

Understanding Biomolecular Markers of Zebrafish Stress

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Zebrafish (*Danio rerio*)

- Small vertebrate fish native to rivers of Pakistan, India and Bangladesh.



- Typically about 3-5 cm in length.

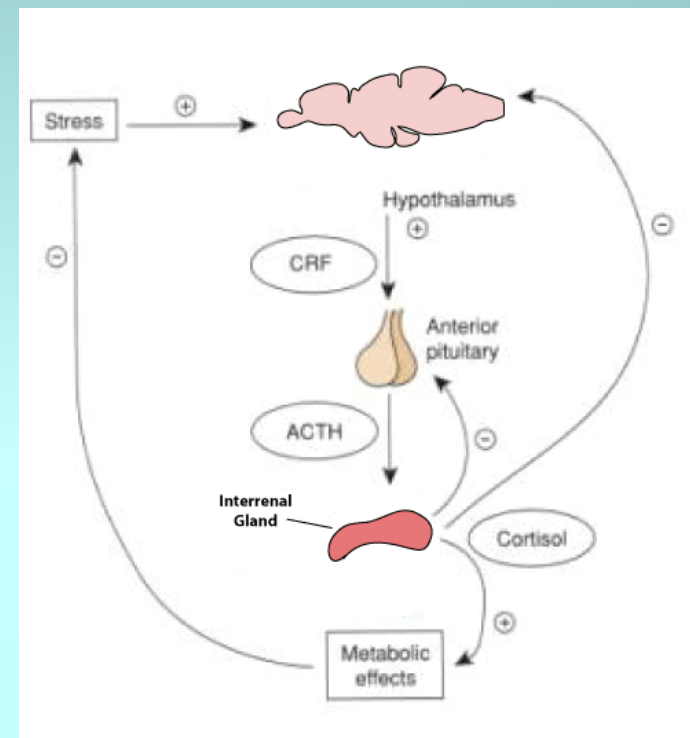
Utility of Zebrafish in Stress Research

- Relatively Simple, Stereotyped Nervous System
- All Classical Vertebrate Neurotransmitters and Neuropeptides
- Reproduce Quickly & Abundantly
- Rapid Development
- Genetic Understanding
- Less Complex Behavior
- Low-Cost Model
- Transparent strains useful in cancer or vascular research



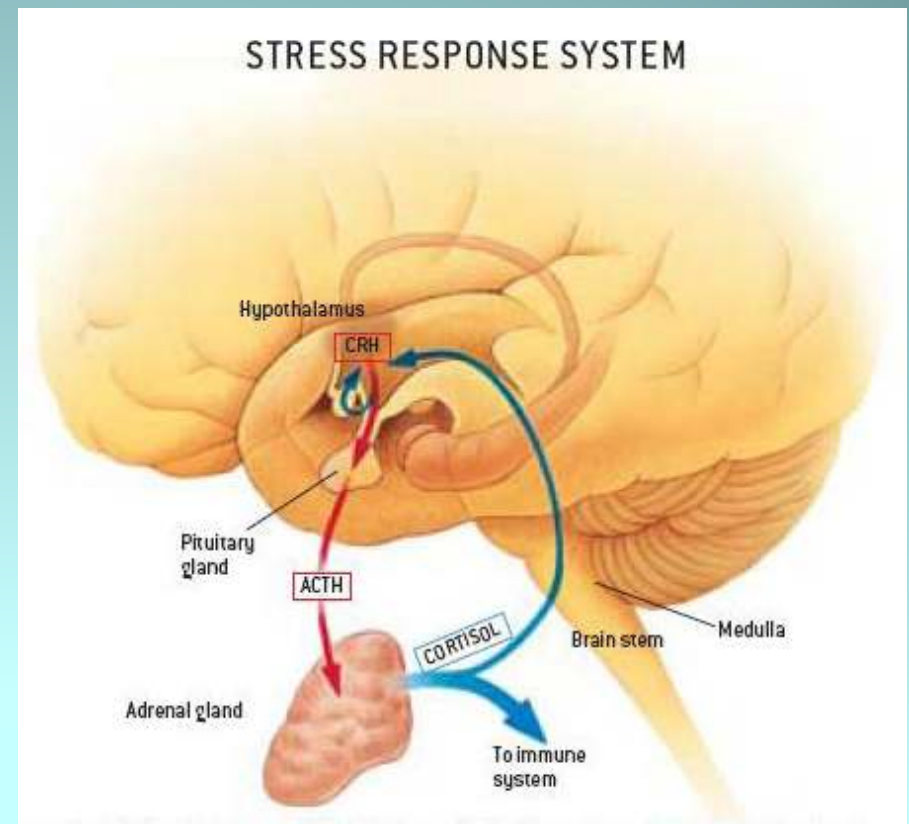
Zebrafish HPA Axis

- Functionally and Morphologically Analogous to Humans
- HPA Axis
 - Mice = Corticosterone
 - Zebrafish = Cortisol



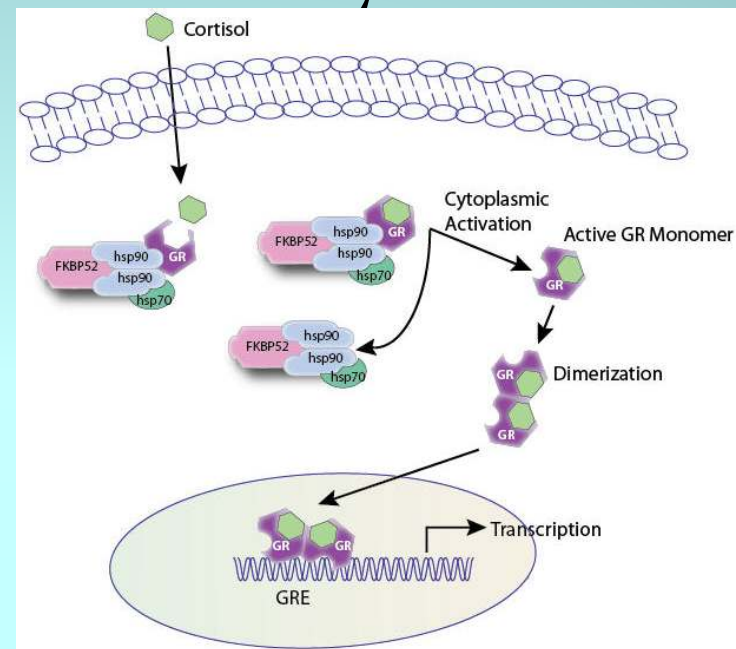
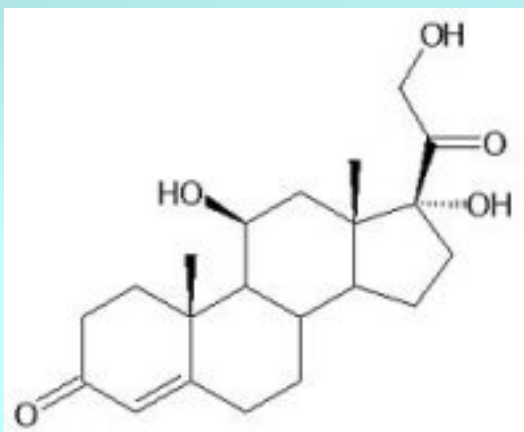
HPA Axis – Endocrine Stress Response

- Hypothalamic-Pituitary-Adrenal Axis
 - Hypothalamus releases CRH
 - CRH stimulates ACTH secretion from Anterior Pituitary
 - ACTH stimulates Cortisol secretion from Adrenal Gland



Cortisol

- Corticosteroid Hormone produced by the Adrenal Cortex
- “Stress Hormone”
- Elevated levels during increased anxiety
- Feedback Loops



Kalueff Lab Research

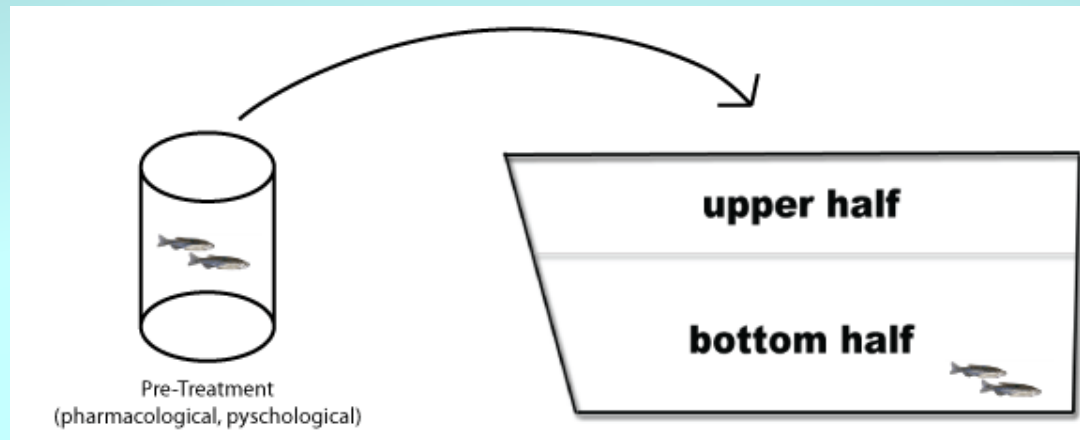
- We analyze stress, anxiety and pharmacological treatment.
- Behavioral and Physiological Endpoints:
 - Novel Tank Diving Test
 - Cortisol Concentrations



Novel Tank Test



- Natural tendency to dive to bottom until “safe” to explore
- Manual and Video Tracking Analysis
- Measurements:
 - Time to Top Half; Number of Top Entries; Total Time in Top; Erratic Movements; Freezing Bouts; Time Frozen



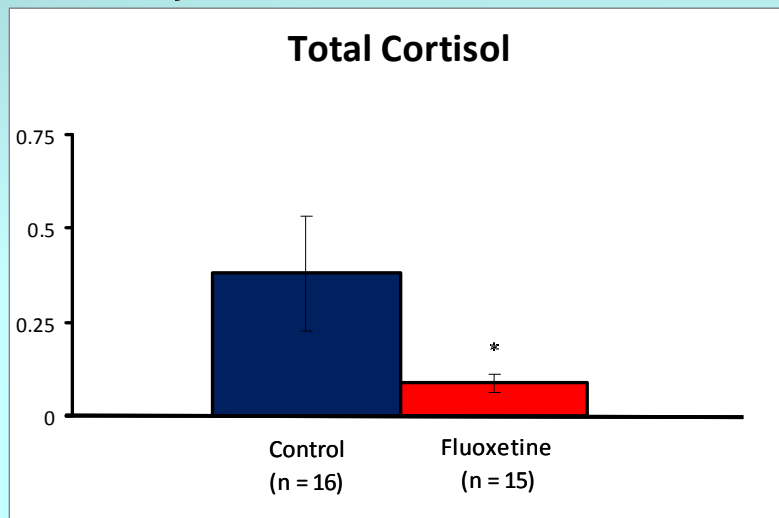
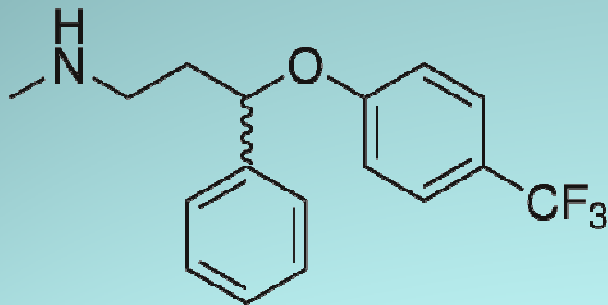
Cortisol Extraction

- Homogenization of Experimental Zebrafish
- Ether Wash 2-3x in glass tubes
- Human Saliva ELISA (Enzyme-Linked Immunosorbent Assay) used to determine zebrafish whole-body cortisol concentrations.



Selective Serotonin Reuptake Inhibitors (SSRI)

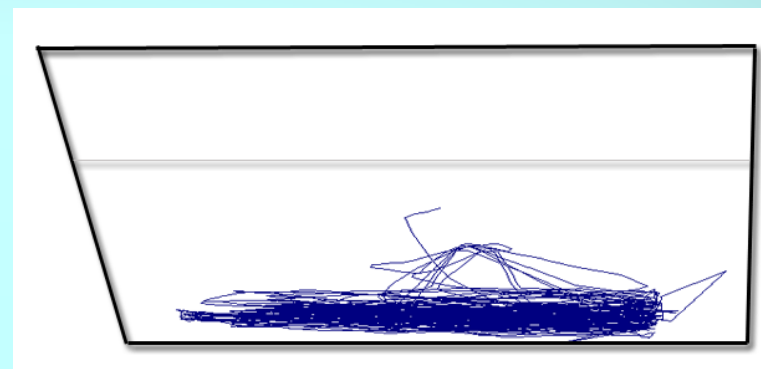
- Fluoxetine (100 µg/L Prozac) given for two weeks prior to Novel Tank Test.



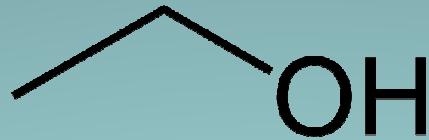
Experimental (SSRI)



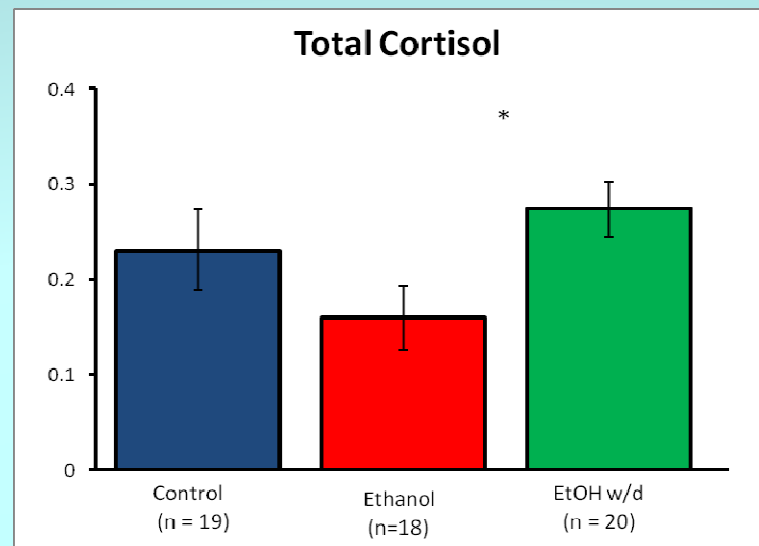
Control



Ethanol

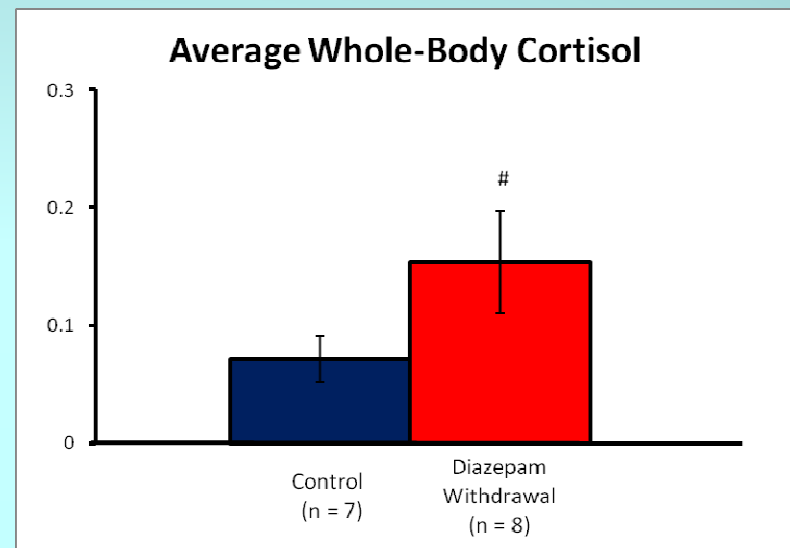
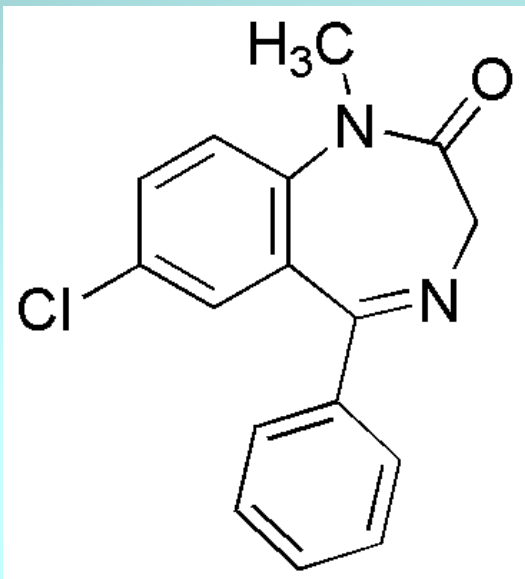


- Chronic EtOH (0.3%) treatment for 14 days
- EtOH Withdrawal = Chronic Treatment ceased 72 hours prior to Novel Tank Test



Diazepam (Valium)

- Chronic Treatment (72 $\mu\text{g}/\text{L}$) for 14 days
- Diazepam Withdrawal = Chronic Treatment ceased 72 hours prior to Novel Tank Test



Summary



- Zebrafish provide an excellent model for brain biochemistry research.
- Zebrafish and humans show strong HPA Axis similarities, as both endocrine systems secrete cortisol.
- Cortisol levels accurately measured through Human Salivary ELISA assay.
- Correlation between cortisol levels and behavioral patterns demonstrate the utility of zebrafish as an experimental stress model.

Thanks



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