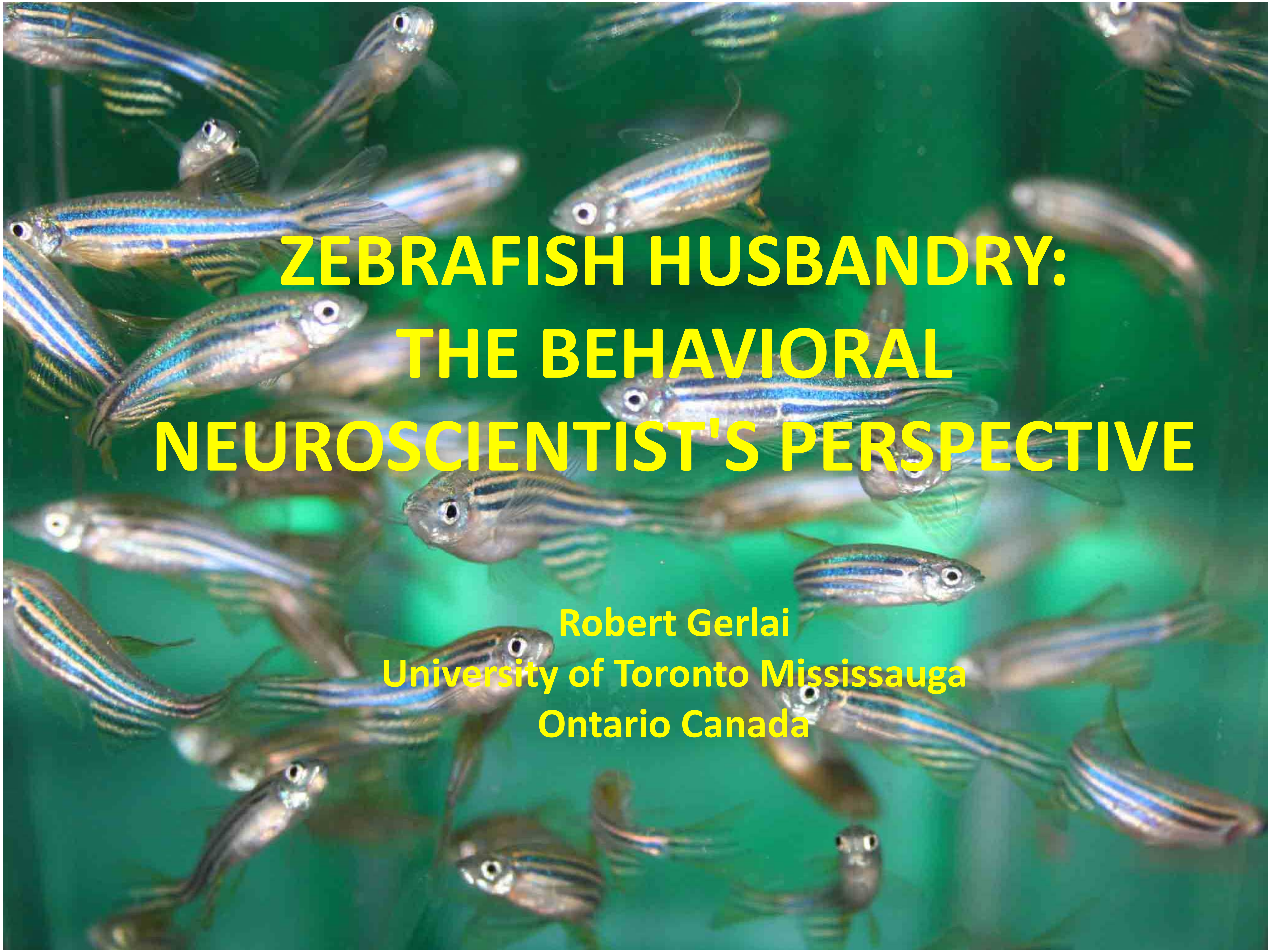
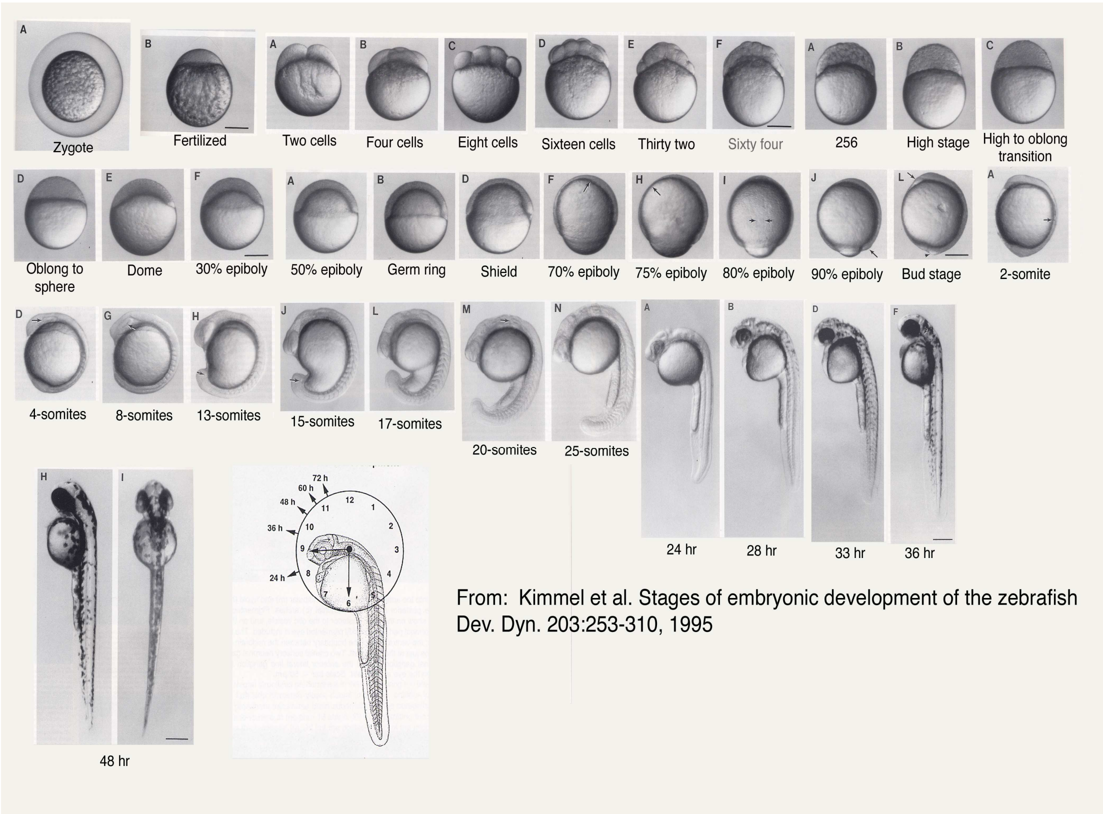
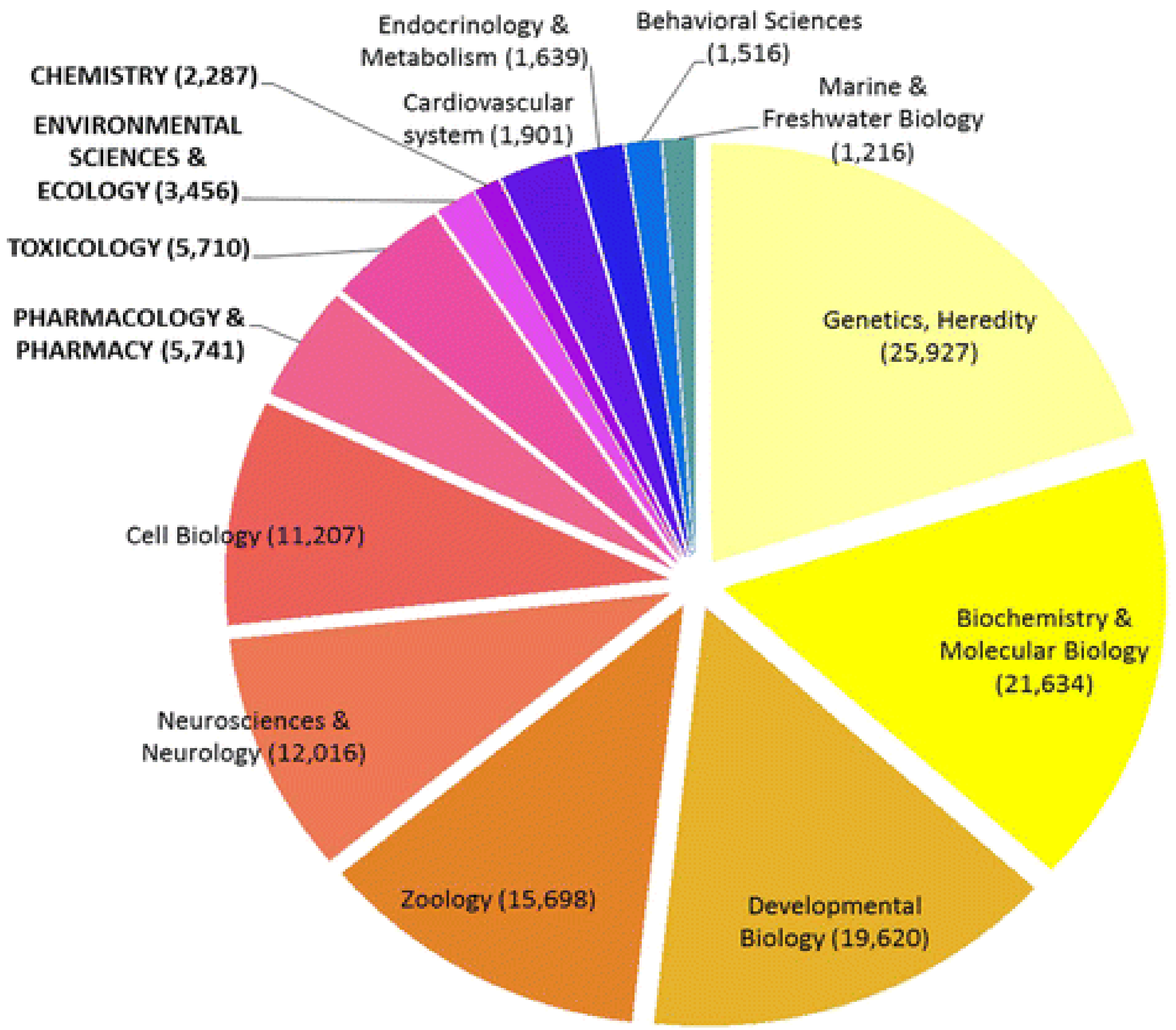

ZEBRAFISH HUSBANDRY:
THE BEHAVIORAL
NEUROSCIENTIST'S PERSPECTIVE
Robert Gerlai
University of Toronto Mississauga
Ontario Canada

Zebrafish for Developmental Biology, The past 4 decades



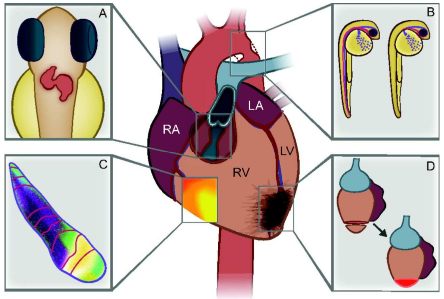
High yield egg production
High throughput screening
Efficient high density housing

The 21st Century & onward: Zebrafish a subject of study in many science fields

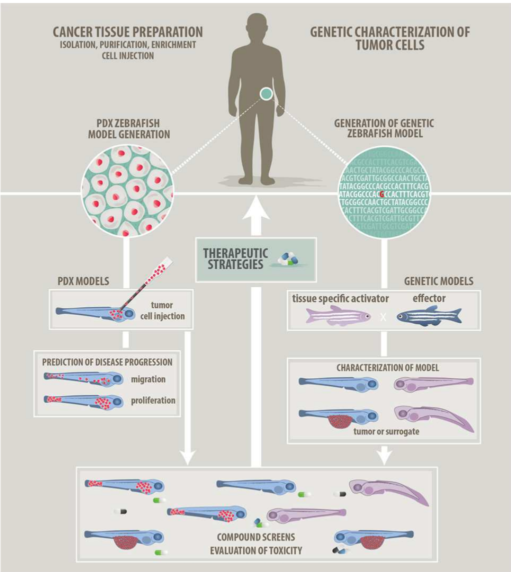


The 21st Century & onward: Zebrafish a subject of study in many science fields

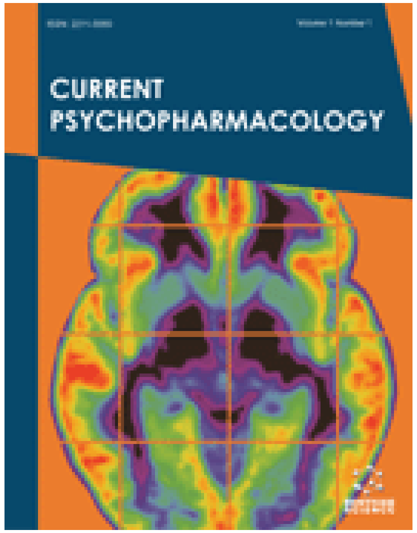
Cardiovascular research



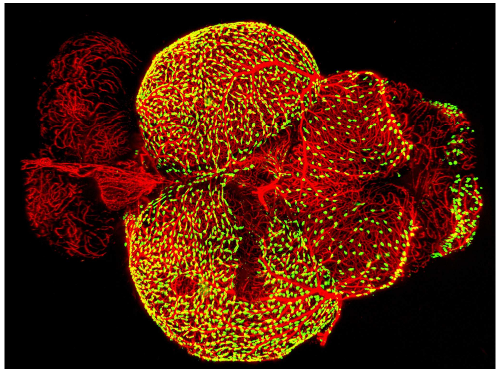
Cancer research



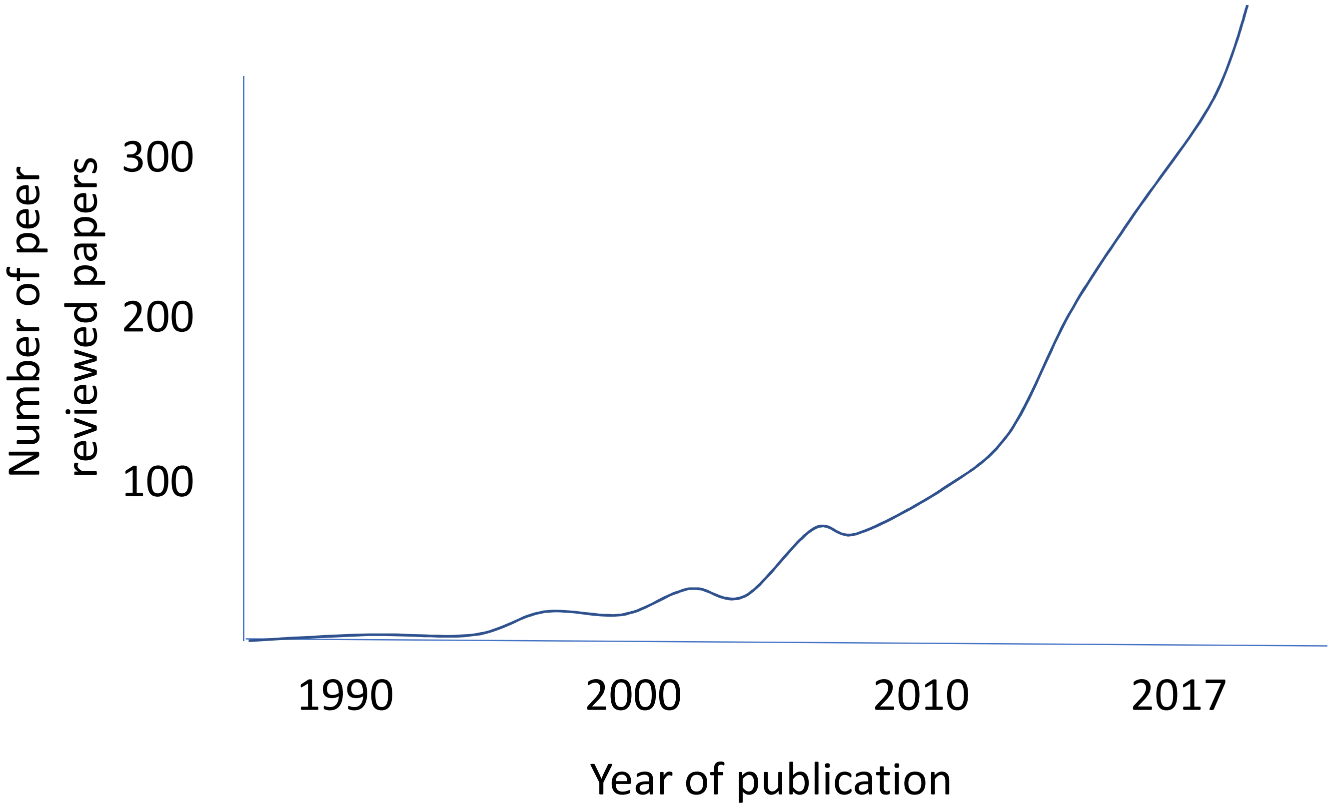
Psychopharmacology



Brain research

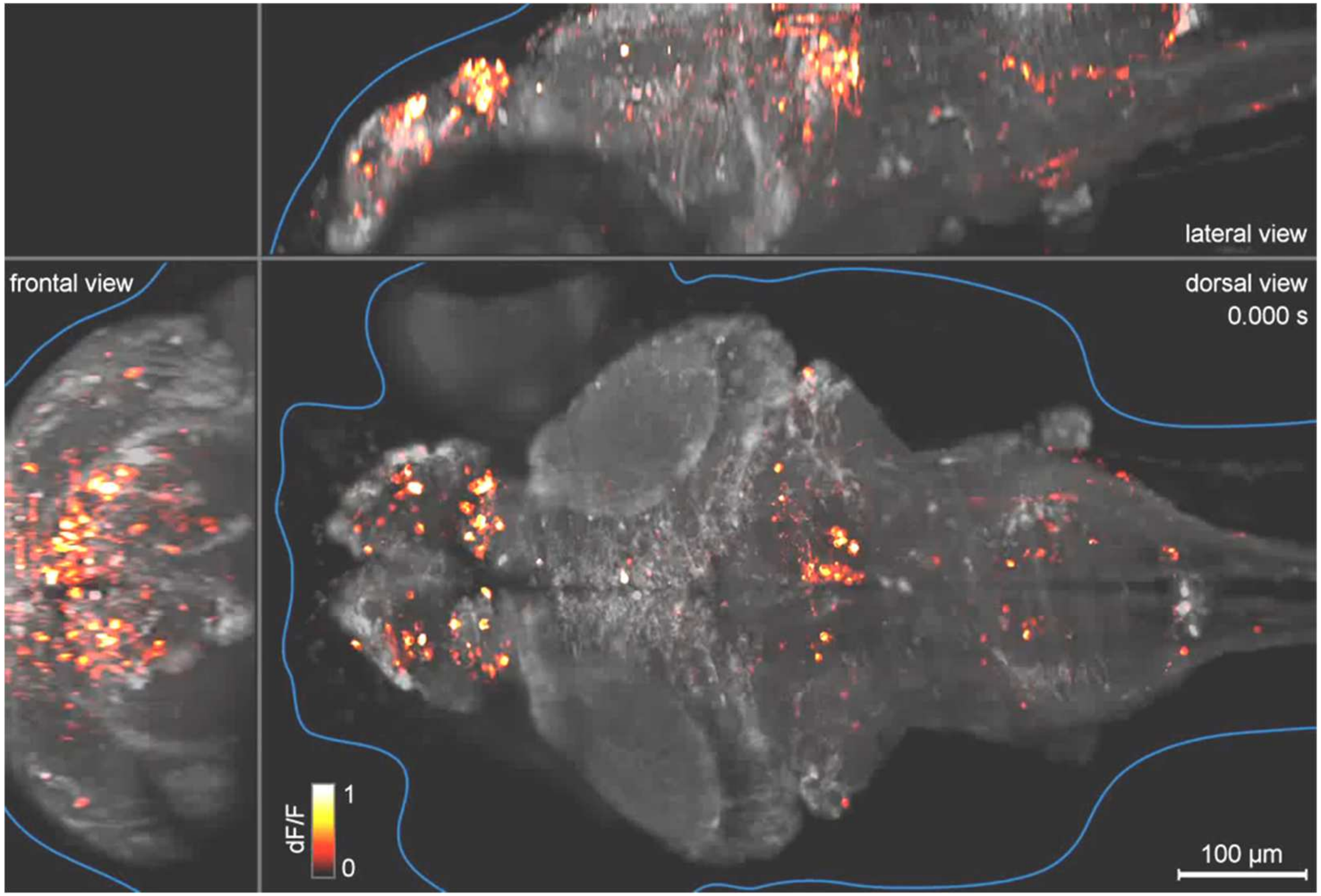


Behavioral neuroscience research in zebrafish is exponentially increasing



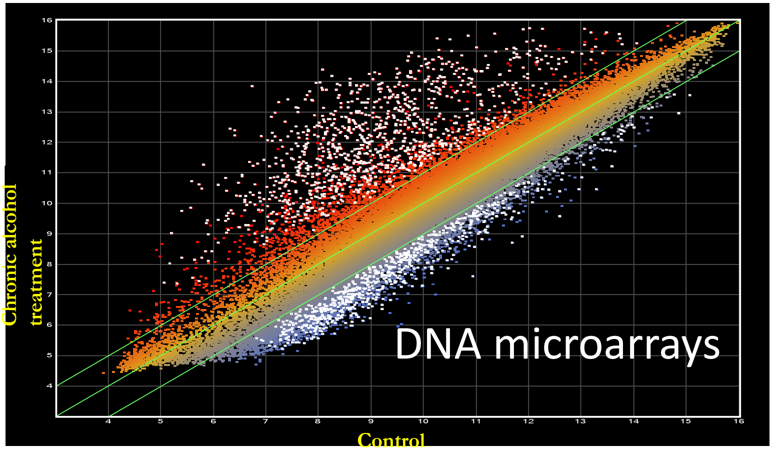
Kalueff AV, Stewart AM, Gerlai R (2014). Zebrafish as an emerging model for studying complex brain disorders. Trends Pharmacol Sci 35: 63-75

Sophisticated neuroscience tools

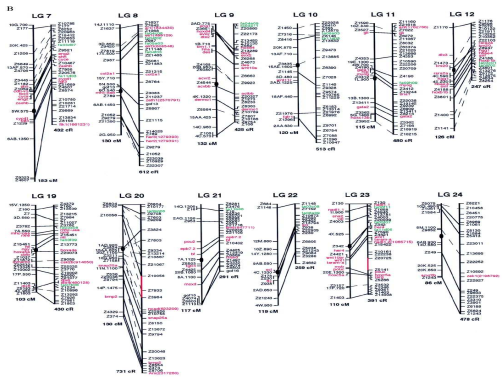


Calcium imaging in live zebrafish

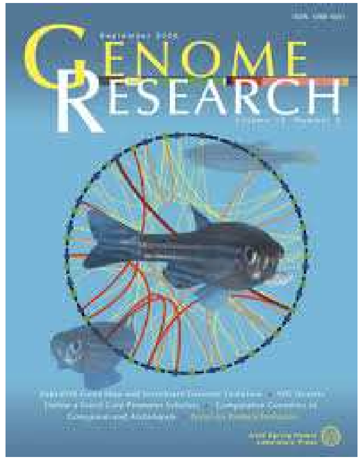
Sophisticated recombinant DNA methods

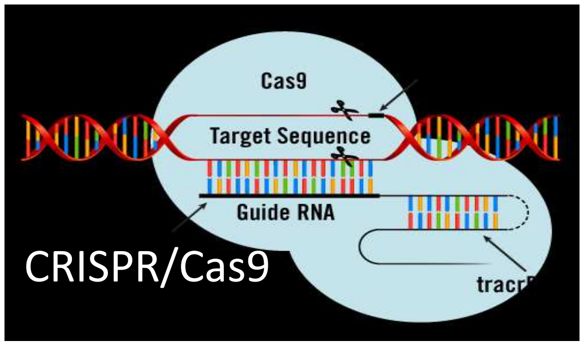


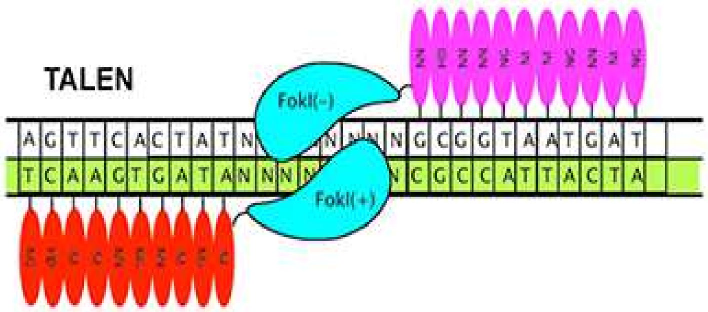
Tens of thousands of genetic markers



Genome sequenced

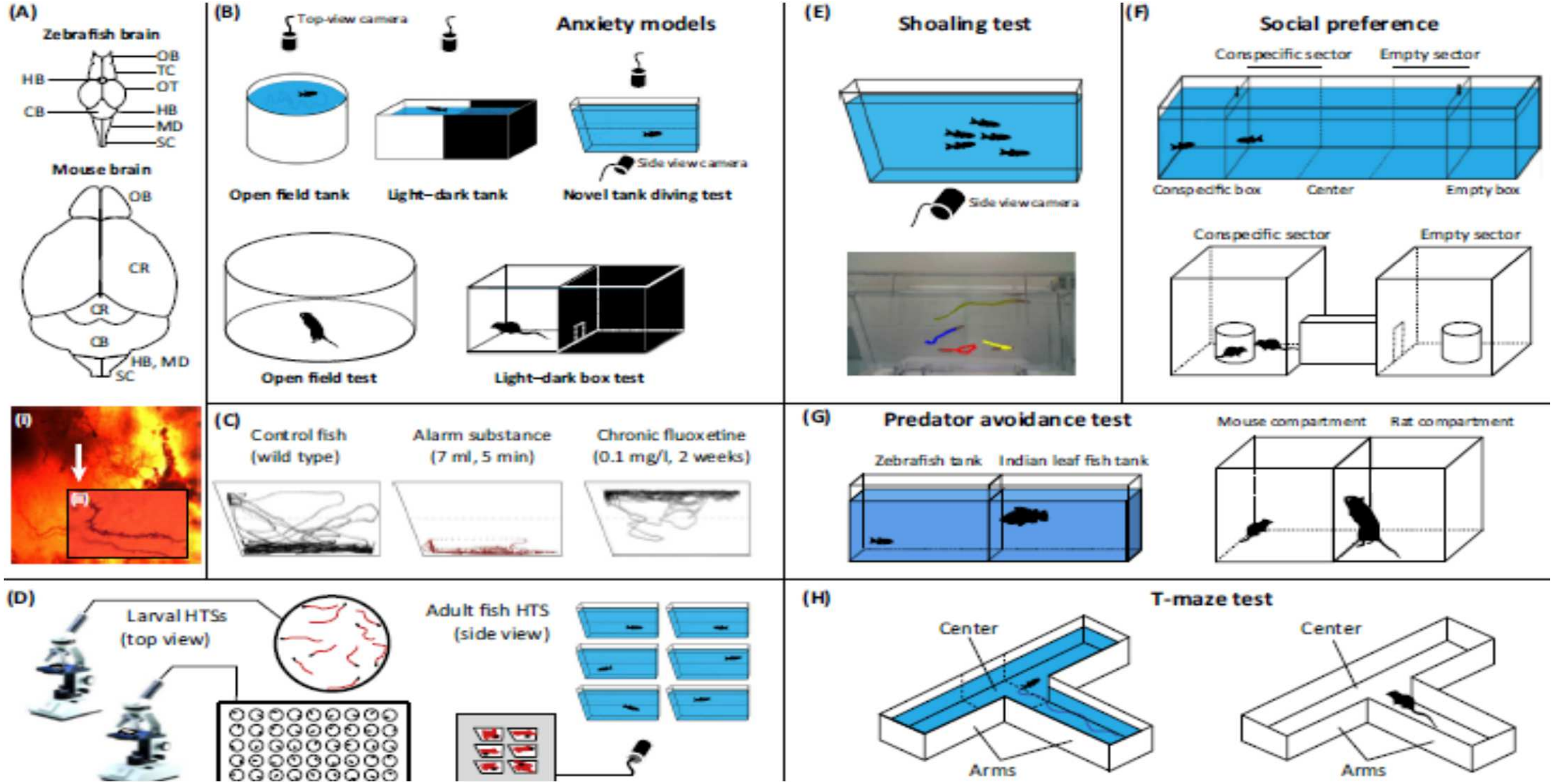






Powerful gene targeting methods

Sophisticated behavioral paradigms



Many zebrafish and mouse equivalents

Kalueff AV, Stewart AM, Gerlai R (2014). Zebrafish as an emerging model for studying complex brain disorders. Trends Pharmacol Sci 35: 63-75

Zebrafish is no longer a high efficiency egg producing tube, but a sensitive model system for behavioral neuroscience & many other science fields

The issue of reproducibility

- More sophisticated models → need for increased sensitivity to detect effects of experimental manipulations
- Increased sensitivity to detect effects of experimental manipulations
  - **higher chance unknown or uncontrolled factors alter experimental group mean → can lead to false positive or negative findings**
  - **higher chance unknown or uncontrolled factors induce detectable error variation → will lead to false negative findings**

Agassi J, Benjamini Y, Chesler E, Crabbe J, Crusio W, Eilam D, Gerlai R et al., (2018). Reproducibility and Replicability of mouse phenotyping in pre-clinical studies. Neuroscience & Biobehavioral Reviews pii: S0149-7634(16)30657-1. doi: 10.1016/j.neubiorev.2018.01.003. (in press)

Gerlai R (2018). Reproducibility and replicability in zebrafish behavioral neuroscience research. Pharmacol Biochem Behav (in press)

NIH Workshop with Leaders of Zebrafish Research, Sep 11-12, 2017

Zebrafish and Other Fish Models: Description of Extrinsic Environmental Factors for Rigorous Experiments and Reproducible Results

Conclusion of the workshop

- We need to improve our knowledge of how to keep and breed zebrafish
- Systematic analyses of environmental factors potentially affecting zebrafish are needed
- A white paper to summarize recommendations reflecting the community's view may be published

This talk: some experimental examples to illuminate the issues

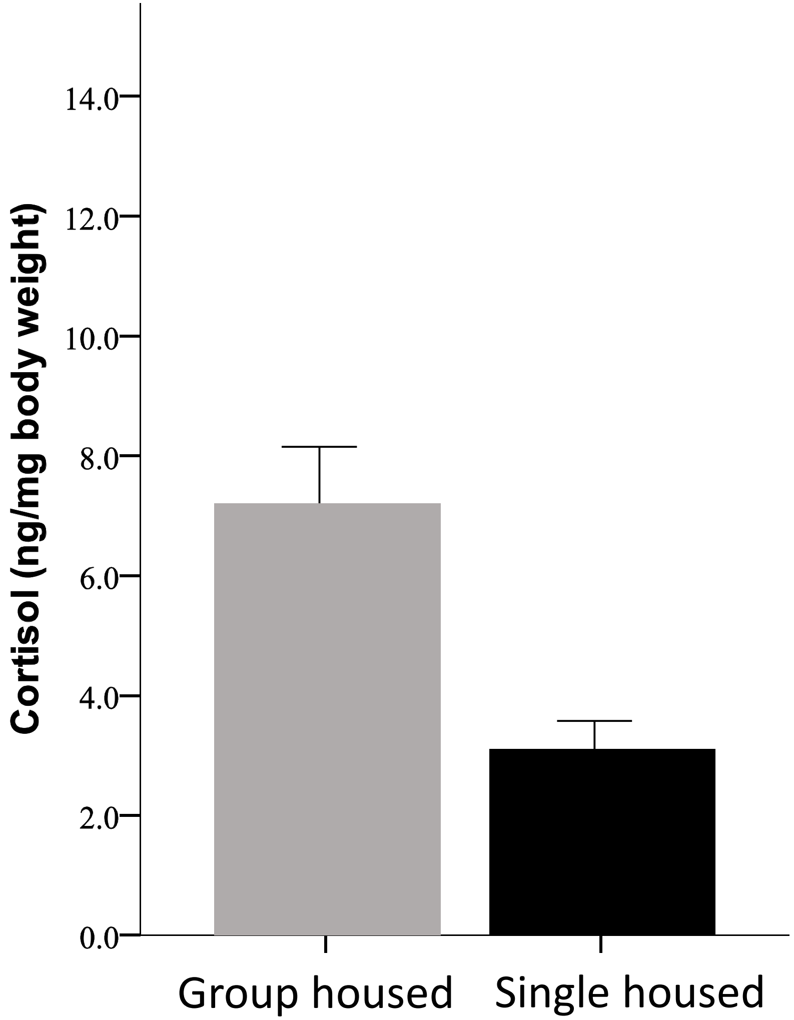
Example 1: Is high density housing the best way to keep zebrafish?

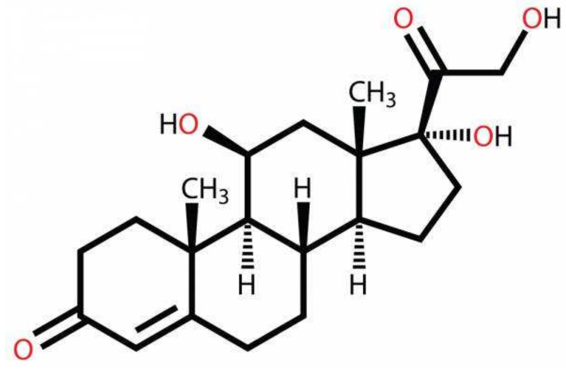
- Yes, if the goal is to produce the largest number of eggs using the smallest amount of space
- No, if the goal is to understand how the zebrafish brain works and if we want to model human brain disorders
  - A surprising tale of an isolation induced stress study

Social Isolation is supposed to be stressful for social species, including the zebrafish

Dr. Soaleha Shams

Surprise: Social isolation reduces cortisol levels





Cortisol, the stress hormone

Isolation:

- chronic 180 day long isolation
- 1 fish per 1.5 L tank
- Tanks separated by grey visual barrier
- Tanks remained on the rack system (no olfactory isolation)

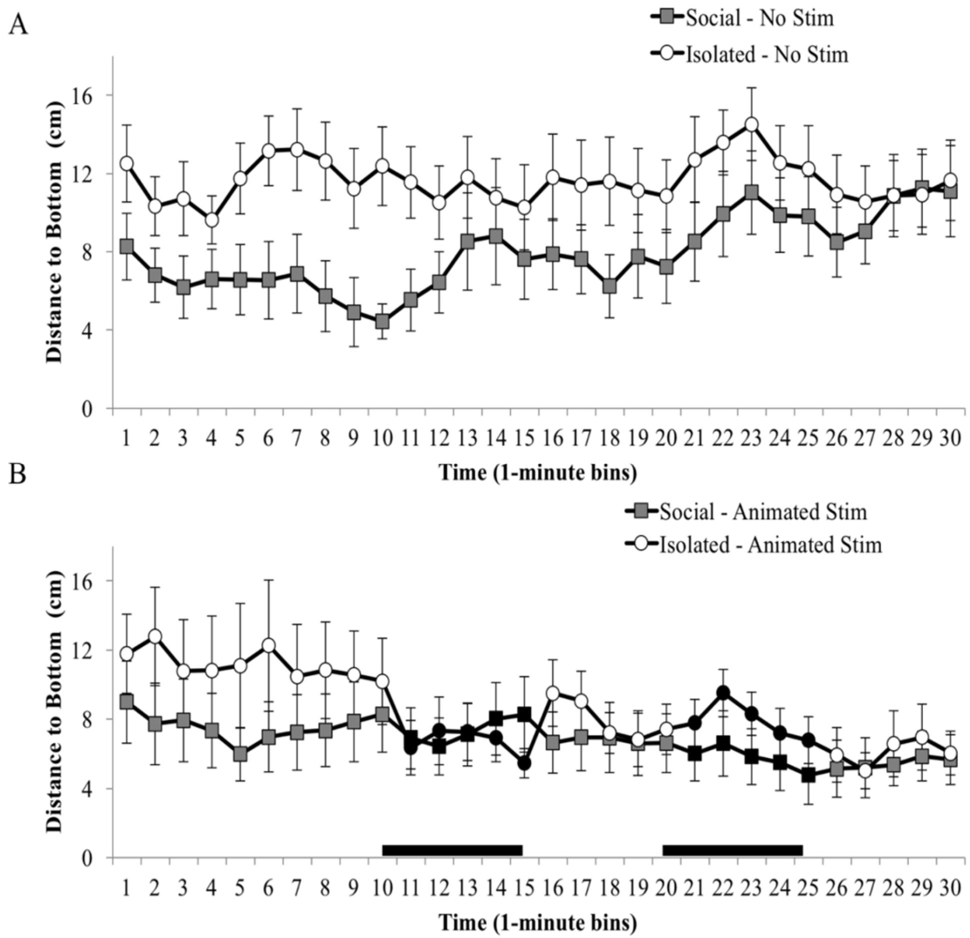
Control:

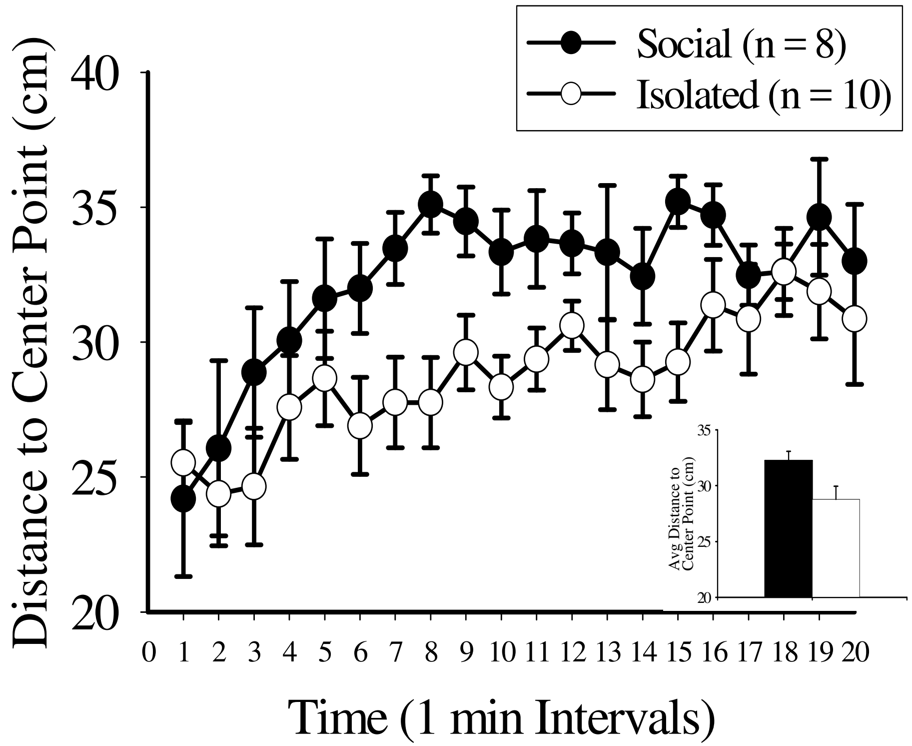
- chronic 180 day long social housing
- 5 fish per 1.5 L tank
- Tanks separated by grey visual barrier
- Tanks remained on the rack system

Shams S, Seguin D, Facciol A, Chatterjee D, Gerlai R (2017). Chronic social isolation affects anxiety-related behaviors, cortisol, and monoamines in adult zebrafish. Behav. Neurosci. 131:492-504.

Could cortisol work the opposite way in zebrafish compared to mammals?

No! Social isolation induced cortisol decrease is coupled with decreased anxiety-like responses in zebrafish





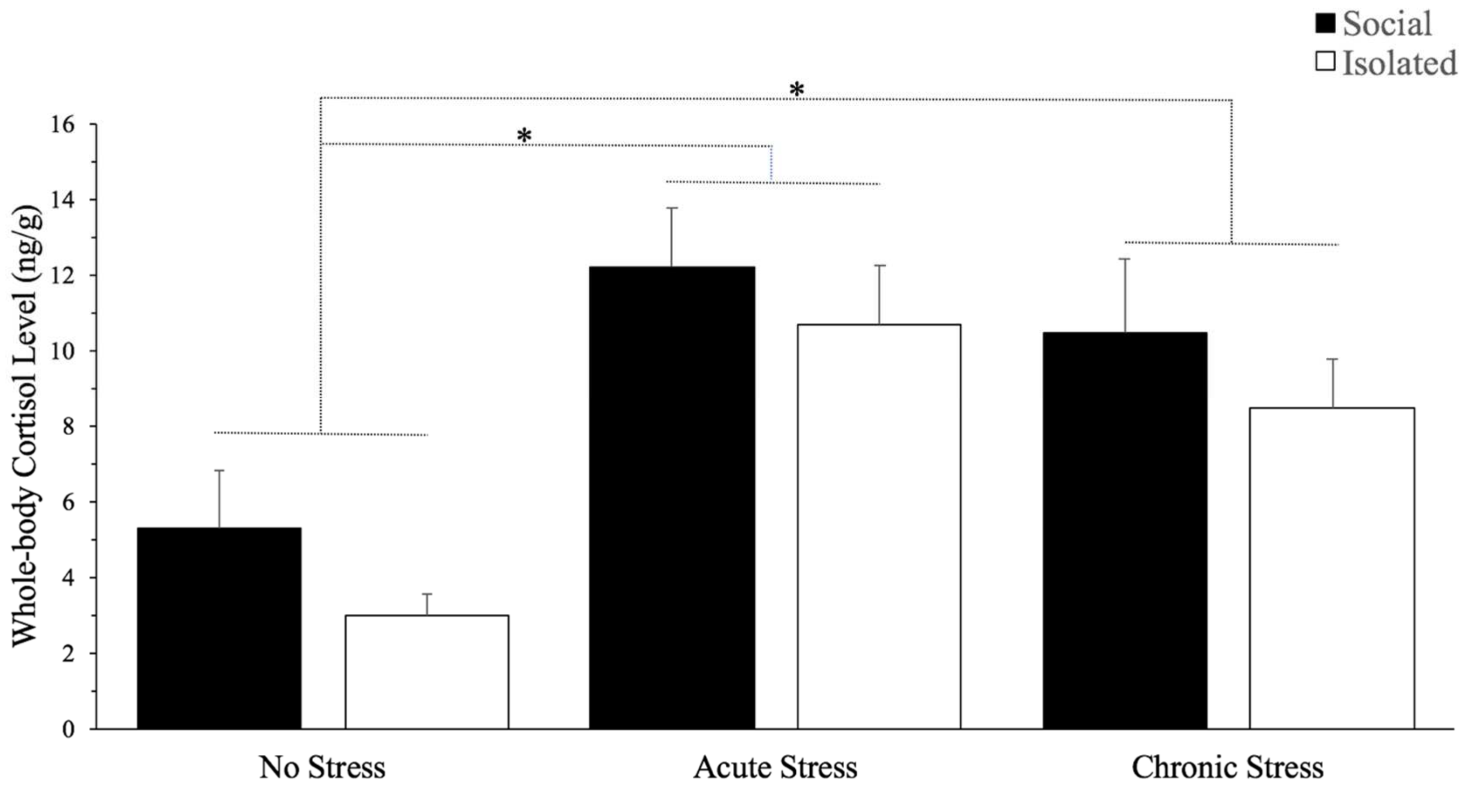
Shams S, Seguin D, Facciol A, Chatterjee D, Gerlai R (2017). Chronic social isolation affects anxiety-related behaviors, cortisol, and monoamines in adult zebrafish. Behav. Neurosci. 131:492-504.

Shams S, Amlani S, Buske C, Chatterjee D, Gerlai R (2018). Developmental Social Isolation affects adult behavior, social interaction, and dopamine metabolite levels in zebrafish. Devel Psychobiol 60: 43-56.

Could it be that we just don’t know how to induce stress/anxiety in zebrafish?

No! Chronic unpredictable stress increases cortisol levels and anxiety-like responses as expected

Chronic unpredictable stress increases cortisol levels



Shams S, Seguin D, Facciol A, Chatterjee D, Gerlai R (2017). Chronic social isolation affects anxiety-related behaviors, cortisol, and monoamines in adult zebrafish. Behav. Neurosci. 131:492-504.

Fulcher N, Tran S, Shams S, Chatterjee D, Gerlai R (2017). Neurochemical and behavioural responses to unpredictable chronic mild stress following developmental isolation: The zebrafish as a model for major depression. Zebrafish 14: 23-34.

Chronic unpredictable stress increases anxiety-like responses as expected

Niveen Fulcher

Fulcher N, Tran S, Shams S, Chatterjee D, Gerlai R (2017). Neurochemical and behavioural responses to unpredictable chronic mild stress following developmental isolation: The zebrafish as a model for major depression. Zebrafish 14: 23-34.

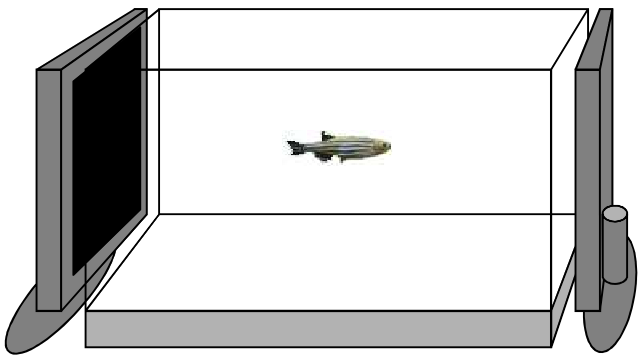
Social isolation reduces stress-related behavioral and neurohormonal responses.

What’s going on?

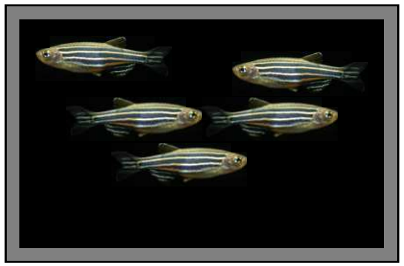
Why does isolation lead to reduced stress and anxiety?

Zebrafish love their neighbors! Highly social fish that should not like being isolated

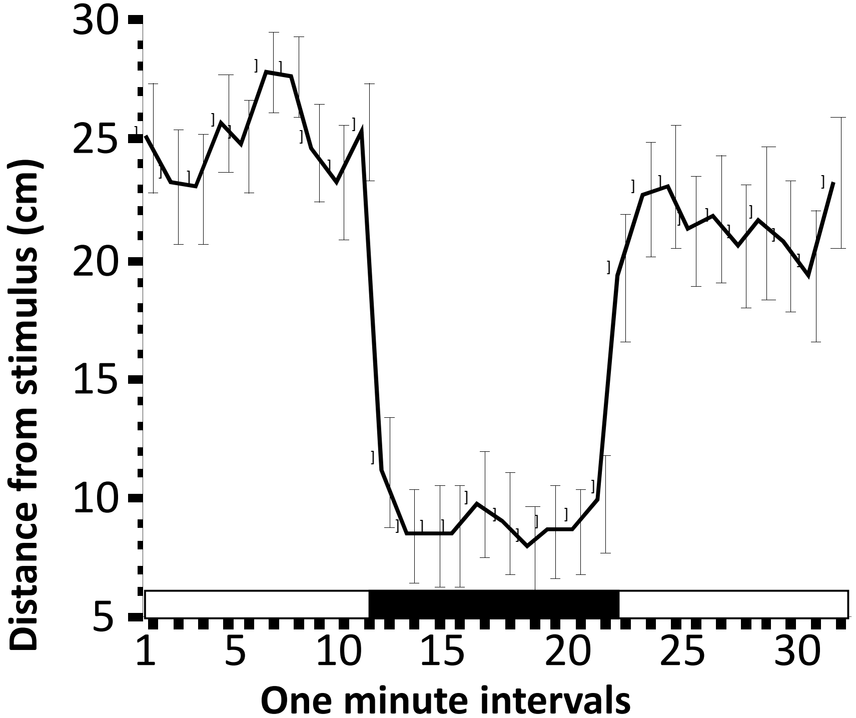
Test apparatus



Animated images of conspecifics



The shoaling response



1. Saverino C, Gerlai R (2008). The social zebrafish: Behavioral responses to conspecific, heterospecific, and computer animated fish. Behav. Brain Res 191:77-87.
2. Al-Imari L, Gerlai R (2008). Sight of conspecifics as reward in associative learning tasks for zebrafish (Danio rerio) Behav. Brain Res 189: 216-219
3. Fernandes Y, Gerlai R (2009). Long-term behavioral changes in response to early developmental exposure to ethanol in zebrafish. Alcoholism: Clinical and Experimental Research, 33: 601-609.

Perhaps isolation in a small standard 1.5 liter tank is still better than being crowded in the same tank

- Crowded housing → Reduced water quality
  - toxic waste products
  - Reduced oxygen levels
- Crowded housing → Reduced amount of space
  - Restraint stress
- Crowded housing → Social stress
  - Dominance-subordinate interactions (aggression)
  - Competition for food
- Impoverished environment of the crowded tank
  - Lack of hiding spaces
  - Lack of variability in the abiotic environment

Do we have to revise how we keep our fish?
What's the optimal tank size and environment?

- Ideal absolute or relative (per fish) volume?
- Ideal shoal size?
- Ideal sex ratio?
- Ideal structural aspects of the environment?

- Other physical parameters

Example 2: Water parameters

Guiding principle: Ethology and Ecology of the species

Gerlai, R., Clayton, N. S. (1999): Analysing hippocampal function in transgenic mice: An ethological perspective. *Trends Neurosci.*, 22: 47-51.

Each species has evolved in particular environments with characteristic ecological features, selection pressures and evolutionary past

Knowing these features should help design our fish husbandry and experimental procedures

Some beautiful examples

Tropheus moori kasakalawe
Lake Tanganyika
Lot of vegetable matter
High pH, High $CaCO_3$, $MgSO_4$
Highly sensitive to nitrates nitrites and NH_3

Pundamelia nyererei
Lake Victoria
Mixed animal and vegetable food
Medium high pH, High $CaCO_3$, $MgSO_4$
Insensitive to nitrates nitrites and NH_3

Enantiopus kilesa
Lake Tangayika
Animal protein based food
High pH, High $CaCO_3$, $MgSO_4$
Extremely sensitive to nitrates nitrites, NH_3
Extremely sensitive to chlorine and cholarime
Requires high oxygen level

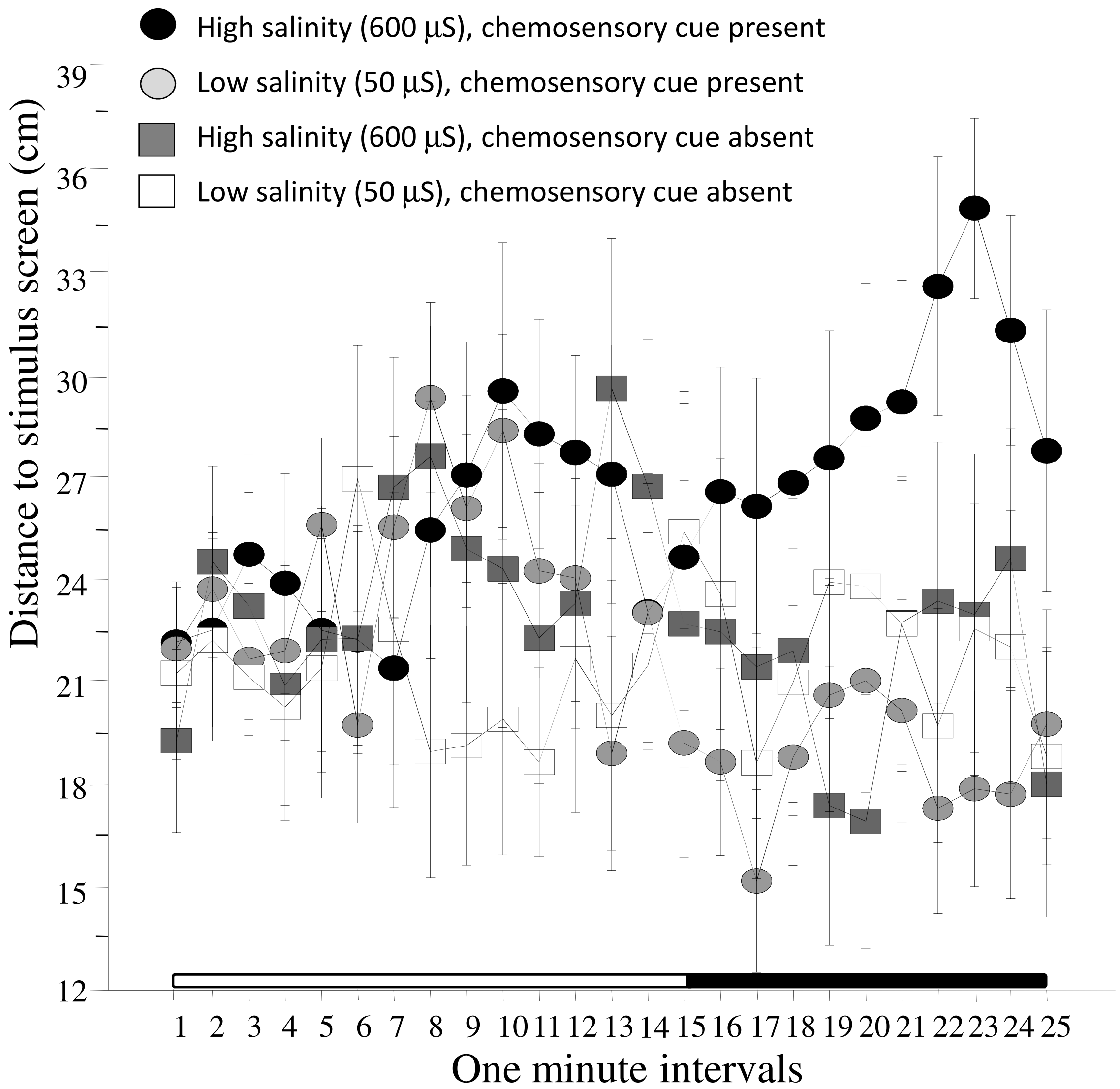
How about zebrafish?

Zebrafish in nature versus the lab

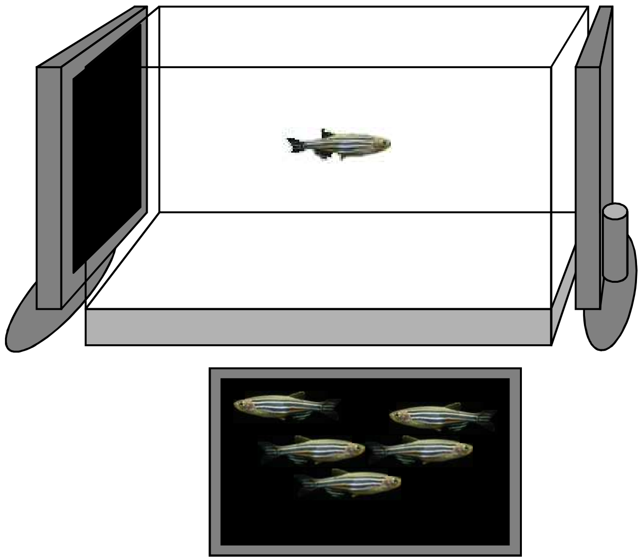
Natural habitat of zebrafish

- Biotic characteristics
- Abiotic characteristics
  - Absolute values and speed of change
  - Light intensity, wavelength-spectrum
  - Temperature and its variation
  - Water chemistry
    - pH: 5.9 to 8.1 → yet we overreact and dump $NaHCO_3$
    - Salinity 10-300 μS yet we employ 900-3000 μS
    - Salt composition: mainly $CaCO_3$ yet we use sea salt (NaCl)

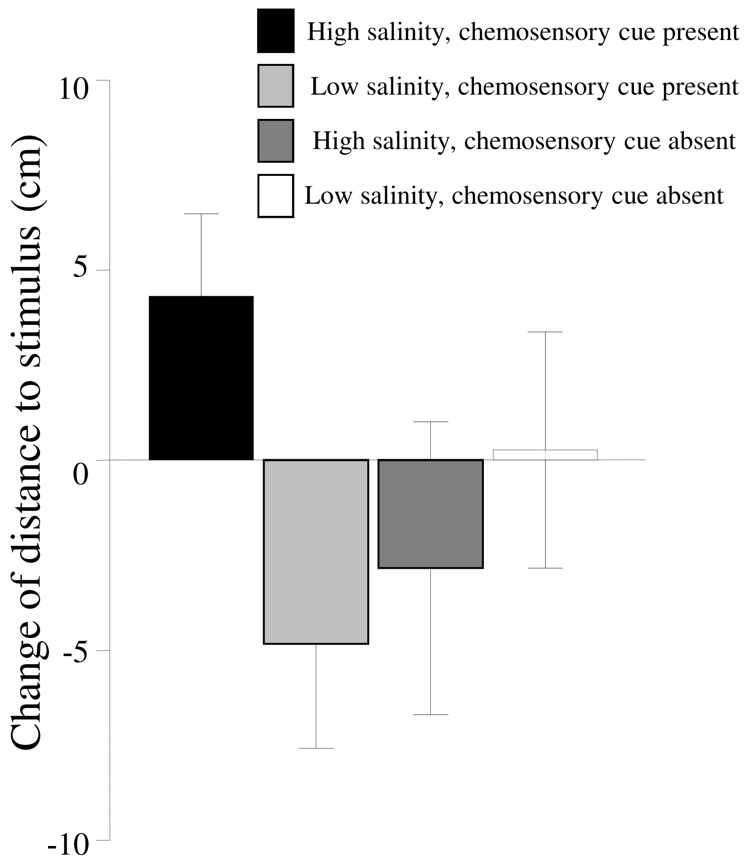
Salinity and the effect of olfactory cues



Samantha Mahabir

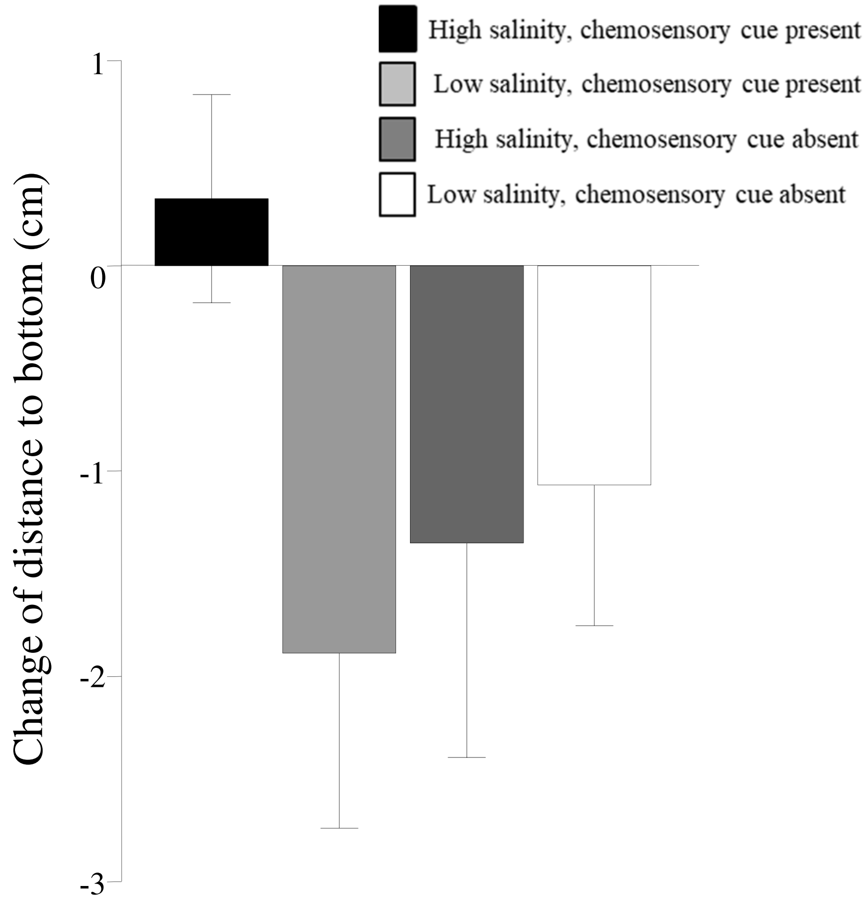


Salinity and the effect of olfactory cues



Mahabir S, Gerlai R (2017). The importance of holding water: Salinity and chemosensory cues affect zebrafish behavior. Zebrafish 4: 444-458

Salinity and the effect of olfactory cues



Mahabir S, Gerlai R (2017). The importance of holding water: Salinity and chemosensory cues affect zebrafish behavior. Zebrafish 4: 444-458

Salinity and olfactory cues interact and affect social and anxiety-related behavioral responses in a complex manner

Example 3: How to use zebrafish to model human Fetal Alcohol Spectrum Disorders

- 1 in 200 newborn with significant physical malformations due to fetal alcohol exposure (FAS)
- Milder cases are 10X more prevalent (1 in 20)

More severe forms

- Hyperactivity
- Impaired executive functioning
- Delinquency
- Self-injury

Milder cases

- Enhanced fear responses/anxiety
- Increased aggression
- Inappropriate sexual behavior
- **Abnormal social skills**

The argument for animal models

- Diagnosing milder FASD cases is difficult
- Mothers often deny they drank alcohol during their pregnancy
- Pregnancy during alcohol consumption is often not yet known
- Biomarkers are not available
- Treatment options are limited
- Mechanistic understanding is limited

Zebrafish FASD: Simplicity of alcohol delivery and other advantages

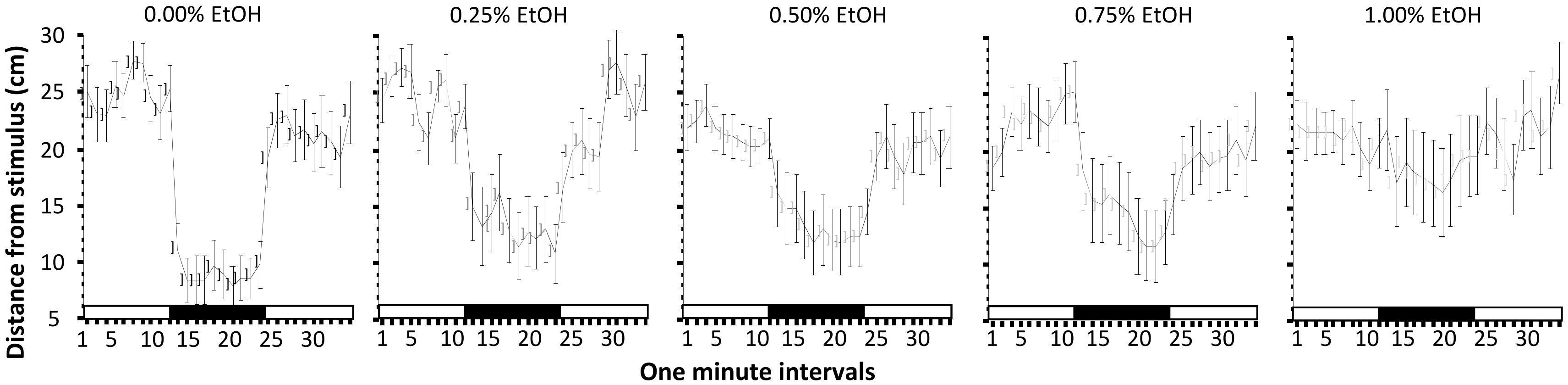
- Embryo develops externally →
  - Precise control over alcohol concentration
  - No complications associated with mother's physiology

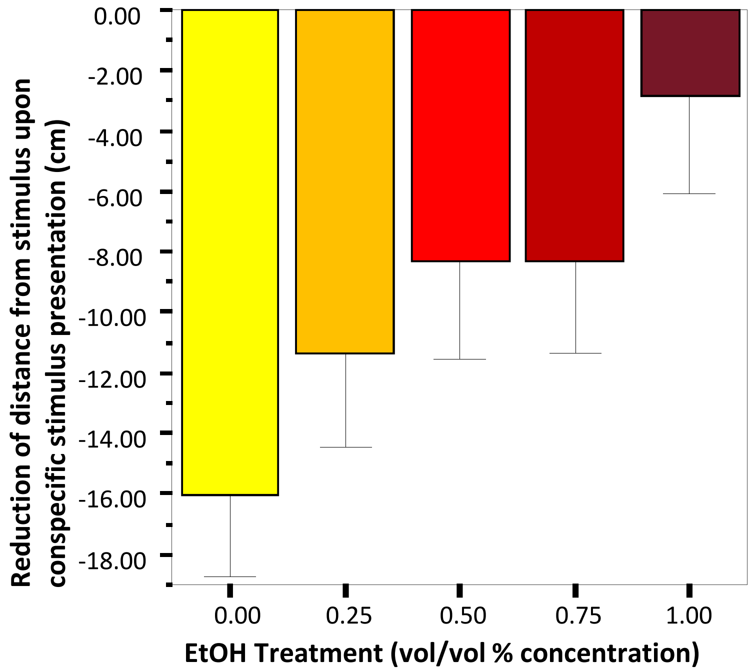
- Sophisticated genetic and neurobiology tool set →
  - Analysis of biological mechanisms

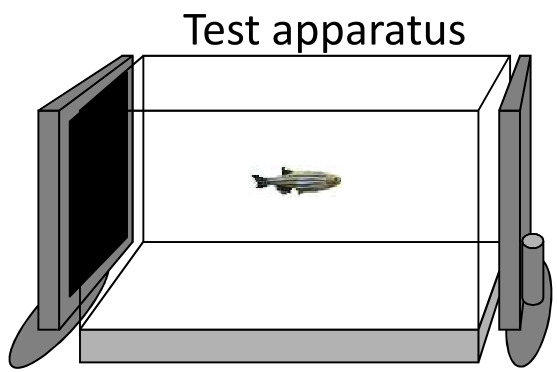
- Complex behavioral repertoire including social behavior →
  - Alcohol induced changes detected easily at the phenotypical level.

Fernandes Y, Gerlai R (2009). Long-term behavioral changes in response to early developmental exposure to ethanol in zebrafish. *Alcoholism: Clinical and Experimental Research*, 33: 601-609

Embryonic alcohol exposure at 24 hpf dose dependently impairs shoaling responses

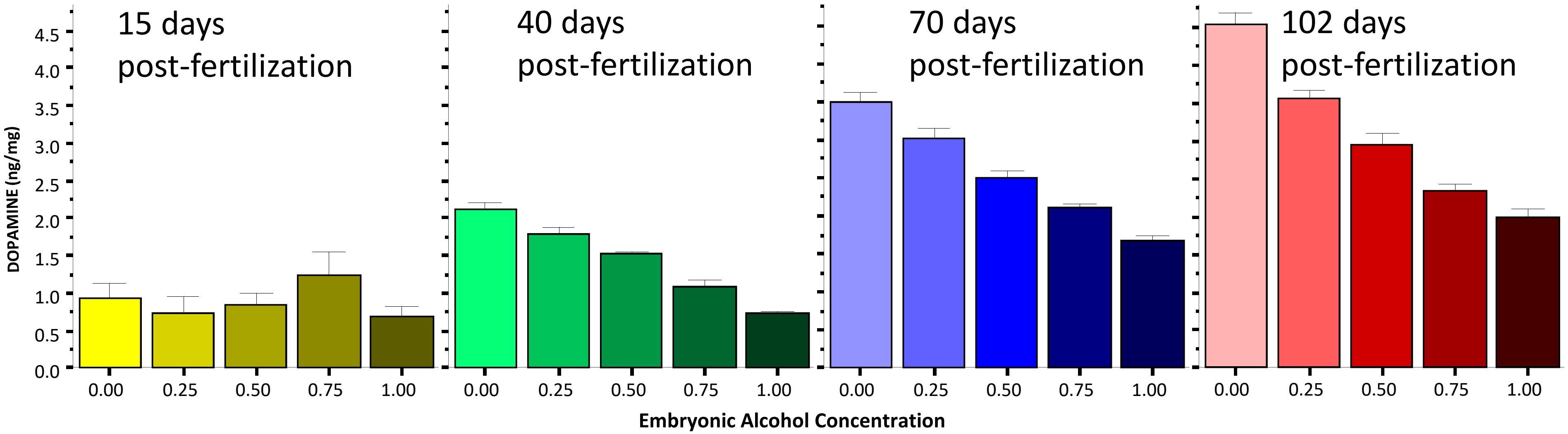






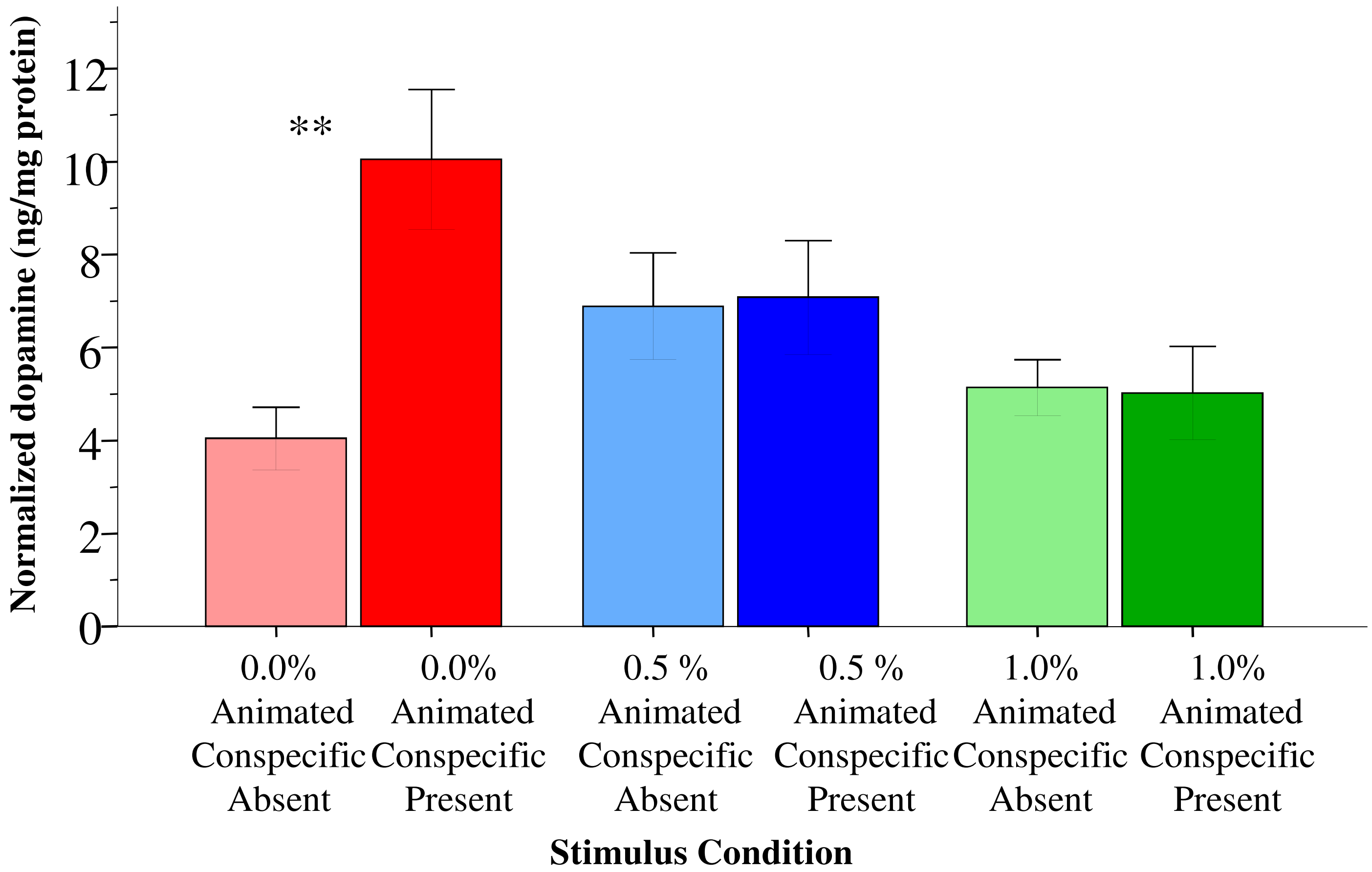
Fernandes Y, Gerlai R (2009). Long-term behavioral changes in response to early developmental exposure to ethanol in zebrafish. *Alcoholism: Clinical and Experimental Research*, 33: 601-609.

Embryonic alcohol exposure impairs the dopaminergic system: dose and age dependent reduction of dopamine levels



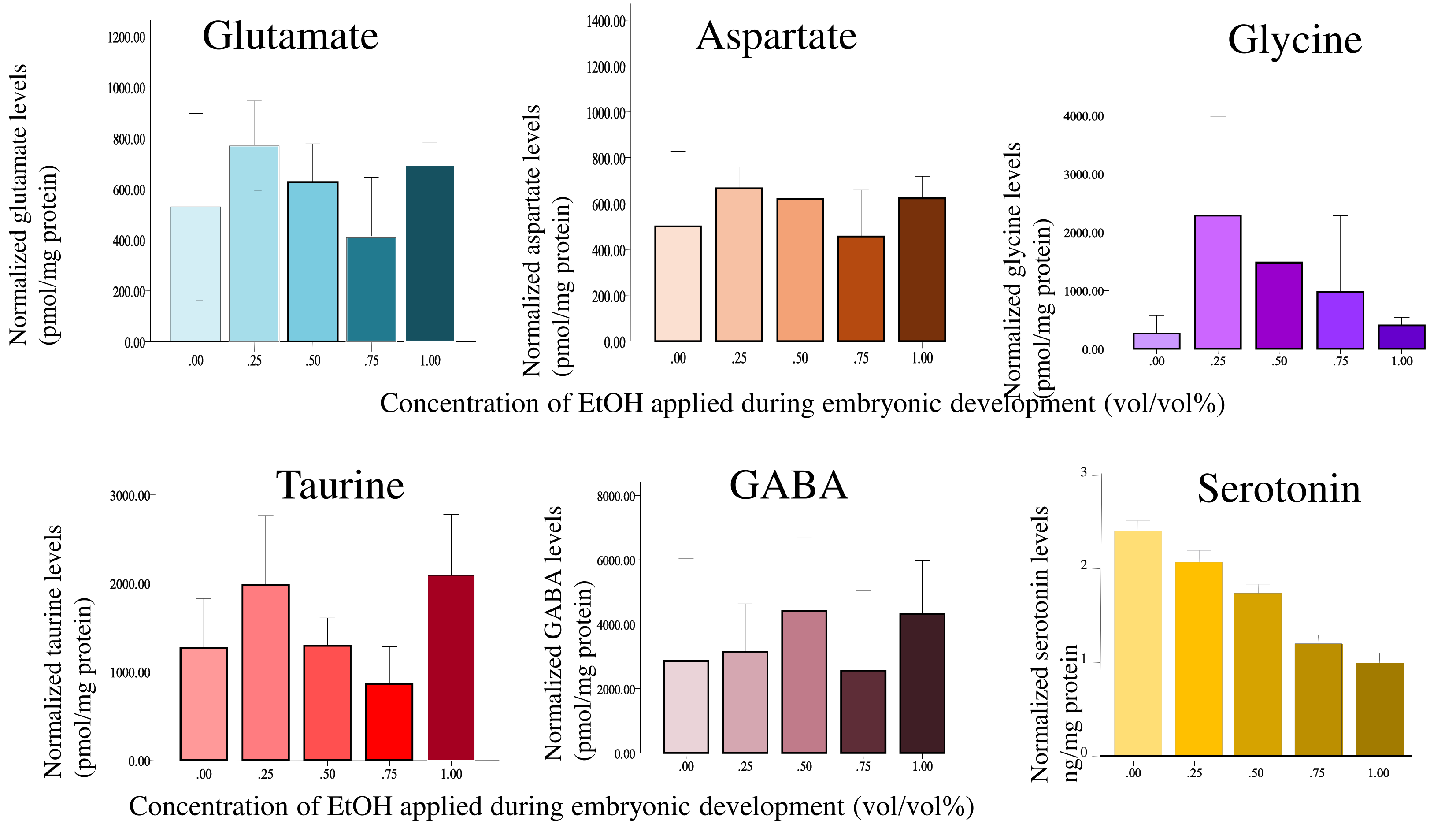
Mahabir S, Chatterjee D, Gerlai R (2013). Strain dependent neurochemical changes induced by embryonic alcohol exposure in zebrafish. *Neurotox Terat* 41:1-7

Conspecific images induce a rapid increase of dopamine levels, and embryonic alcohol treatment abolishes this response in adult

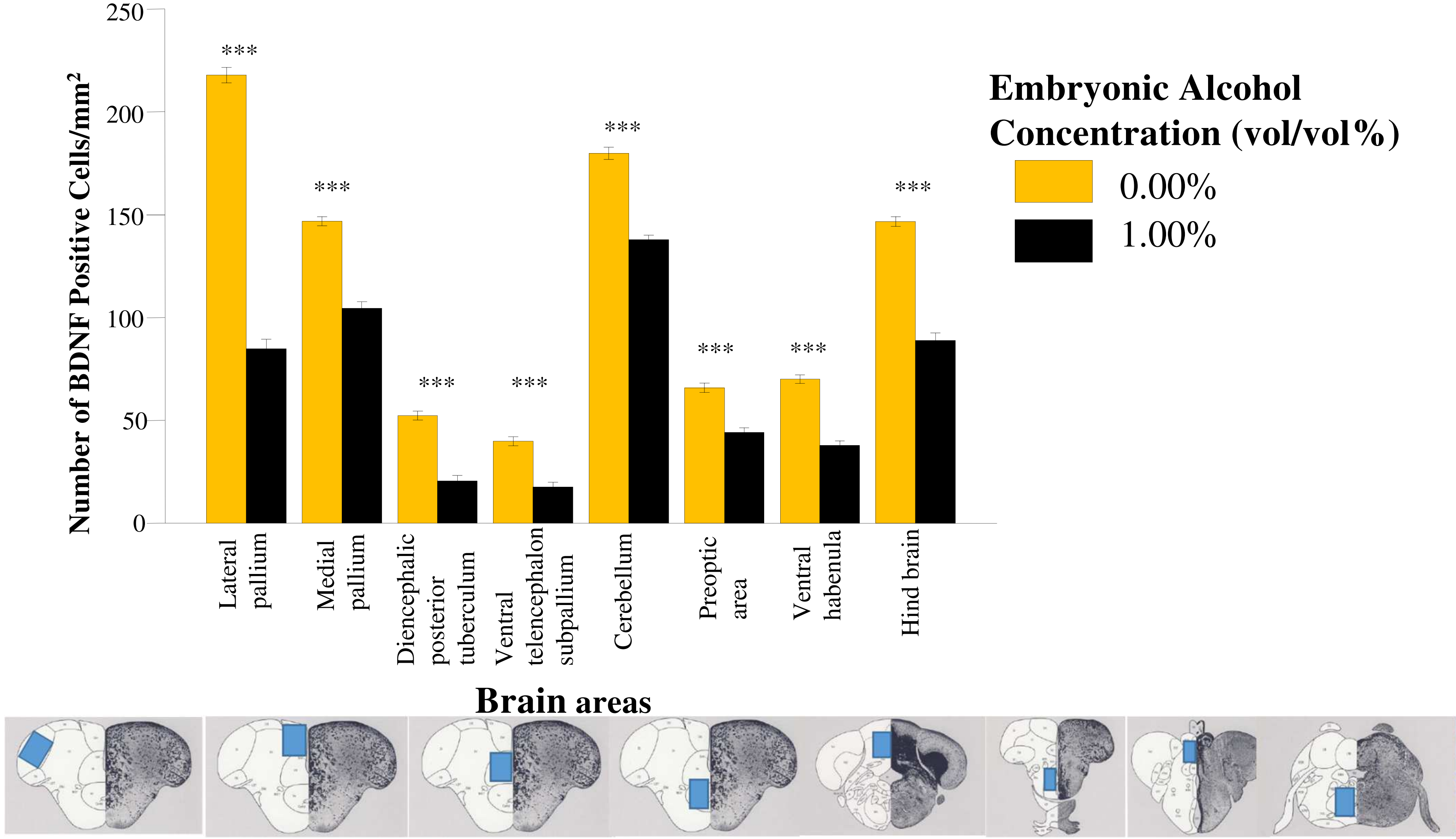


Fernandes Y, Rampersad M, Gerlai R (2015). Embryonic alcohol exposure impairs the dopaminergic system and social behavioural responses in adult zebrafish. The International Journal of Neuropsychopharmacol 18(6) 1-8.

All other neurotransmitter systems (glutamate, aspartate, glycine, taurine, GABA), except serotoninergic, remain unaltered in adult zebrafish



Number of cells expressing BDNF is reduced in the brain of adult zebrafish exposed to embryonic alcohol

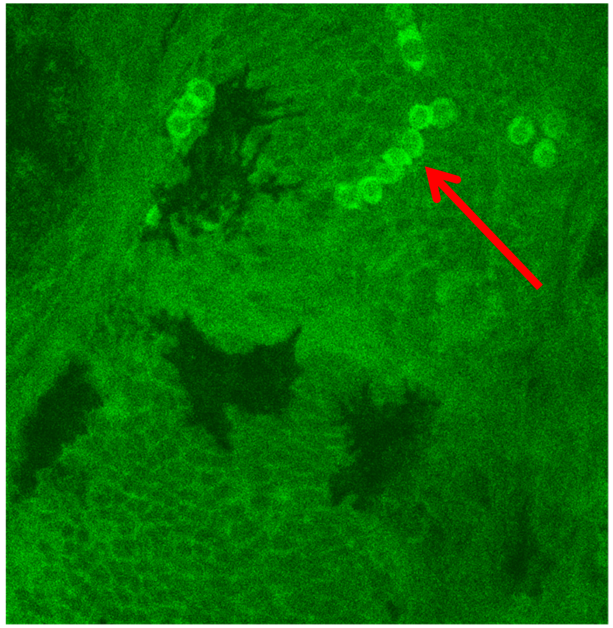


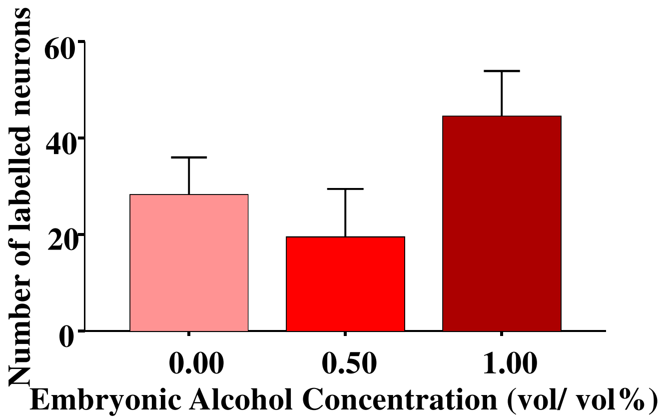
Alcohol exposure increases apoptotic cell death in the brain of the zebrafish embryo

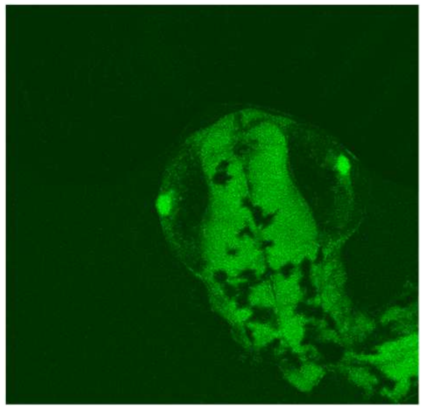
0.00% EtOH

0.50% EtOH

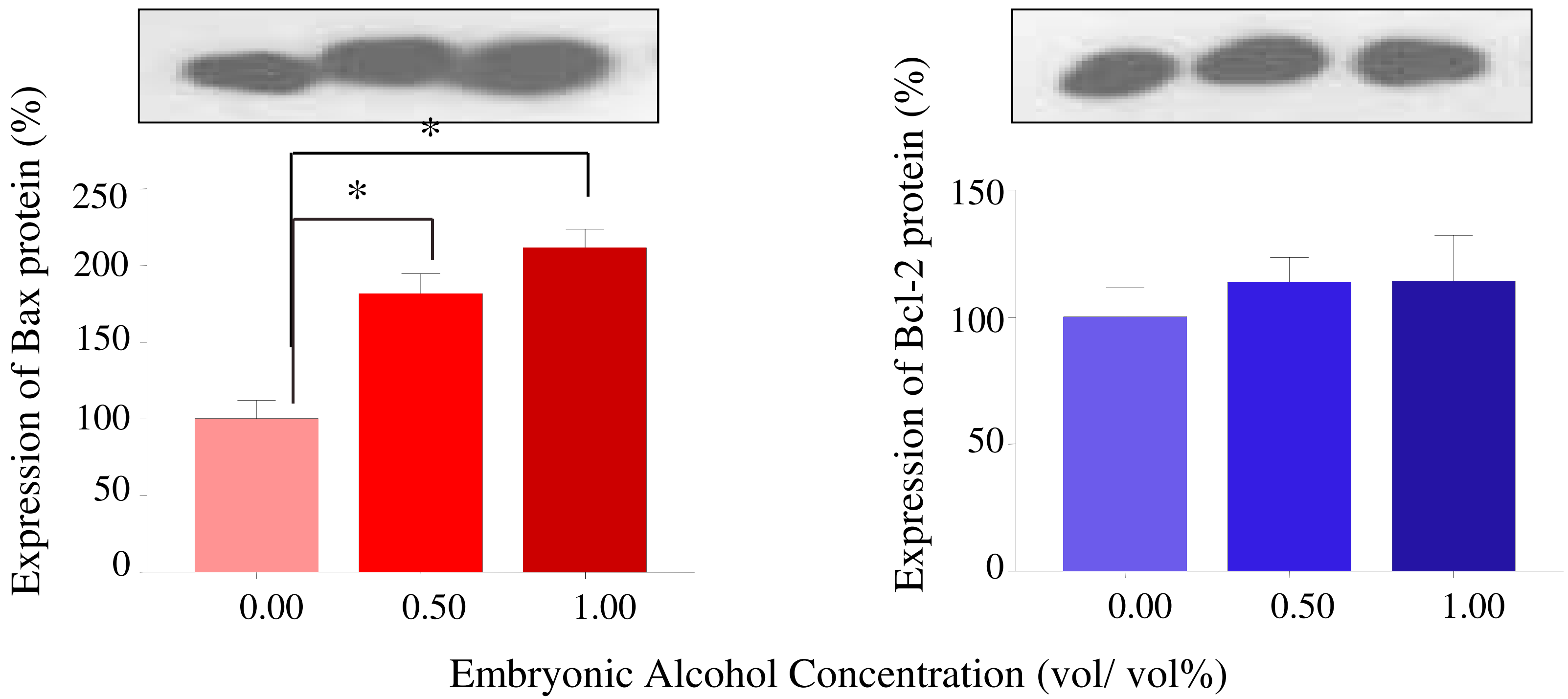
1.00% EtOH







Embryonic alcohol exposure increases pro-apoptotic protein expression without affecting anti-apoptotic protein expression in the embryonic zebrafish brain



Our current theory:

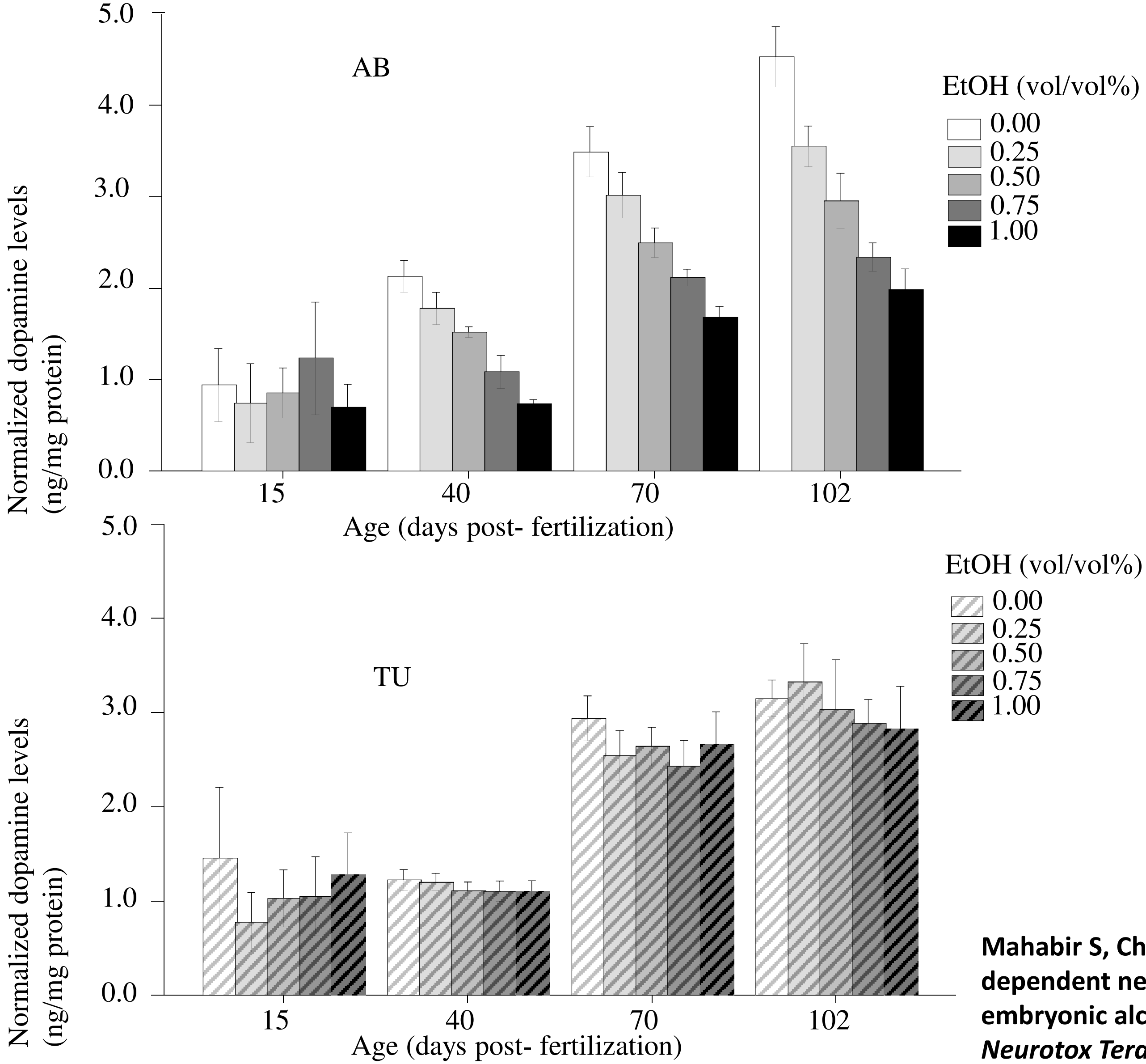
- **Alcohol increases apoptotic cell death in the embryonic zebrafish brain**
- **While the actual number of dopaminergic and/or serotoninergic neurons may not change (?), the enhanced cell death may lead to reduced connectivity/neuronal communication.**
- **When engaged, the activity of the dopaminergic (and maybe also the serotoninergic) system of the adult fish is blunted.**
- **The impaired activity of this (these) neurotransmitter system(s) leads to reduced expression of proteins involved in neuronal communication and plasticity.**
- **Reduced neuronal communication and plasticity results in impaired responding to complex social stimuli.**

But, all these alcohol induced changes are absent when the fish are raised in “enriched” environment

- Accidental finding
- We do not yet know what “enriched” means

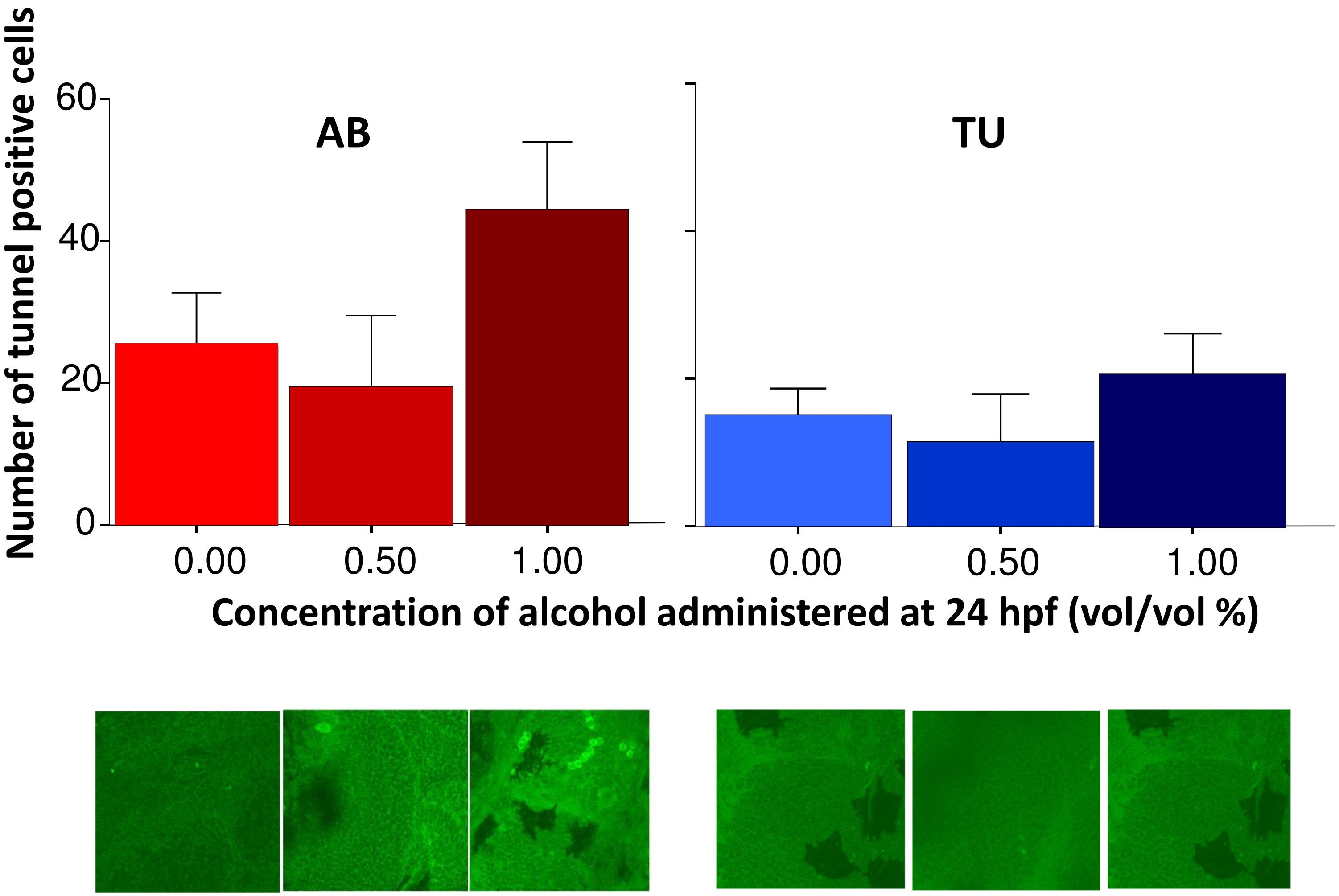
Genes also exert their effects on embryonic alcohol induced changes

Dose- and Age-dependent Decrease In Dopamine in AB but not in TU zebrafish



Mahabir S, Chatterjee D, Gerlai R (2013). Strain dependent neurochemical changes induced by embryonic alcohol exposure in zebrafish. *Neurotox Terat* 41:1-7

Strain differences in apoptotic cell death induced by embryonic alcohol exposure: AB responds, TU is protected



Overall summary

- Sophisticated models and methods
- Increased sensitivity to known and unknown environmental and genetic confounding factors
- Systematic analyses of optimal maintenance conditions is needed
- Learning from features of the natural habitat of zebrafish likely will speed up progress
- Few known changes recommended are:
  - Larger tanks, enriched environment, lower salinity, calcium carbonate based salts, speed of water parameters change more important then absolute value.

Acknowledgements

Many talented students & collaborators: over 100 fish papers in the past 6 years from the Gerlai lab

NIAAA
National Institute on Alcohol Abuse and Alcoholism

National Institutes of Health

NSERC
CRSNG

Facciol A, Tran S, Chow H, Tsang B, Gandhi P, Desai P, Fulcher N, Shams S, Chatterjee D, Nowicki M, Seguin D, Rampersad M, Fernandes Y, Luchiari AC, Gomez-Laplaza L, Muraleetharan A, Jia J, Salajan DC, Talpos A, Markovic S, Buske C, Abraham E, Qin M, Wong A, Pannia E, Mahabir S, Cheung E, Saif M, Miller N, Greene K, Dydynski A, Ahmed TS, Karnik I, Scerbina T, Pan Y, Luca R, Sison M, Ahmed O, Grella S, Kapur N, Parra KV, Adrian JC, Pereira T, Pather S, Sawashima T, Krishnannair R, Prajapati S, Saverino C, Al-Imari L, Speedie N, Bass SLS, Kalueff AV, Stewart AM, Gebhardt M, Stewart AM, Cachat JM, Brimmer M, Chawla JS, Craddock C, Kyzar EJ, Roth A, Landsman S, Gaikwad S, Robinson K, Baatrup E, Tierney K, Shamchuk A, Norton W, Miller N, Nicolson T, Braubach O, Gilman CP, Pittman J, Rosemberg DB, Gerlai R, Echevarria D, Lamb E, Neuhauss SCF, Weng W, Bally-Cuif L, Schneider H.