Bully Prevention Program: supression of GABA-(A) in *A. burtoni*

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GABA-(A) neuroreceptors have been implicated in the regulation of social behavior in male *Astatotilapia burtoni* cichlids. We investigated whether GABA-(A) receptors are crucial to the expression of dominance in these fish.

Male cichlids are known to be very territorial, and subordinate fish are often the victims of incessant bullying

A. burtoni live in communities with defined social hierarchies

- Males compete to defend territories used for courtship and breeding
- Dominant males show aggressive behaviors including biting, chasing, outspread fins, and mouth-to-mouth contact
- Vibrant coloring and black stripes on the face are also visual signs of dominance
- Males can lose their dominant status if challenged and beaten by a subordinate fish
- Such reversal in behavior is known to be controlled by neurological pathways in the brain

Will the suppression of GABA-(A) receptor activity result in reversal of social hierarchies?

HYPOTHESIS: Suppression of GABA-(A) activity will decrease display of dominant behaviors

Experimental Design:

Male cichlid pairs were observed to establish social hierarchies. The dominant male was injected with (+)-Bicuculline, a GABA-A antagonist. Pre- and post-injection behavior scores were compared to identify changes in dominant behavior.

Table 1. Aggressive and submissive cichlid behaviors with corresponding scores for dominance.

BEHAVIOR	SCORE
Fight	5
Threat	4
Bite	3
Chase	2
Approach	1
Swim	0
Sift/feed	0
Still	0
Avoid	-1
Flee	-2
Hide	-3

- 12 fish were paired into 6 groups (3 control, 3 experimental) according to size and weight
- Pre-injection focal sampling observations of the male pairs were made using Jwatcher software event recorder over 5 minute intervals.
- Behavior scores were calculated to identify the dominant male in each pair.
- 50 µl of PBS was injected into control group
- 50 µl of (+)-Bicuculline diluted with 100% ETOH was injected into each dominant male.
- 3 hours post-injection, observations were repeated and new dominance scores were calculated.

Results:

Due to high frequency of fish deaths, results were only obtained for one experimental group.

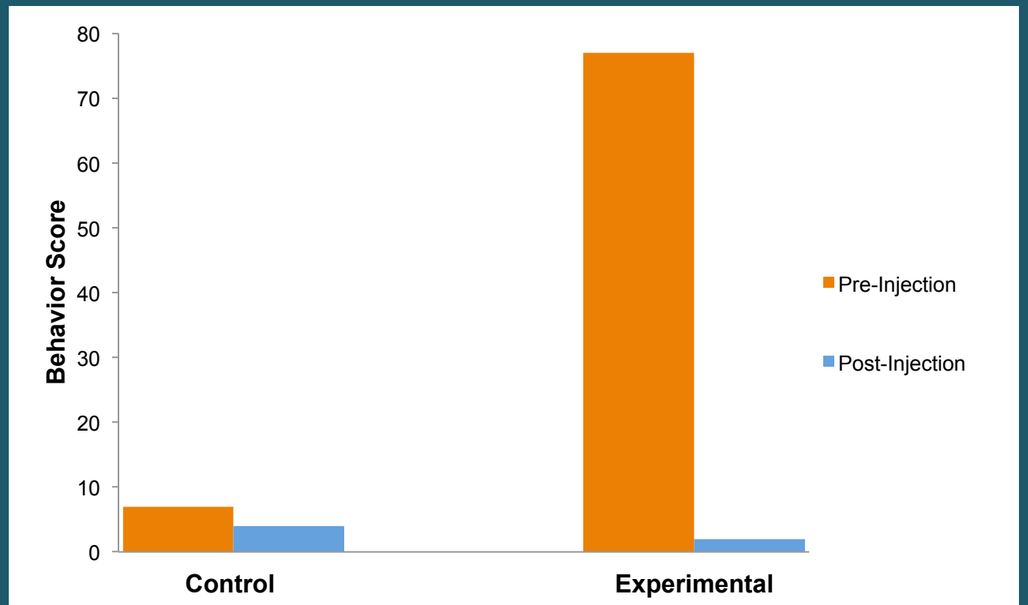

Figure 1. Behavior scores pre- and post-injection for two remaining dominant male cichlids, one control and one experimental. Behavior score of control fish decreased by 3 after injection and score of experimental fish decreased by 75.

The Problem with Bullying:

- Fish were frequently found to be injured or dead
  - Had to replace and repeat observations for new pairs
- 6 out of 12 fish died before injections could be reliably conducted

Injection Complications:

- One of two remaining experimental dominant males died post-injection, likely from accidental puncture
- (+)-Bicuculline insoluble in water and only partially soluble in ETOH
  - ETOH may have confounded observed effects on behavior

Qualitative Observations:

- Aggressive behavior in experimental male and interaction with subordinate fish was decreased post-injection
- Injected male no longer occupied terracotta “territory” and often stayed in corner of tank
- Overall activity of subordinate fish was observed to increase, and subordinate fish showed more coloration including black lines on the face.

Observed decline in aggression may have been influenced by factors other than (+)-Bicuculline injection, such as shock or unknown effects of ETOH.

Because of limited results, we cannot confirm or reject that suppression of GABA-(A) affects social behavior.

Conclusions:

GABA-(A) suppression may be involved in decreased aggression of *A. burtoni*
However, the importance of GABA-(A) to the expression of dominant behavior remains largely unknown

Future Directions:

- Conduct further tests with larger sample sizes to confirm the role of GABA-(A) in regulating dominance
- Neurological studies of (+)-Bicuculline effect on GABA-(A) receptor activity may provide better understanding of the neural mechanisms underlying the reversal of social behavior
- Test the effects of other GABA-(A) antagonists such as Metrazol or benzodiazepine.

References:

(1) Renn, S.C.P, Aubin-Horth, N. and Hofmann, H. (2008) Fish N' Chips: Functional Genomics of Social Plasticity in African Cichlid Fish. Journal of Experimental Biology 2111:3041-3056.
(2) Ganguli I (2006). Getting on top, genetically. http://stanford.edu/group/fernaldlab/The%20Scientist.pdf
(3) http://www.jwatcher.ucla.edu/

Images:
(1) http://www.segrestfarms.com/index.cfm?fuseaction=catalog.productDetail&productID=11375&CICHLID-A-BURTONI-LRG-ASTATOTILAPIA-BURTONI.html
(2) http://lemihacioglu.net/photography/category/aquatic

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