



# **SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety**

## **Zebrafish as an Alternative Species for Developmental Neurotoxicity Testing that can Provide Hazard Identification and Mechanistic Information**



**Randall Peterson  
Massachusetts General Hospital  
Harvard Medical School  
Broad Institute**

# Disclosures and Funding

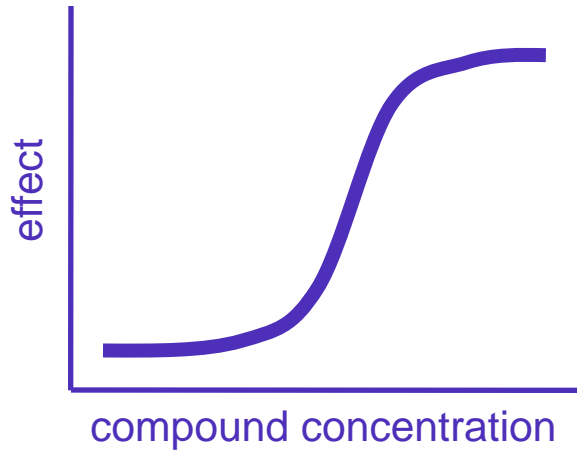
Randall Peterson holds equity in Teleos Therapeutics, Keros Therapeutics and Tolero Pharmaceuticals.

The Peterson laboratory has received funding from:

- Hoffmann La Roche
- NIH
- AHA
- USDA
- US Army
- Novartis
- Merck



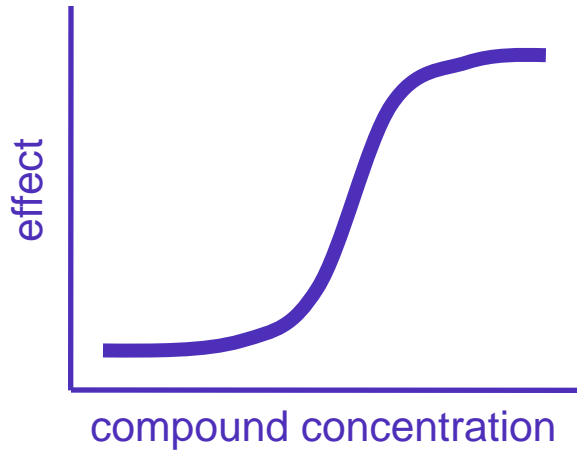
# Defining Compound Activity: Simplicity Versus Complexity



- single, well-defined target
- single, quantifiable activity



# Defining Compound Activity: Simplicity Versus Complexity

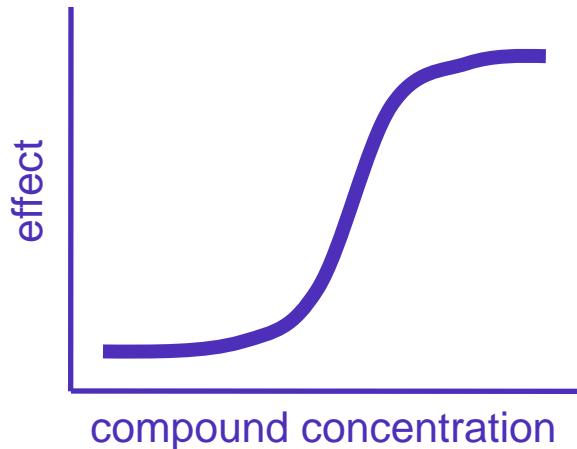


- single, well-defined target
- single, quantifiable activity



- multiple targets, often unknown
- complex, interconnected activities

# Defining Compound Activity: Simplicity Versus Complexity



- single, well-defined target
- single, quantifiable activity



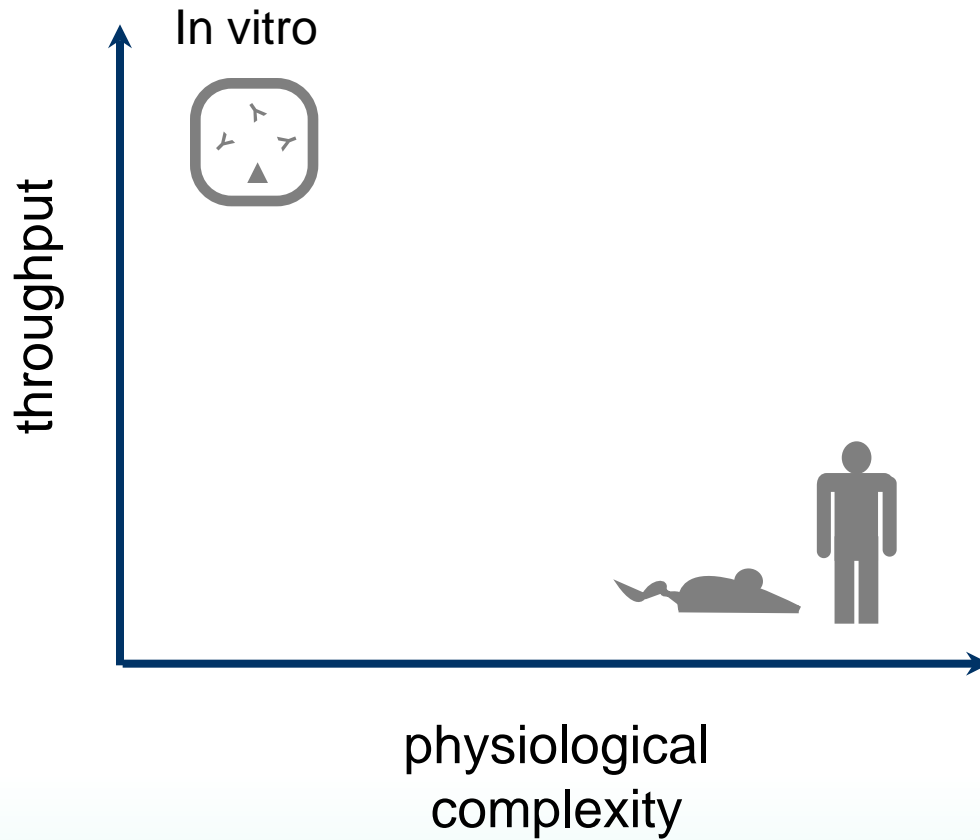
- multiple targets, often unknown
- complex, interconnected activities

*How might we better define the activity of compounds on complex systems?*

*How might we identify compounds with detrimental effects on the nervous system?*

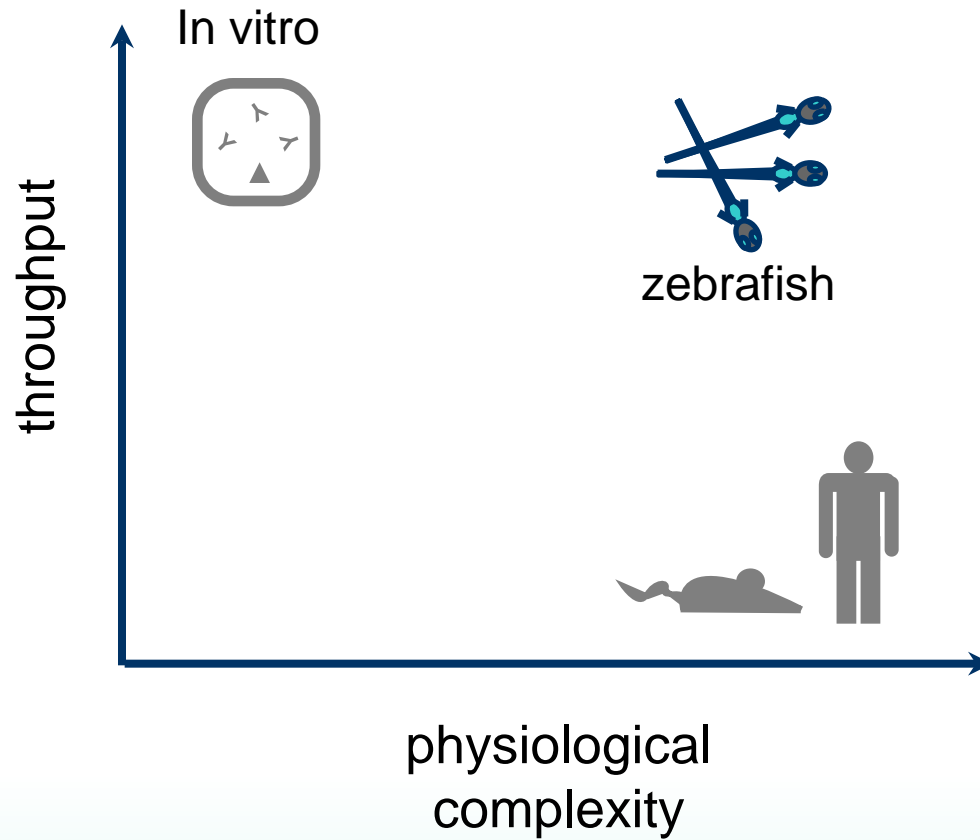


# Scale Versus Complexity





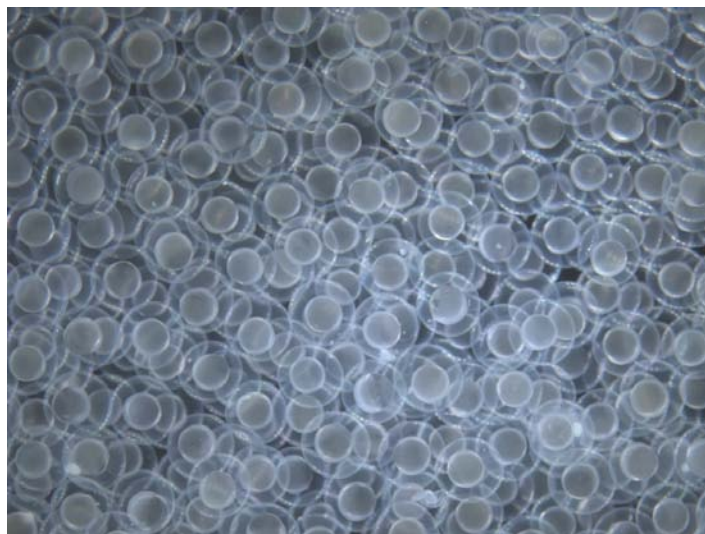
# Combining the Best Features of *In Vitro* and *In Vivo* Approaches



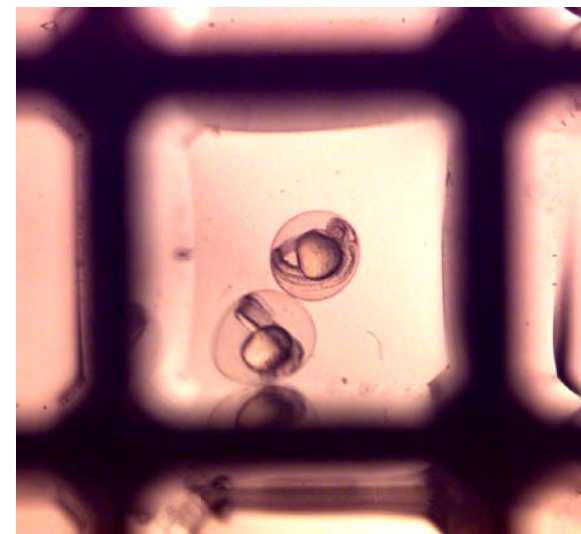
# *In Vivo* Biology at *In Vitro* Scale and Cost



inexpensive housing



high fecundity

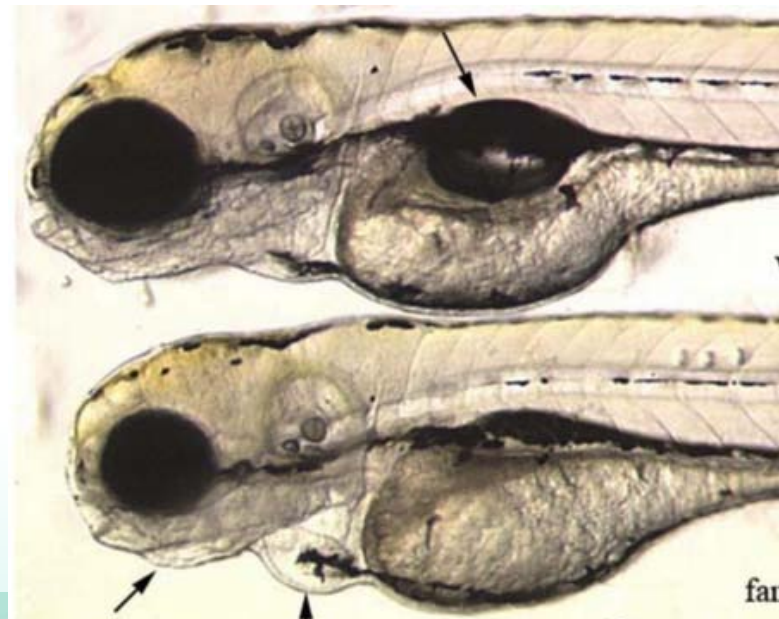
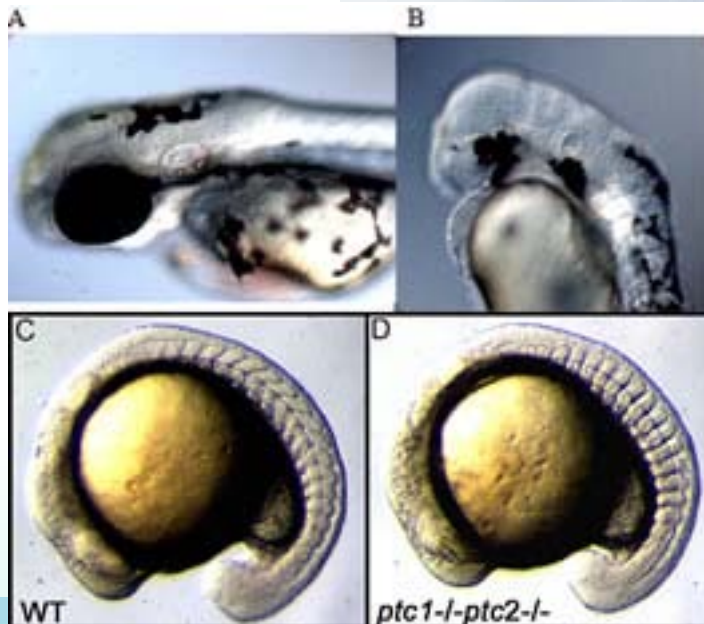
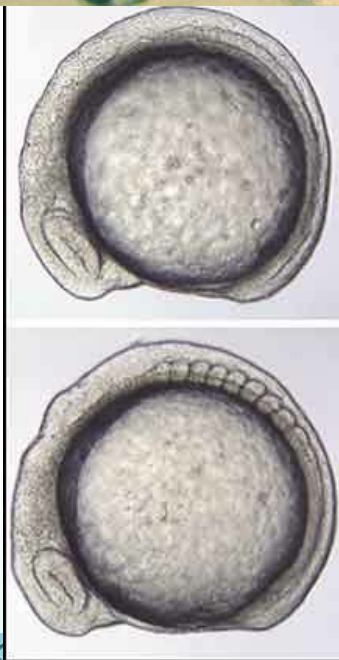
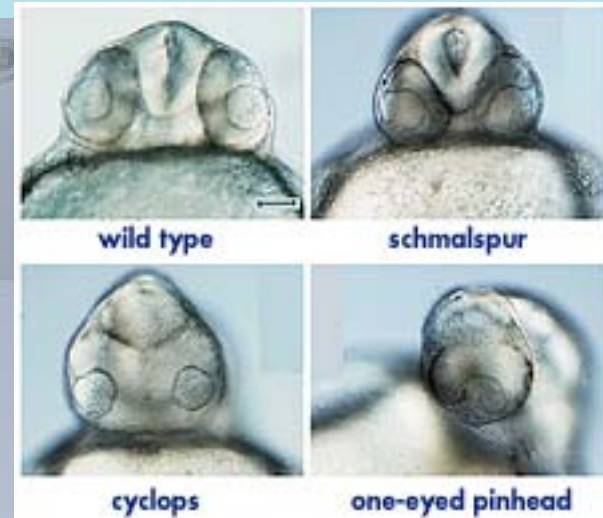
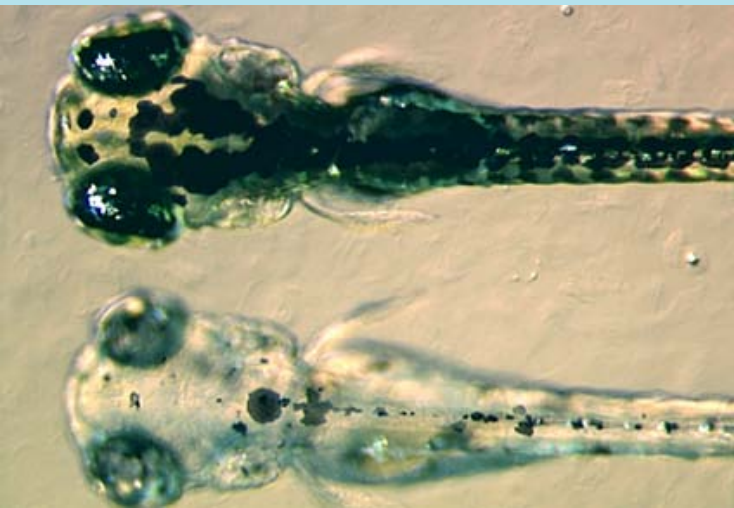


miniature scale

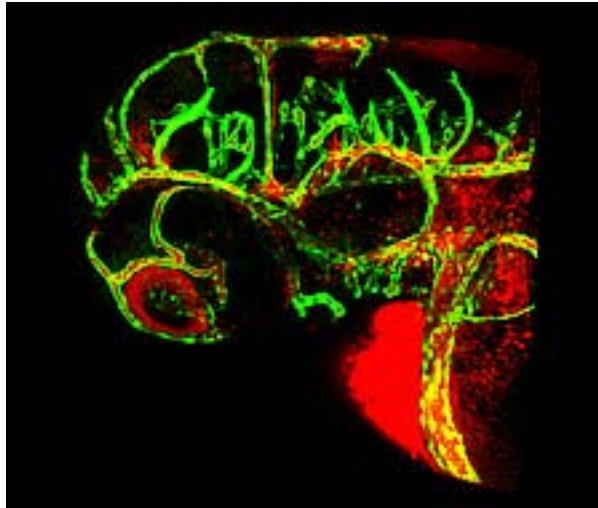




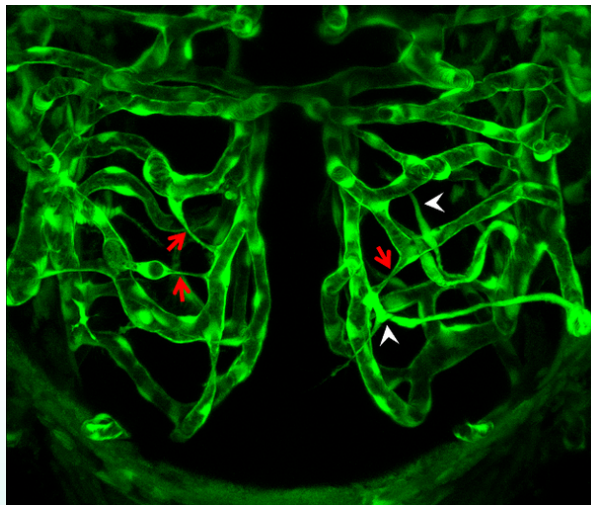
# A Rich Repertoire of Phenotypes



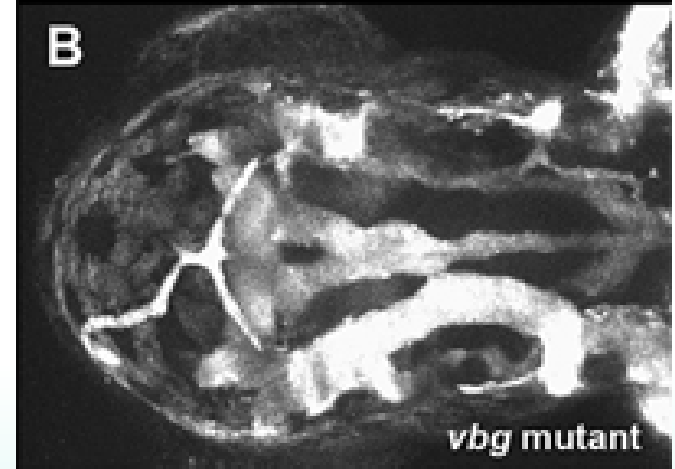
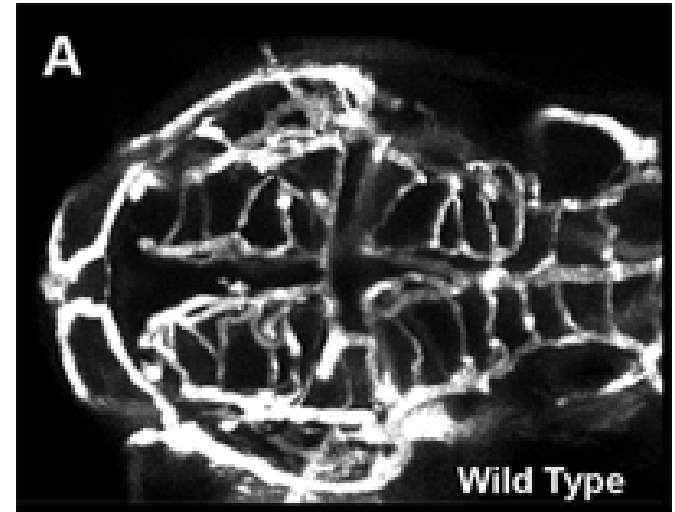
# Transparency Enables Facile Visualization



Markus Affolter



Jiu-lin Du



Brant Weinstein



# Zebrafish are Increasingly Used in Toxicology

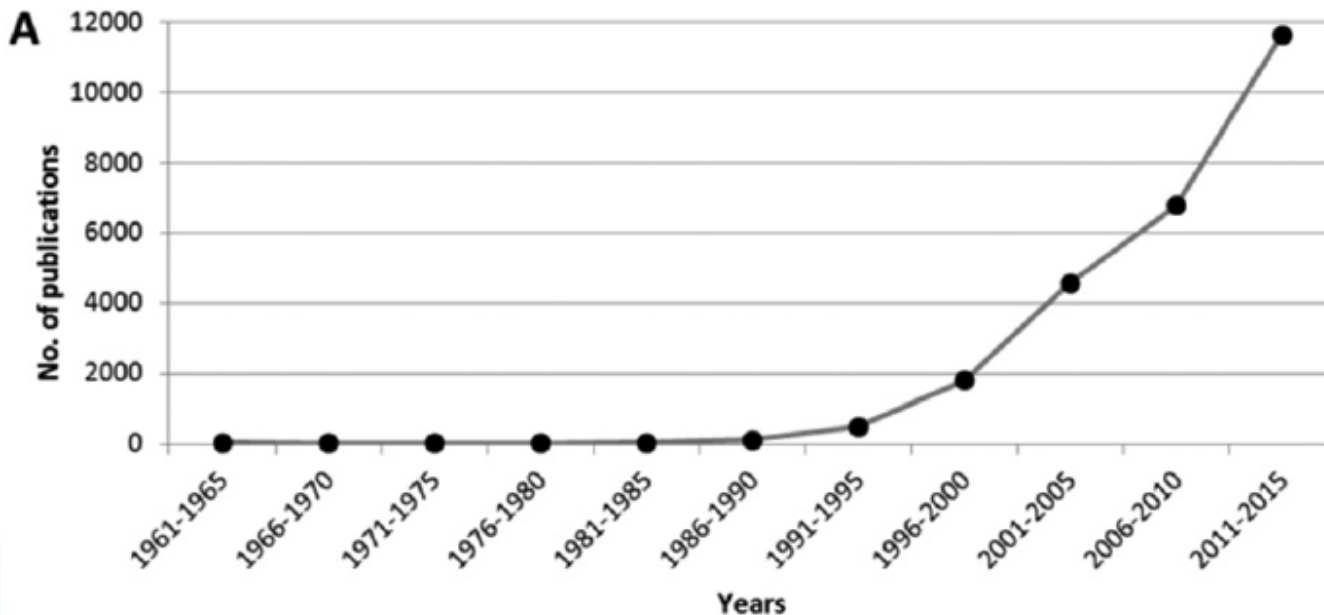
Ecotoxicology

Developmental toxicology

Cardiotoxicity – QT prolongation

Organ toxicity – liver, kidney, GI, blood

Nervous system



Caro et al, Food Funct., 2016, 7, 2615



# Applications in Food Safety and Efficacy

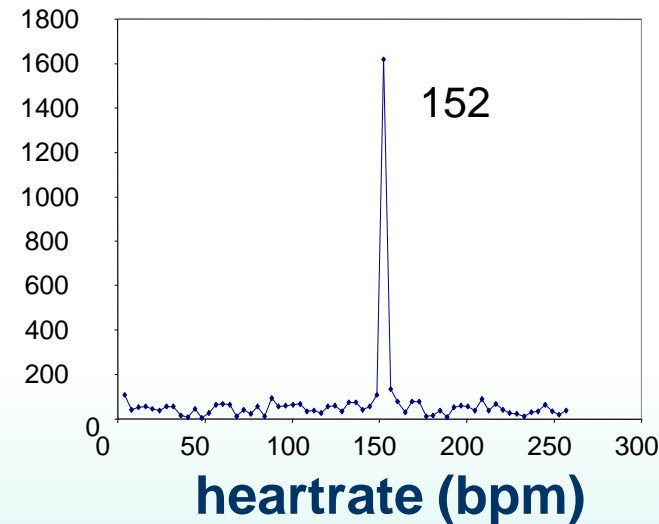
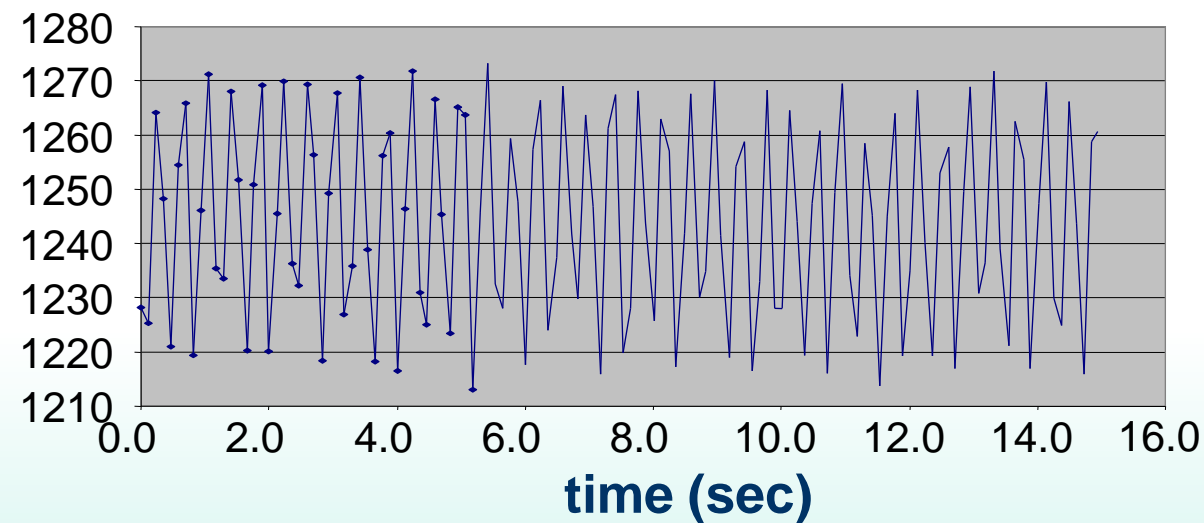
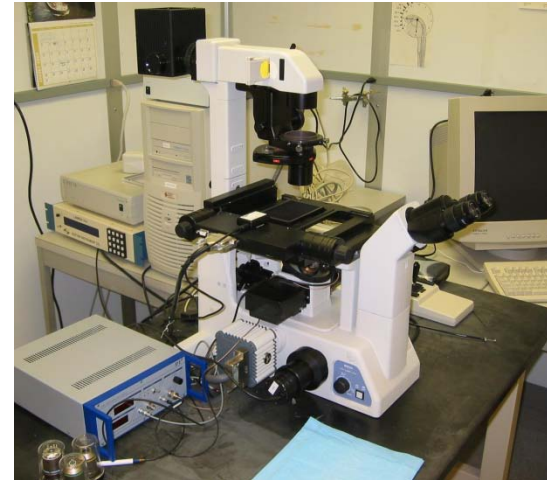
- Food contaminants
  - pesticides e.g. atrazine
  - endocrine disruptors e.g. bisphenol A
  - natural products e.g. mycotoxin
  - nanomaterials e.g. silver nanoparticles
  - byproducts e.g. acrylamide
- Food efficacy
  - antioxidants e.g. flavenoids
  - neuroprotectants e.g. quercetin
  - immunomodulators e.g. cinnamon extract
  - metabolic modulators e.g. coffee polyphenols

Caro et al, Food Funct., 2016, 7, 2615





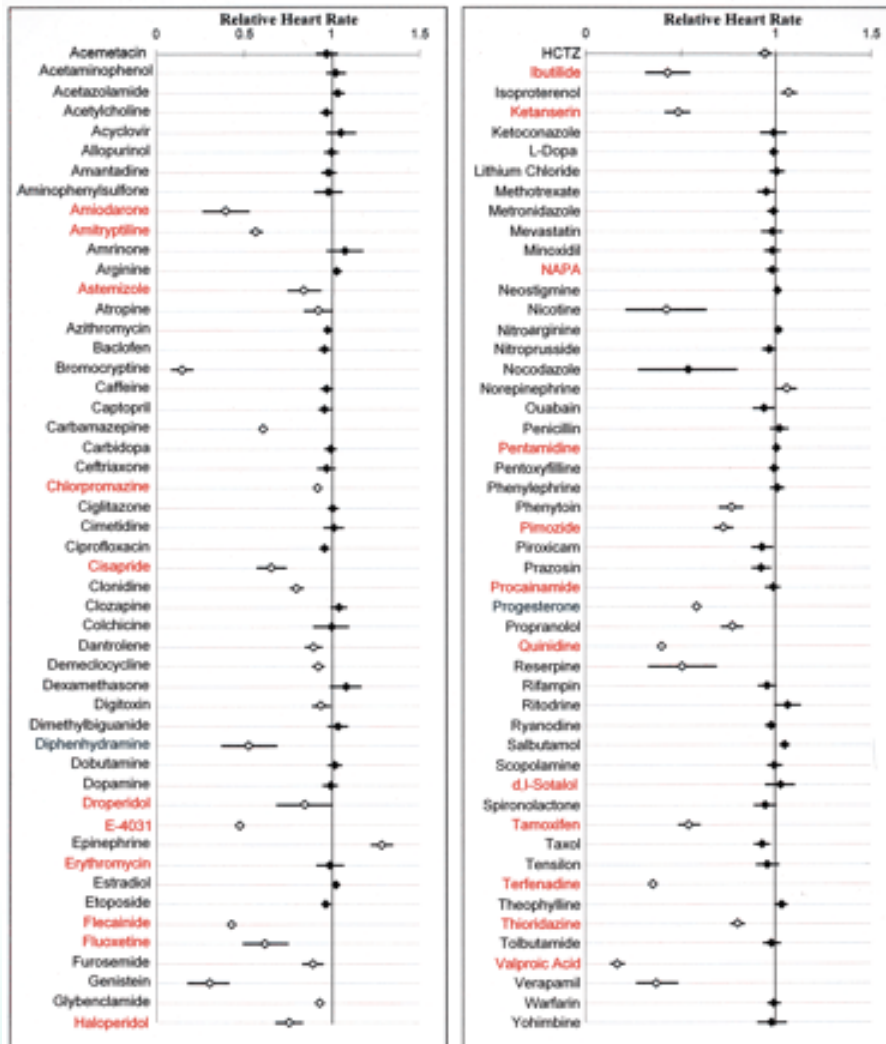
# Automated Phenotyping in 384-Well Format



David Milan, Calum MacRae, Geoff Burns



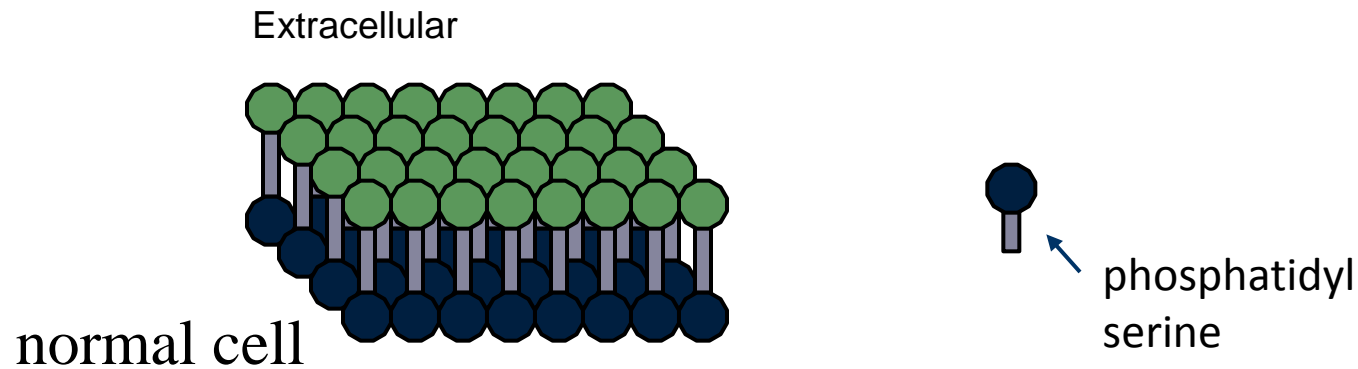
# High Percentage of Conservation Between Zebrafish and Human Cardiotoxicity



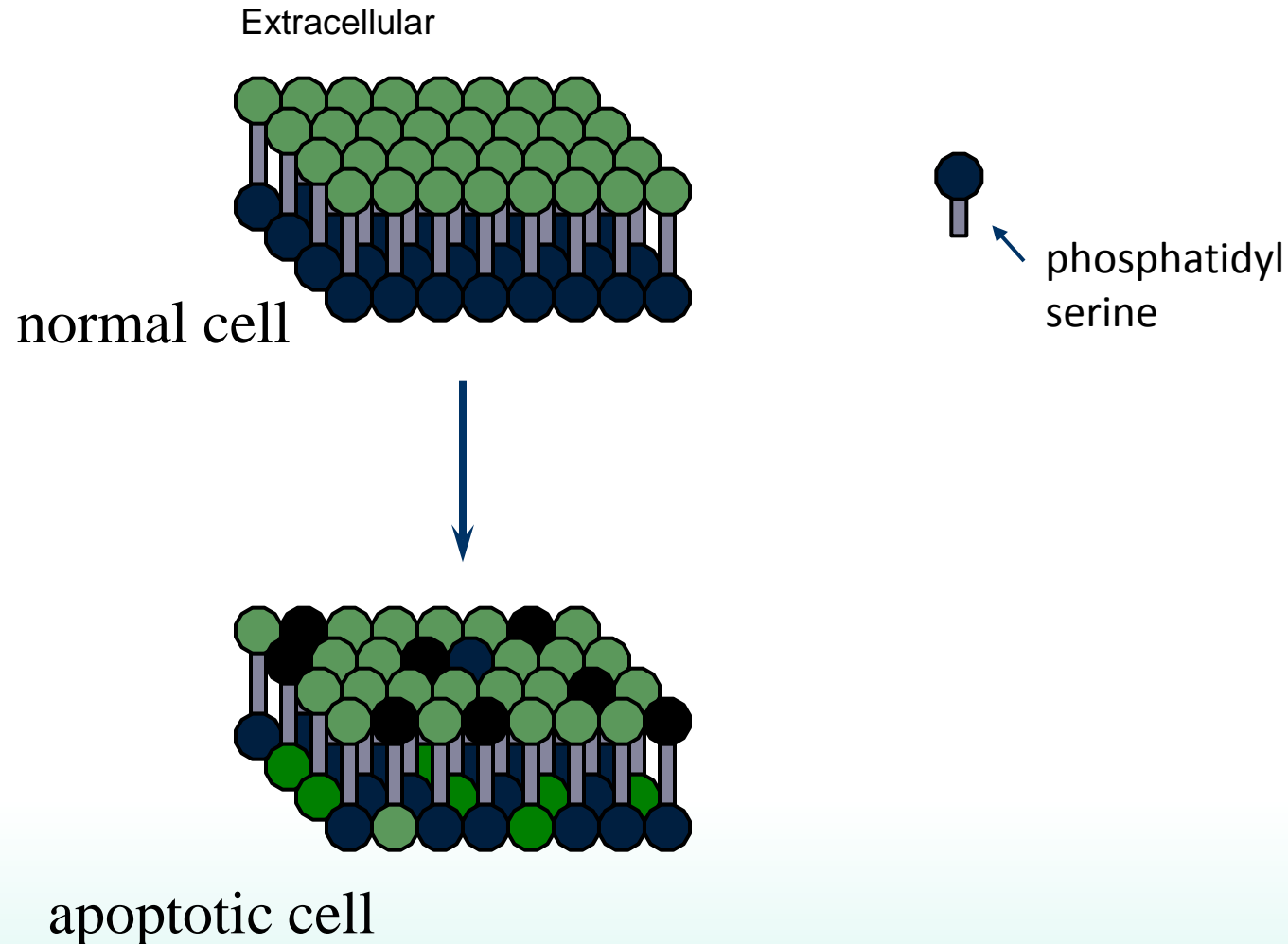
Milan et al, Circulation.  
2003;107:1355-1358



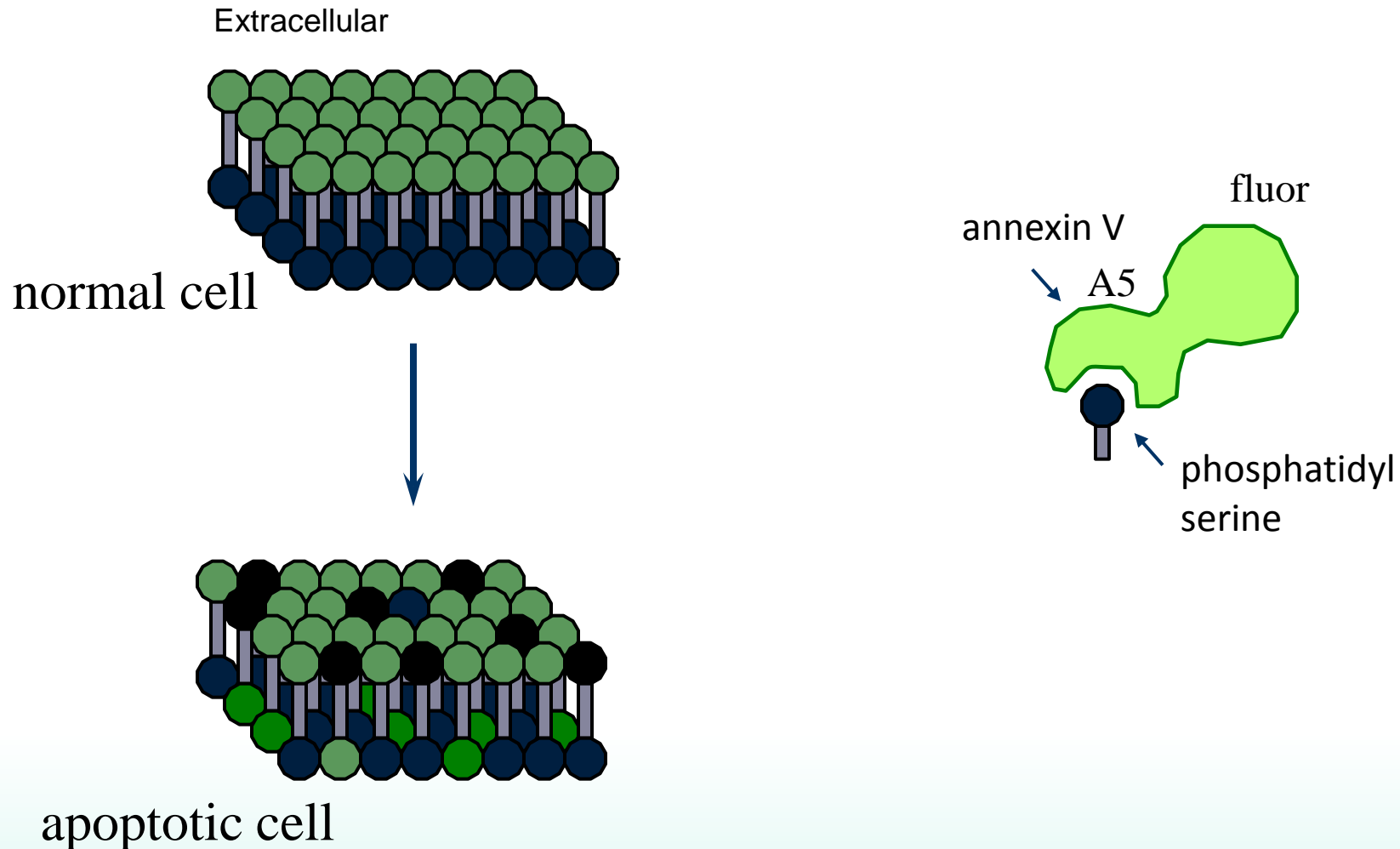
# Exploiting Loss of Membrane Asymmetry for Apoptosis Detection



# Exploiting Loss of Membrane Asymmetry for Apoptosis Detection

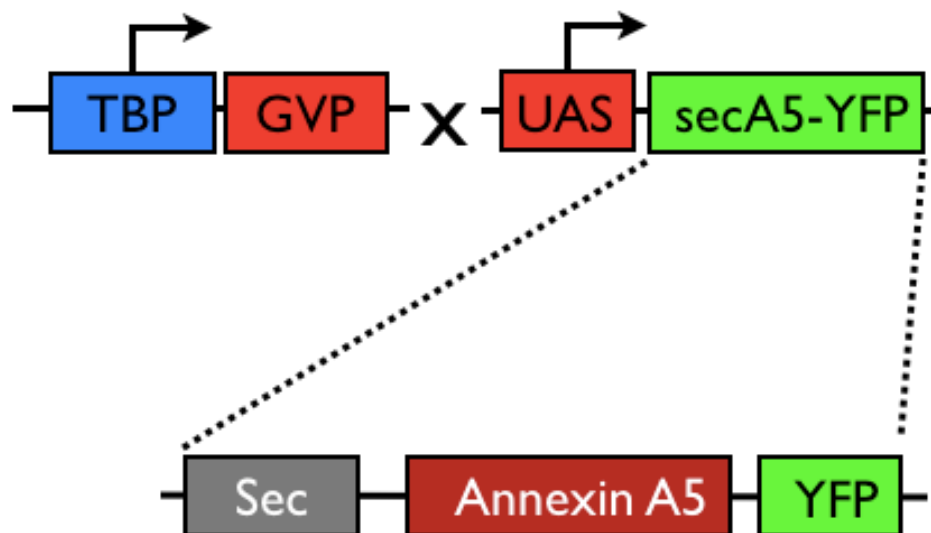


# Exploiting Loss of Membrane Asymmetry for Apoptosis Detection



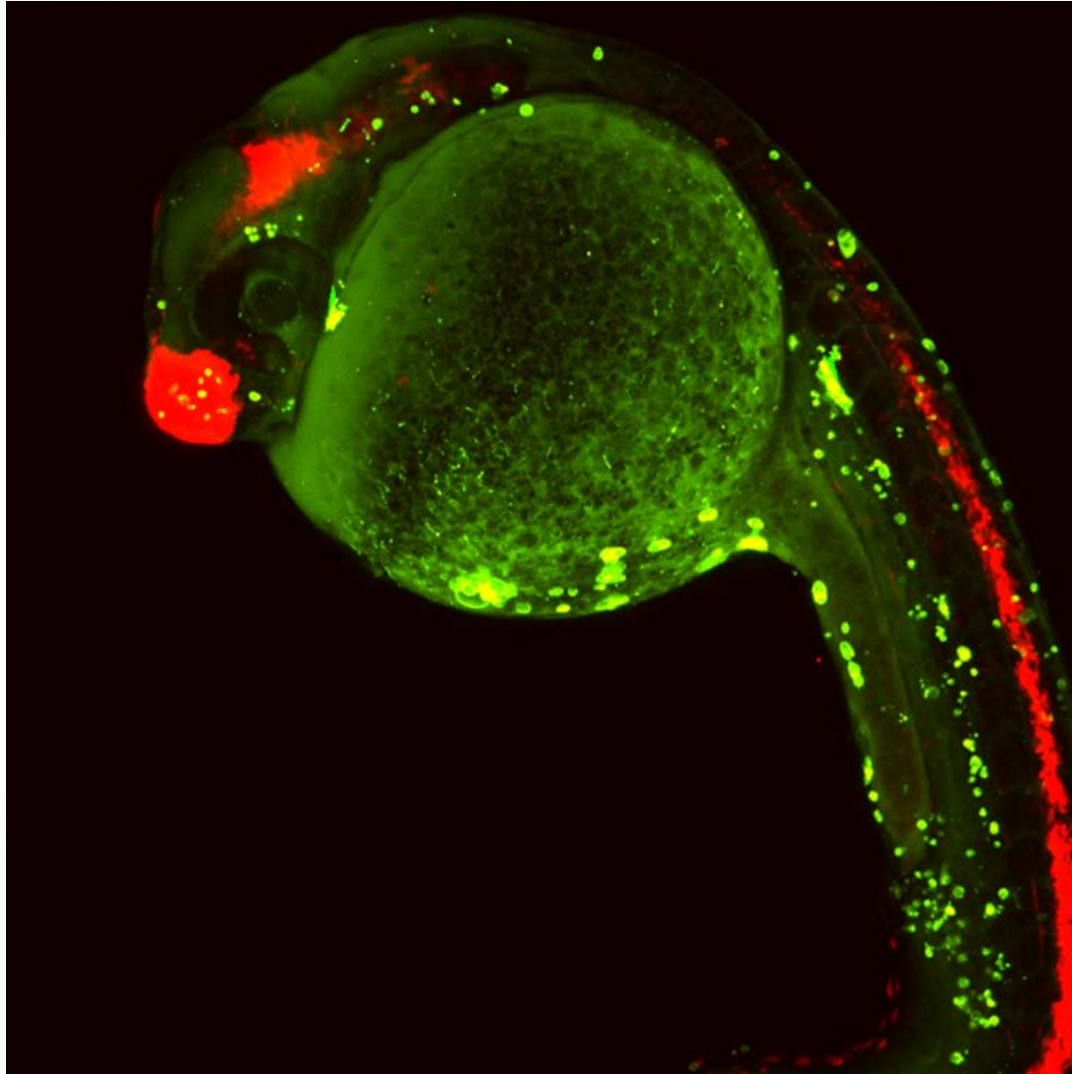
# Creation of an *In Vivo* Apoptosis Reporter

Expression system:



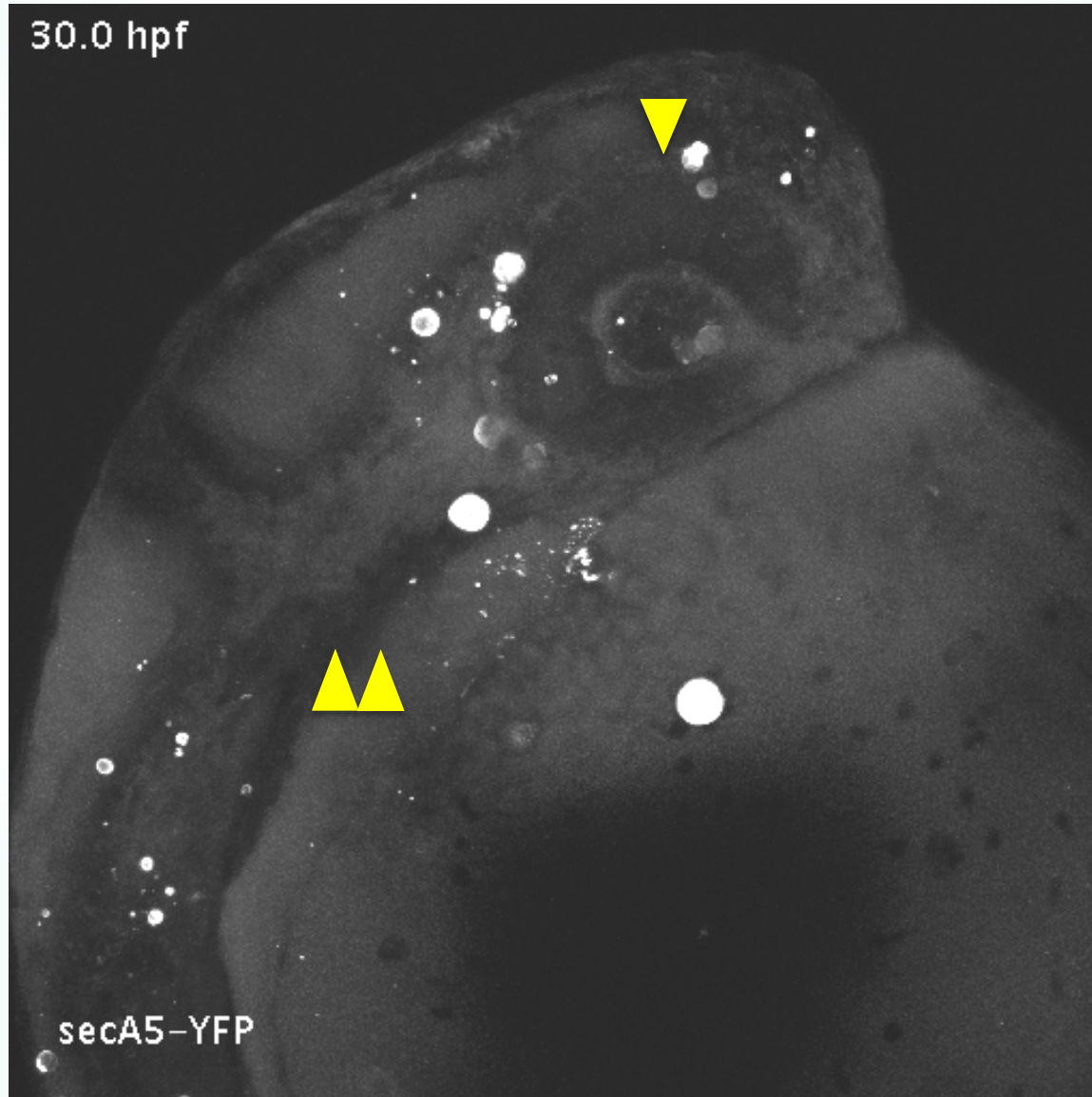


# Facile Detection of Apoptotic Cells in Zebrafish



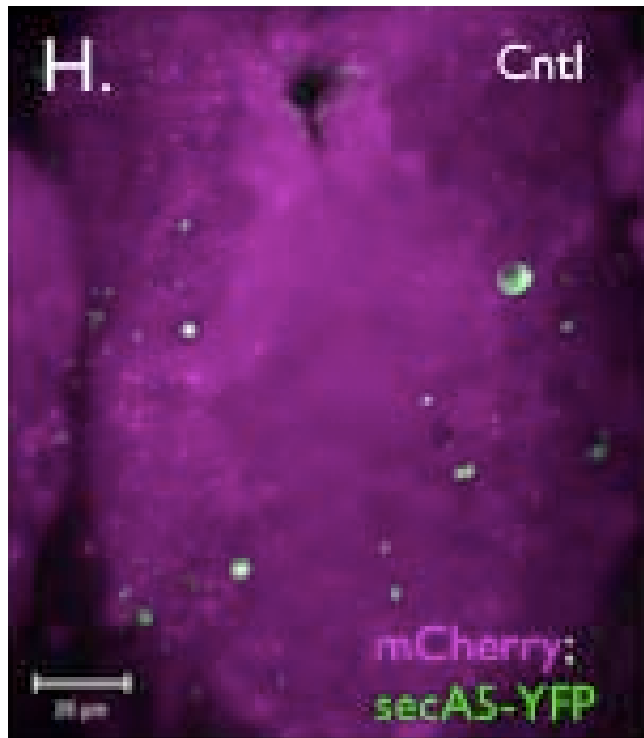
Tjakko van Ham

# secA5-YFP Fish Exhibit Dynamic, Fluorescent Cells

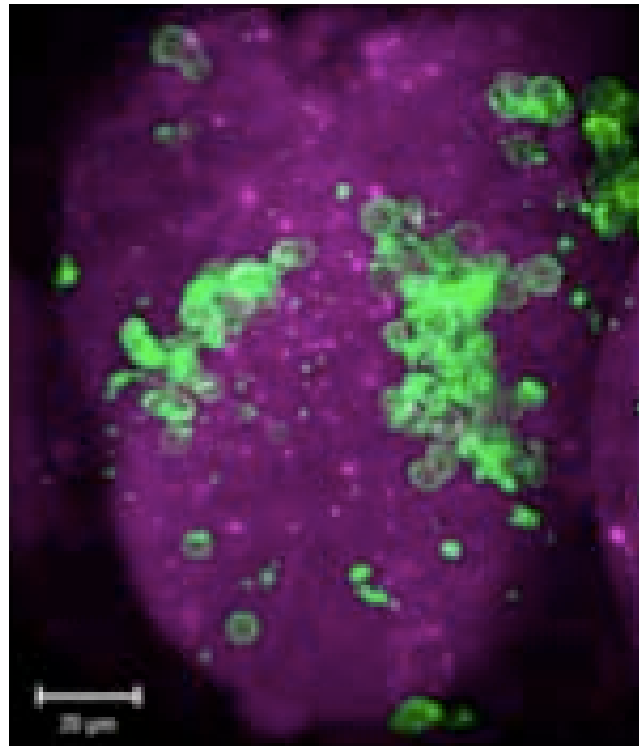


Video posted  
on [vimeo.com](https://www.vimeo.com)

# Detection of Localized Apoptosis in the Forebrain



control



pu.1 knockdown

Tjakko van Ham

# High-Throughput Assessment of Compound Effects on CNS Function

## Zebrafish exhibit a rich repertoire of behaviors

- Stimulus-response behaviors (light, sound, food, pain, etc.)
- Sleep and wakefulness
- Associative learning
- Non-associative learning
- Fear
- Aggression
- Addiction
- Social interaction



*The genes, cells, and circuits that enable these behaviors are very similar to those involved in human CNS disorders.*

# High-Throughput Behavior-Based Chemical Screening

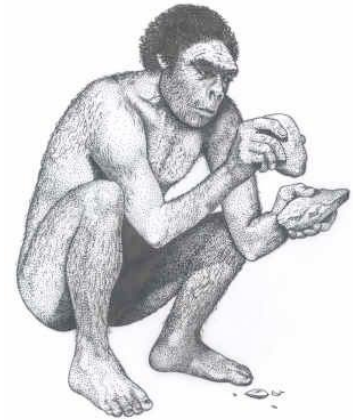


Dave Kokel



Andrew Rennekamp

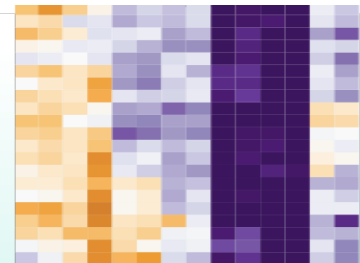
tools



assays



analytics





# Sauron: A Tool for Automated Behavioral Analysis

1 day

20 plates

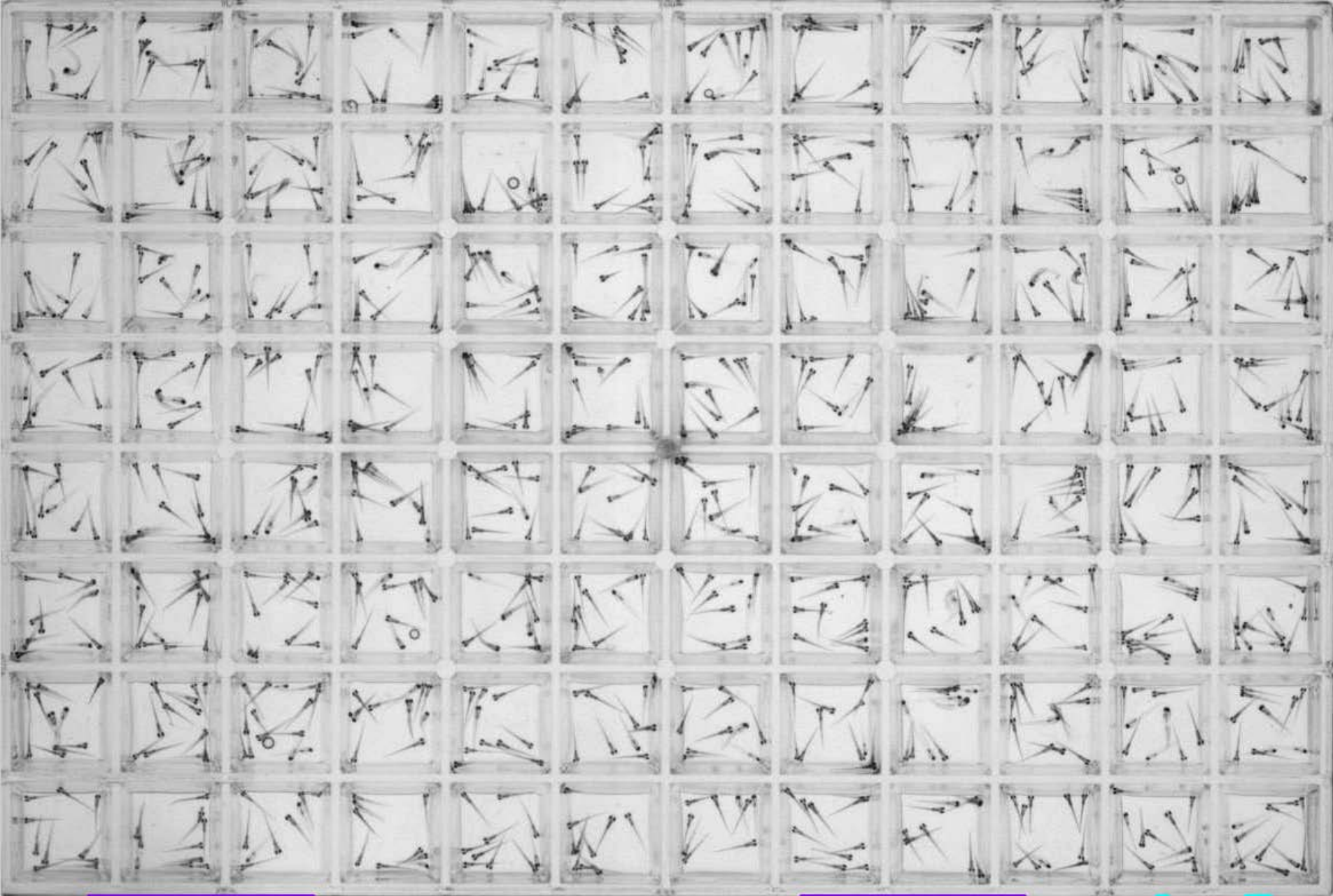
10 behavioral assays

2,000 compounds

20,000 fish

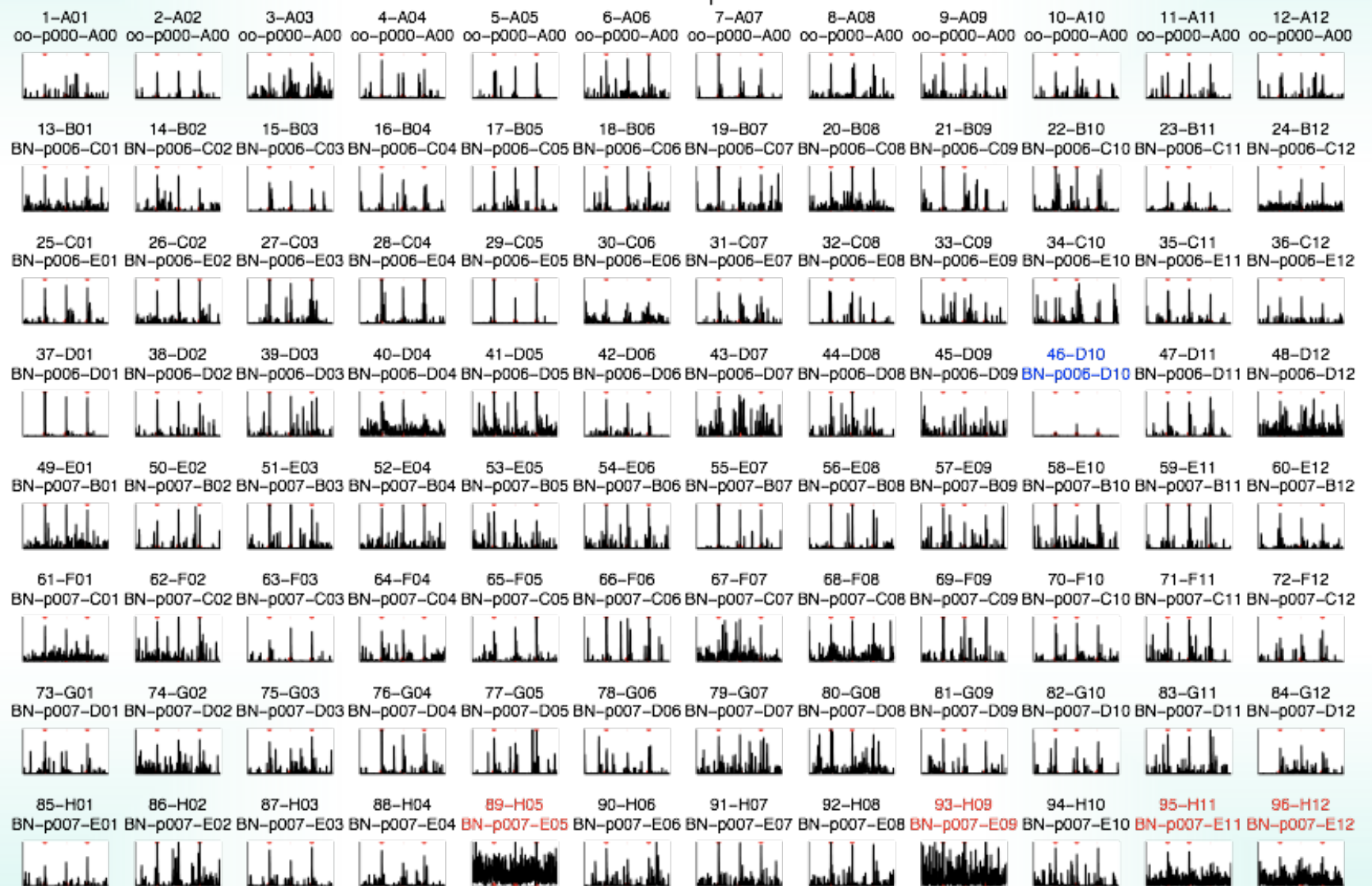
David Kokel





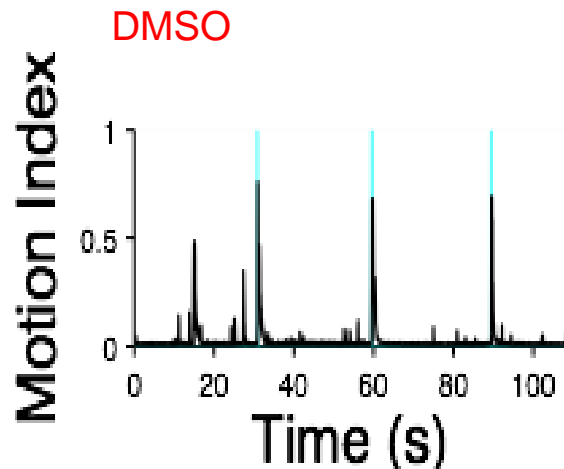
Video posted on [vimeo.com](https://vimeo.com)

# High-Throughput Extraction of Behavioral Features

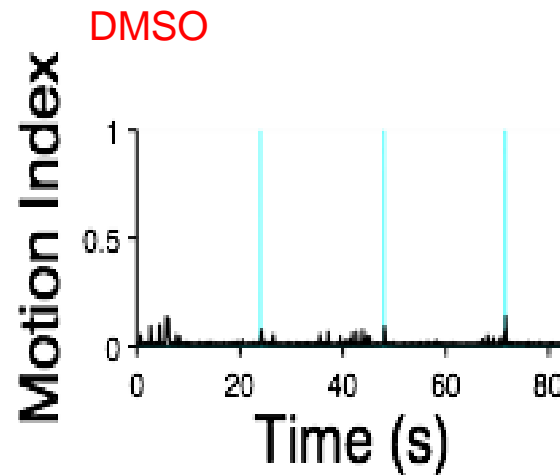


# Multiple Behavioral Features can be Measured for Each Specific Assay

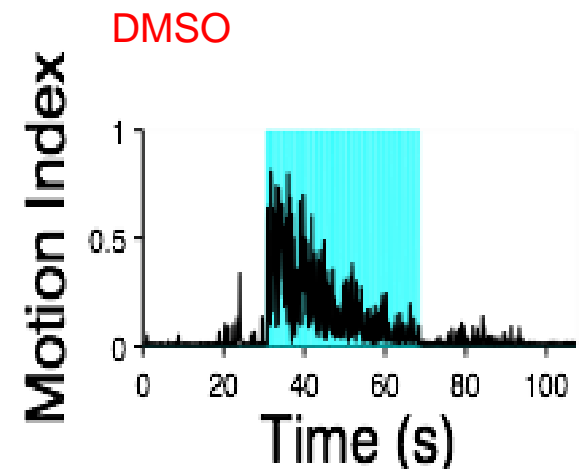
Startle assay  
(Full-strength stimuli)



Sensitization assay  
(Sub-threshold stimuli)

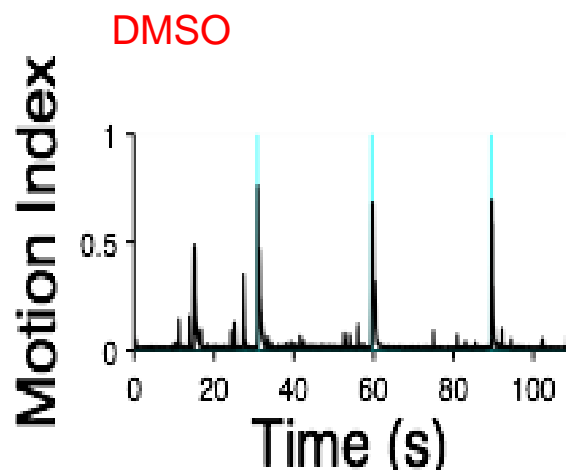


Habituation

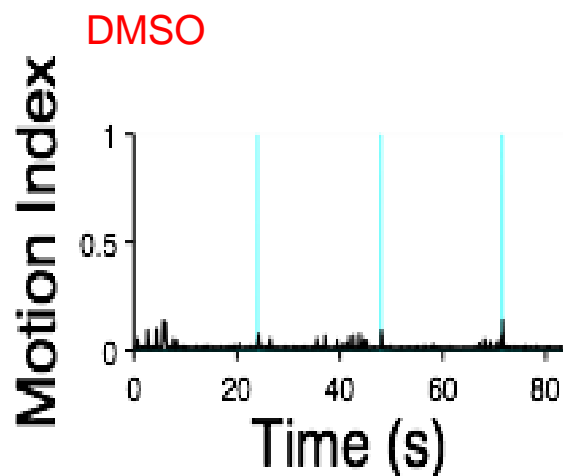


# Small Molecules with Diverse Effects on Acoustic Responses

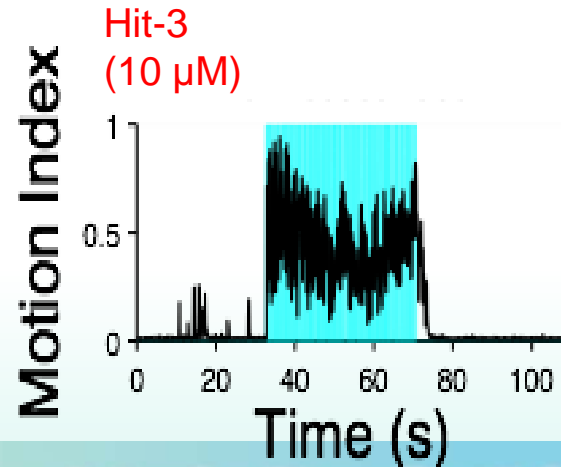
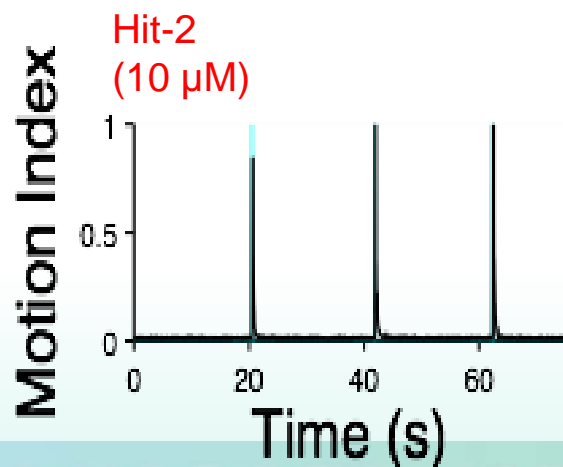
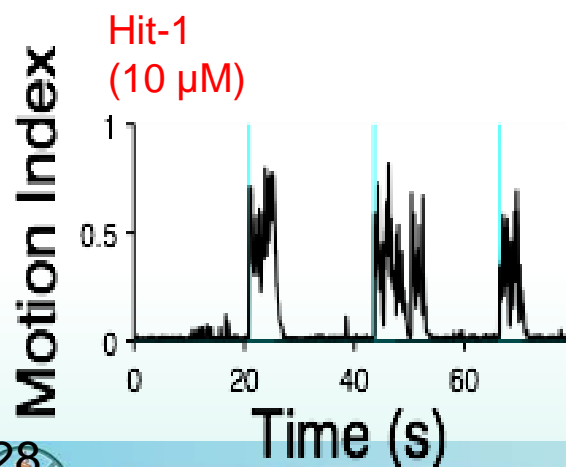
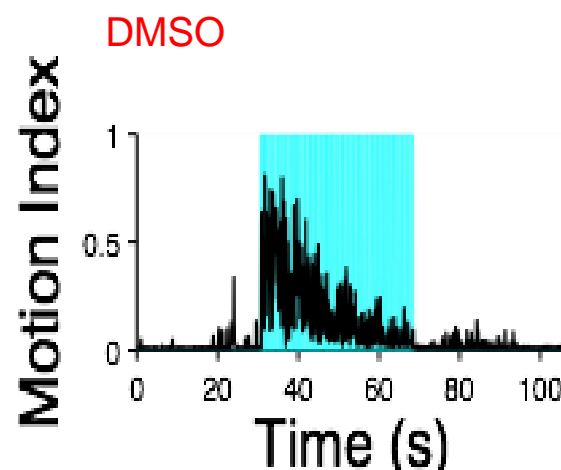
Startle assay  
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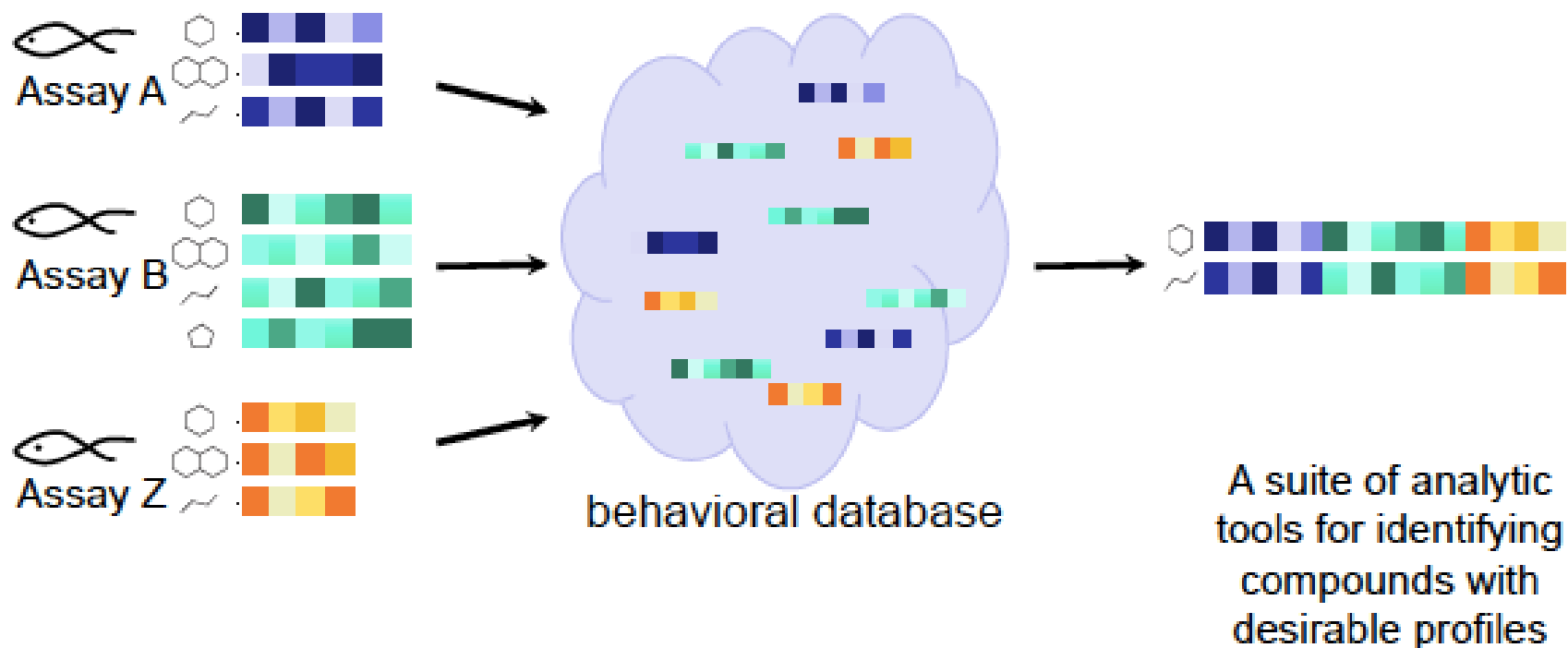


Habituation

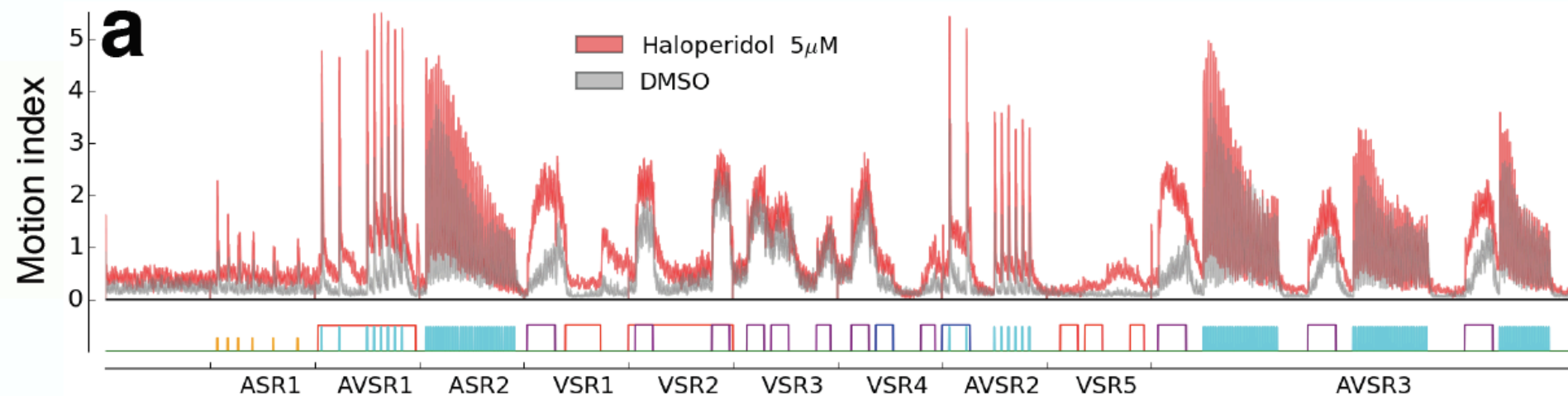




# New Databasing and Searching Tools Enable Mining Behavioral Dataset



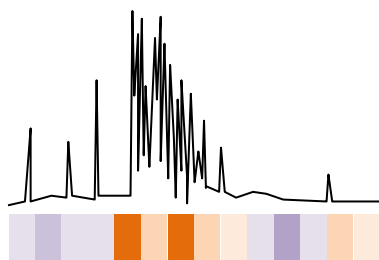
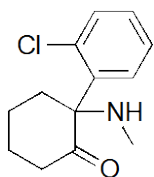
# Perturbations (Chemical or Genetic) Cause Specific, Characteristic Changes in Behavior



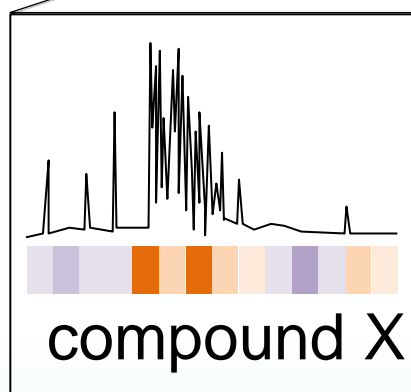
An example of a behavioral profile associated with a drug:  
haloperidol

# 'PhenoBlast' Approach Enables Discovery of Novel Compounds that Phenocopy a Desirable Drug Signature

Drug with desirable clinical activity

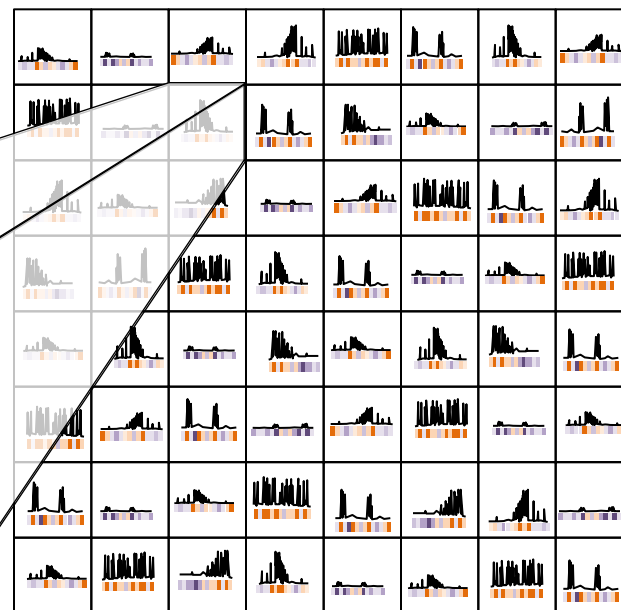


Behavioral signature



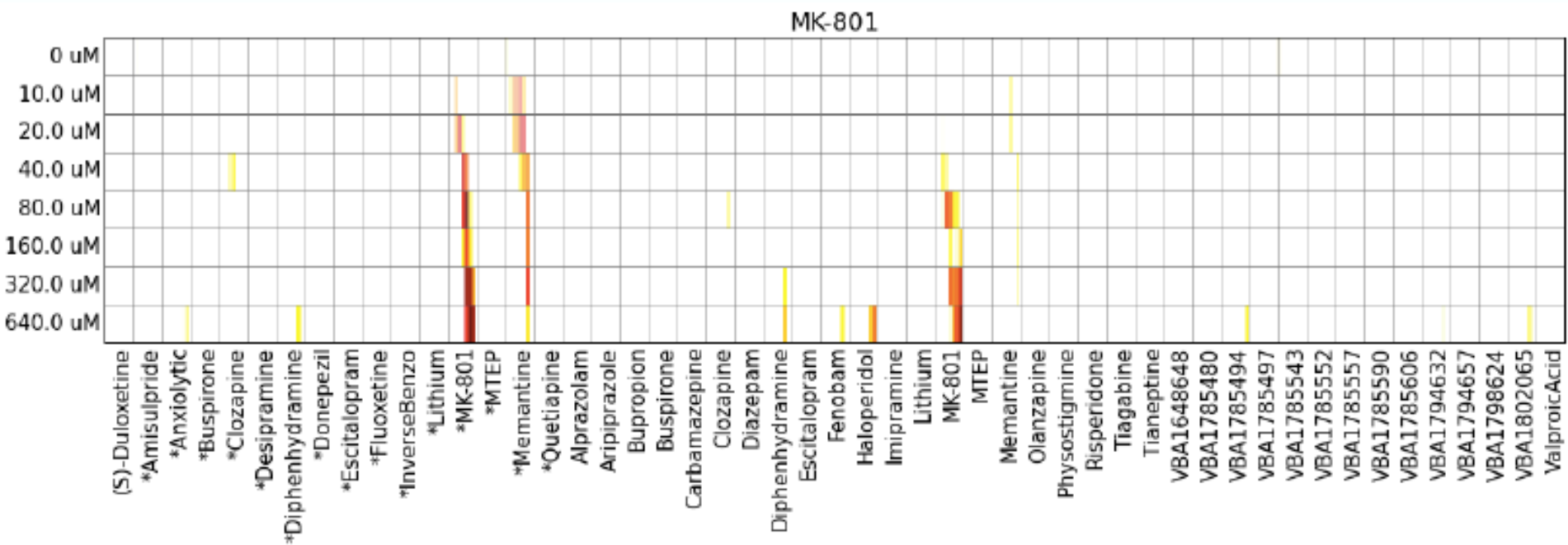
compound X

Compound library with unknown pharmacology

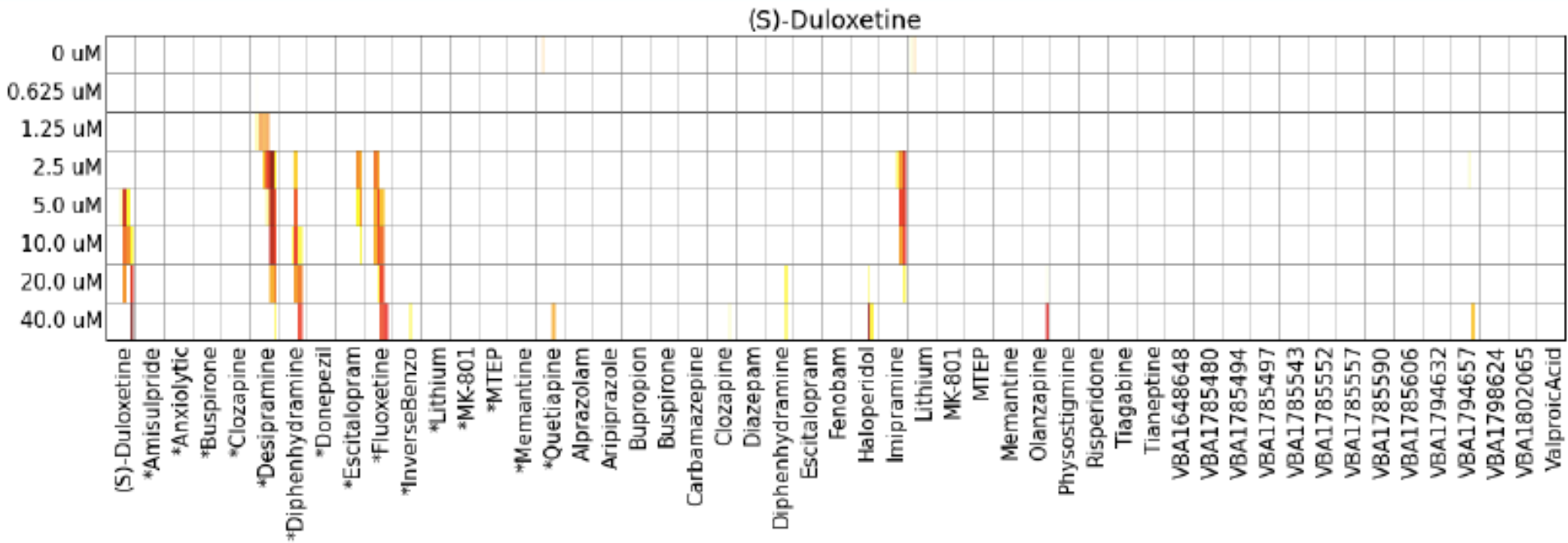


Behavioral signatures for chemical library

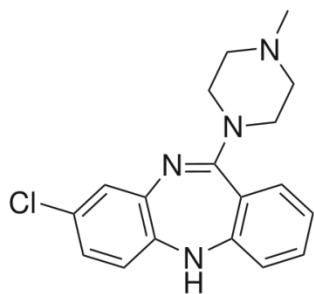
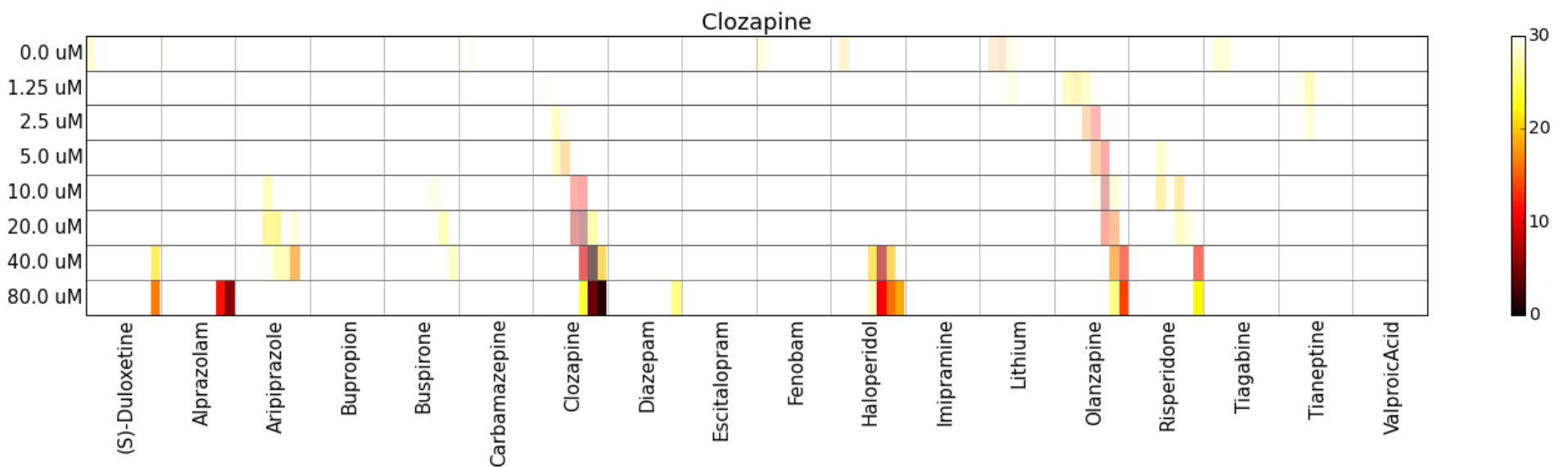
# The Behavioral Profile of NMDAR Antagonist MK801 Matches Other NMDAR Antagonists



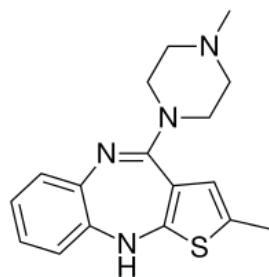
# The Behavioral Profile of Antidepressant Duloxetine Matches Other Antidepressants



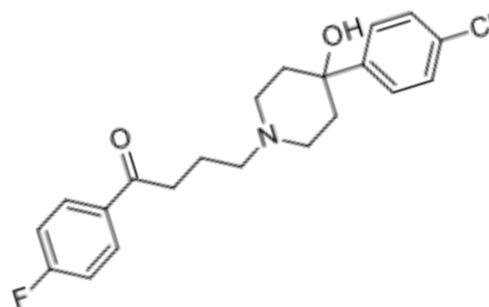
# Drug Signatures are Compound Specific but Share Features within a Therapeutic Class



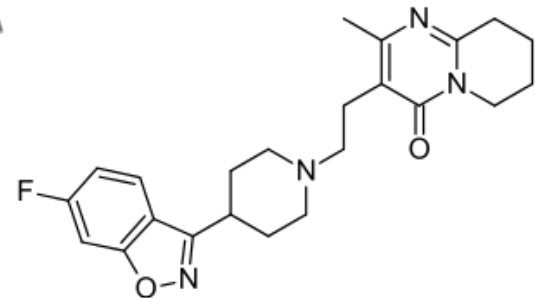
clozapine



olanzapine



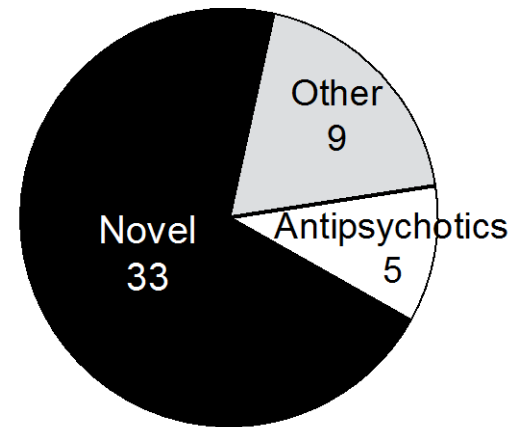
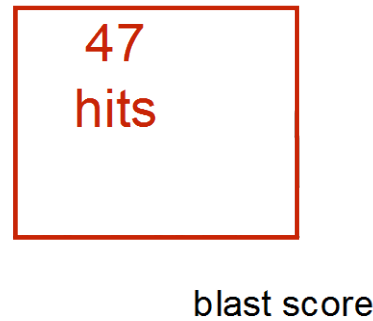
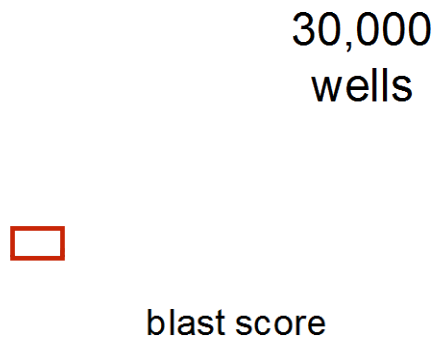
haloperidol



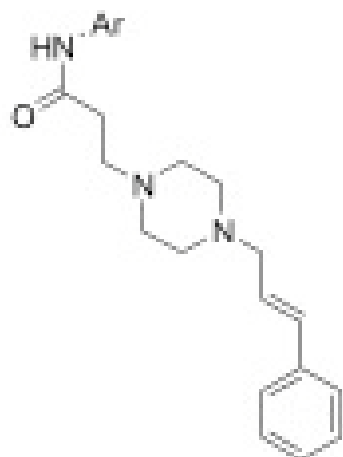
risperidone



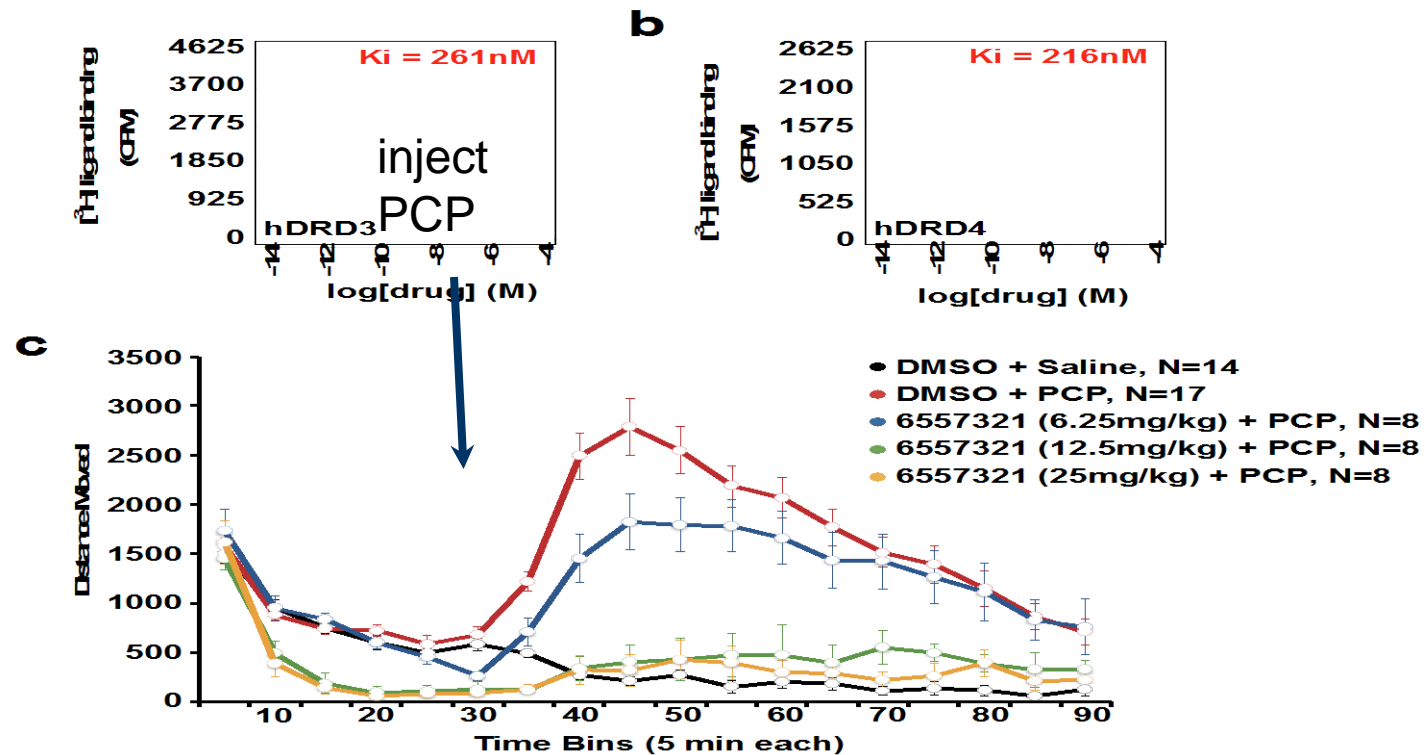
# Phenoblasting Haloperidol Profile against a Database of 30,000 Compound Behavioral Signatures



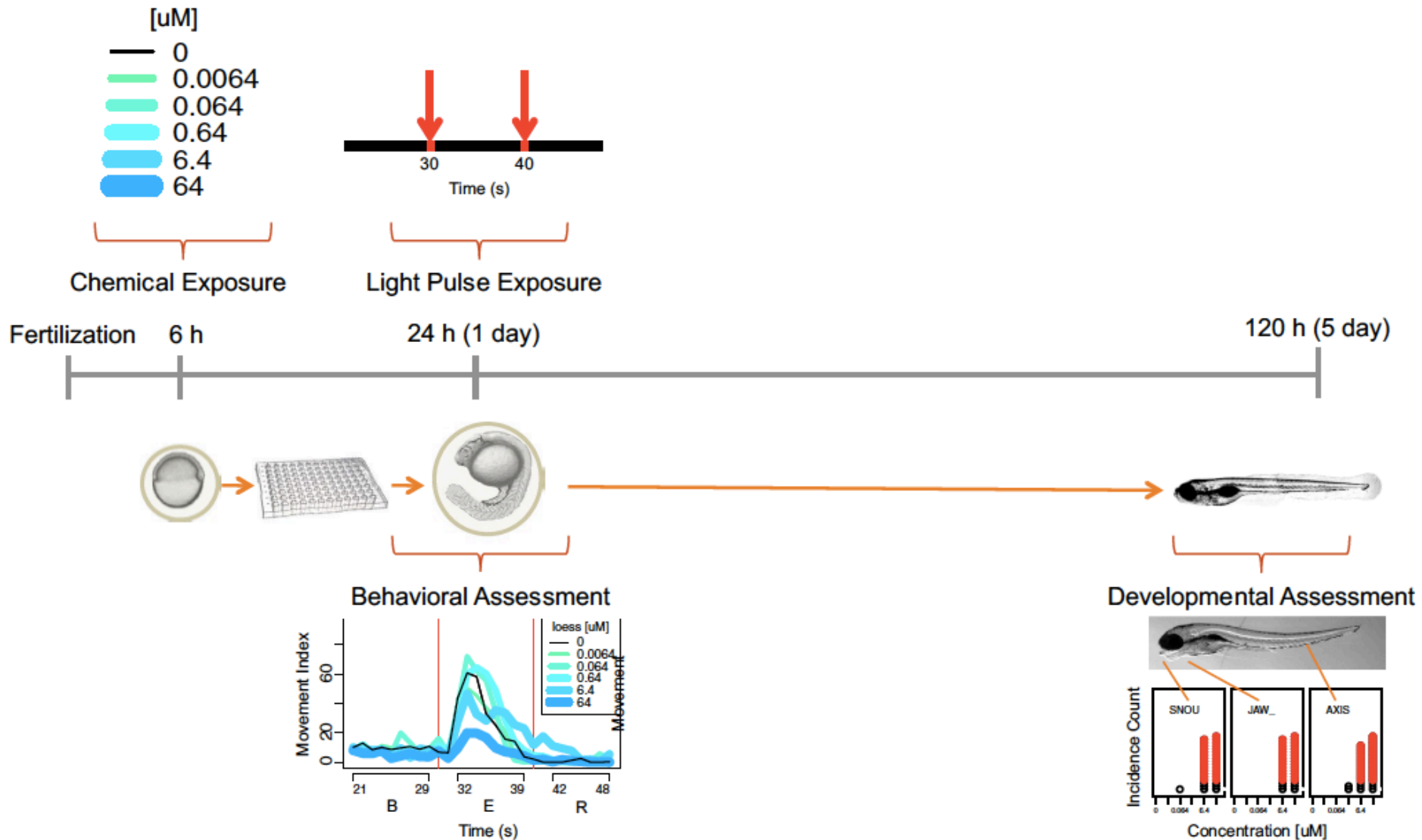
# A New Class of Haloperidol Mimetics

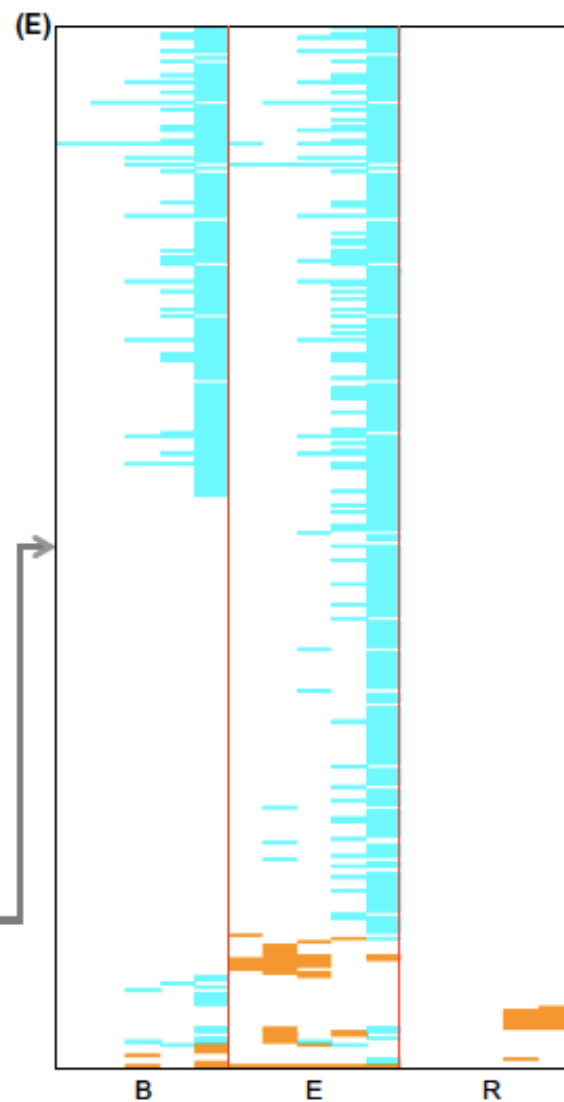
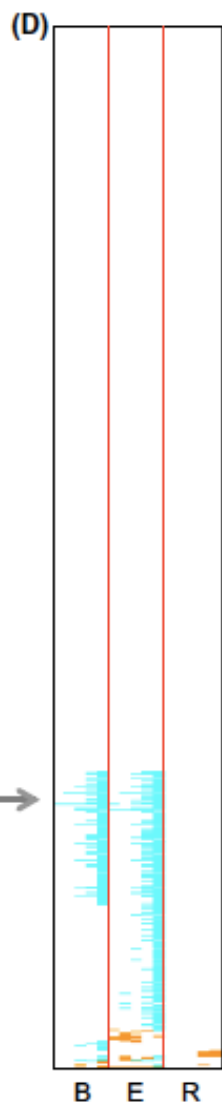
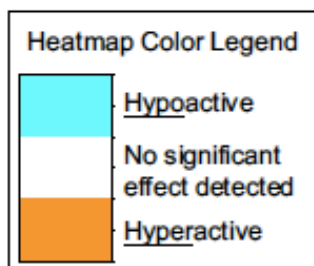
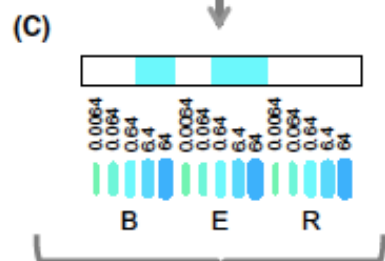
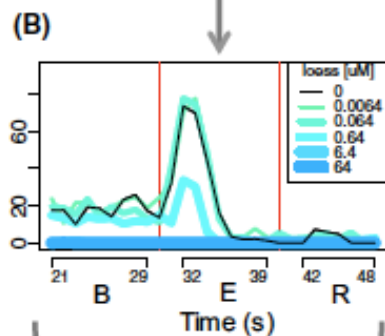
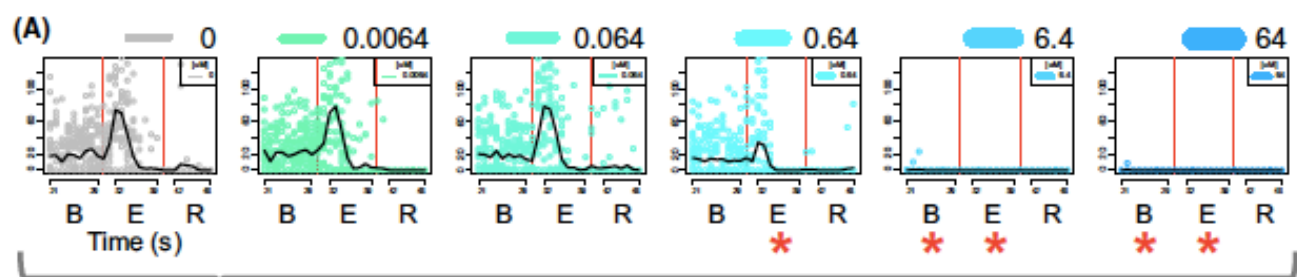


# Finazine is Active in a Mouse Model of Schizophrenia



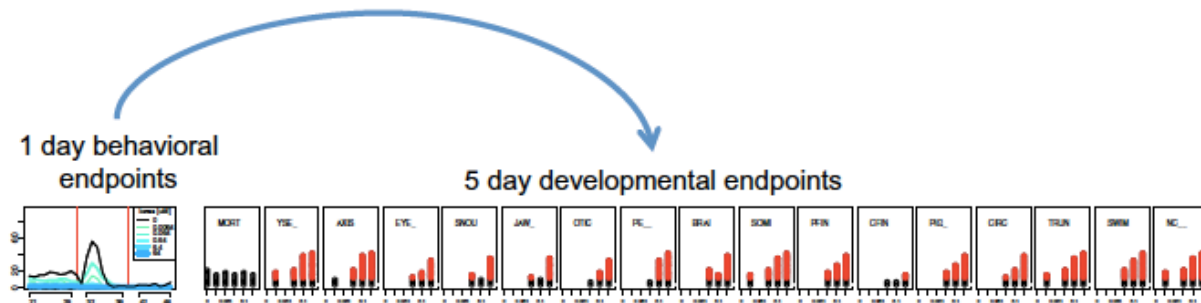
# HT Assessment of 1060 ToxCast Chemicals in Behavioral and Developmental Assays





Reif et al, Arch Toxicol.  
2016; 90: 1459–1470.

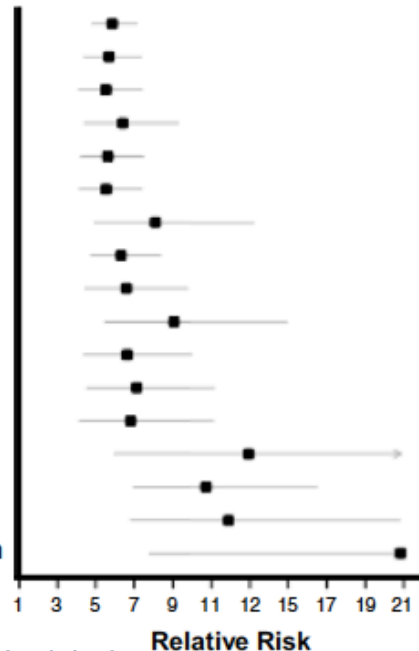
# Early Behavioral Deficits Predict Later Developmental Phenotypes



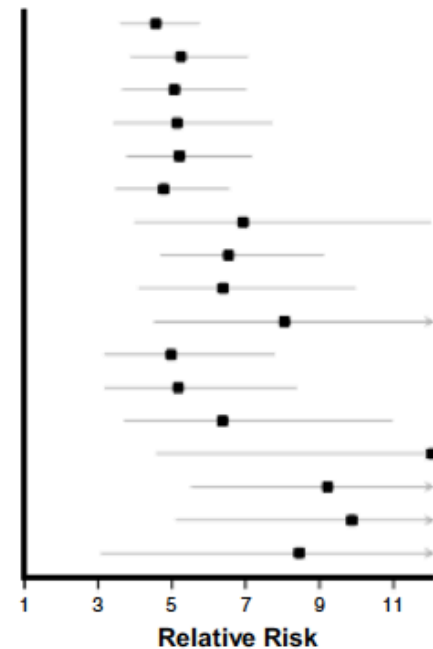
## Specific developmental endpoints at 5dpf

MORT: mortality  
YSE: yolk sac edema  
AXIS: bent body axis  
EYE: eye  
SNOU: snout  
JAW: jaw  
OTIC: otic  
PE: pericardial edema  
BRAI: brain  
SOMI: somite  
PFIN: pectoral fin  
CFIN: caudal fin  
PIG: pigmentation  
CIRC: circulation  
TRUN: trunk length  
SWIM: swim bladder  
NC: notochord distortion

## Background (B) hypoactivity at 24hpf

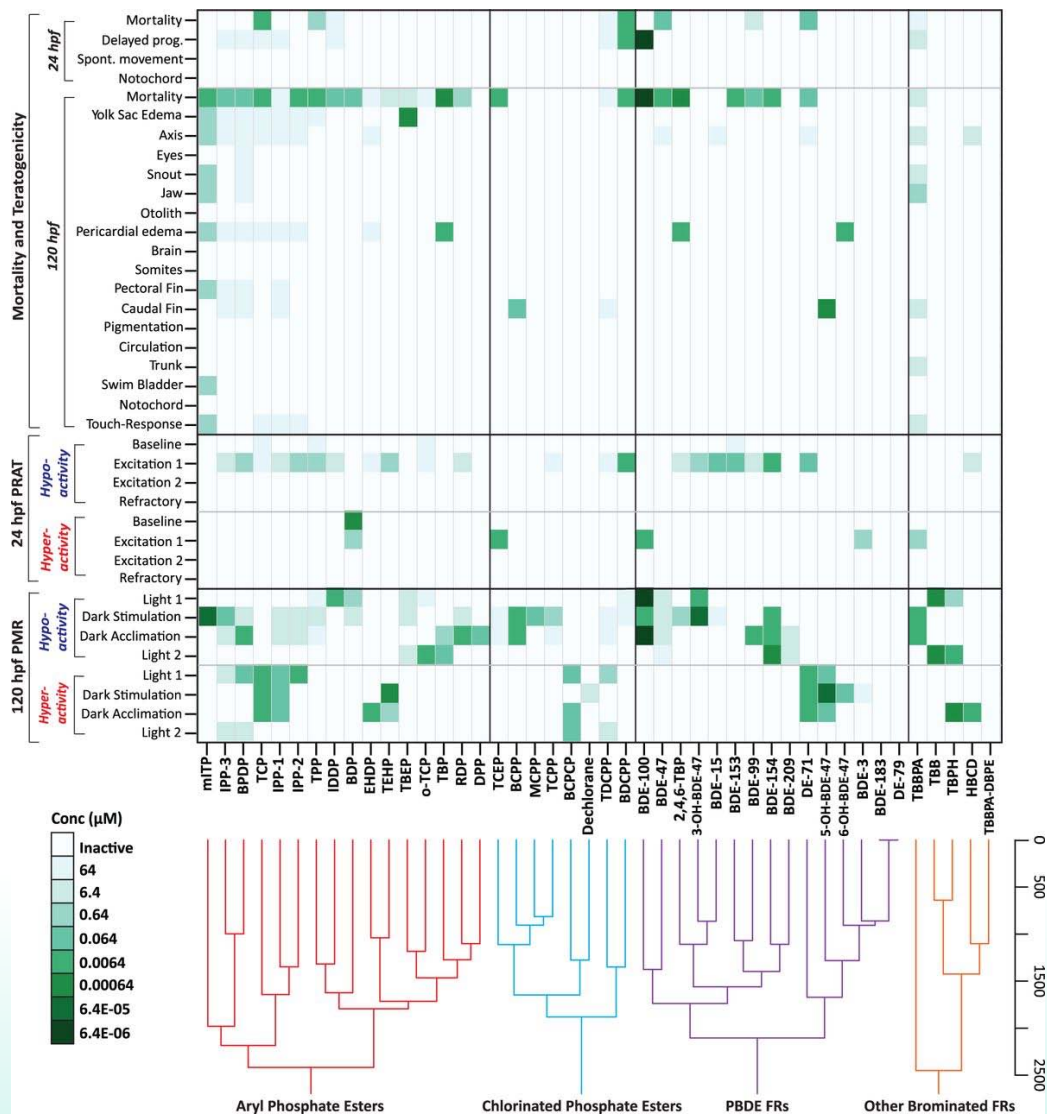


## Excitatory (E) hypoactivity at 24hpf



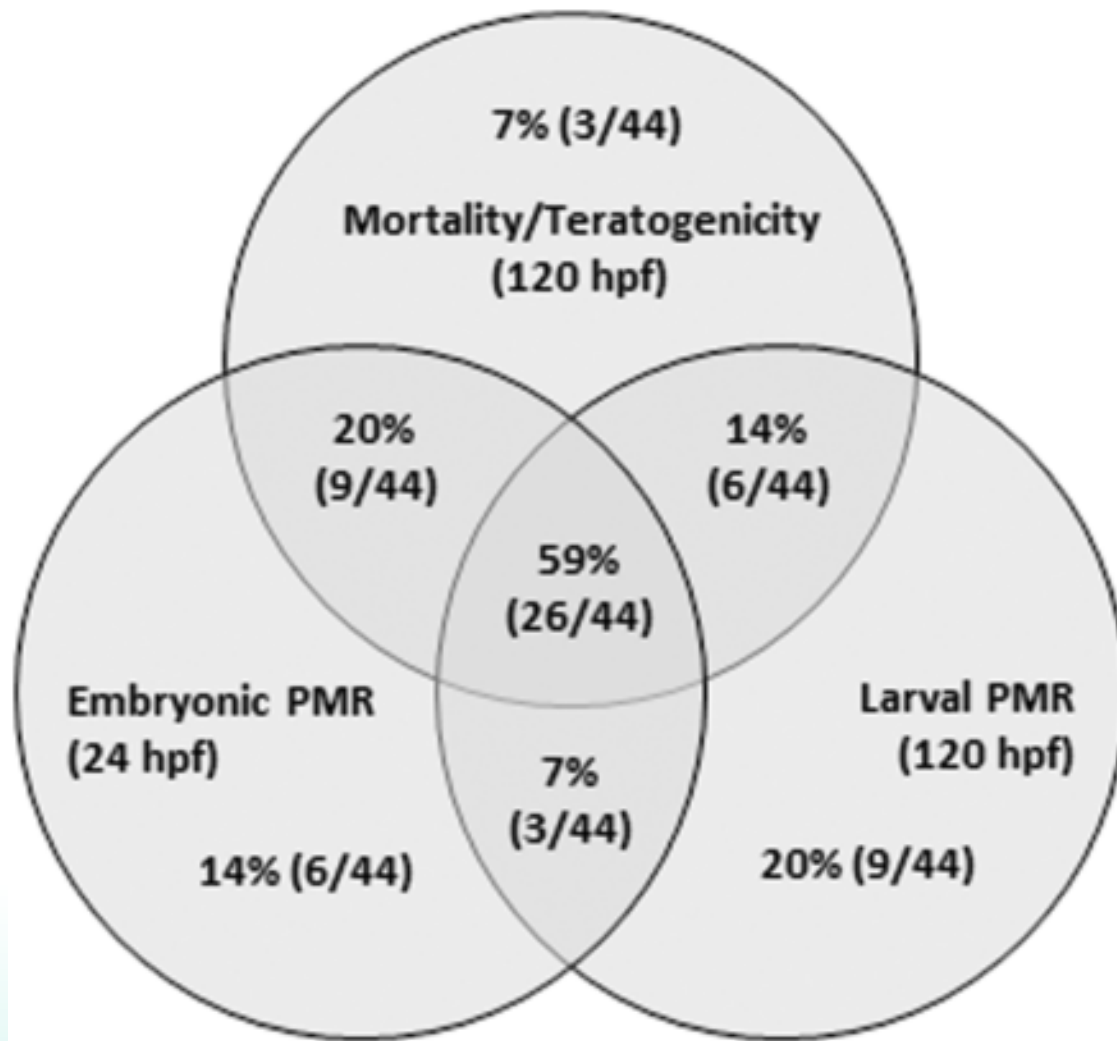


# HT Assessment of 44 Flame Retardants in Behavioral and Developmental Assays

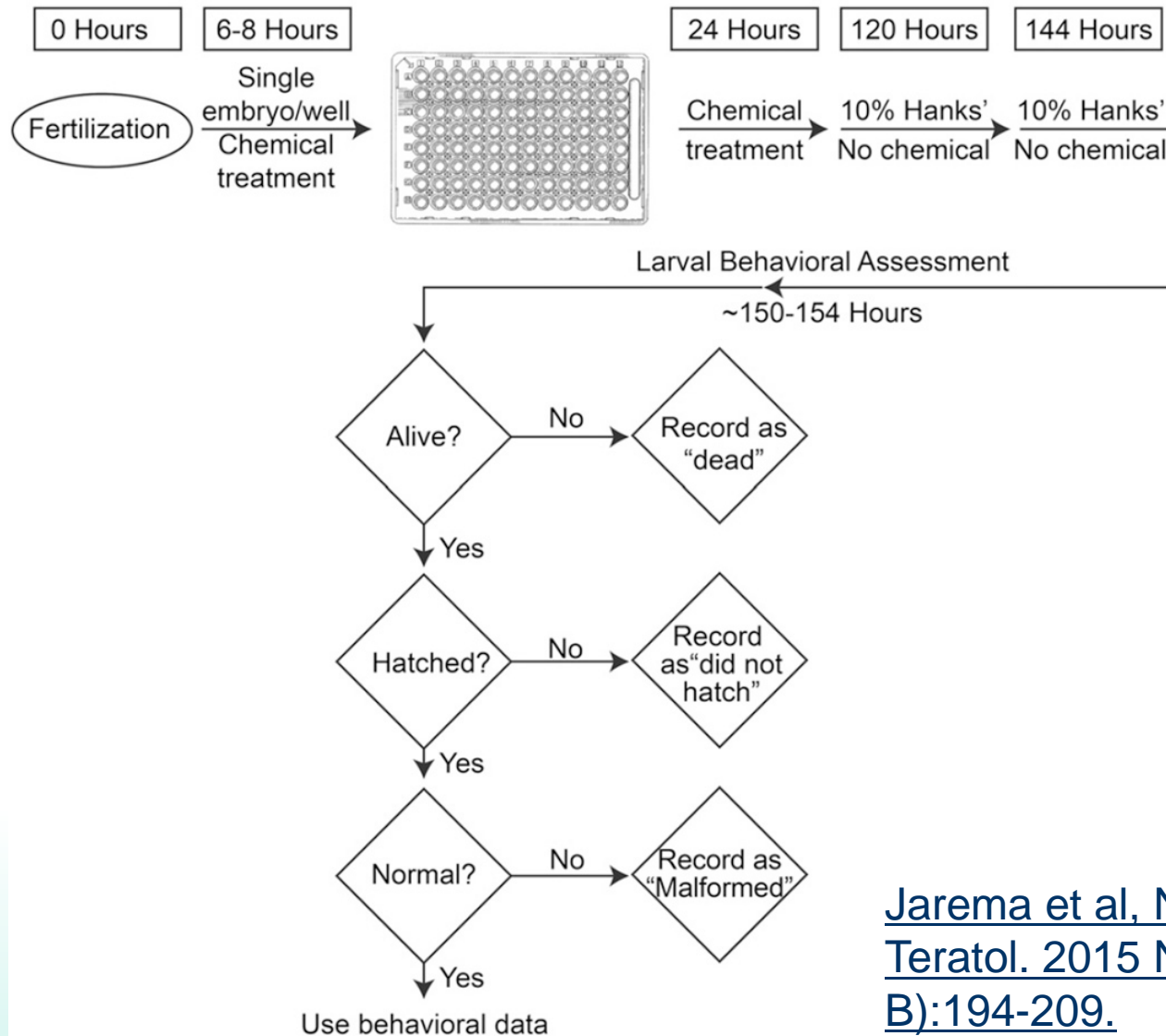


Noyes et al, Toxicol. Sci. (2015) 145 (1): 177-195.

# HT Assessment of 44 Flame Retardants in Behavioral and Developmental Assays

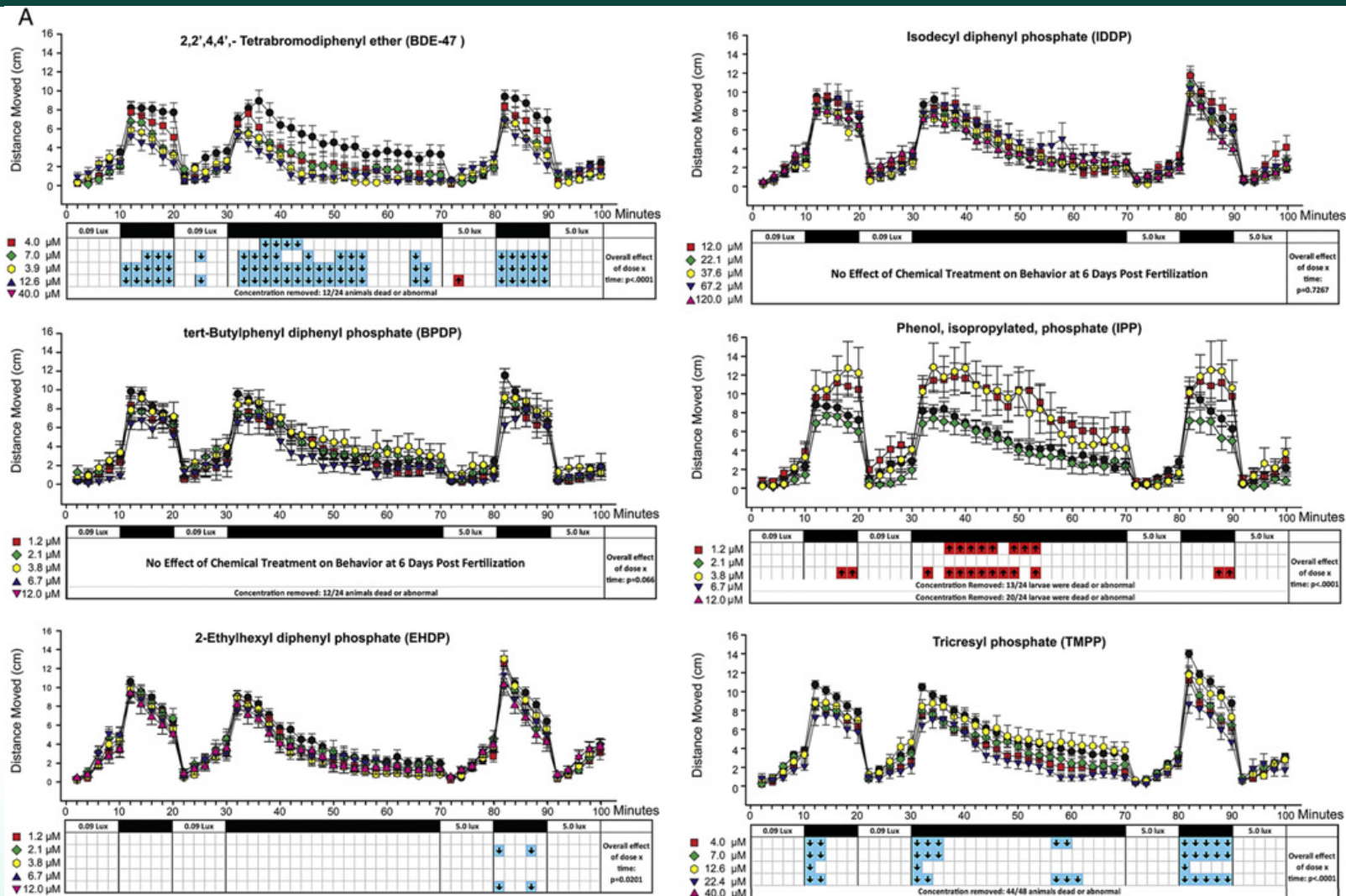


# A Scheme for Neurodevelopmental Assessment

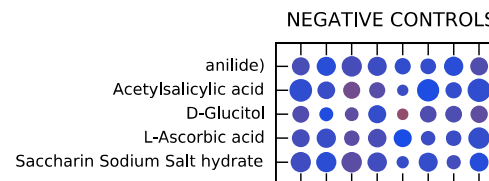
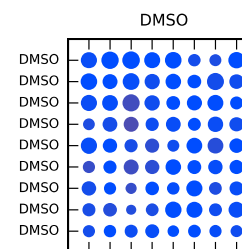
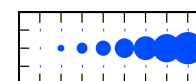
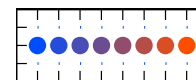
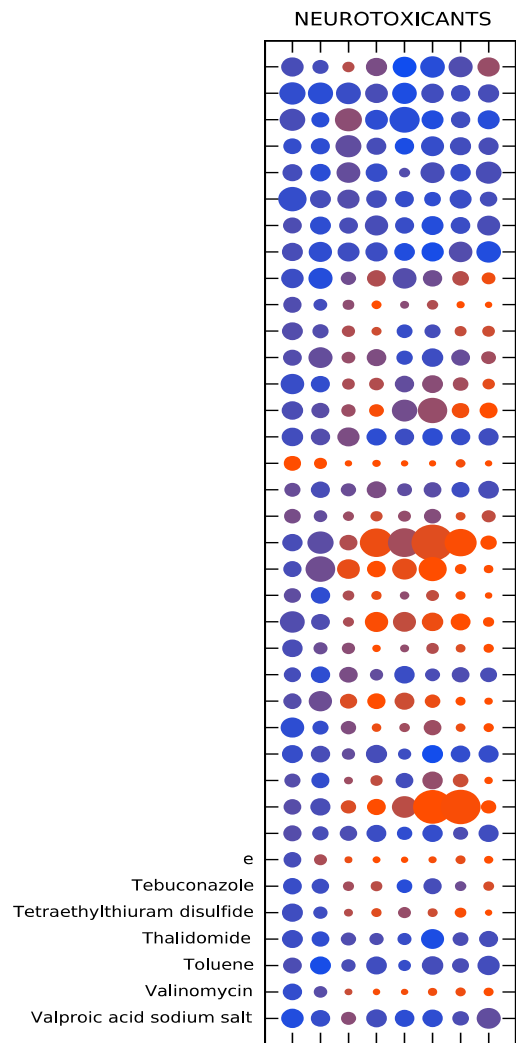


Jarema et al, Neurotoxicol Teratol. 2015 Nov-Dec;52(Pt B):194-209.

# A Scheme for Neurodevelopmental Assessment



# Automated Testing of Known Neurotoxicants in Teleos Behavioral Battery



♥ Tested at: 9.8nM, 39nM, 156nM, 2.5μM, 10μM, 40μM, 160μM

◇ Tested at: 9.8nM, 39nM, 156nM, 2.5μM, 10μM, 40μM, 160μM

♣ Tested at: 1nM, 3.9nM, 16nM, 63nM, 250nM, 1μM, 4μM, 16μM

♣ Tested at: 73pM, 0.3nM, 1.2nM, 4.7nM, 19nM, 75nM, 300nM, 1.2μM

§ Tested at: 78nM, 3.1nM, 12.5nM, 50nM, 200nM, 800nM, 3.2μM, 12.8μM

# Conclusions:

1. Systematic screening in zebrafish can identify compounds with diverse *in vivo* effects, including alterations in physiology and CNS function.
2. Conservation of toxic effects between zebrafish and humans is high-- ~95% in some cases.
3. A phenocopy approach can accurately predict compound mechanism of action.
4. Systems-level analyses can identify compounds with desirable profiles or with novel mechanisms of action.





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