Larval Zebrafish as a Model System for Learning and Memory

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Introduction

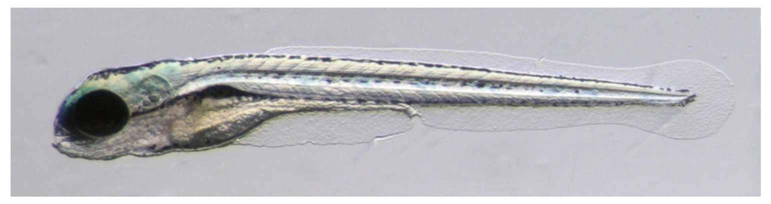

Figure 1. A larval zebrafish at 5 days post fertilization (DPF) [7]

Zebrafish (*Danio rerio*) provide a reductionist model for studies of learning and memory. [2]

Why study zebrafish? [1,3]

- ✓ Genetic homology to humans
- ✓ Optical transparency
- ✓ Simple neural circuits
- ✓ Mapped genome
- ✓ Amenable to genetic alteration
- ✓ High-throughput screening applications

Past research has shown that zebrafish exhibit sensitization, a form of non-associative learning characterized by an enhanced response to all stimuli.[4] Furthermore, allyl thiocyanate (mustard oil) was shown to increase locomotor activity in larval zebrafish, suggesting that mustard oil may be used to induce sensitization.[5]

In our study, we asked whether larval zebrafish could be used to study nonassociative learning by using allyl isothiocyanate (mustard oil) as an aversive stimulus.

Our main research questions included:

1) What is the effective dose of mustard oil necessary to induce a significant behavioral response?
2) How does development relate to mustard oil responsiveness?
3) Can mustard oil be used to induce sensitization of the C-start reflex to an auditory stimulus?

Methods and Materials

To assess learning, two behaviors were recorded and measured via high speed camera:

1) Tail Flick Activity
- Fish 5-6 days post-fertilization (DPF)
- Restrained using low-melting point agarose
- Mustard oil or E3 (embryo water) is administered via pipette
- Head and tail portion cut free for visualization
- Recorded at 120 frames/sec

2) Startle Response (C-start)
- Fish 5-6 DPF
- Auditory stimulus used to induce startle (500 Hz 0.002 s)
- Individual fish freely swimming in 24 well plate
- Recorded at 1000 frames/sec

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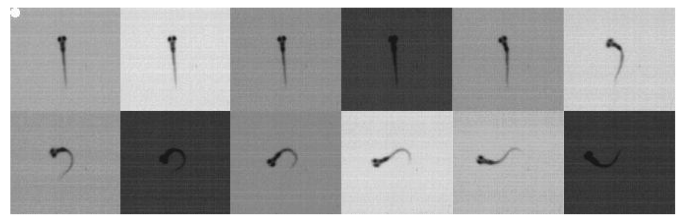

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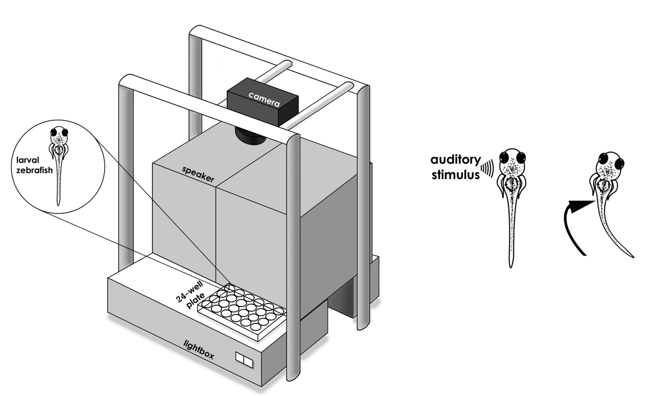
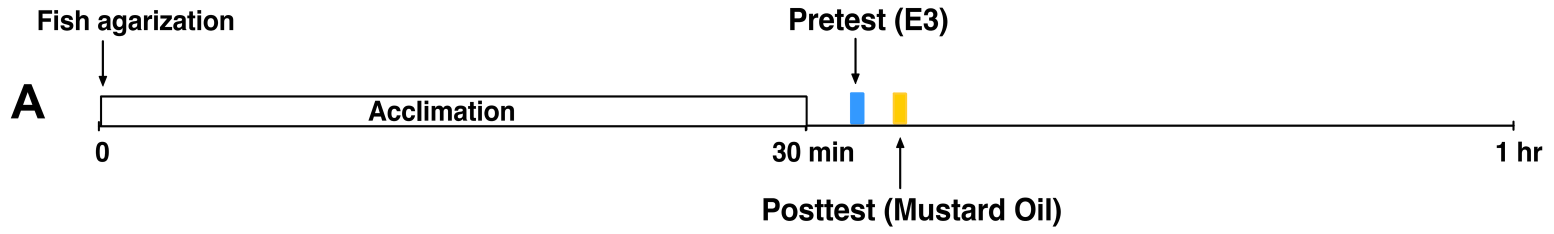


Figure 2. A. The recorded C-start, a characteristic fear response where the zebrafish bends into a C-shape, is shown frame by frame.
B. The experimental set-up for recording C-start responses is depicted.[6]

Dose-Response Curve

A



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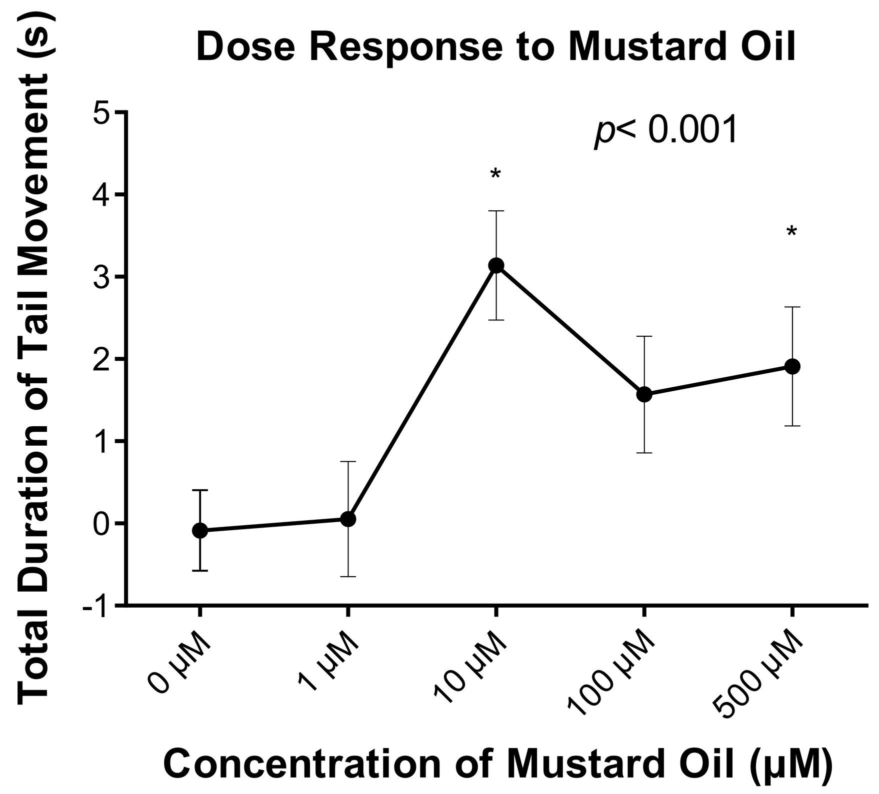
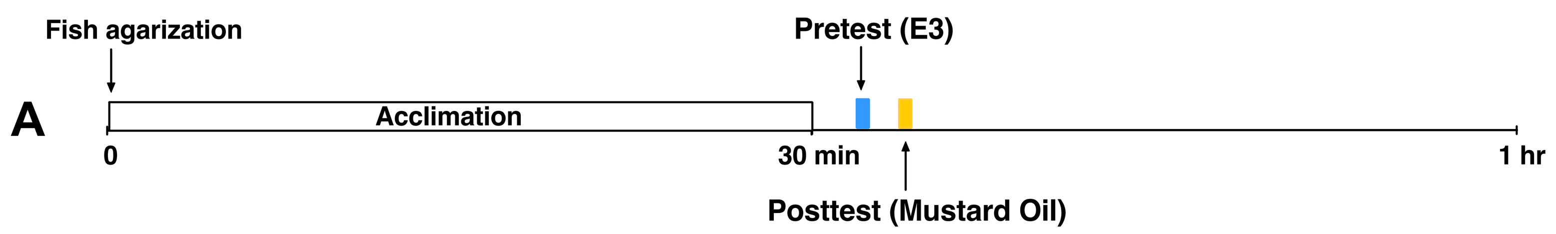


Figure 3. A. Timeline of experimental protocol is shown. Procedure was repeated using mustard oil at 0, 1, 10, 100 and 200 μM.
B. Larval zebrafish aged 5 and 6 DPF, treated with mustard oil, had significantly increased tail flick durations ($F_{4,50}$= 8.843, p < .001). Post-hoc analysis indicated significance at 10 μM and 500 μM total concentrations.

Developmental Curve

A



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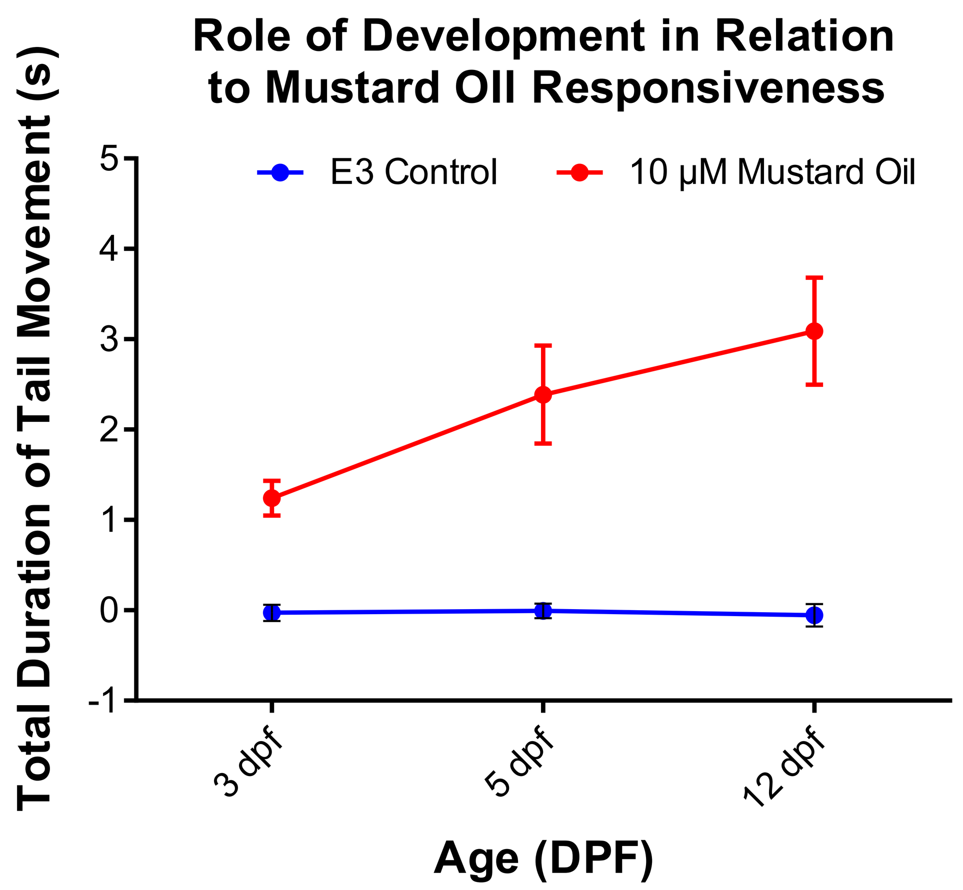
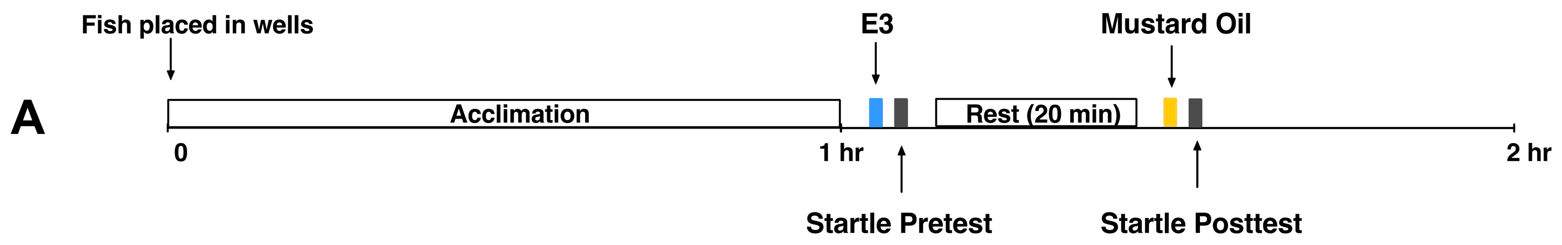


Figure 4. A. Timeline of experimental protocol is shown. Procedure was repeated with zebrafish aged 3, 5, and 12 DPF. Experimental groups received mustard oil in posttest at 10 μM while control groups received E3 at posttest.

B. Comparison of mustard oil responsiveness between zebrafish aged 3, 5 and 12 DPF showed statistically significant differences 1) between control, which received only E3 and experimental animals which received E3 and mustard oil ($F_{1,44}$= 67.58, p <.0001) 2) between experimental groups at different ages ($F_{2,44}$= 3.820, p =.0295) 3) in the interaction between exposure to mustard oil and zebrafish developmental stage ($F_{2,44}$= 3.987, p =.0256)

Sensitization

A



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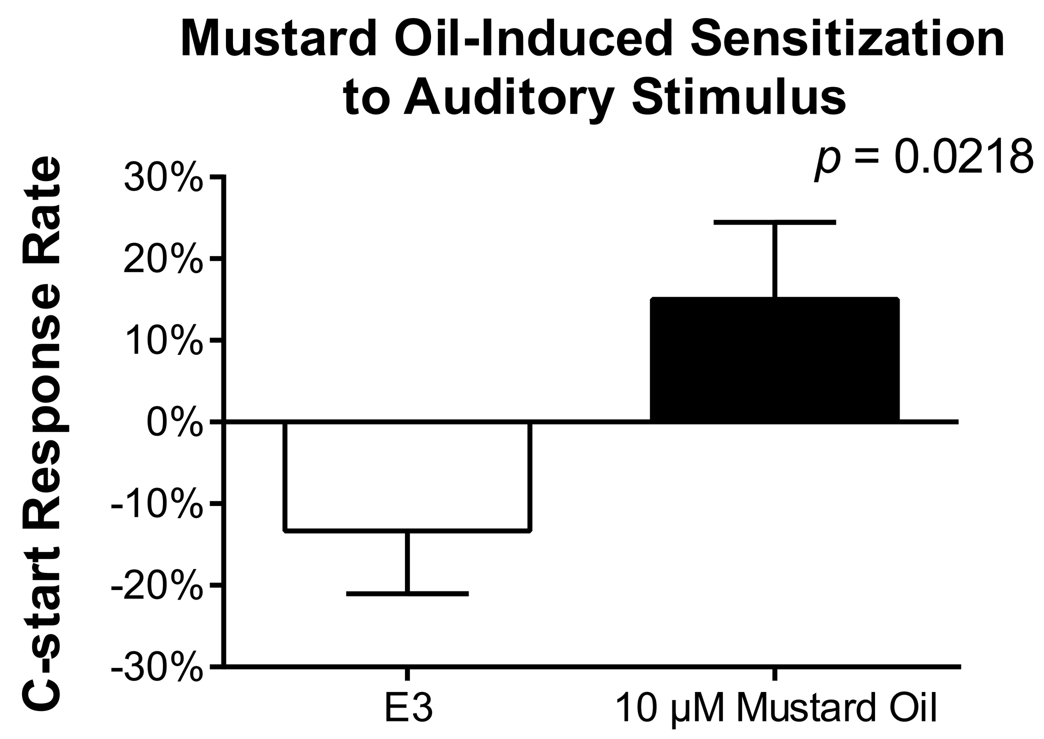

Figure 5. A. Timeline of experimental protocol is shown. At pre- and posttest, an auditory stimulus was used to induce startle. Control animals received E3 prior to posttest while experimental animals received mustard oil prior to posttest.

B. Animals treated with 10 μM of mustard oil (M=15, SD=9.456) had a significantly higher startle response rate to an auditory stimulus (t [118]=2.325, p = .0218) compared to control animals that received E3 treatment alone (M=-13.33, SD=7.690)

Conclusions

- ✓ Mustard oil at 10 μM is the effective dose necessary to induce a significant increase in the duration of tail flick activity in zebrafish aged 5 to 6 (DPF) ($F_{4,50}$= 8.843, p<0.001).
- ✓ Using zebrafish at age 3, 5, and 12 DPF, we showed a positive linear relationship between development and mustard oil responsiveness ($F_{2,44}$= 3.820, p=0.0295)
- ✓ Mustard oil can induce a sensitized C-start response by increasing the likelihood of a C-start to an auditory stimulus in zebrafish aged 5 to 6 DPF (p<0.05).

Future Work

Next steps in our research include identification of the mechanism of mustard oil effects via pharmacological antagonism and the development of associative learning paradigms using mustard oil as a noxious stimulus. Future experiments may also include optogenetic technology to image dynamic activity in the zebrafish brain pre- and post-learning.

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