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Evidence of rattlesnake crotalpine effects on tetrodotoxin-sensitive Na channels of mouse dorsal root ganglion neurons

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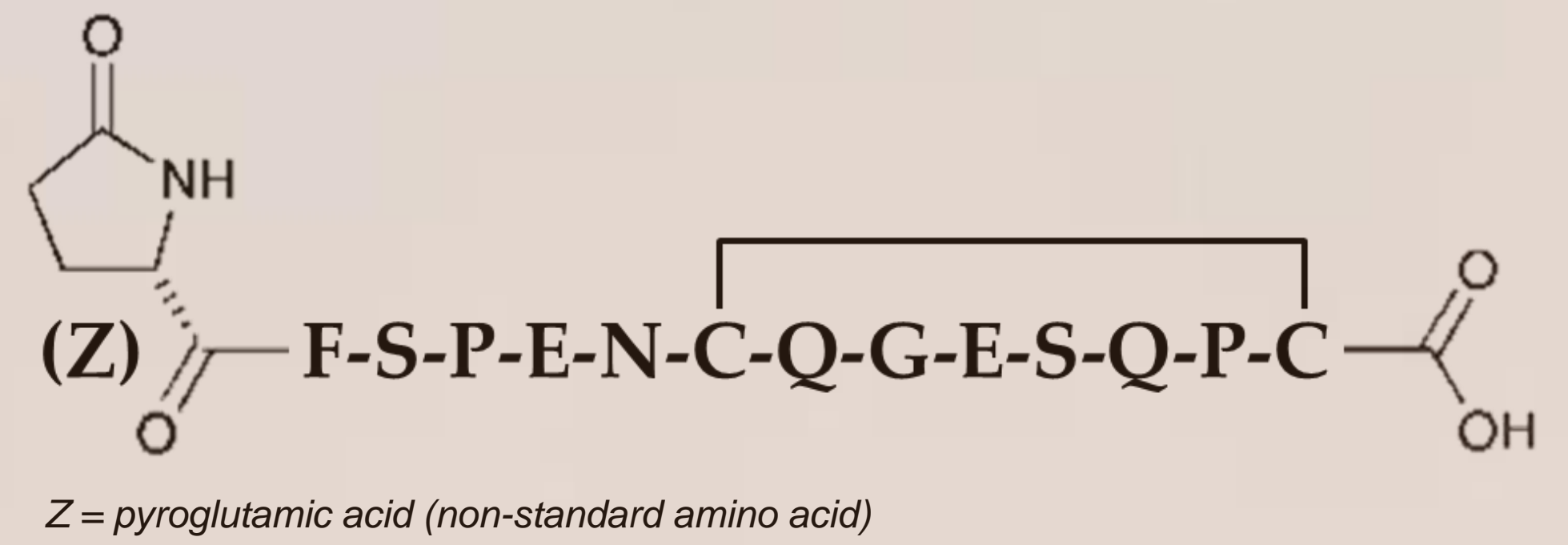
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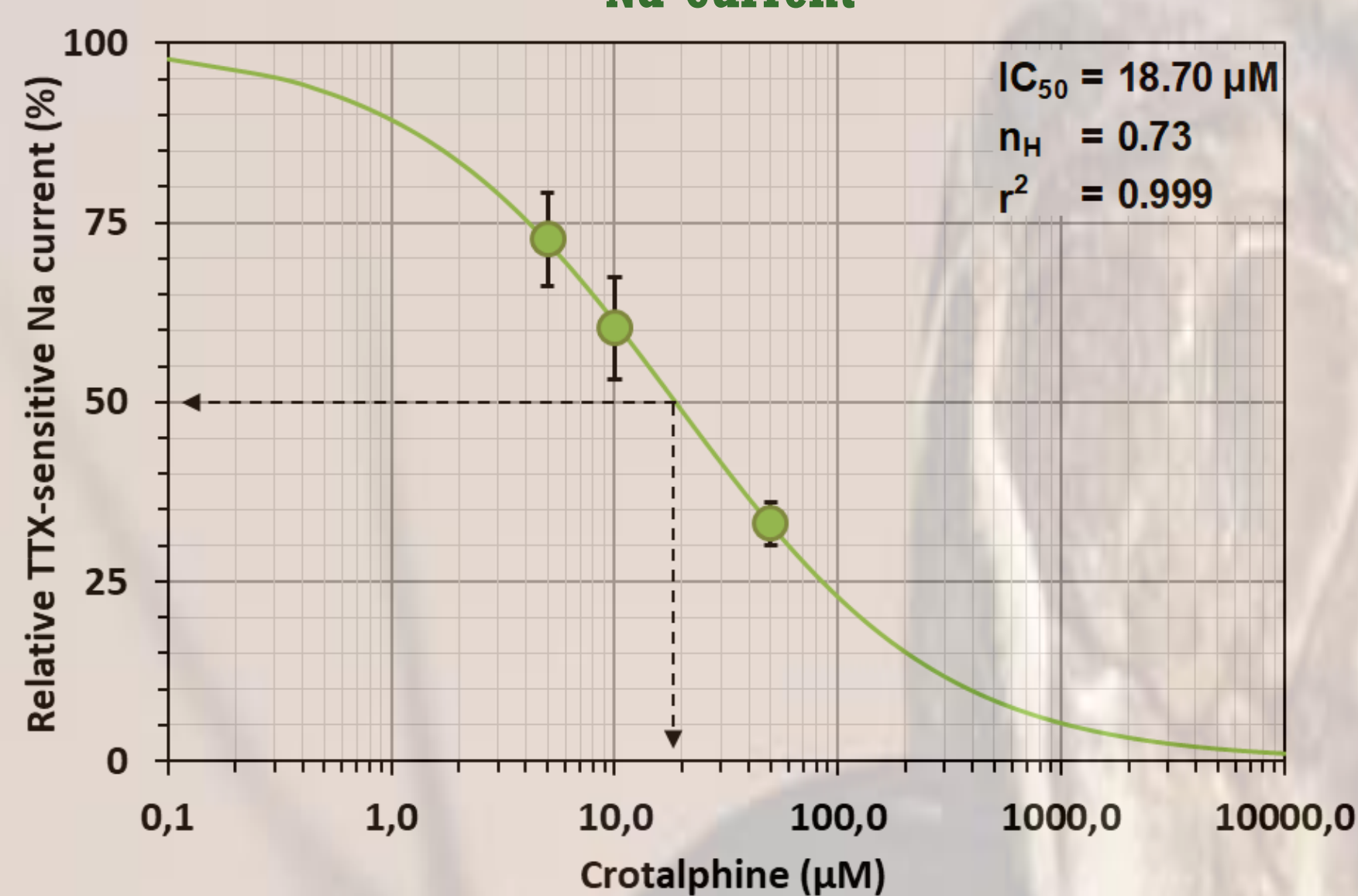
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INTRODUCTION. Crotalpine is an analgesic peptide identified in the venom of the South American rattlesnake *Crotalus durissus terrificus*. Although its antinociceptive effects are well documented, its direct mechanisms of action are still under investigation. The aim of the present work was to study the action of the crotalid peptide on the sodium channel subtype Na_v1.7, a genetically validated pain target. To this purpose, the effects of crotalpine were evaluated on the tetrodotoxin (TTX)-sensitive Na current of dorsal root ganglion (DRG) neurons of adult mice, using the whole-cell patch-clamp configuration, and on the cell viability, using the propidium iodide fluorescence and trypan blue assays.



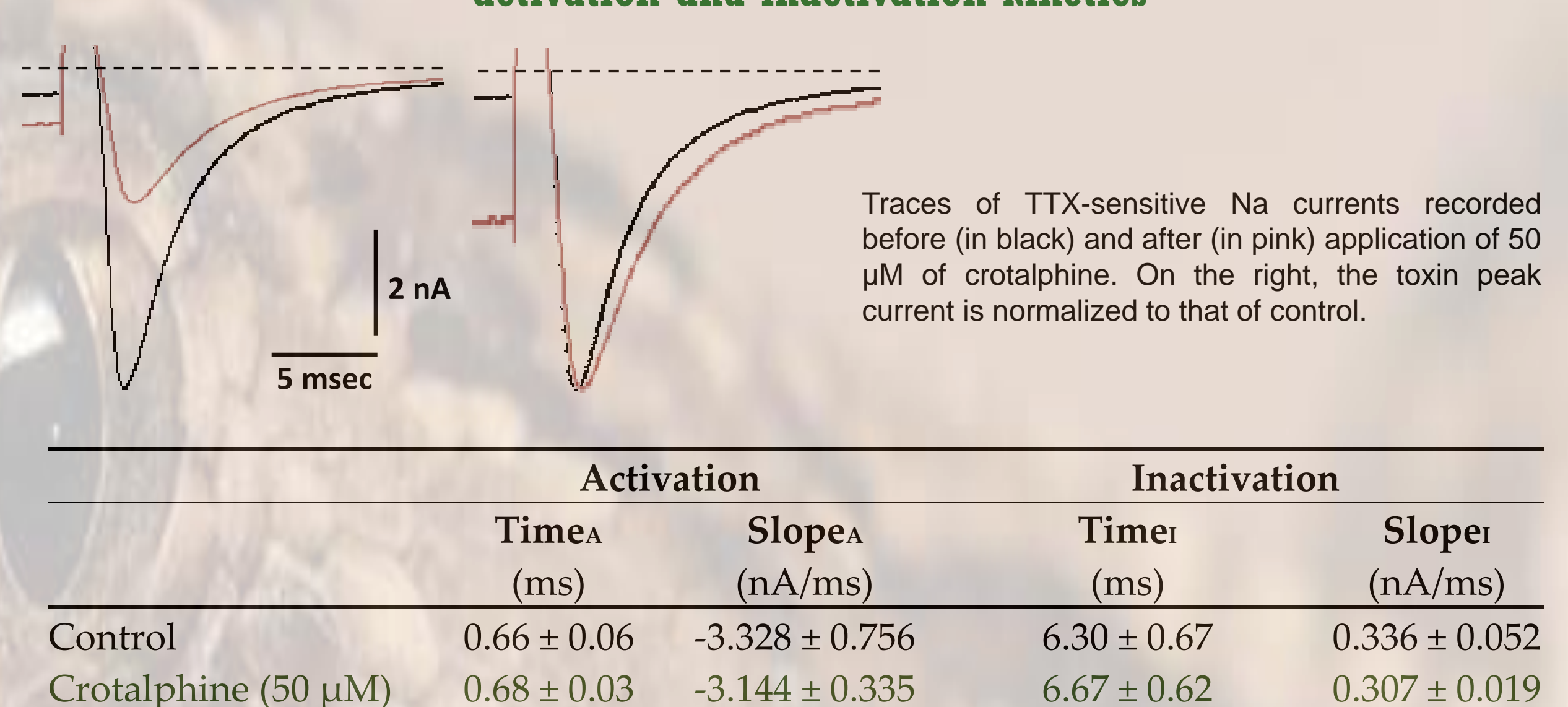
RESULTS. Crotalpine inhibited the TTX-sensitive Na current of mouse DGR neurons with a micromolar range affinity ($IC_{50} = 18.70 \mu M$) (1). In addition, the toxin had no effect on activation or inactivation kinetics (2) and voltage-dependence of the TTX-sensitive Na current (3). Thus, crotalpine appears to block the channel pore without affecting its biophysical properties. The leakage current amplitude was significantly enhanced with increasing blockade of TTX-sensitive Na current (4), likely due to a decreased DRG neuron viability (5).

1. A crotalpine concentration of 18.70 μM is necessary to inhibit 50% of Na current



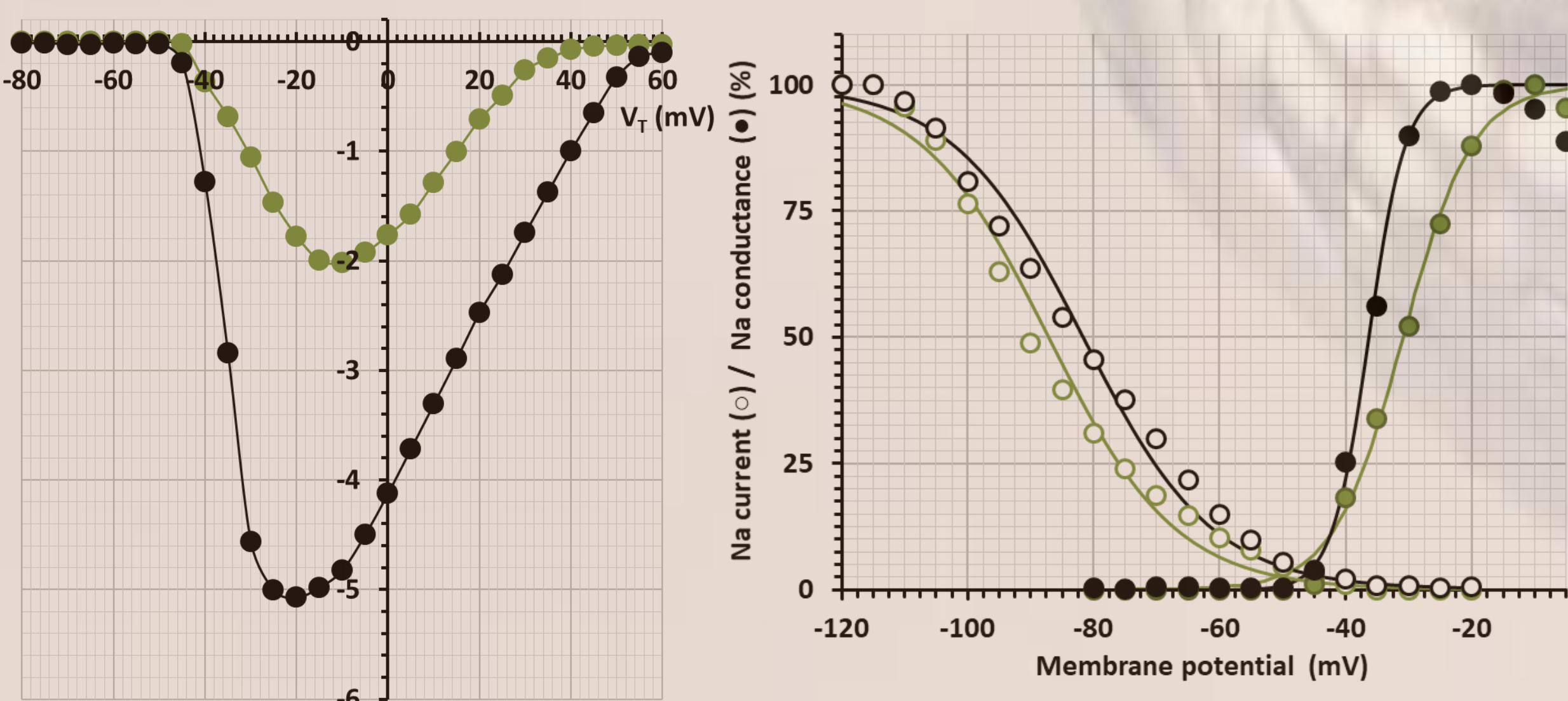
Concentration-response curve of crotalpine steady-state effects on TTX-sensitive Na current. Each value is expressed as percentage of that determined before toxin application.

2. The blocking effect occurs without any marked change in the current activation and inactivation kinetics



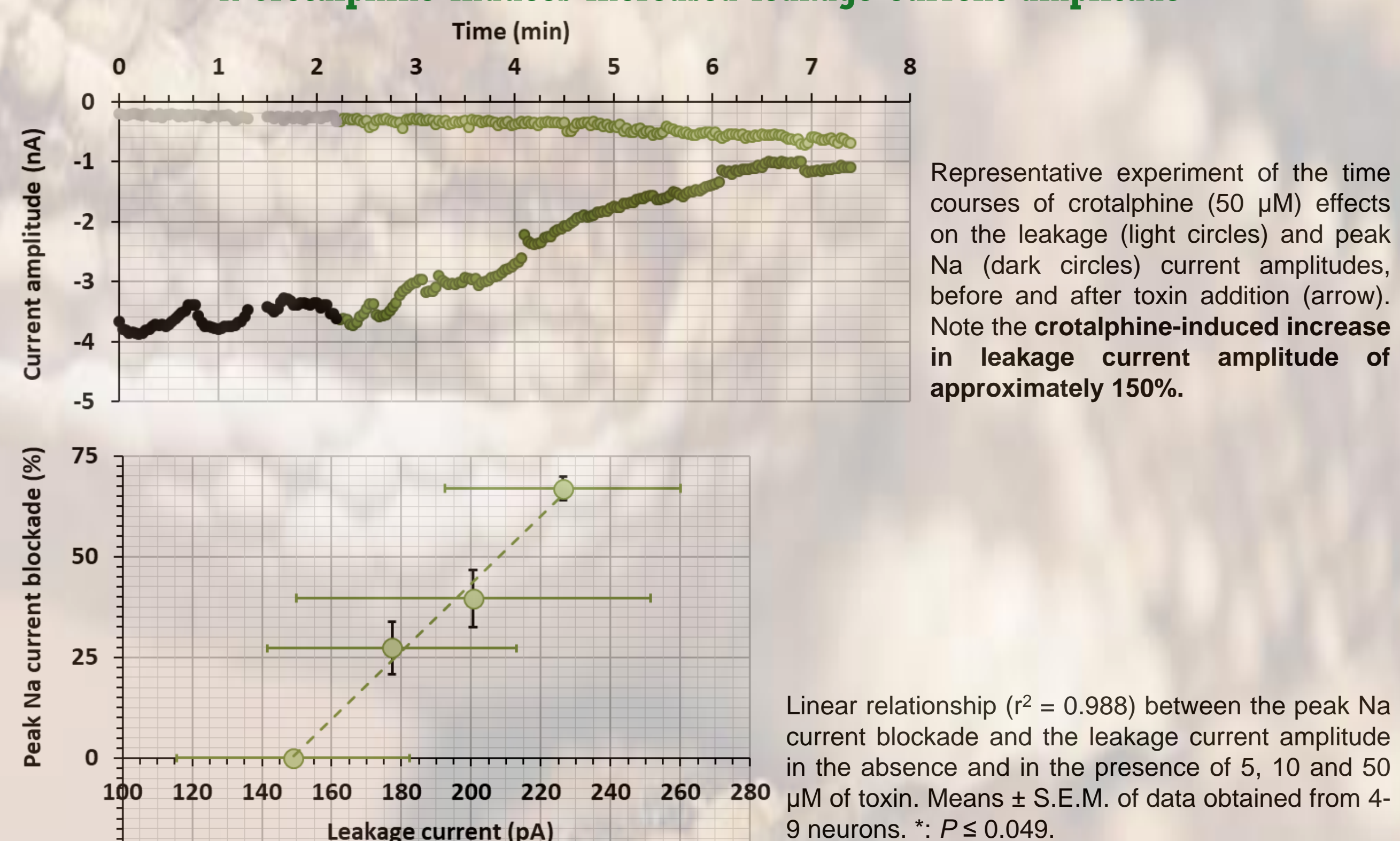
Kinetic parameters of activation (Time_A and Slope_A) and inactivation (Time_I and Slope_I) of TTX-sensitive Na current recorded without and with 50 μM of crotalpine (means ± S.E.M. of 4 neurons).

3. The blocking effect occurs without any marked change in the current activation and inactivation voltage-dependencies



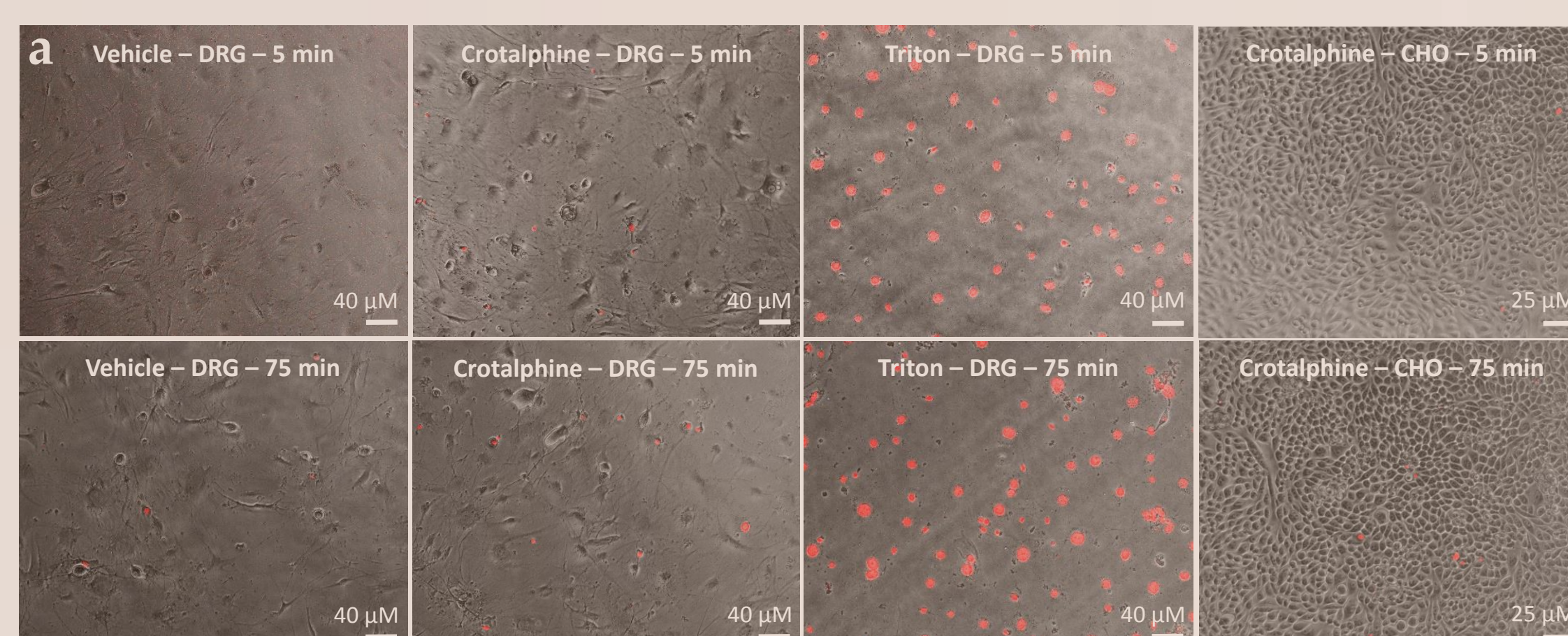
Current-voltage relationships (left panel), and steady-state inactivation- (open circles) and conductance- (filled circles) voltage relationships (right panel), before (black circles) and after (green circles) exposure of neurons to 50 μM of crotalpine. Each value is expressed as percentage of maximal peak amplitude of current at strongly negative pre-pulse voltages or maximal conductance calculated at strongly positive test-pulse voltages.

4. Crotalpine induces increased leakage current amplitude

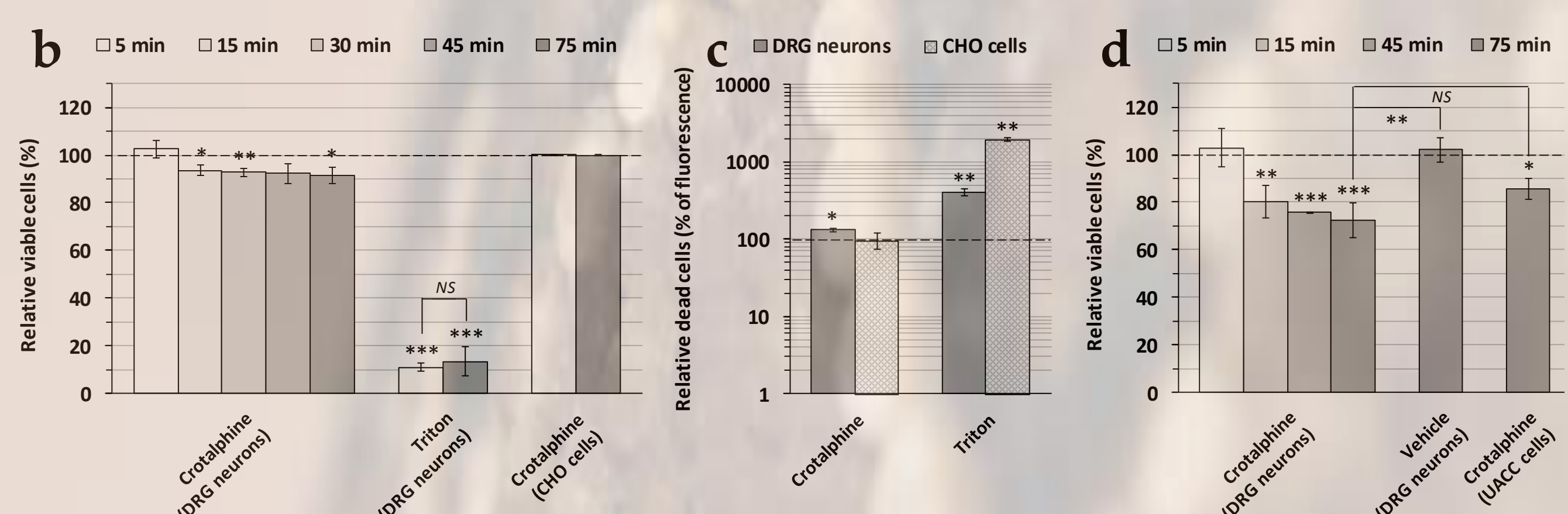


Linear relationship ($r^2 = 0.988$) between the peak Na current blockade and the leakage current amplitude in the absence and in the presence of 5, 10 and 50 μM of toxin. Means ± S.E.M. of data obtained from 4-9 neurons. *: $P \leq 0.049$.

5. Crotalpine induces decreased DRG neuron viability



A decrease of DRG neuron, but not of CHO cell, viability is detected in the presence of crotalpine (50 μM), using the propidium iodide fluorescence assay (a,b,c). Similarly, a decrease of DRG neuron and UACC-257 cell viability is detected in the presence of crotalpine (50 μM), using the trypan blue assay (d). Note that the toxin induces a maximal decrease of 28% of cell viability. Means ± S.E.M. of data from 3-12 cell cultures. *: $P \leq 0.05$, **: $P \leq 0.005$ and ***: $P \leq 0.0005$ (b,c,d).



CONCLUSION. Taken together, these results point out, for the first time, the effectiveness of crotalpine to act on the Na_v1.7 channel subtype, directly and/or indirectly by affecting cell membrane integrity. This mechanism of action could contribute to the peptide analgesic properties.

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