



3Z Pharmaceuticals
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PENTYLENETETRAZOL Exposure Model of Epilepsy in Larval Zebrafish

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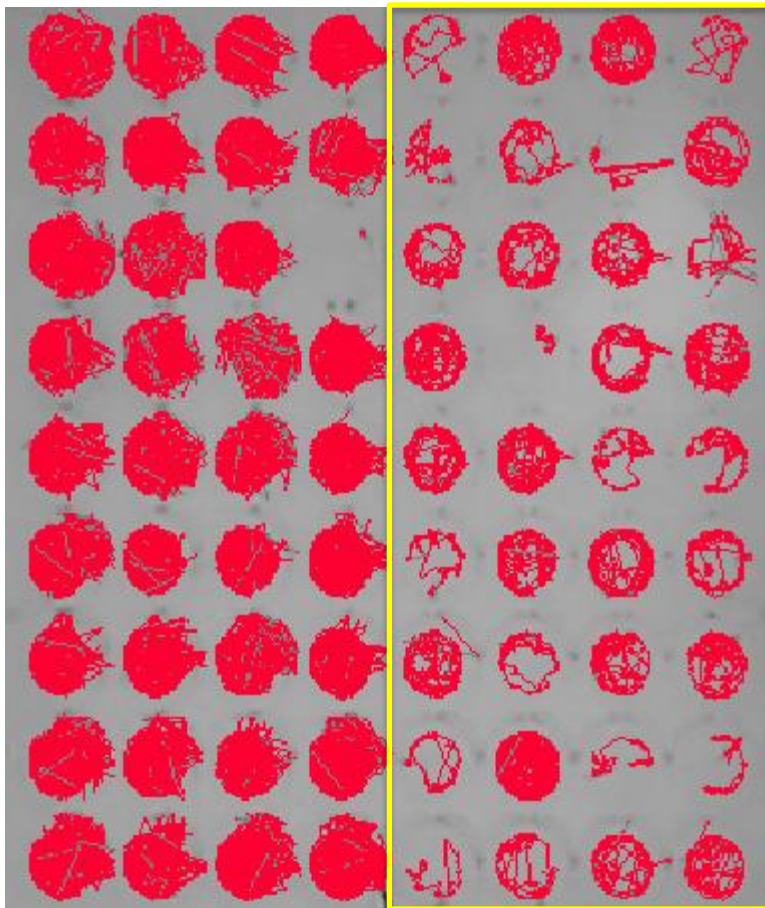


Following PTZ exposure zebrafish reliably exhibit behavioral and electrographic hallmarks of status epilepticus

- Exposure of PTZ reliably induces seizures in larval and adult zebrafish
- At 2.5 mM exposure zebrafish exhibit increased movement and low frequency of seizures
- At 5.0 mM exposure zebrafish exhibit increased movement and high frequency of seizures
- At 15.0 mM exposure zebrafish exhibit high frequency of seizures and catatonia
- PTZ induced seizures can be readily reversed using common anticonvulsants such as retigabine or diazepam

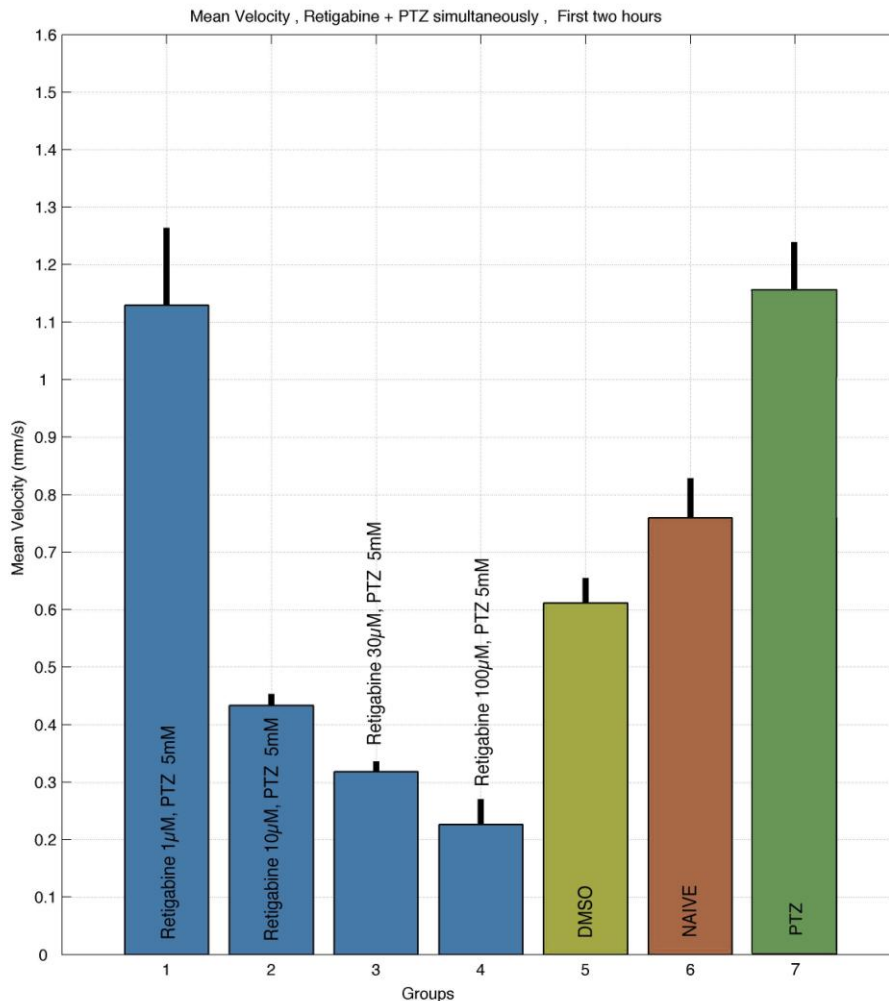
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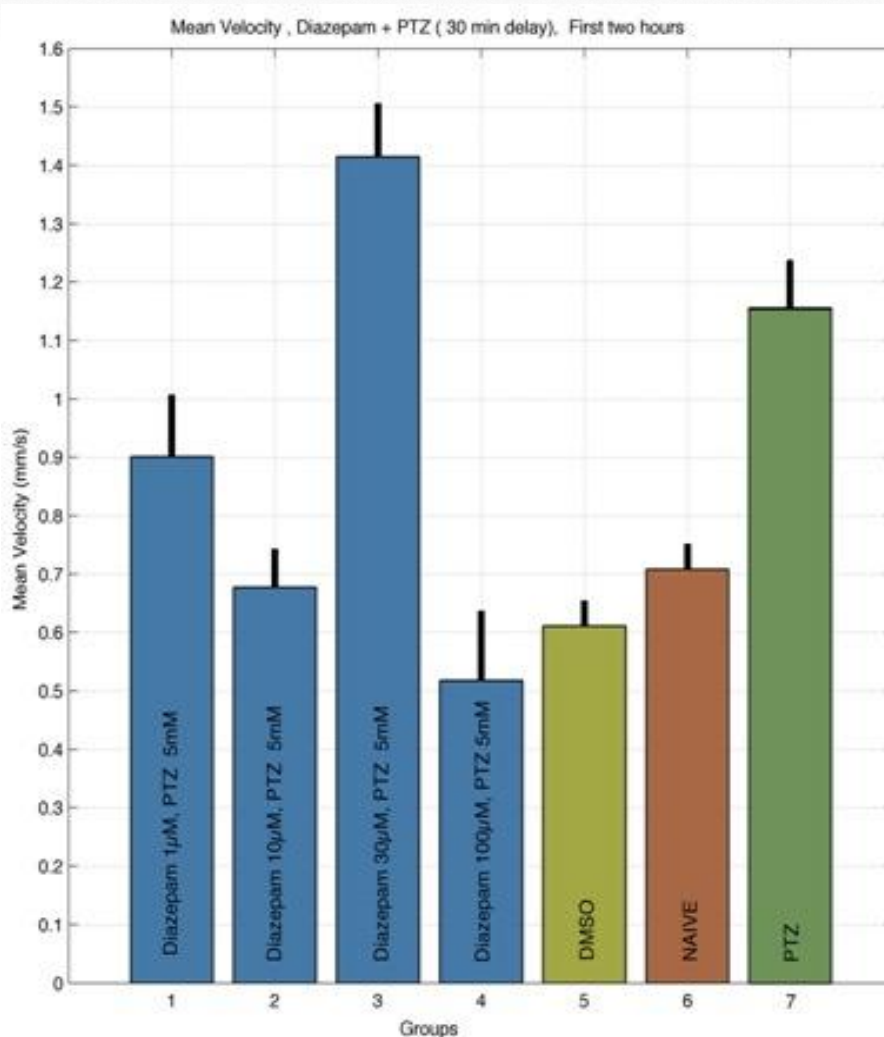
Behavioral traces from two groups of larval zebrafish. *Four columns on the left received 5 mM PTZ. The four columns on the right (enclosed in a yellow square) received 5 mM PTZ and 100 μM diazepam. Both groups received the compounds at the same time and were recorded for one hour. Red lines represent the 60 min trajectories of each larvae.*

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Quantification of average velocity after application of PTZ and retigabine in larval zebrafish. The columns depict average velocity following application of 5 mM PTZ and simultaneous application of 5mM PTZ with 1,10,30 and 100 µM retigabine. Also depicted are naïve and DMSO larvae ($n=16$). PTZ group differs significantly from all other groups ($p<0.05$) except retigabine 1µM-PTZ 5mM.

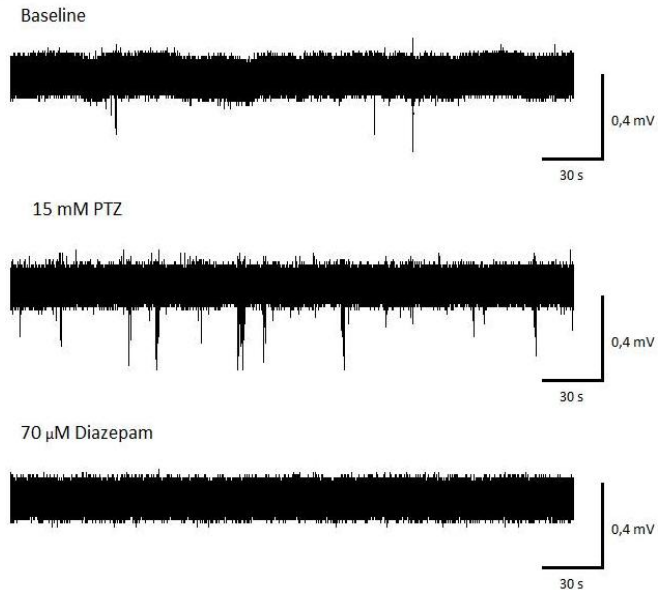
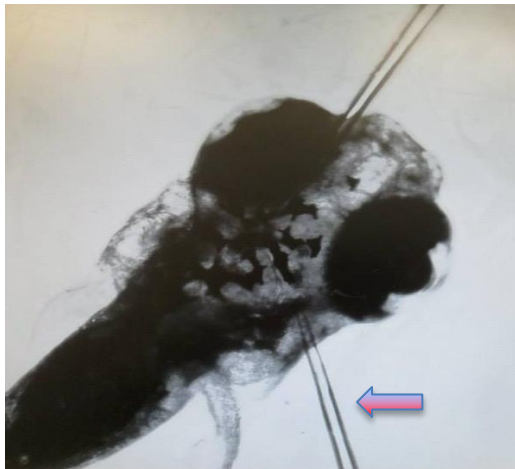
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Quantification of average velocity after application of PTZ and diazepam in larval zebrafish. The columns depict average velocity following application of 5 mM PTZ and simultaneous application of 5mM PTZ with 1,10,30 and 100 µM diazepam. Also depicted are naïve and DMSO larvae (n=16). PTZ group differs significantly from all other groups ($p < 0.05$).

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Electrophysiological verification of the stimulation and recovery protocol. The picture on the left depicts agarose-embedded 7dpf larva during recording. Red arrow indicates the recording electrode (1.5 mOhm resistance, current-clamp) placed in the tectum opticum. Recording traces to the right depict (from above): baseline recording, recording following application of PTZ and recording following application of diazepam (traces recorded less than 5 min apart). Following application of PTZ interictal spikes are clearly evident but are absent following application of diazepam.

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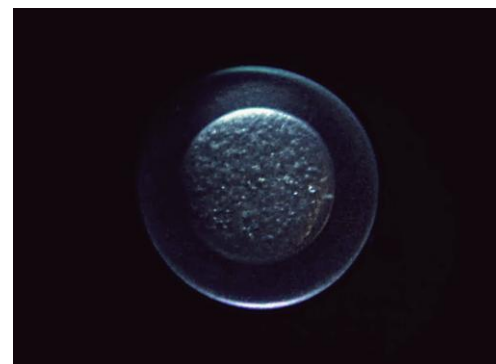
Overview of screening protocol

1. Screens for novel anticonvulsants (or drug reprofiling screens) are run on 7-dpf larvae in a 96-wellplate format in which up to 2304 larvae are run in parallel.
2. Following PTZ application test compounds are added to microwells to generate a behavioral read-out.
3. Hits (compounds that significantly reduce seizures) are recorded (without PTZ) for 24 hrs to evaluate toxicology (LC50) and off-target effects.
4. Anticonvulsant effects of hits from steps 1 and 2 are verified using electrophysiology.

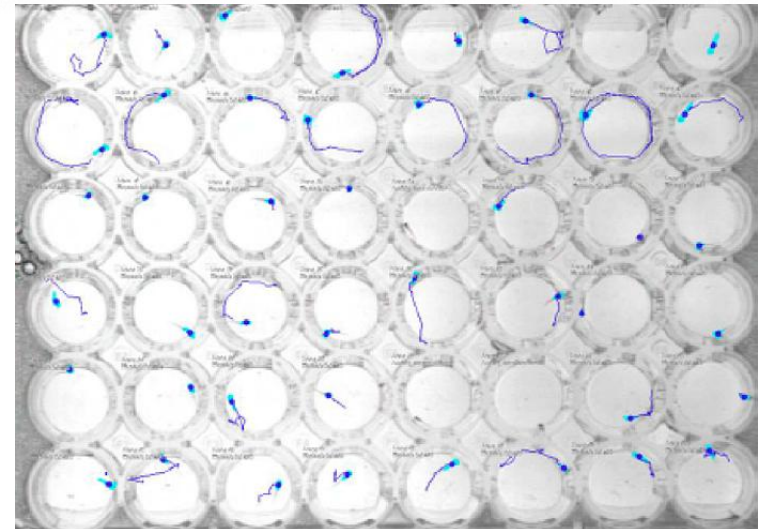
ANTICONVULSANT SCREENING SERVICES



- Approximately 100 compounds tested per week, servicing screening projects of up to 10,000 compounds; expected throughput to reach 600 compounds per week.
- Behaviorally-based screen yields reliable parameters of potential novel anticonvulsants.
- Customers receive a concise overview of the effects of each compound enabled by a user-friendly interface.



CNS DISCOVERY SERVICES



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