

Thirty-Second Net Stressor Task in Adult Zebrafish

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[Abstract] Zebrafish have become a popular animal model for behavioral neuroscience (Gerlai, 2014). Recent studies have demonstrated that brief experimental handling prior to euthanizing animals can subsequently alter biological measures quantified post-mortem (e.g. cortisol levels) (Ramsay *et al.*, 2009; Tran *et al.*, 2014). Here we provide a detailed protocol for a simple 30-sec net stressor task for adult zebrafish that increases whole-body cortisol levels without altering the levels of whole-brain dopamine, 3, 4-dihydroxyphenylacetic acid, serotonin, and 5-hydroxyindoleacetic acid (Tran *et al.*, 2014).

Materials and Reagents

1. Adult zebrafish
2. System water: Reverse osmosis purified water supplemented with 60 mg/L instant ocean sea salt
3. 2.8 L zebrafish housing tank and lid (Aquaneering, catalog number: ZT280)

Equipment

1. Fish net (soft material)
2. Timer
3. Zebrafish housing (single sided, five shelf stand alone rack) (Aquaneering)

Procedure

1. Adult zebrafish were individually isolated in 2.8 L housing tanks and placed on a stand alone rack system with filtration.
2. Zebrafish were allowed to habituate to being in isolation for 7 days prior to the stressor (Tran *et al.*, 2014).
3. On the day of the stressor, the housing tank was moved to the testing room and zebrafish were allowed to acclimatize to the new environment for 1 h.
4. Zebrafish were netted out of the tank using a fish net and suspended above the tank and fully exposed in air for 30 sec, with the lid covering the net.

5. Following the 30 sec net stressor, zebrafish were placed back in their housing tanks.
6. Zebrafish behavioral responses can be recorded and/or tissue samples can be collected after 15 min post-stressor.

Representative data

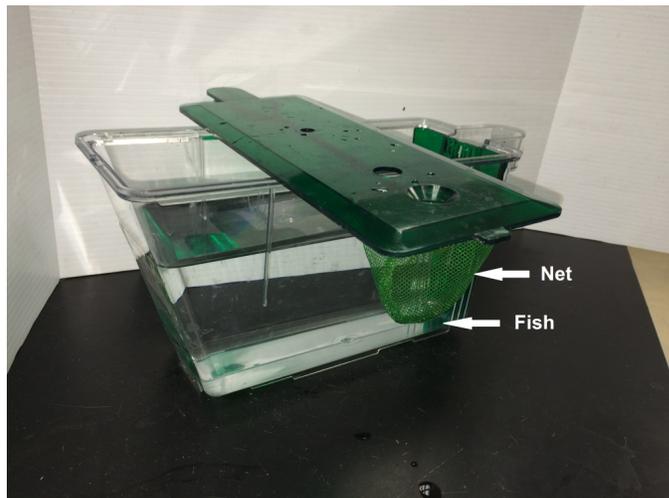


Figure 1. Note that the fish is exposed to air in the net held in place by the tank lid on top

Notes

Zebrafish were isolated because the stressor and subsequent behavioral recordings are conducted on individual zebrafish. In the case of multiple days of testing, it is recommended that the stressor be applied at the same time of the day to minimize the effects of circadian rhythms on endogenous cortisol levels.

Acknowledgments

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References

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2. Ramsay, J. M., Feist, G. W., Varga, Z. M., Westerfield, M., Kent, M. L. and Schreck, C. B. (2009). [Whole-body cortisol response of zebrafish to acute net handling stress](#). *Aquaculture* 297(1-4): 157-162.
3. Tran, S., Chatterjee, D. and Gerlai, R. (2014). [Acute net stressor increases whole-body cortisol levels without altering whole-brain monoamines in zebrafish](#). *Behav Neurosci* 128(5): 621-624.