Viral-mediated transgenesis of MAOA and AVP increases territorial aggression in stickleback

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Introduction

How do genes influence behavior?

We developed viral-mediated transgenesis to test the effects of increased expression of candidate genes in the brain on behavior in sticklebacks.

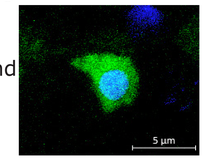
Why sticklebacks?

This classic model for studying behavior features:

- Numerous independently-derived populations
- Seasonal territorial aggression
- Fully sequenced genome
- Established behavioral assays
- Many candidate genes for behavior from multiple QTL & RNAseq studies

Why viral-mediated transgenesis?

- Fast: stable transgenic lines aren't required
- Flexible: different promoters target different locations or time frames
- Directly establish a **causal relationship** between genes and behavior
- Allows powerful repeated measures designs: measuring the same individuals before and after transgenesis **preserves** individual variation and **reduces** the sample size



Study Goals

Demonstrate detectable behavior-level changes resulting from increased expression of candidate genes

Candidate genes for aggression

- Arginine vasopressin (AVP), a highly conserved pituitary hormone important for social behavior, learning, and memory
- Monoamine oxidase (MAOA), an enzyme that processes dopamine, norepinephrine & serotonin; associated with Brunner syndrome, depression and antisocial disorders

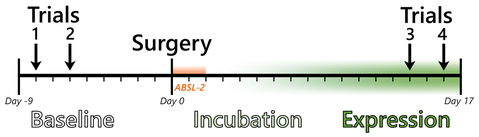
Hypotheses

1. Increased expression of arginine vasopressin (AVP) within the socio-behavioral network of the brain should **increase aggression** because AVP regulates the HPA axis through ACTH signaling to gonadal hormones.

2. Since lower monoamine oxidase is associated with increased aggression, higher MAOA was expected to **decrease aggression** through serotonin turnover.

Methods

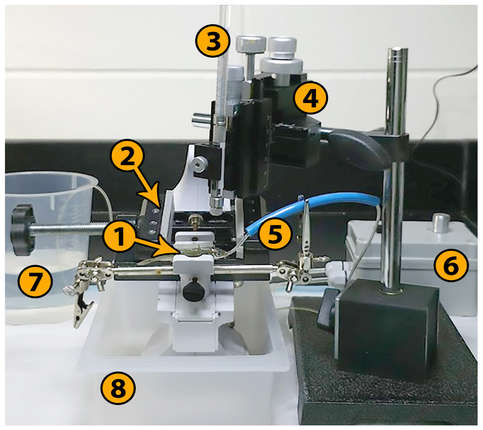
Aggression toward a confined intruder was quantitatively assayed in two baseline trials, eight and six days prior to surgery. Breathing rate was assayed prior to intruder exposure. In a ten minute surgical procedure, a randomly-assigned viral construct was administered to the anterior diencephalon of the brain via 2 transcranial injections. The construct used a broadly-expressed promoter to drive expression of a selected gene. After an incubation period, strong expression of the payload gene occurred in the transfected tissue. Behavior and breathing were reassessed in two further trials at 14 and 16 days after surgery.



Constructs

- EYFP (fluorescent control), n=16
- AVP (human), n=18
- MAOA (human), n=20

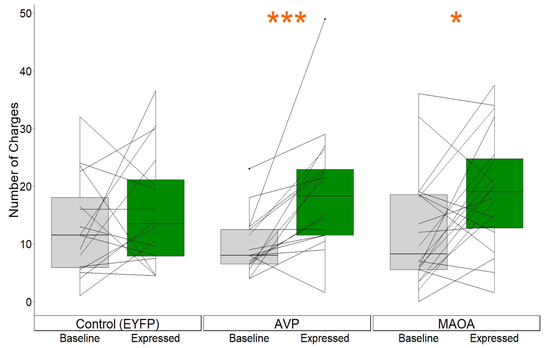
Mammalian cDNA ORF clones or a novel fluorescent protein were packaged in a replication-deficient herpes virus (HSV-1). Three promoters were piloted; hCMV was choosen due to its long-term window of effect (2-5 weeks). Mammalian homologs were selected for their wide availability in plasmid form. As a result, this technique requires minimal molecular expertise.



1. Stickleback in padded clamp
2. Alternative clamp for larger fish
3. Neuros syringe, 5 µL
4. Three-axis manipulator
5. Oral cannula and guide tube
6. Peristaltic cannula pump, 100 $^{mL}/_{min}$
7. Pump source reservoir
8. Drip tray

Results

Increased Aggression from MAOA and AVP

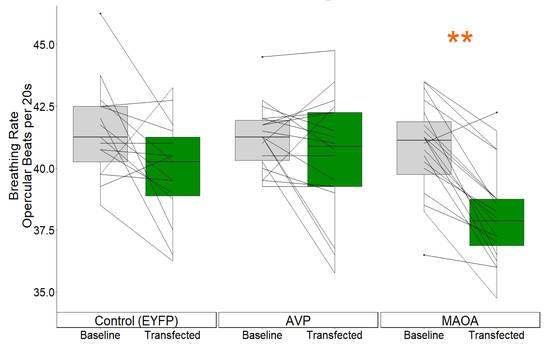


- Aggression increased in transgenic fish that received constructs driving expression of either candidate gene.

  AVP: Z = -3.1, p = 0.001
  MAOA: Z = -2.1, p = 0.02

- No change in any aggressive behavior occurred in control fish.

MAOA decreases breathing rate

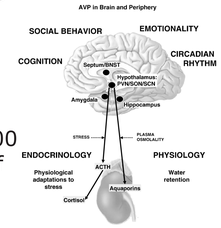


- Only increased expression of MAOA caused a decrease in breathing rate
  - significant decrease after expression
    Z = -3.6, p = 0.0001
  - significant difference between treatment groups
    Z = 2.6, p = 0.01 vs. EYFP
- No change in breathing rate for either the control or AVP transfected fish
- All groups had similar baseline breathing rates

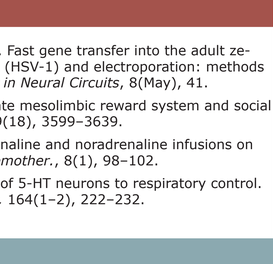
Expected Mechanism
↑ MAOA leads to ↓ 5-HT/NE resulting in ↓ breathing

Conclusions

1. AVP confirms viral-mediated transgenesis as a viable method for establishing causal links between genes and behavior.
   - Repeated measures design drastically reduces required sample size
   - Between group comparison would have required 150-200 fish to detect a difference of this magnitude



2. Overexpression of MAOA increased aggression in stickleback fish.
   - Decreased breathing rate shows that MAOA is functioning as expected – enzymatically lowering levels of dopamine and norepinephrine
   - Increased aggression might be due to dopamine's "inverted-U" effects or increased anxiety



Acknowledgments

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Full dataset avalible at:

References

Zou, De Koninck, Neve, & Friedrich (2014). Fast gene transfer into the adult zebrafish brain by herpes simplex virus 1 (HSV-1) and electroporation: methods and optogenetic applications. *Frontiers in Neural Circuits*, 8(May), 41.

O'Connell & Hofmann (2011). The Vertebrate mesolimbic reward system and social behavior network. *J Comp Neurol.*, 519(18), 3599–3639.

Whelan & Young (1953). The effect of adrenaline and noradrenaline infusions on respiration in man. *Br J Pharmacol Chemother.*, 8(1), 98–102.

Hodges & Richerson (2008). Contributions of 5-HT neurons to respiratory control. *Respiratory Physiology & Neurobiology,* 164(1–2), 222–232.