UNIVERSITY OF NEBRASKA AT OMAHA

Investigating COMT Influence on the Proactive-Reactive Stress Coping Axis in Zebrafish

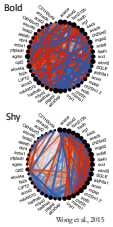
Sean T. Bresnahan, Ryan Y. Wong
Department of Biology, University of Nebraska at Omaha

Background

Proactive-Reactive Stress Coping Axis

http://animalhall.xyz/sitemap/gallery-f/

	Proactive	Reactive
Exploratory Behavior	High	Low
Aggression	High	Low
Stress Sensitivity	Low	High

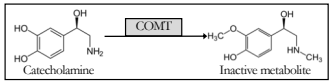


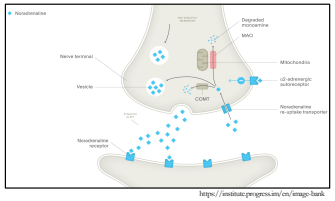
Wong et al., 2015

Individuals must cope with stressors in their environments; within species, responses to similar stressors may vary.

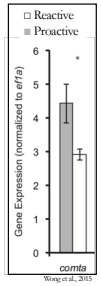
Stress coping styles often consist of correlated suites of behaviors. One set of alternative stress coping styles found in many species consist of behaviors on a proactive (bold) and reactive (shy) axis, which emerge out of an array of neural and genomic mechanisms.

Catechol-O-methyltransferase

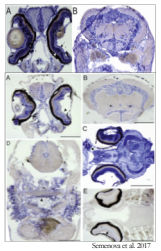




https://institute.progress.im/en/image-bank



Wong et al., 2015



Semenova et al. 2017

Catechol-o-methyltransferase (COMT) – a regulator of stress-relevant signaling molecules in the brain – is one neural mechanism which may play a role in stress coping style.

comta (a zebrafish isoform of COMT) baseline expression is higher in proactive zebrafish compared to reactive individuals.

Hypothesis

Higher *comta* expression may confer on proactive individuals an increased ability to cope with stress. Knockout of *comta* by Cas9 will result in proactive individuals with similar sensitivity to stress (stationary behavior in novel environments) as reactive conspecifics, and reactive individuals with increased sensitivity to stress compared to controls.

Materials and Methods

Doudna & Mali, 2016

Design comta sgRNA and target region primers

Synthesize comta-Cas9/gRNA mRNA

Inject 1-cell-stage embryos

Monitor survival

Validation 1: HRMA

Validation 2: on-target analysis

Validation 3: off-target analysis

Generate F1 lines

Stress-behavior assay

in situ hybridization

Results

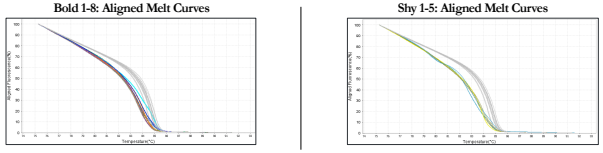
Spawning and Post-Treatment Survival Rates

Breeding attempts	Times eggs observed	Spawning probability
155	50	32.25%

Eggs saved	Survived at 72hpf	% survival at 72hpf	Mean per attempt
1,200	285	23.75%	1.8

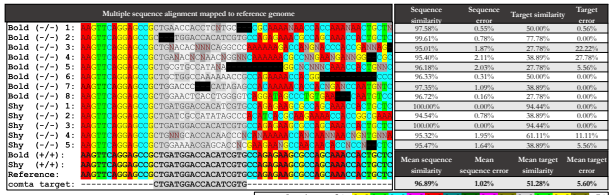
Survived at 72hpf	>72hpf or used	% >72hpf or used	% of total
285	87	30.53%	7.25%

Initial detection of variants by High Resolution Melt Curve Analysis (HRMA)

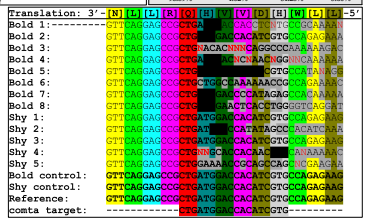


HRMA indicates variants in *comta* target region for Cas9-gRNA injected individuals compared to controls. Genomic DNA from 6 month old -/- (8 proactive, 5 reactive) and +/+ individuals (4 proactive, 4 reactive) assessed for variation in the target region. Gray curves represent +/+ and colored curves represent -/- individuals.

On-target analysis by Sanger sequencing and bioinformatic determination of mutation type



11/13 Cas9-gRNA injected individuals display mutations in target sequence. *(Top left):* Target region genomic sequence alignment for 8 proactive (bold) and 5 reactive (shy) -/- individuals with relative controls, mapped to the GRCz11 zebrafish reference genome. Target sequence highlighted in grey. (N) = base-call errors. Possible missing bases highlighted in black. *(Top right):* whole sequence and gRNA target similarity scores. *(Bottom right):* reading frame/translation analysis. Columns highlighted for corresponding codon. Many samples were heterozygous; alternative variants shown; mutation types vary from top left.



Targeting efficiency

Potential -/- tested	Confirmed by HRMA	Confirmed by Sequencing	% -/- confirmed
23 (of 42)	18	11	84.61%

Conclusions and Future Directions

23.75% survival of Cas9-gRNA injected larvae at 72 hours post fertilization

- 285/1200 -/-? survived at 72hpf – a common timepoint in similar studies (Varshney et al., 2016).
- Survival rate increased over trials as cytotoxicity and rearing issues were worked out.

18/23 individuals (78.26%) verified for genomic variants by HRMA

- 23 -/-? individuals (data not shown for 10/23) assessed by HRMA.
- 18/23 verified for variants in the target region compared to controls.
- Melt curves for 5/23 individuals aligned with those of controls; individuals removed from the study.

11/13 individuals (84.61%) verified for *comta* knockout by sequencing

- 13/18 HRMA verified individuals assessed by sanger sequencing.
- Ensemble and Clustal Omega alignments revealed 100% identity upstream of the target sequence.
- Within and downstream of the target sequence, various mutation types were revealed.
- Several samples displayed heterozygosity; sequencing files were duplicated and base-calls manually modified to distinguish between variants.
- Across variants, 11/18 individuals displayed successful frameshift-causing and/or other mutation types.

Current efforts: generation of F1 line, behavioral testing, and *in situ* hybridization

- Off-target analysis of the 11/18 *comta* -/- individuals is currently being conducted.
- Individuals verified to be without off-target mutations will be cross-bred to generate an F1 line.
- At 6-8 months old, F1's will be subjected to a battery of stress-behavior assays, and brains stored for visualization of *comta* expression by *in situ* hybridization.

Acknowledgements

A very special thanks to Ryan Wong for training and mentorship throughout this project; to Rebecca Patterson for assistance in breeding efforts; to Matthew Baker for assistance on histology-related components; to Danny Reevers, Nagat Mohamed, and Amber Park for zebrafish husbandry; and all members of the Wong lab for many useful critiques and discussions as well as ample encouragement.

This study was supported by a Fund for Undergraduate Scholarly Experiences (FUSE) award to STB, funds from the National Institutes of Health (R15MH113074), Nebraska EPSCoR First Award (OIA-1557417), Nebraska Research Initiative, and University of Nebraska Omaha start-up grants to RYW.

References

Coppens, C. M., de Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: towards underlying mechanisms. *Philosophical Transactions of the Royal Society of London: Biological Sciences*, 365(1560), 4021-4028.

Doudna, J. A., & Mali, P. (2016). *CRISPR-Cas: A laboratory manual.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Eiden, L. E. (2013). Neuropeptide-Catecholamine Interactions in Stress. *Adv. Pharmacol.* 68: 339-404

Godwin J., Sawyer S., Perrin F., Oxendine S.E., Kezios Z.D. (2012) Adapting the Open Field Test to Assess Anxiety-Related Behavior in Zebrafish. In: Kalueff A., Stewart A. (eds) Zebrafish Protocols for Neurobehavioral Research. *Neuromethods*, vol 66. Humana Press.

Semenova, S., Rozov, S., Panula, P. (2017). Distribution, properties, and inhibitor sensitivity of zebrafish catechol-O-methyl transferases (COMT). *Biochem Pharmacol*, 1(145): 147-157

Varshney, G. K., Carrington, B., Pei, W., et al. (2016). A high-throughput functional genomics workflow based on CRISPR/Cas9-mediated targeted mutagenesis in zebrafish. *Nature Protocols*. 11: 2357-2375

Wong, R. Y., Lamm, M. S., & Godwin, J. (2015). Characterizing the neurotranscriptomic states in alternative stress coping styles. *BMCG*, 16:425.

University of Nebraska Omaha