

Effects of the anti-migraine drug Sumatriptan on larva zebrafish seizure-like behaviour



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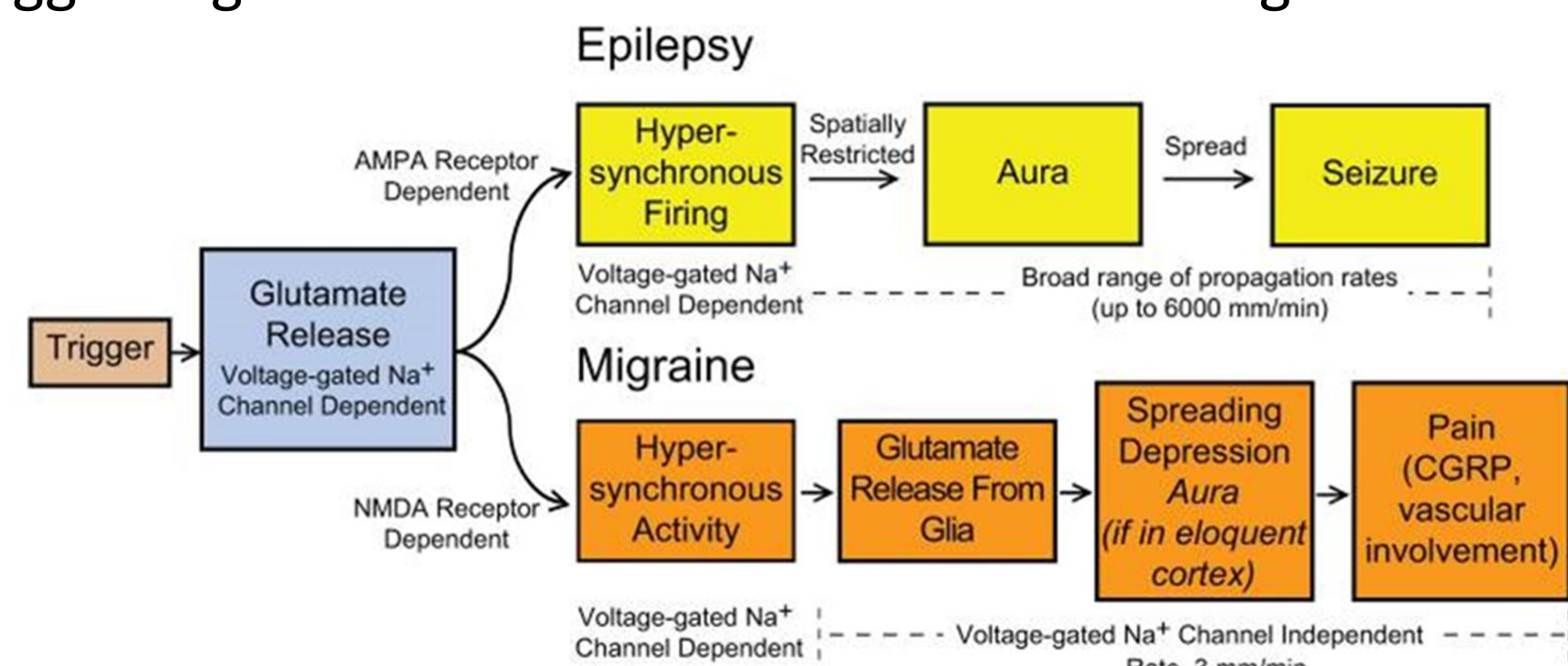
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Abstract

- Comorbidity of epilepsy with migraine and the prophylactic effect of anti-epileptic drugs (AEDs) on migraine led to the hypothesis that a sub-population of these diseases might share pathophysiology.
- We investigated the effects of sumatriptan (SUM), a 5-HT_{1B/1D} agonist anti-migraine drug, on seizure-like behaviour zebrafish. Larvae were incubated in diazepam (DZP), SUM, or embryo medium before adding PTZ and video recording.
- Seizure-like behaviour progressed from thigmotaxis and rapid whirlpool-like movement to stiffening and loss of posture. Temporal patterns of cyclic high and low velocity episodes were evident during ictal-like and post-ictal-like periods, respectively.
- Distance travelled, mean velocity and percentage of time in motion were significantly reduced by SUM and DZP, suggesting anti-seizure activity.
- Using zebrafish as a model, we found that seizure-suppressive effects of SUM were comparable to DZP, making it a potential candidate for a novel AED.

Introduction

- Epilepsy affects at least 70 million people worldwide. After an epileptic seizure, some patients experience post-ictal headache with migraine-like symptoms.
- Hyperexcitability of neuronal networks is a shared feature of both migraines and epileptic seizures.
- Some anti-epileptic drugs (AEDs) have prophylactic effect on migraine. Post-ictal headache responds to anti-migraine drugs such as sumatriptan, suggesting some common mechanisms with migraine



Comparisons between epilepsy and migraine pathophysiology (Rogawski, 2012)

Objectives

- We investigated the effects of sumatriptan (SUM) on zebrafish pentylenetetrazole (PTZ)-induced seizure-like behaviour.
- SUM is a 5-HT_{1B/1D} agonist that causes cerebral vasoconstriction and inhibits release of pro-inflammatory factors.
- Our hypothesis was that SUM would suppress seizure-like behaviour with effects comparable to diazepam (DZP).

Materials and methods

Animals. After mating 2 females and 1 male, eggs were kept in embryo medium (E3) at 28 °C.

PTZ range-finding test: 7 dpf larvae were exposed to 2mM to 26 mM pentylenetetrazole and recorded. Seizure stages were scored and compared to negative control.

Maximum-tolerated concentration (MTC) test for Diazepam and Sumatriptan: 6 dpf larvae were incubated in serial dilutions from the maximum dissolvable concentration for 18 hrs. They were checked for startle response and toxicity signs. MTC was the maximum concentration at which no signs of toxicity were observed in all of larvae.

PTZ-induced seizure-like behaviour tests: 6 dpf were incubated in 6 µM DZP, 1.8 mM SUM, or embryo medium for 18 hrs in a 96-well plate. PTZ was then added to a final concentration of 14 mM. A video was recorded and larvae behaviour tracked and quantified using open-source MATLAB applications [LSRtrack and LSRanalyze: Zhou et al., (2014). Nat. Prot.].

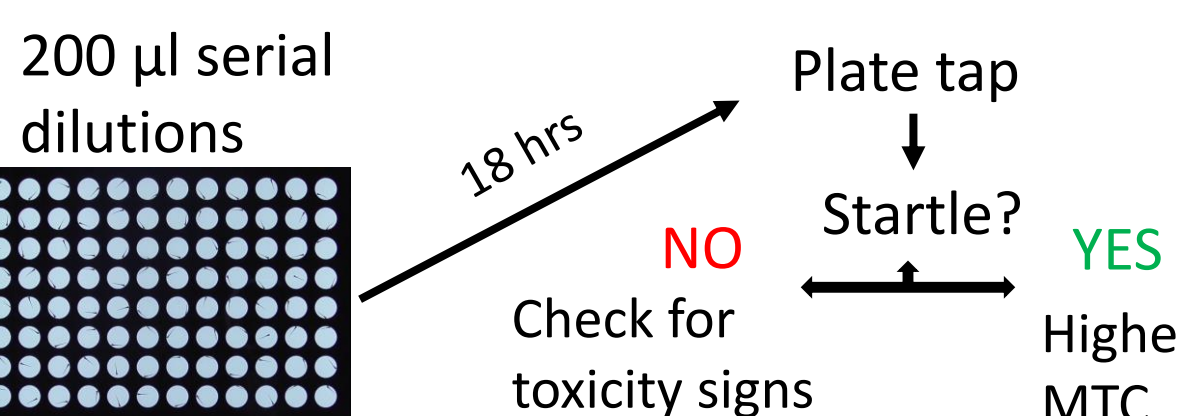
Sedative effects test for DZP and SUM: 6 dpf were incubated in 6 µM DZP, 1.8 mM SUM, or embryo medium for 18 hrs in a 96-well plate and their behaviour recorded. Tracking and quantification was done using open-source MATLAB applications.

PTZ range-finding test

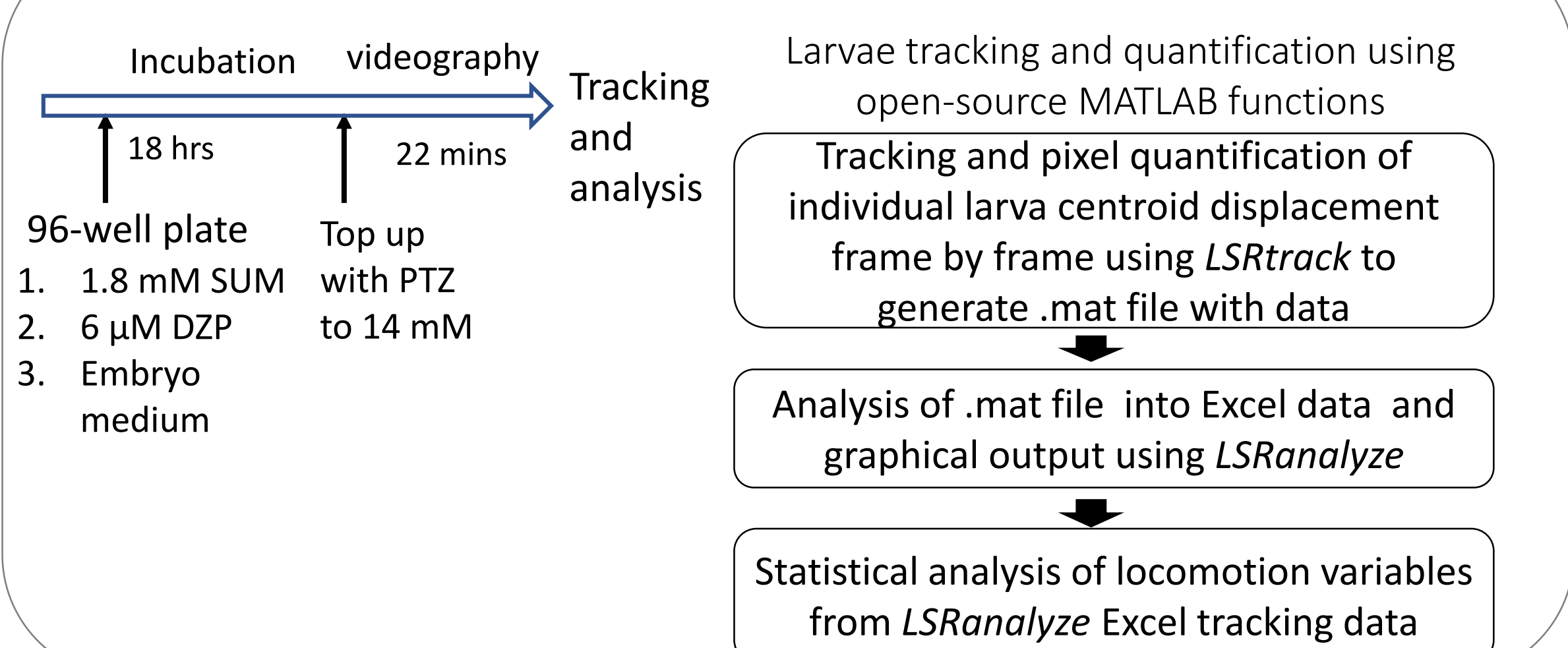
2, 5, 8, 11, 14, mM PTZ

- 7 dpf larvae
- Seizure scoring
- Comparison with control

Maximum Tolerated Concentration test for DZP and SUM



PTZ-induced seizure behaviour tests



Results

Larvae seizure behaviour

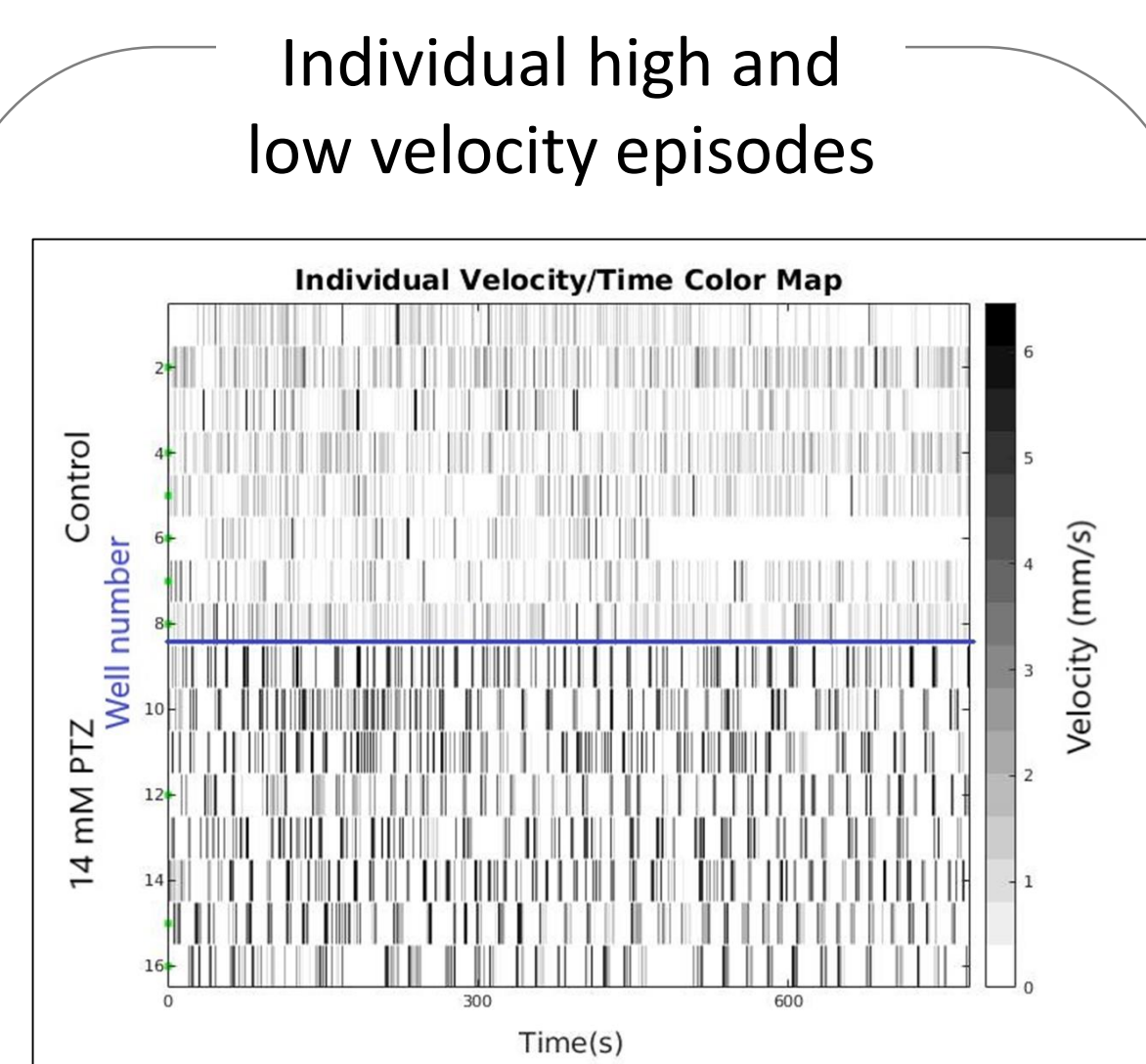
Larvae seizure progression (Baraban et al., 2005)

Stage 1: Increased swimming activity

Stage 2: Rapid whirlpool-like circular swimming

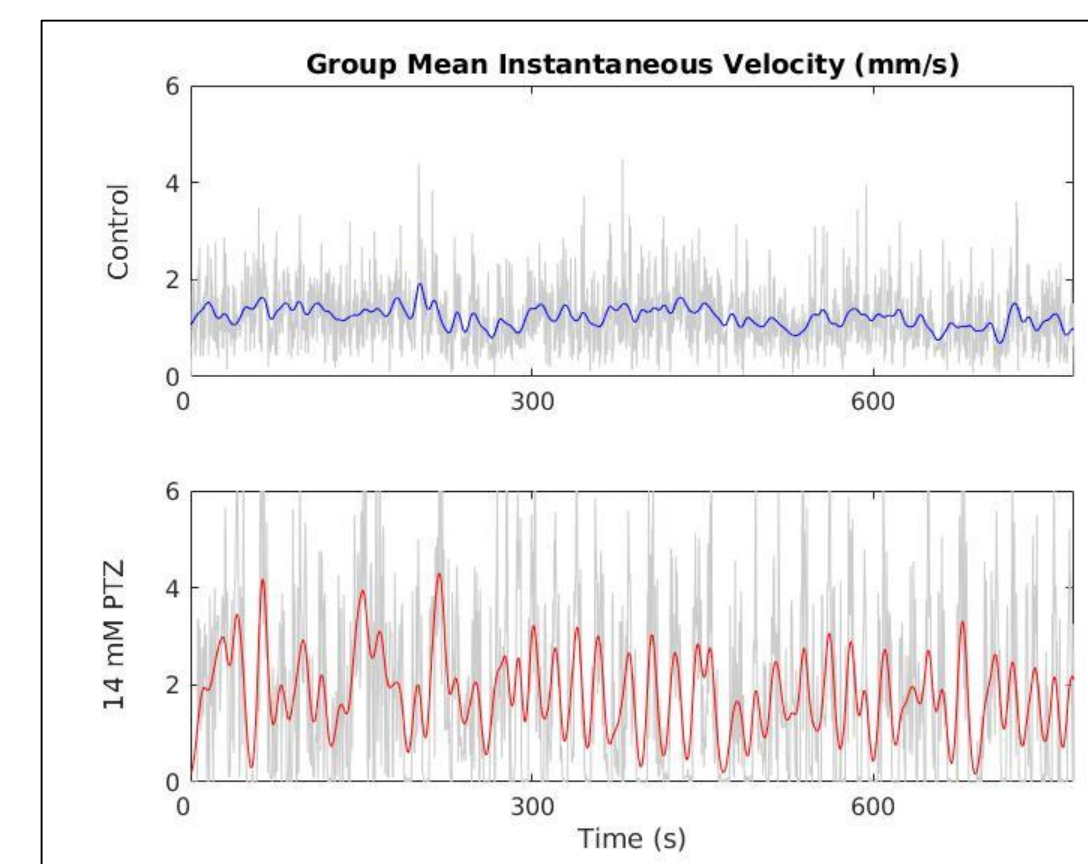
Stage 3: Brief clonus-like convulsions leading to a loss of posture

14 mM pentylenetetrazole-treated larvae showed seizure progression with strong contrast in locomotor activity against untreated group



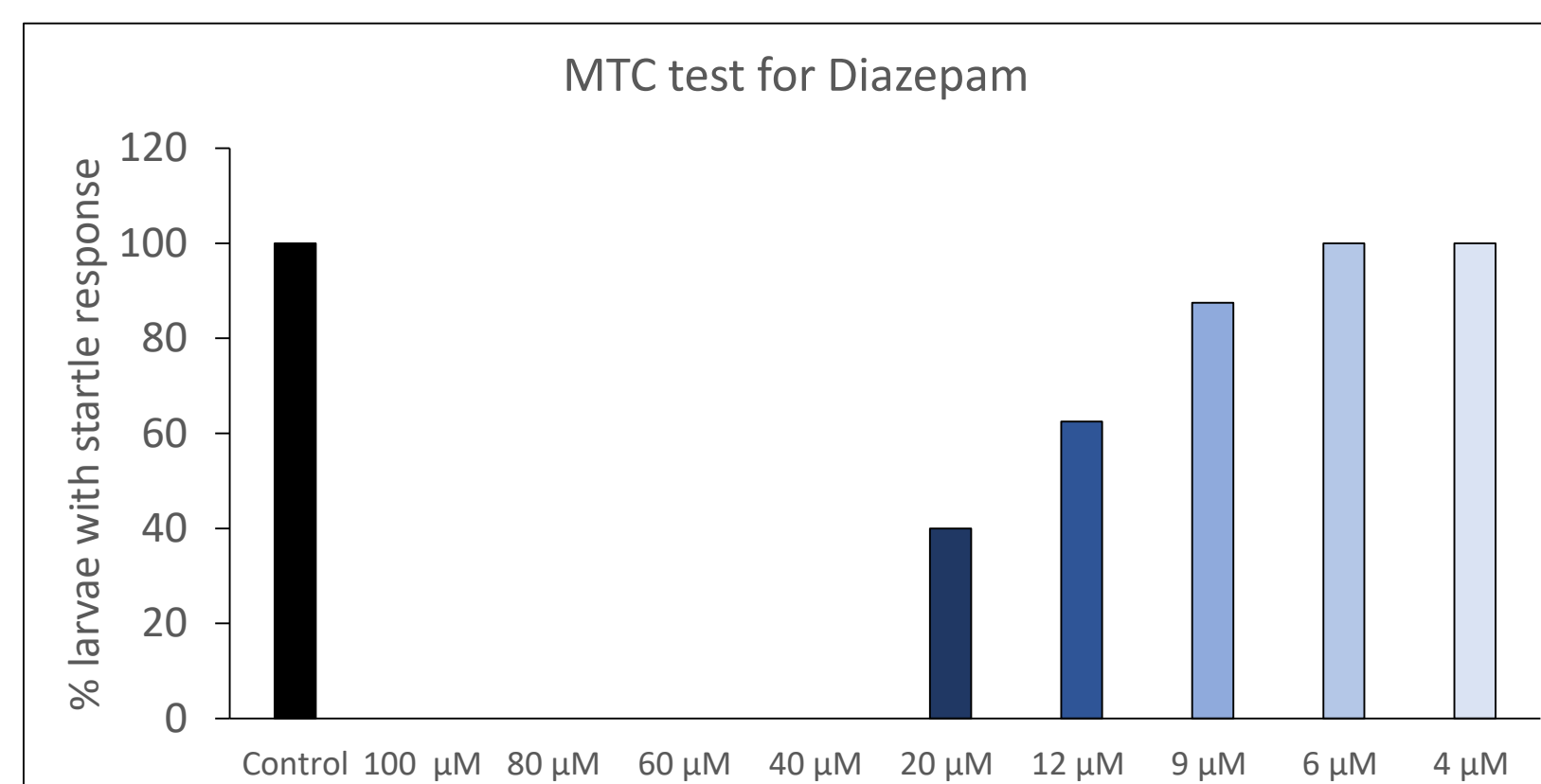
- High velocity ↔ ictal-like phase
- Low velocity ↔ post ictal-like phase

Group motor activity

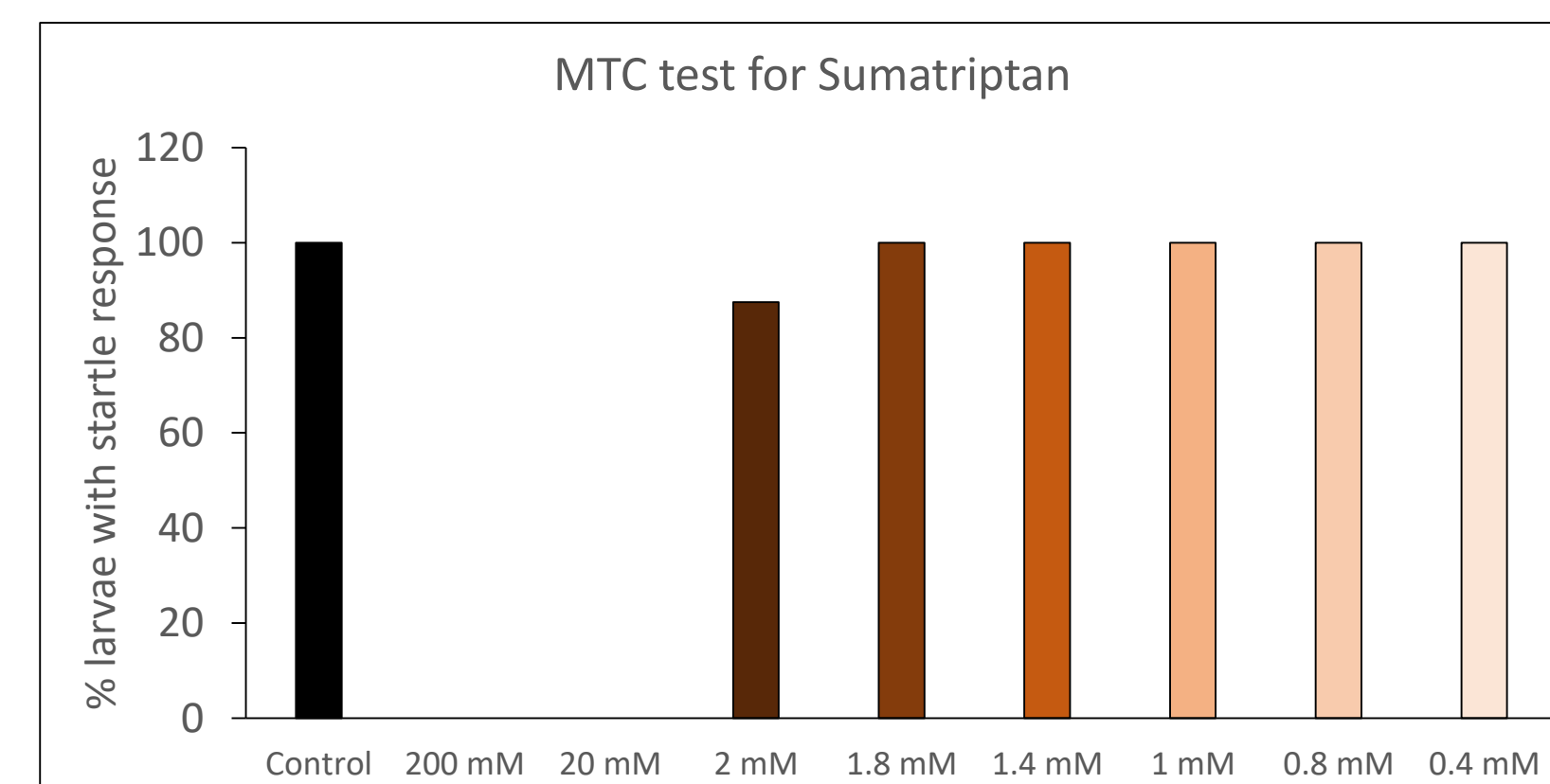


Cyclic group displacement at time points caused by pentylenetetrazole treatment

Maximum Tolerated Concentrations

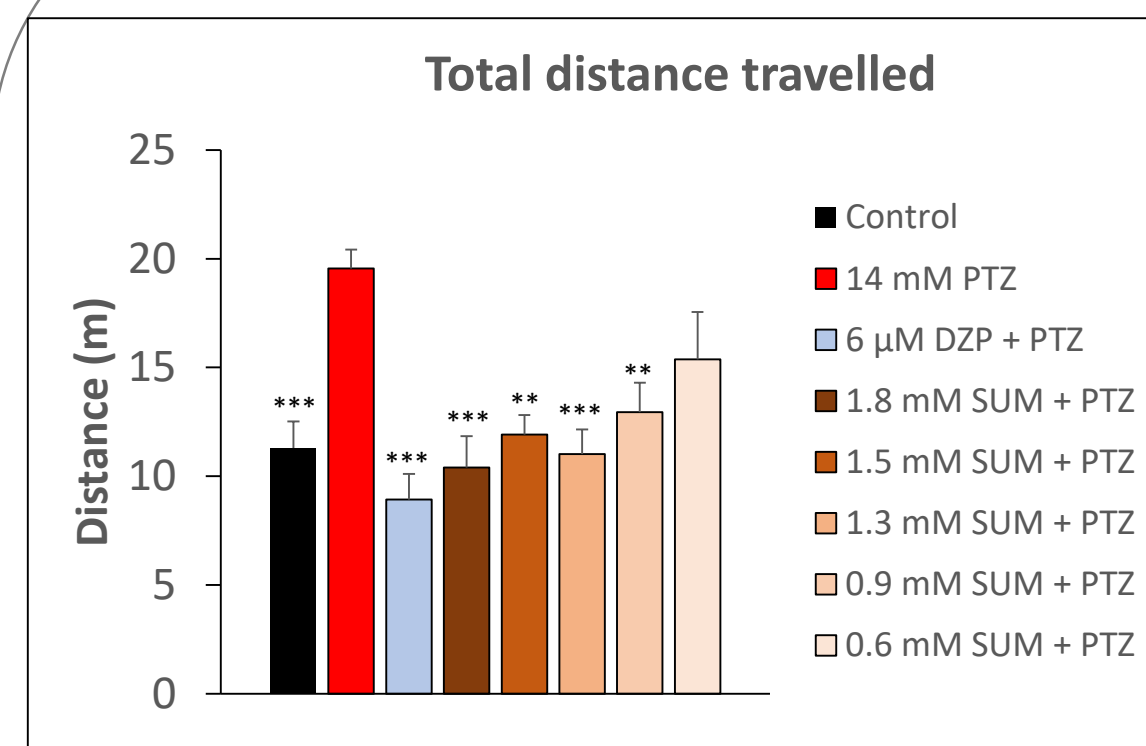


- Highest concentration with no signs of toxicity was 6 µM
- 6 µM was then used to check for anti-seizure effects

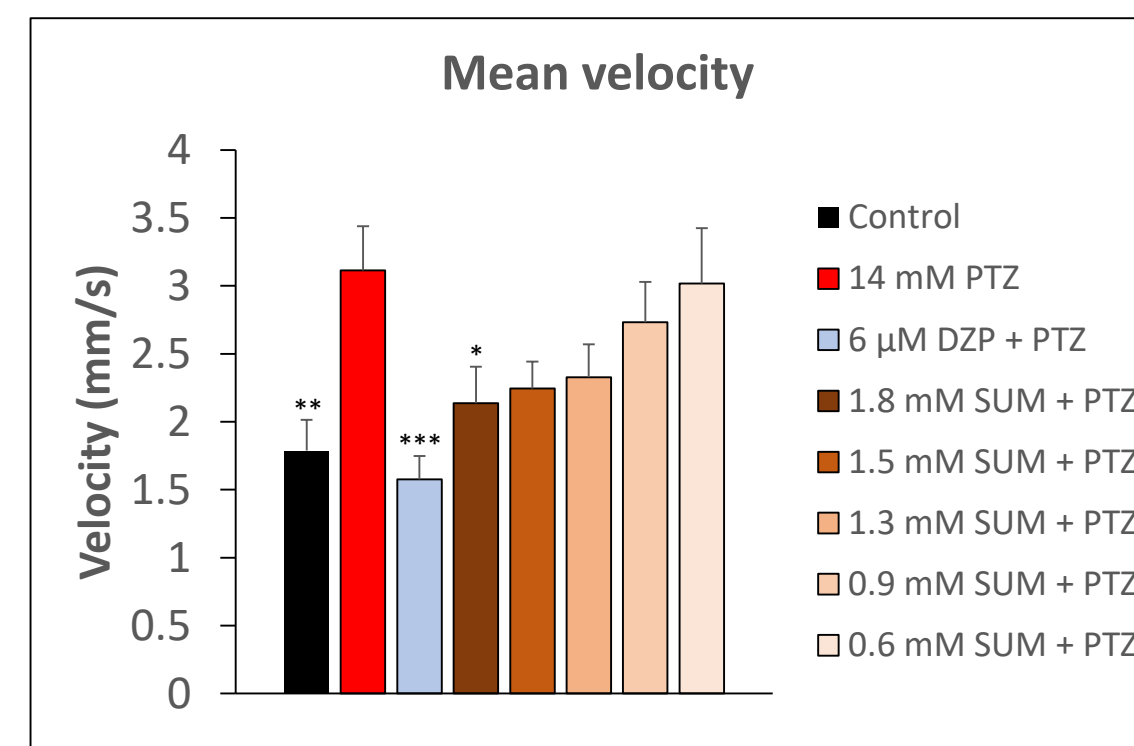


- Highest concentration with no signs of toxicity was 1.8 mM
- Serial concentrations from 1.8 mM were then screened for effects on seizures

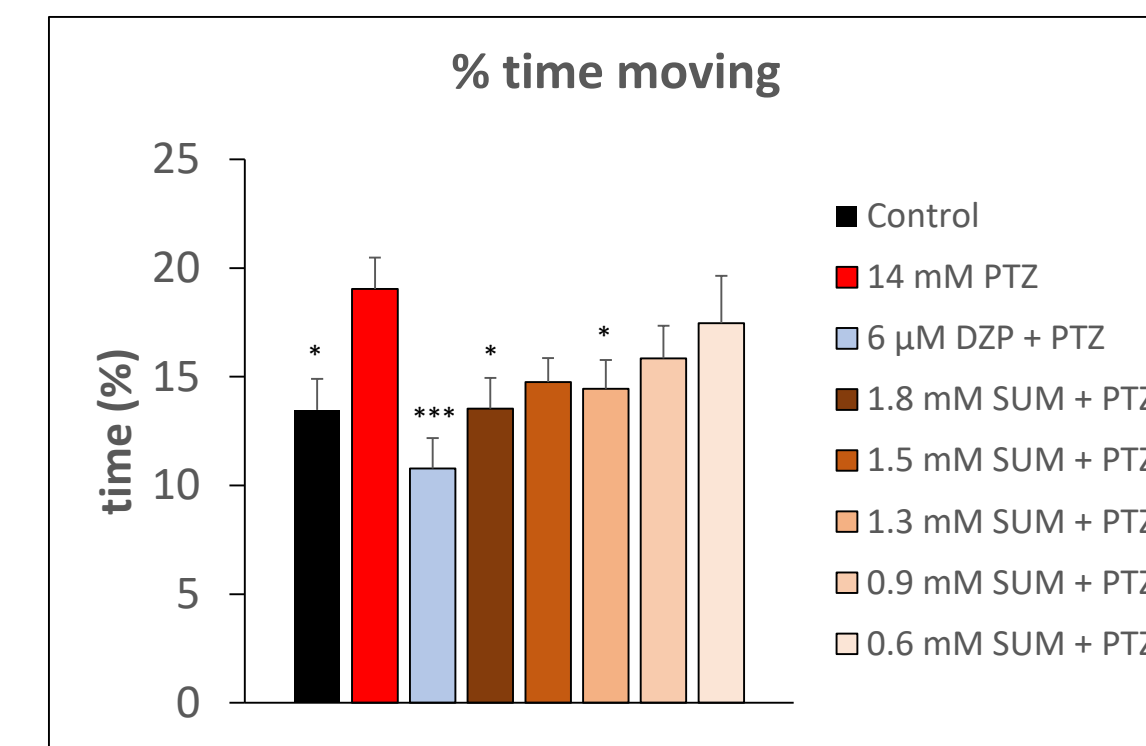
Sumatriptan showed dose-dependent anti-seizure effects



- Sumatriptan, down to 0.9 mM, significantly decreased the total distance travelled by larvae compared to the PTZ-only group.
- These effects were comparable to DZP.



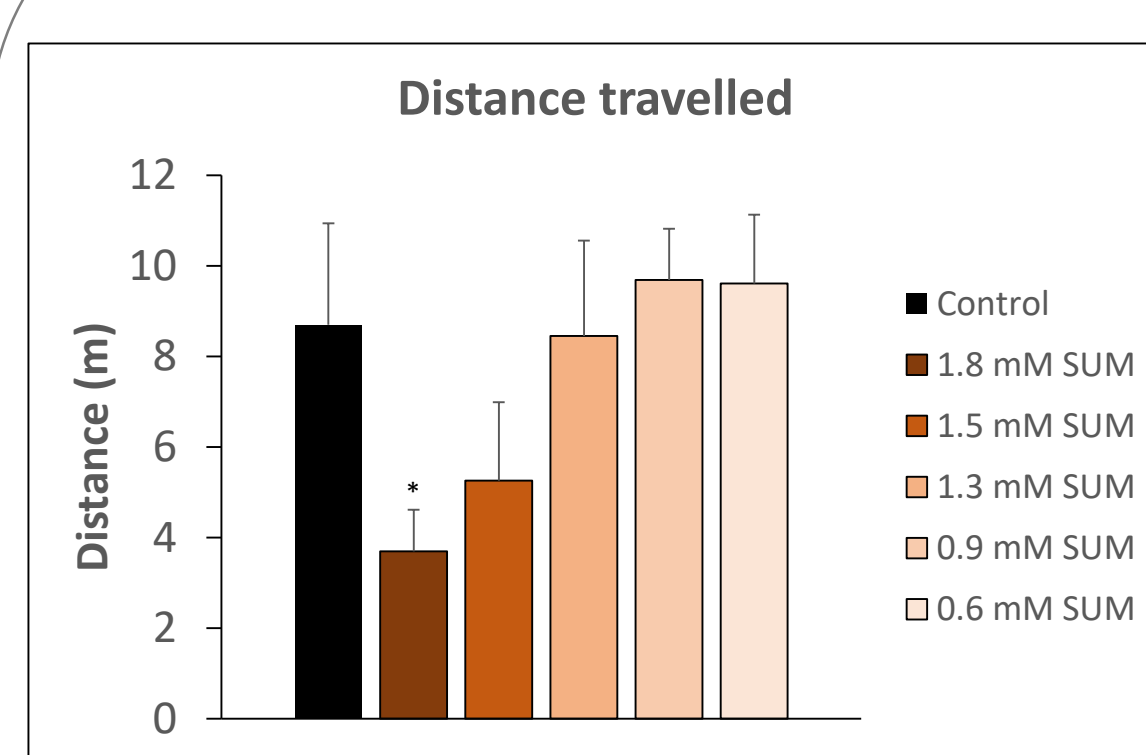
- Only the highest concentration of 1.8 mM significantly reduced the mean velocity of larvae



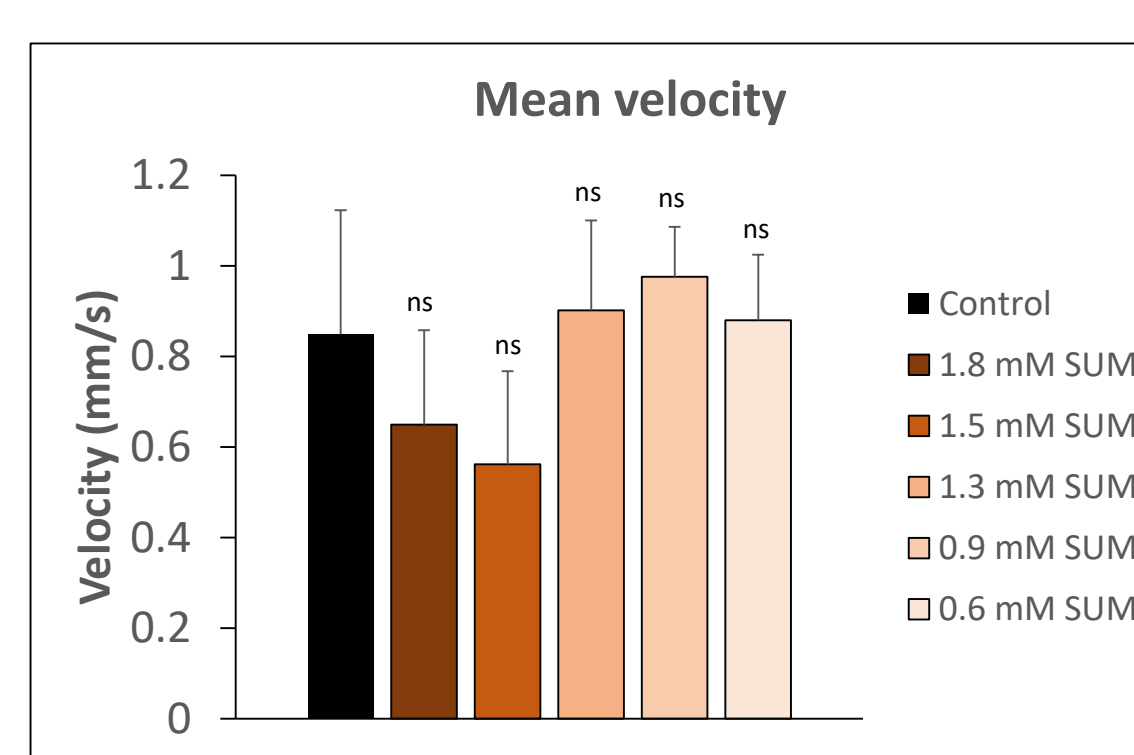
- The PTZ-only group predictably spent a higher percentage of time in motion
- Higher concentrations of sumatriptan lowered the % time spent moving

Kruskal-Wallis with Dunn Post-Hoc test and Bonferroni correction. All contrasts are made against the 14 mM PTZ group as reference
* p < 0.05, ** p < 0.01*** p < 0.001, N = 8

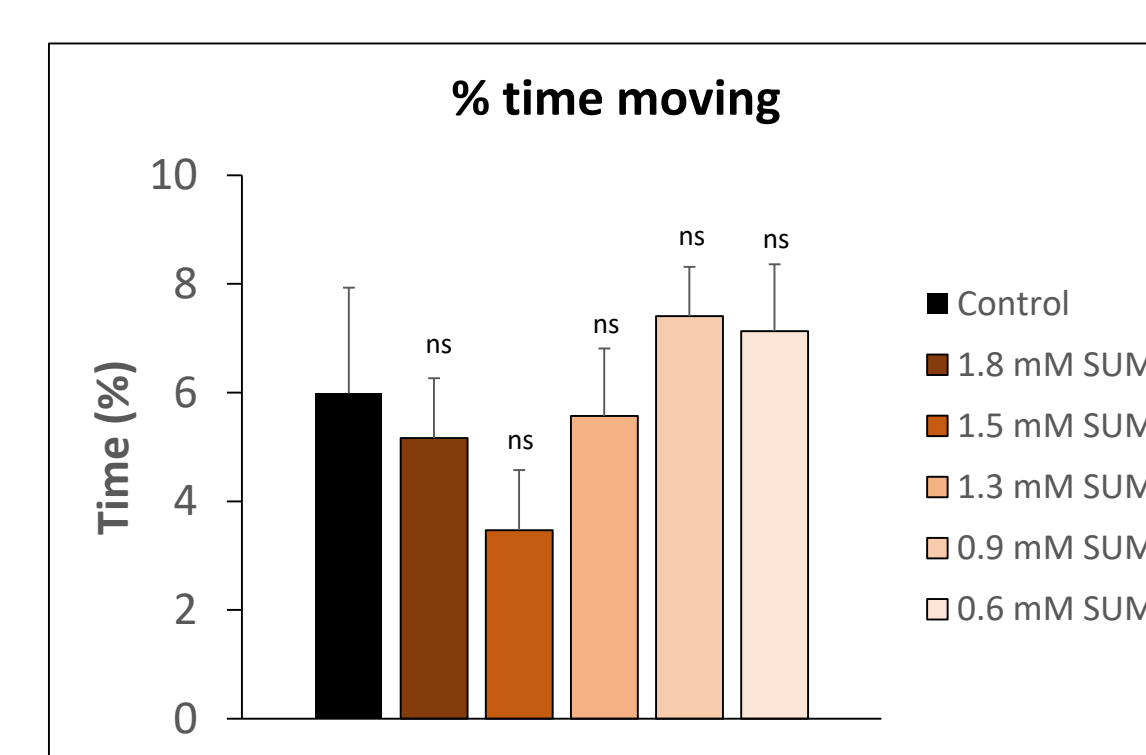
Sumatriptan caused mild sedation in larvae after an 18-hr incubation



- Compared to the control group, 1.8 mM Sumatriptan significantly reduced distance travelled by larvae
- Sumatriptan caused mild sedation



- The concentrations of Sumatriptan used did not affect the mean velocity of larvae



- % of time spent moving was not altered by Sumatriptan treatment

Kruskal-Wallis with Dunn Post-Hoc test and Bonferroni correction. All contrasts are made against the control group as reference
ns Not significant, * p < 0.05,

Conclusions

- SUM showed anti-seizure effects comparable to those of diazepam
- Mild sedative effects of sumatriptan may contribute to anti-seizure effects.

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The authors declare no conflicts of interest

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