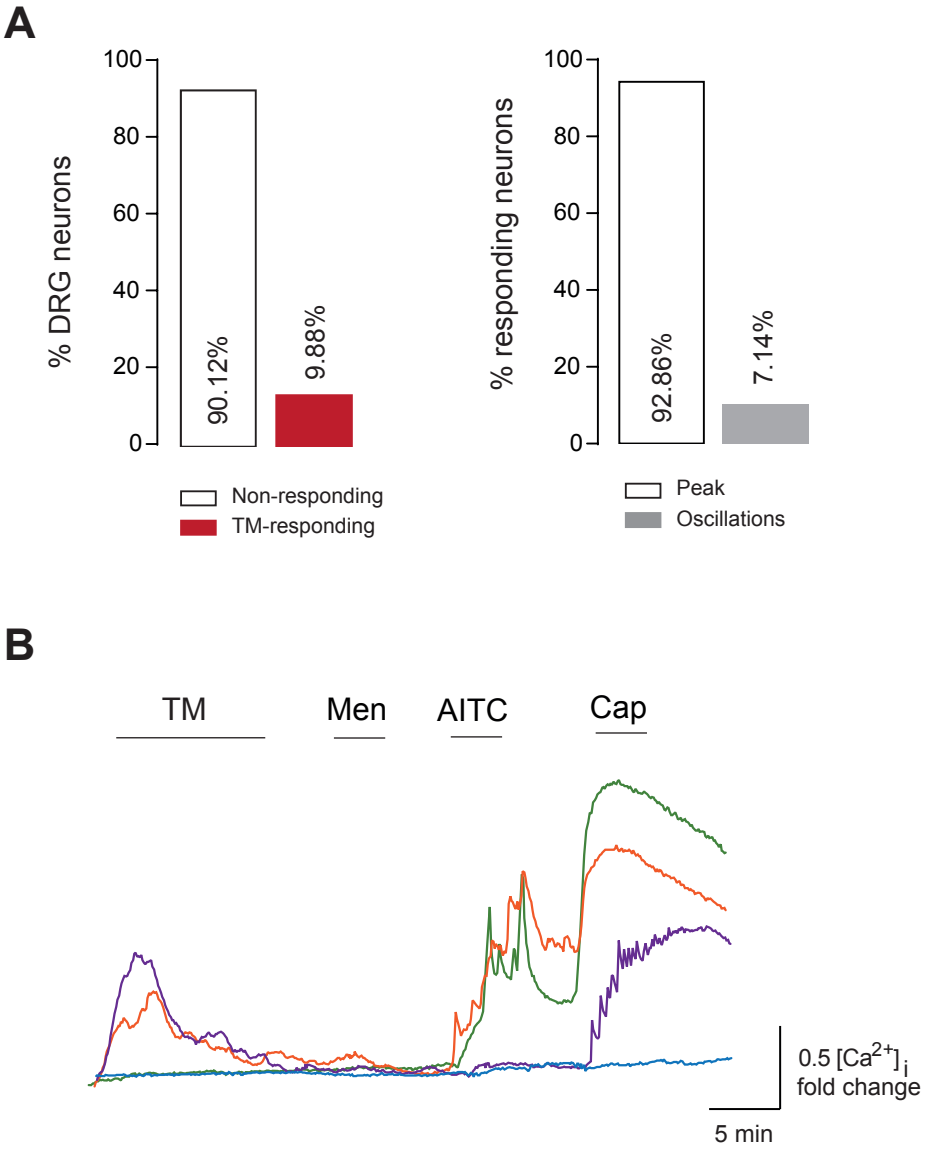
Supplementary Figure 2: Tetramethrin inhibits TRESK current in membrane cell patches

Left: Representative recording of a patch clamp recording in the outside-out configuration obtained in a HEK293 cell transfected with mouse TRESK. The TRESK current present in the membrane patch was recorded with a voltage ramp from -100 to +50 mV. Holding current was -60 mV. Solutions used were as described in methods for whole-cell patch clamp (physiological conditions). Tetramethrin (20 µM) application produced a significant reduction of the current. *Right:* quantification of TM inhibition of TRESK current. **p<0.01 paired student's t-test (n=7 independent patches).


Outside-out
patch
200
150
100
-50
-100
pA
-100
-75
-50
-25
25
50
mV
Baseline
Tetramethrin
+50 mV
-60 mV
-100 mV
60
40
20
0
Current density
at +45 mV (pA/pF)
**
Baseline
Tetramethrin

Supplementary Figure 3: TRESK current enhancement by cloxyquin prevents neuronal activation by tetramethrin

A. DRG wild-type neurons stimulated with tetramethrin (20 µM) in the presence of cloxyquin (50 µM), a TRESK channel activator. Neurons were pretreated for 5 min and cloxyquin was kept in the bath for the whole duration of the experiment. Left: Quantification of the percentage of neurons responding to tetramethrin from a total of 141 cells. Right: Percentage of tetramethrin-responding cells showing an oscillatory calcium pattern or responding with a single calcium peak. **B.** Representative traces of wild-type mouse DRG sensory neurons pretreated with cloxyquin. Neurons were exposed to tetramethrin (20 µM), menthol (Men, 100 µM), AITC (100 µM), capsaicin (Cap, 1 µM) and KCl (not shown) and intracellular calcium was measured by Fura-2 ratiometric imaging.


A
100
80
60
40
20
0
% DRG neurons
90.12%
9.88%
Non-responding
TM-responding
% responding neurons
92.86%
7.14%
Peak
Oscillations
B
TM
Men
AITC
Cap
0.5 [Ca2+]i
fold change
5 min