Molecular mechanisms for persistence of the effects of developmental toxicants: The fetal basis of adult disease/dysfunction, and potential for transgenerational inheritance

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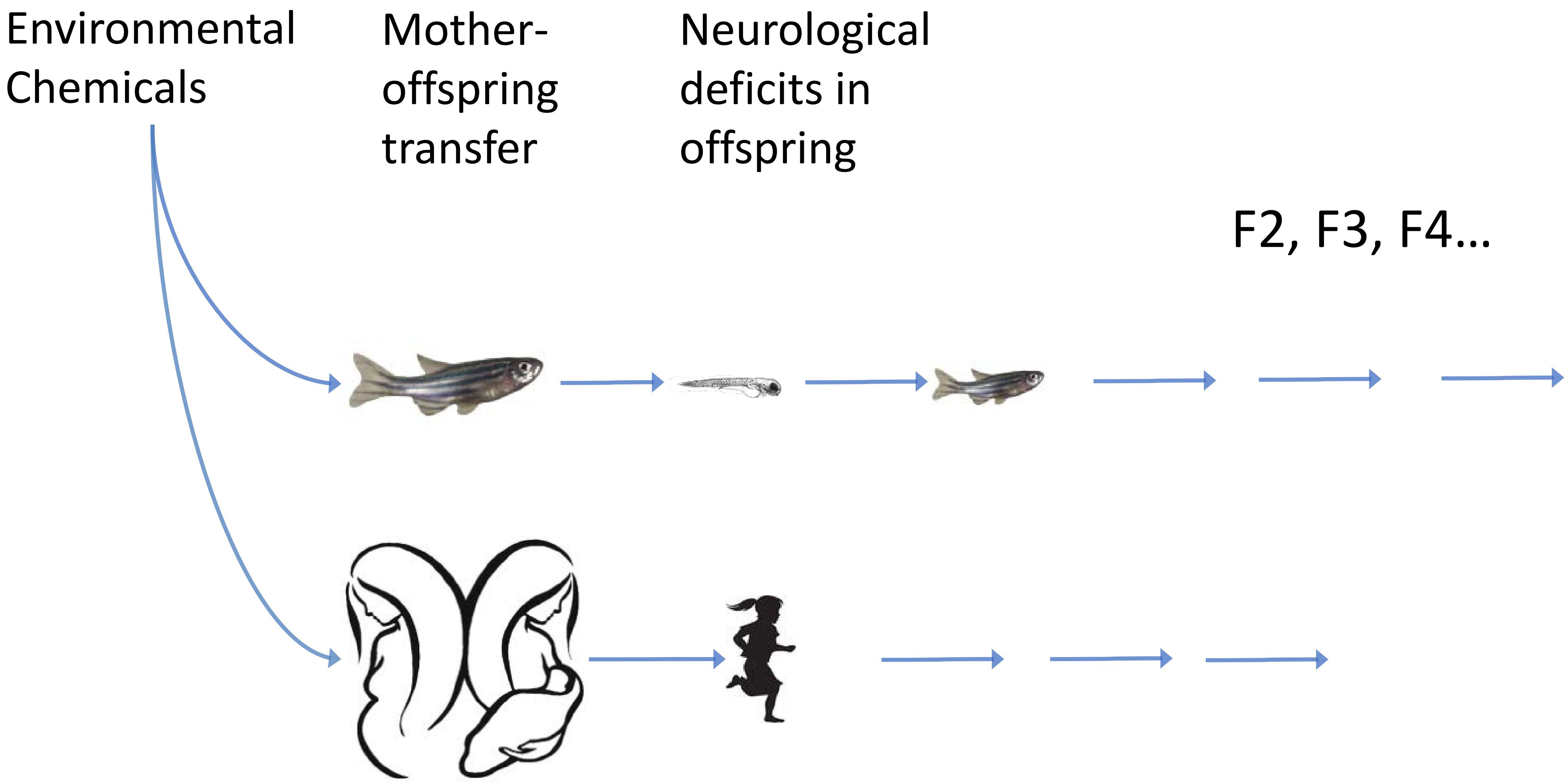
Barker Hypothesis

The proposition that a baby's nourishment in utero and during infancy determines the subsequent development of risk factors such as high blood pressure, blood clotting biochemistry and glucose intolerance and is thus a major determinant of coronary heart disease later in life.

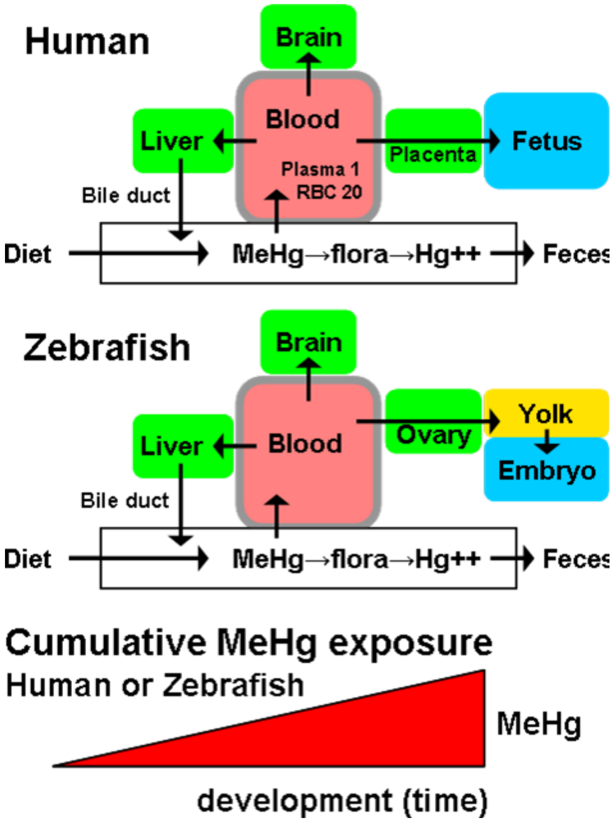
Barker et al. The Lancet 1986, 1989, 1993

Developmental Origins of Health and Disease

Laboratory Focus



MeHg Exposure Over Developmental Period



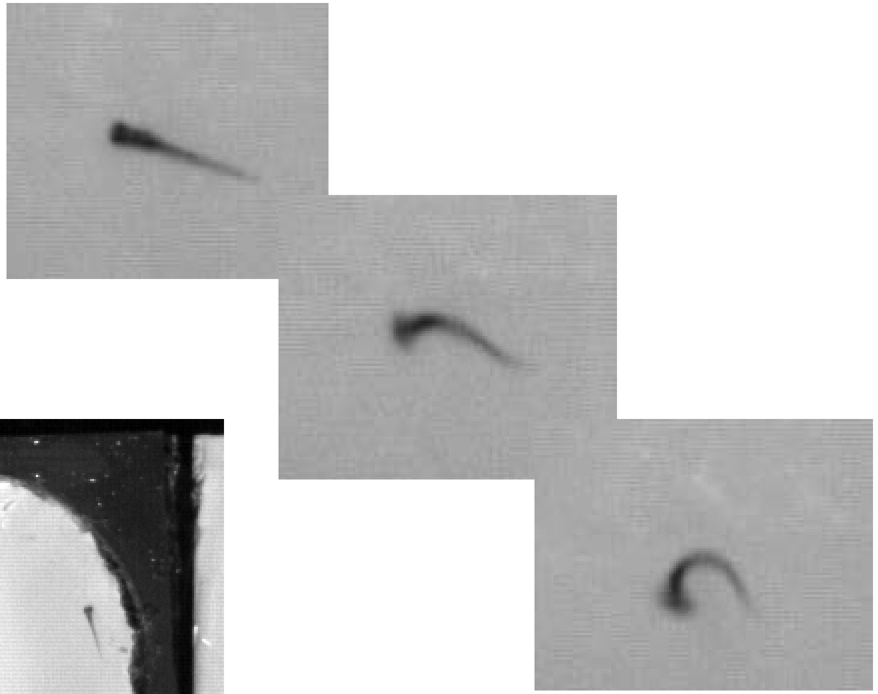
unpublished

For lipophilic chemicals with a long-half life:

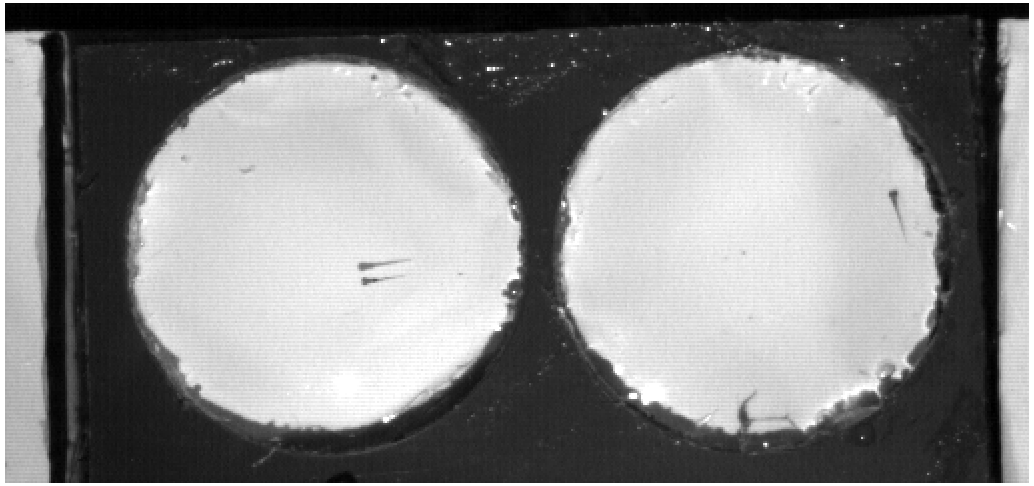
Yolk partitioning upon direct exposure (**4-24 hpf**), with subsequent distribution to embryo during growth and yolk utilization, creates a developmental exposure similar to that of mammals WITHOUT the influences of maternal metabolism

Acoustic/Vibrational Startle Reflex in Zebrafish

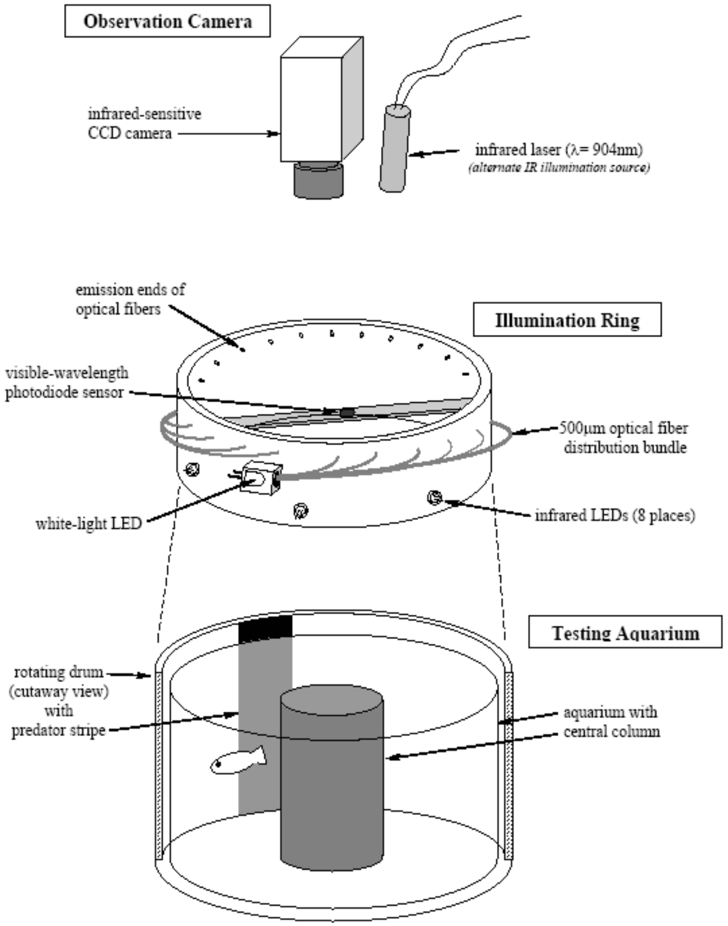
- Simple reflex
  - Stimulus (acoustic/vibrational, touch, visual), Receptors, Mauthner cell, Interneurons, Trunk muscles

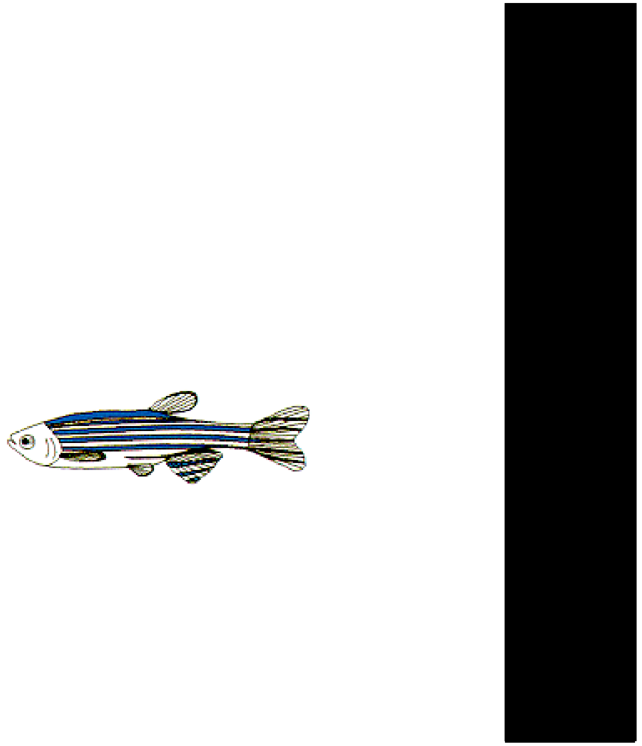


- C-start
  - Latency of response
  - Angle of flexion
  - Escape velocity
  - Habituation

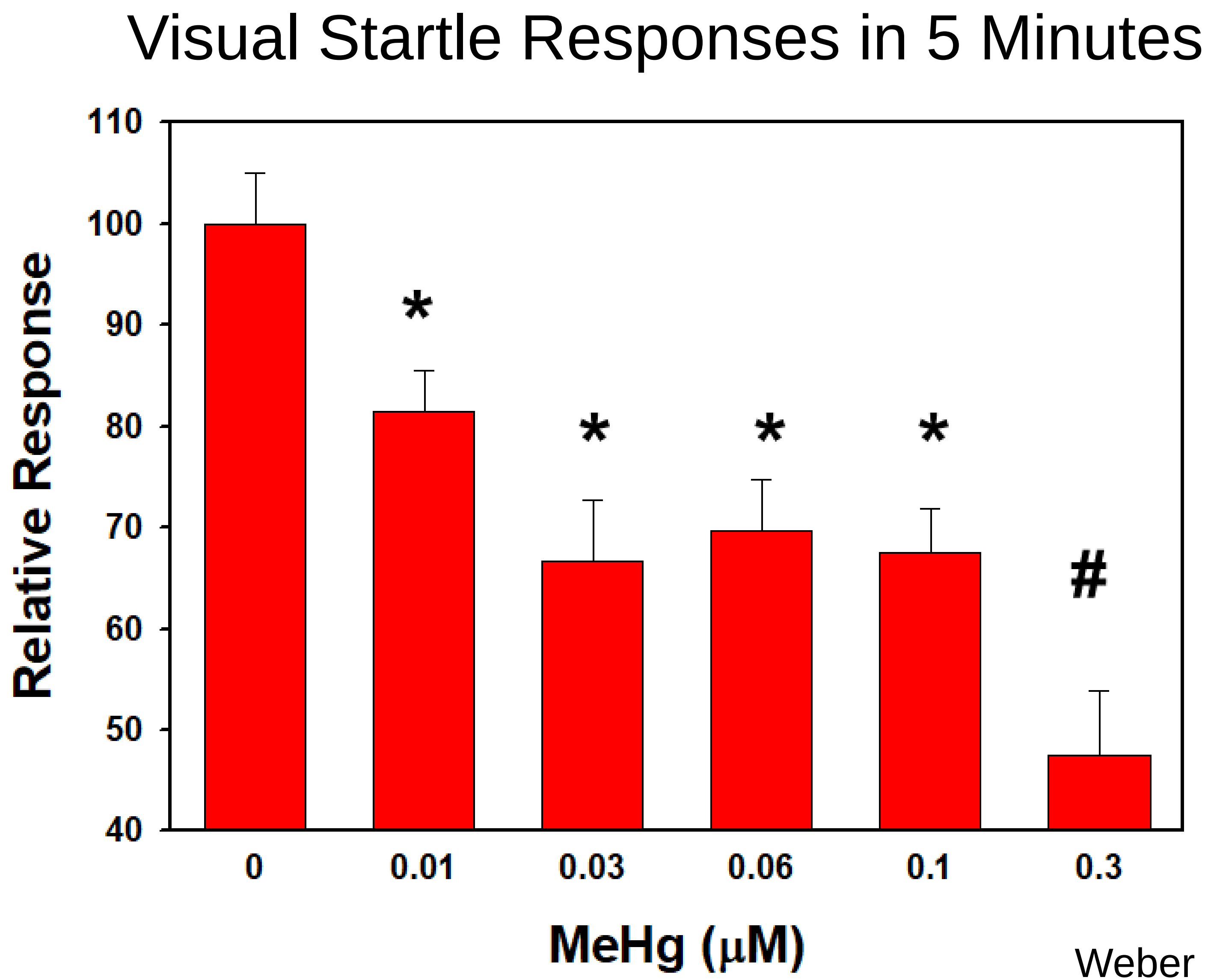


Adult Visual Startle Response





Adult Visual Startle—Direct Exposure (4-24 hpf)



Weber et al., Physiol Behav 2008.

Possible Mechanisms for Deficit

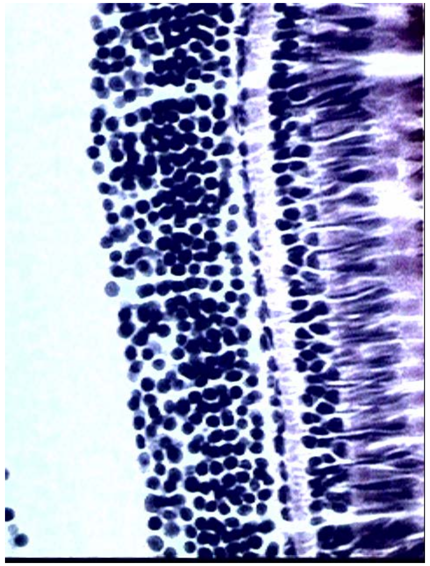
Sites of concern:

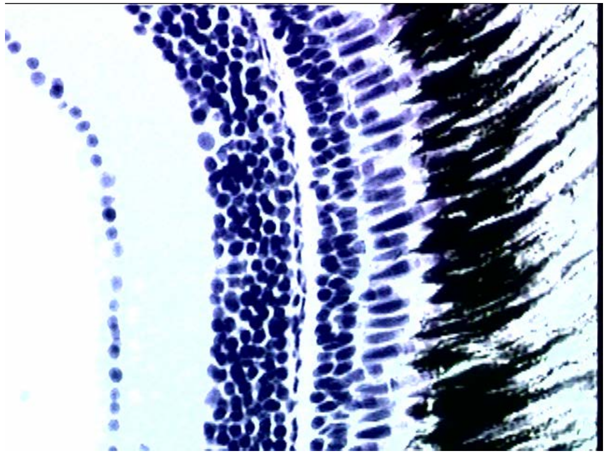
Retina, Optic Nerve, Optic Tectum

Locomotor Pathways

- Permanent structural damage during development?
- Mistakes in neuronal development?
- Permanent changes in neuronal function?

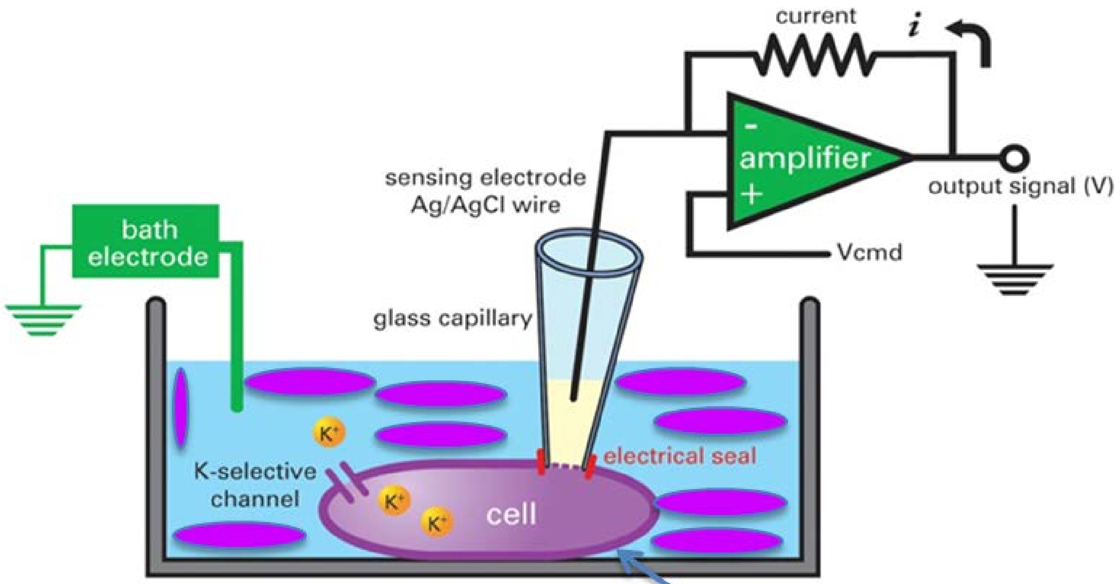
untreated

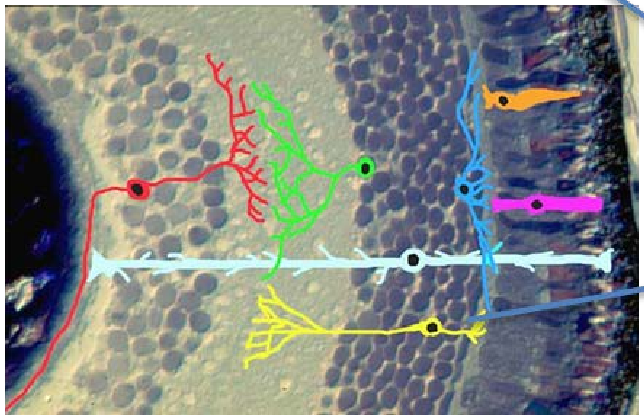




0.3μM MeHg

Retinal Electrophysiology

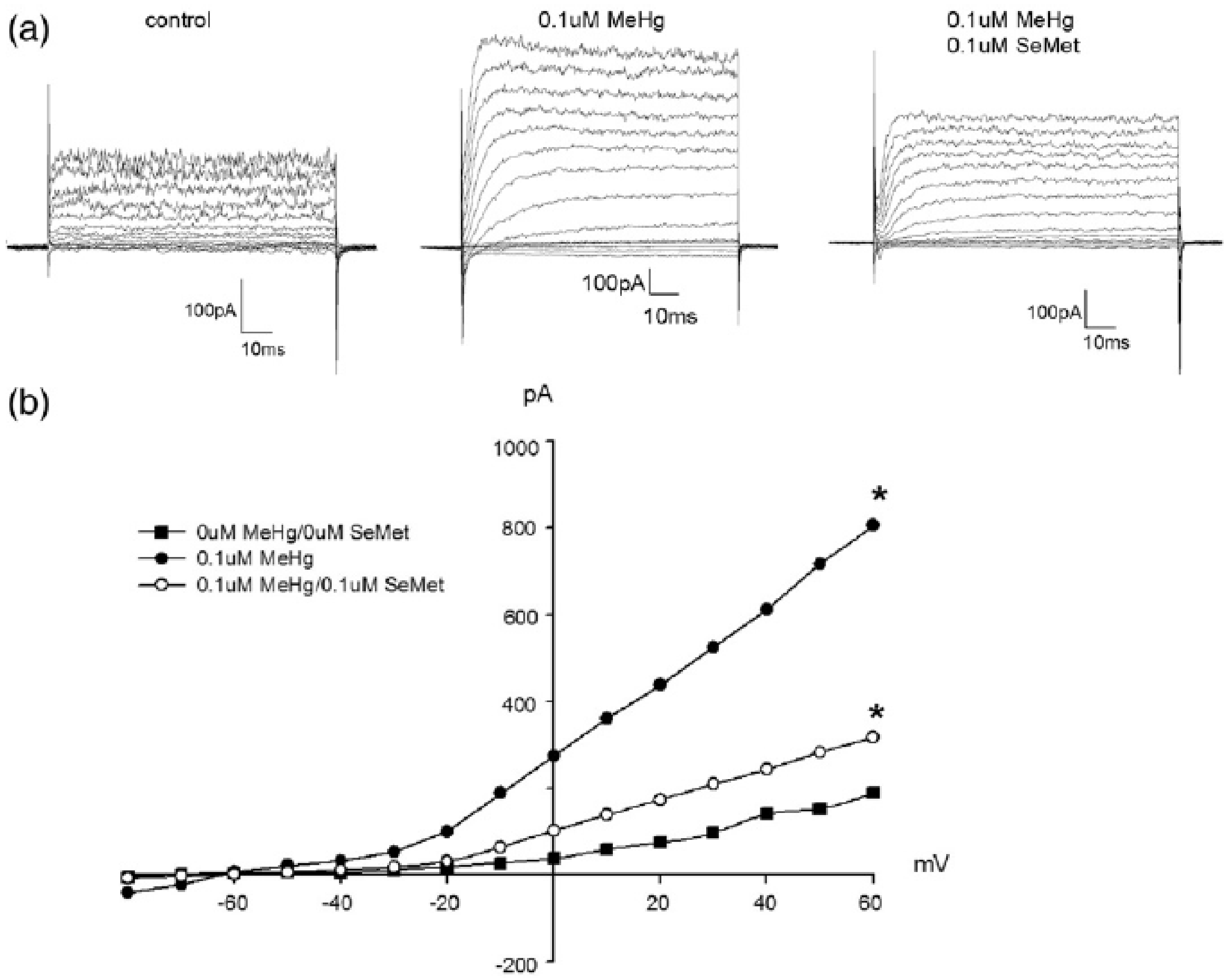




Bipolar cells of retina

http://www.pdn.cam.ac.uk/staff/harris/cell.jpg

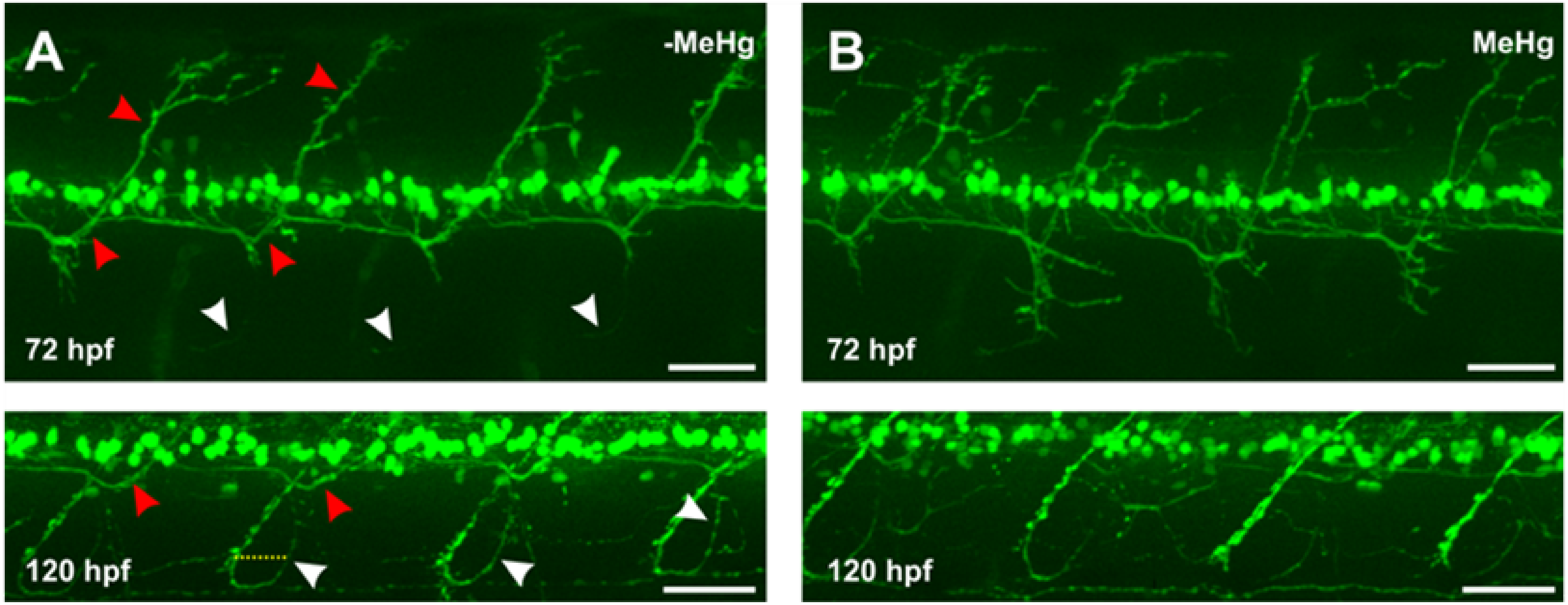
Effects on Adult Retinal Potassium Currents



Delayed rectifying (I_K) current is enhanced in adult zebrafish following developmental exposure to MeHg.

Weber et al., Physiol Behav 2008.

Effects on Spinal Motorneurons

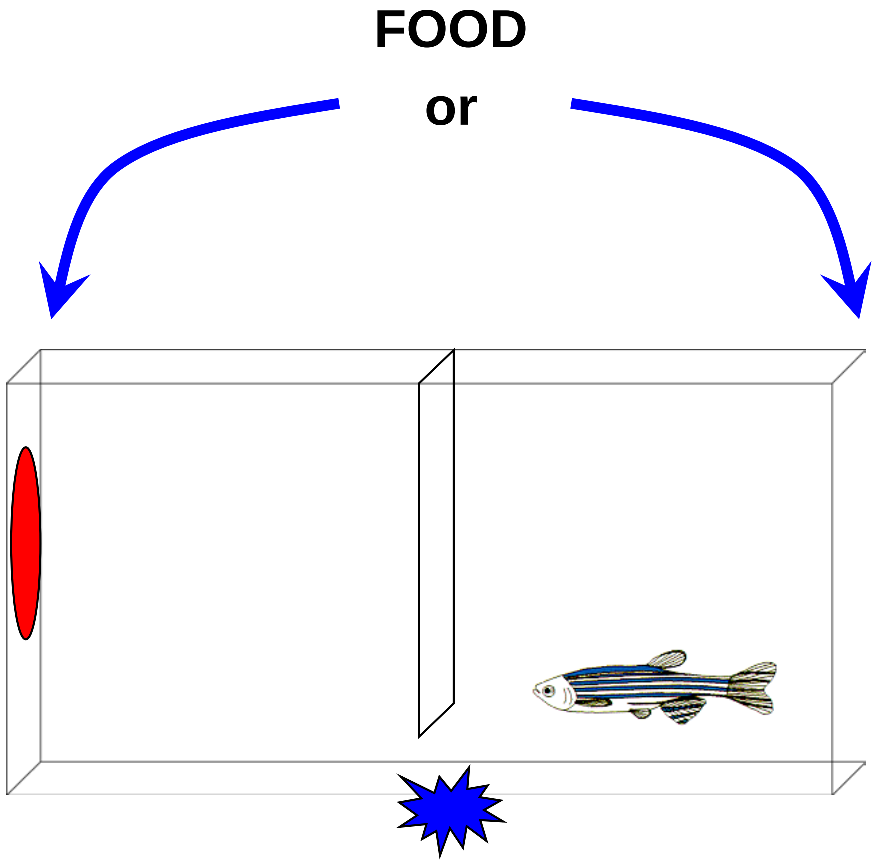


Tg (*Isl1:gfp*)

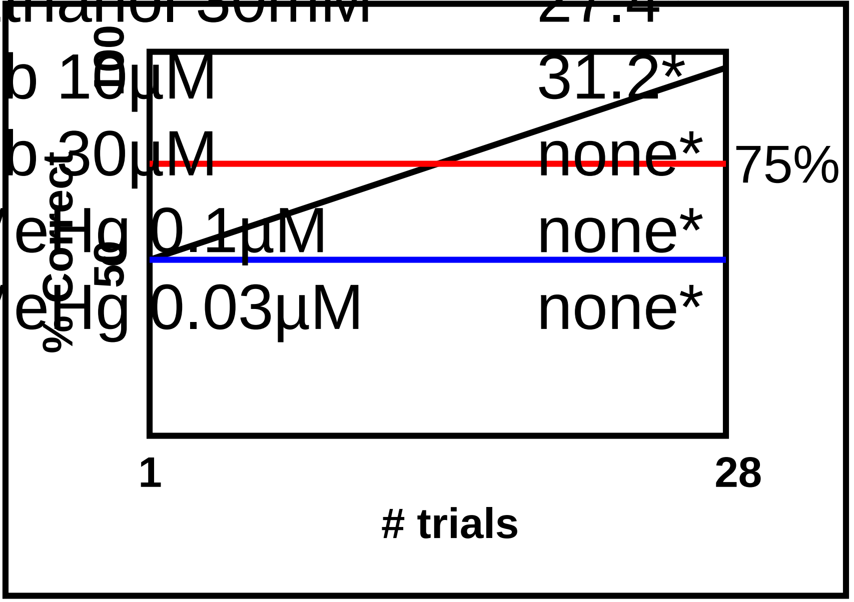
0.1 μM MeHg

And they are HYPERACTIVE

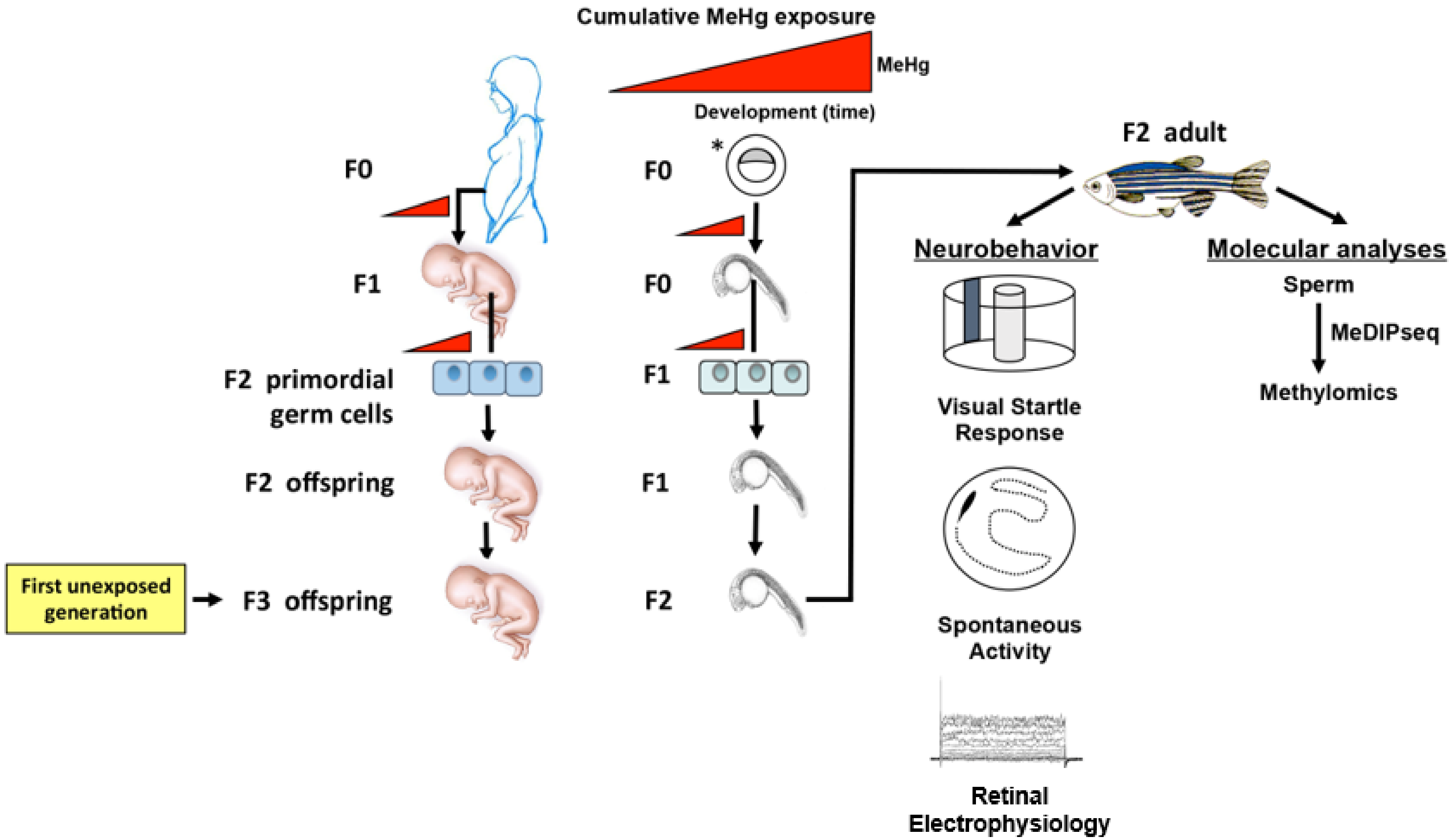
Spatial Alternation Task



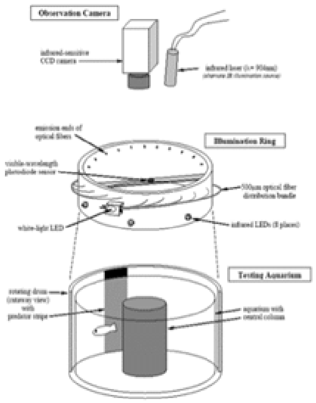
Treatment	trials to 75%
Untreated	13.9
Ethanol 10mM	21.7*
Ethanol 30mM	27.4*
Pb 10µM	31.2*
Pb 30µM	none*
MeHg 0.1µM	none*
MeHg 0.03µM	none*



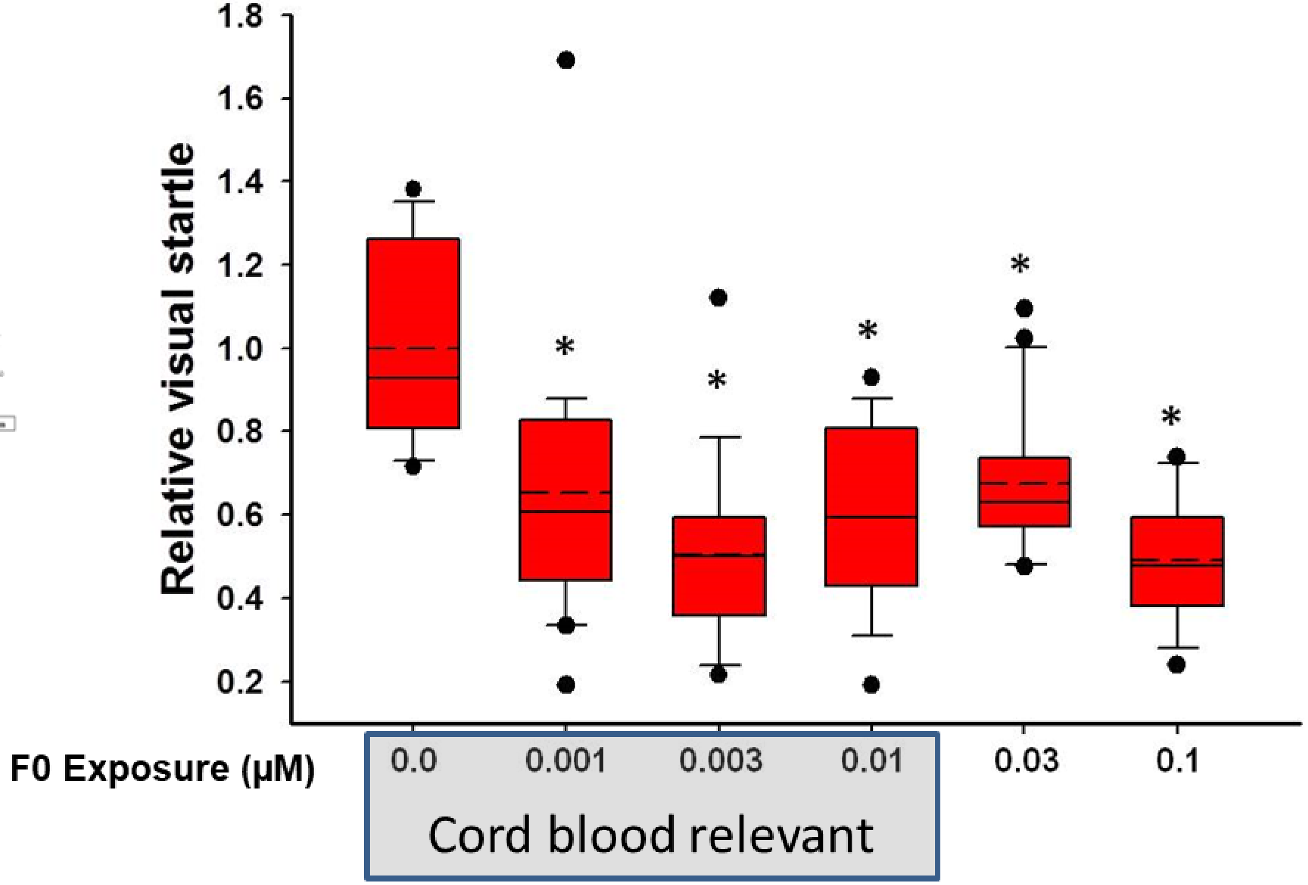
Mercury- Induced Transgenerational Inheritance of Abnormal Phenotypes



Visual Startle in Adult F2 Lineages



F2 Visual Startle Responses in 5 Minutes



Carvan et al., PLoS ONE 2017

Effects on Retinal Potassium Currents in F2 Lineages

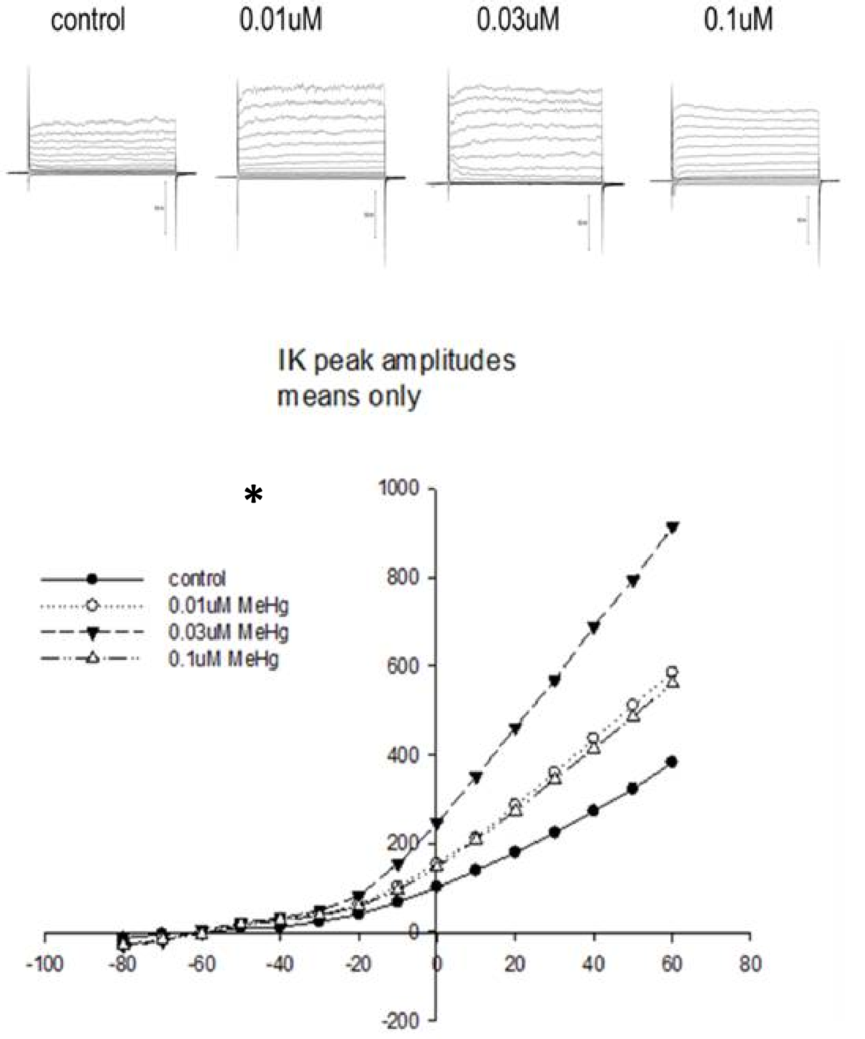
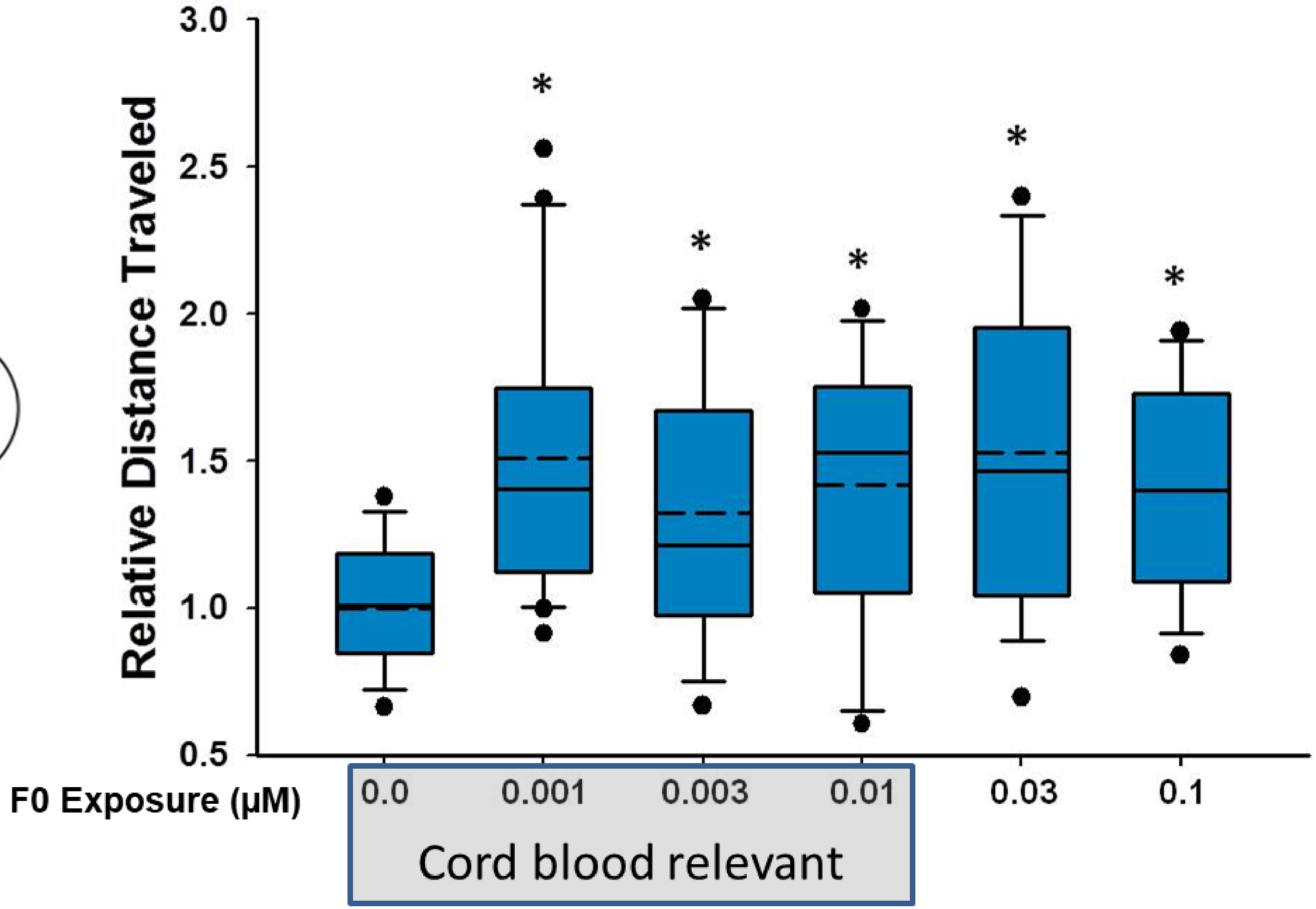


Figure 4. Change in peak amplitude of I_K current recorded from bipolar cells in retinas exposed to various concentrations of MeHg. Representative current traces are given at the top. The graph at the bottom plots mean peak currents elicited at different voltage steps from a holding potential of -60mV.

Carvan et al., PLoS ONE 2017

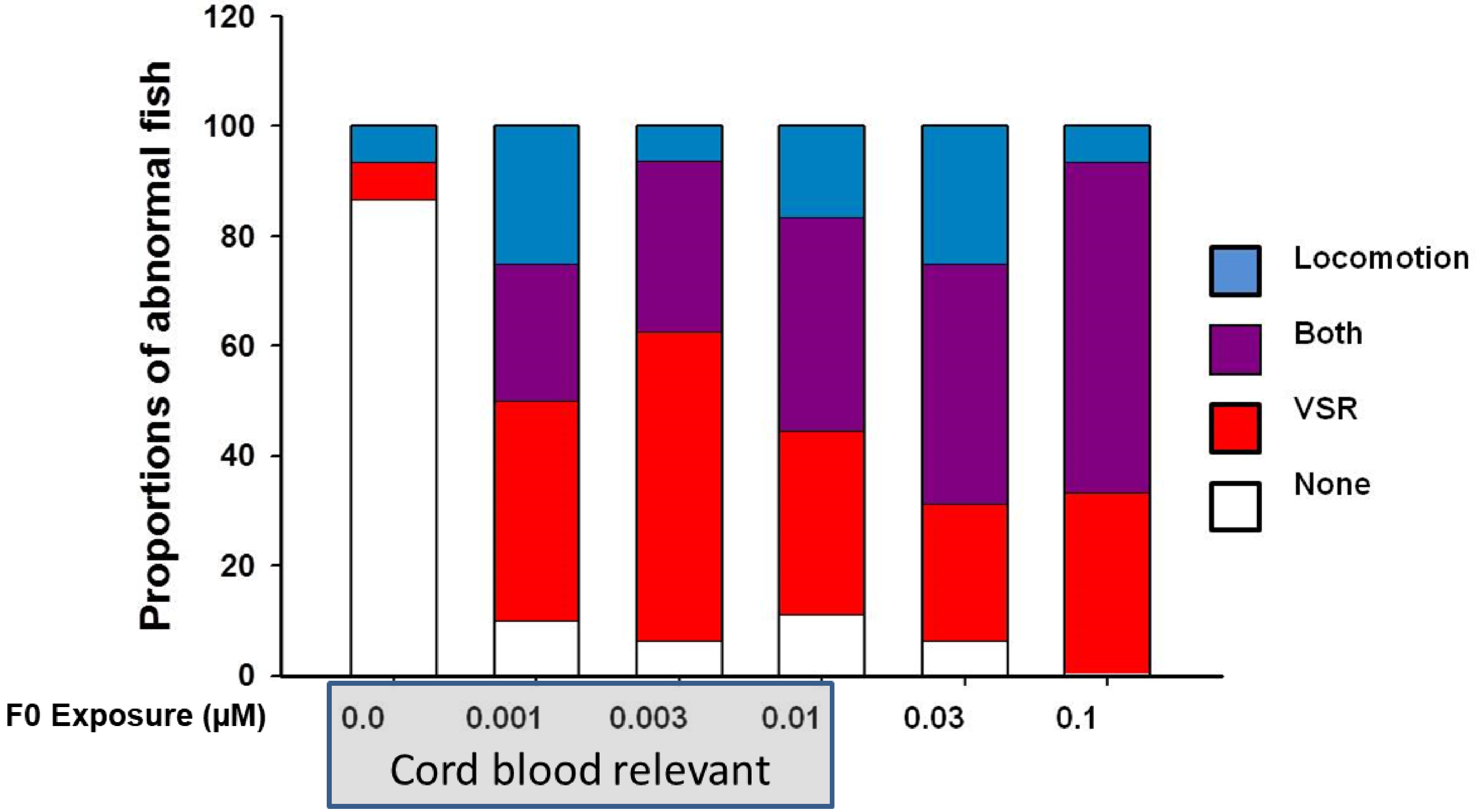
Spontaneous Activity in F2 Lineages



They've lost their "glide"

Carvan et al., PLoS ONE 2017

Distribution of Phenotypes in F2 Lineages



Independent inheritance

Carvan et al., PLoS ONE 2017

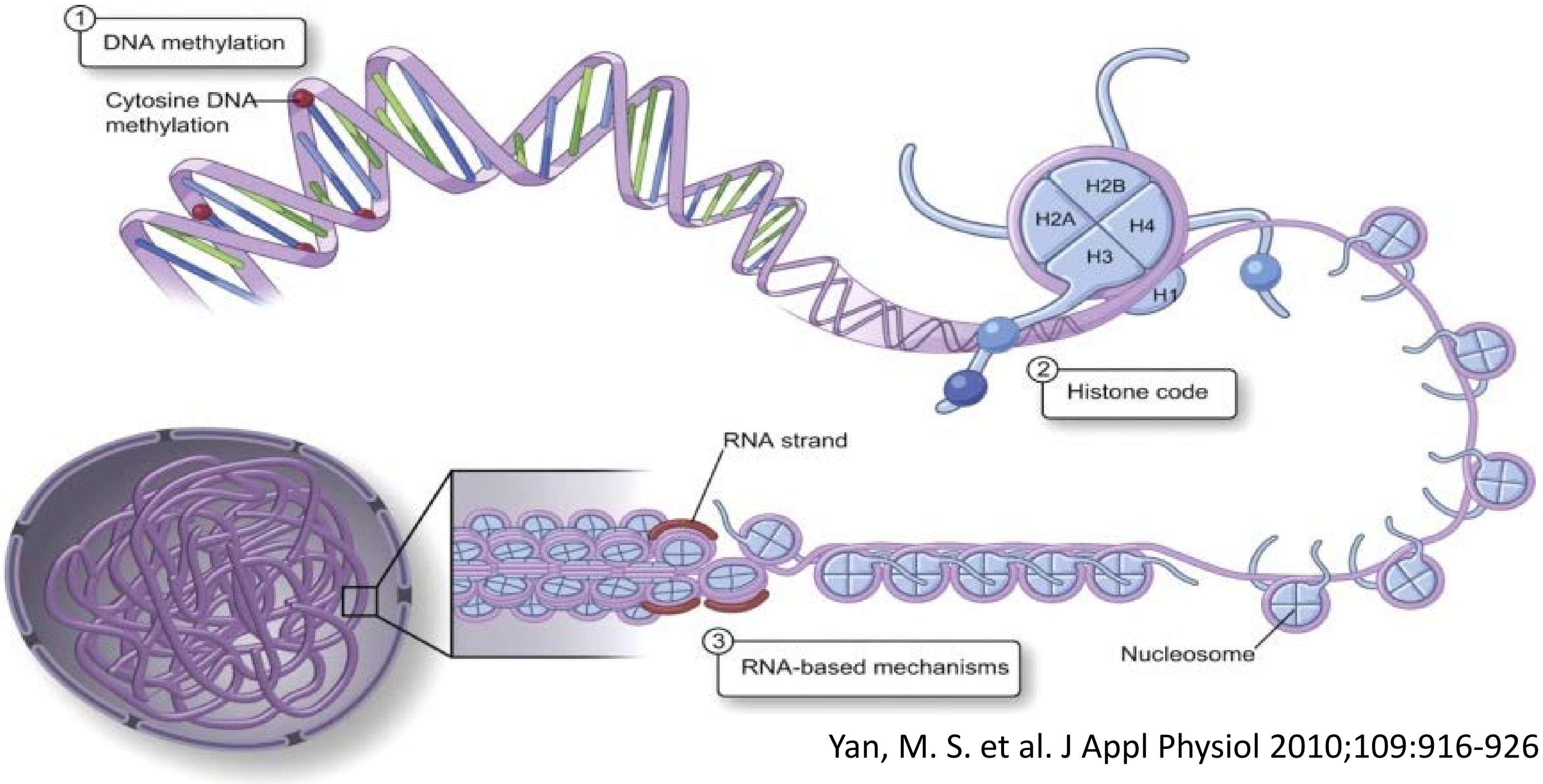
Independent inheritance

Supplemental Table S2: Evaluation of expected versus observed inheritance of neurobehavioral phenotypes.

F2 lineage (nM)	n	Neither Phenotype	Visual deficit	Hyperactivity	Both Phenotypes		Chi-square
					Expected	Observed	
0	15	87% (13)	7% (1)	7% (1)	0% (0)	0% (0)	□2=0.067 df=4 **p=0.999**
1	20	5% (1)	65% (13)	65% (13)	42% (8)	35% (7)	
3	16	6% (1)	93% (14)	38% (6)	35% (6)	31% (5)	
10	18	6% (1)	72% (13)	61% (11)	44% (8)	39% (7)	
30	17	6% (1)	71% (12)	71% (12)	50% (9)	47% (8)	
100	15	0% (0)	93% (14)	60% (9)	56% (8)	53% (8)	

Carvan et al., PLoS ONE 2017

Mechanism? Epigenetic Inheritance



Yan, M. S. et al. J Appl Physiol 2010;109:916-926

miRNA
lncRNA
Other?

DNAmet epimutations have been shown to be heritable and associated with transgenerational inheritance in several species.

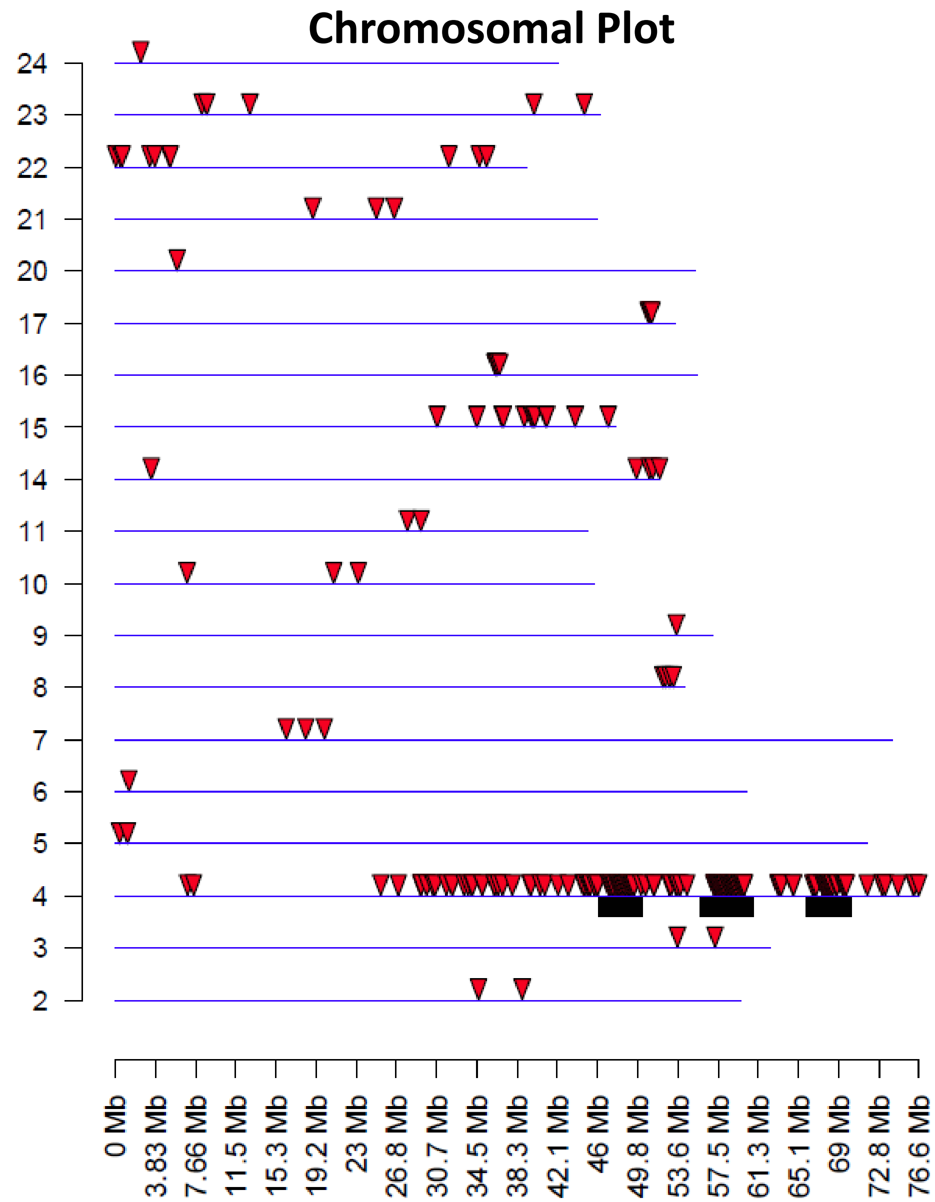
Transgenerational Inheritance in Zebrafish

Chemical	Citation	Phenotypic endpoint(s)	Epigenetics
TCDD	Baker TR et al. 2014	Skeletal develop, sex ratio, male-mediated reproduction	None
Bisphenol A	Lombó et al. 2015	Heart failure	Global DNAmet in testes, sperm
	Akhter, et al. 2018	Reproductive abnormalities	None
Methylmercury	Carvan et al. 2017	Neurobehavior, bipolar cell electrophysiology	MeDIPseq in sperm
Mono(2-ethylhexyl) phthalate 5-azacytidine	Kamstra et al. 2017	None	Locus-specific methylation (6 and 2, respectively)
Benzo[a]pyrene	Knecht et al. 2017	Neurobehavior, BMI, heartbeat, mitochondrial function	Global DNAmet

Differentially Methylated Regions in F0 Sperm

Number of DMRs using different EdgeR p-values curroff thresholds

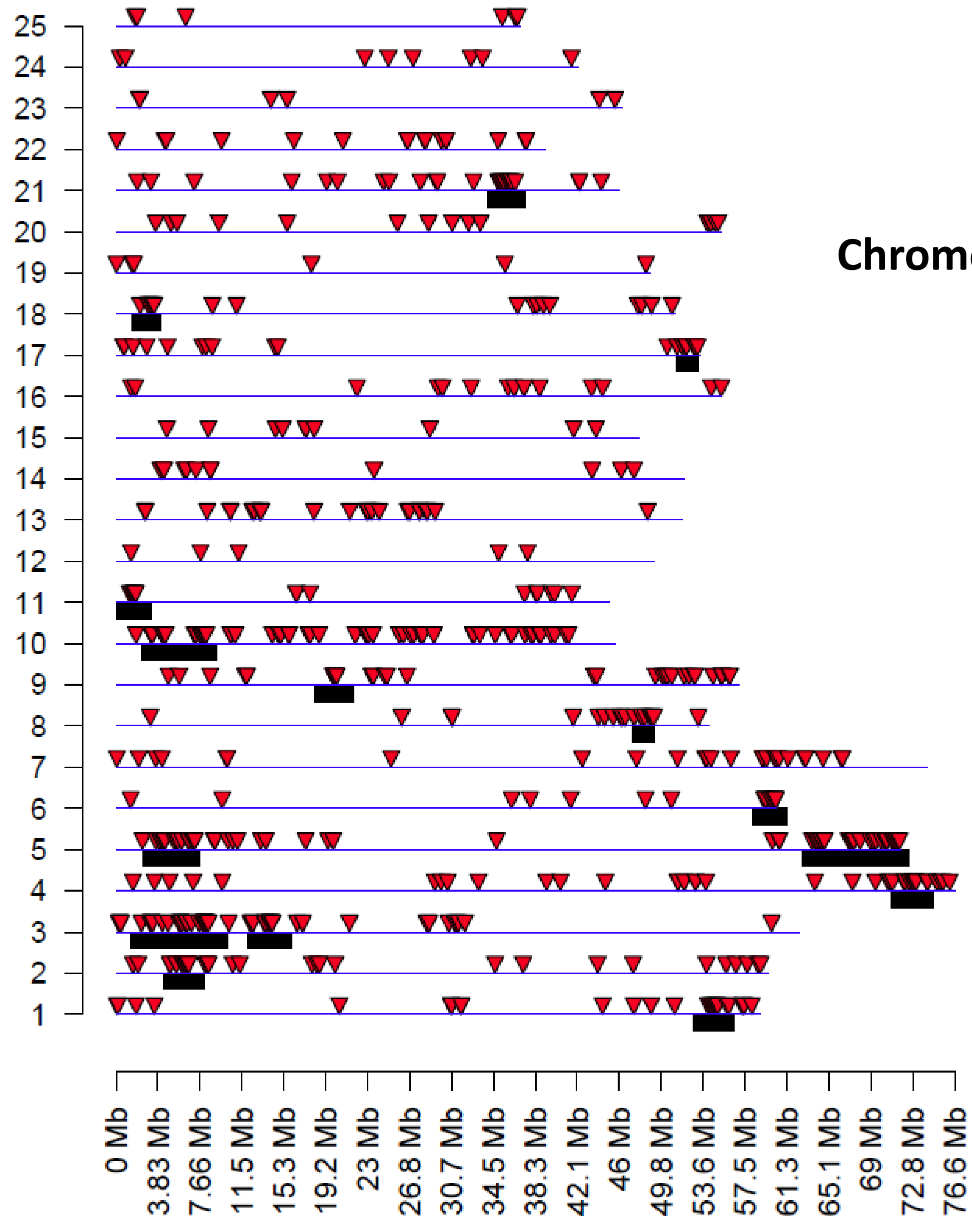
p-value	allWindow	twoWindow
$1x10^{-3}$	10125	2966
$1x10^{-4}$	3005	1171
$1x10^{-5}$	1383	634
$1x10^{-6}$	811	413
$1x10^{-7}$	533	291



Differentially Methylated Regions in F2 Sperm

Number of DMRs using different EdgeR p-values curroff thresholds

p-value	allWindow	twoWindow
$1x10^{-3}$	22877	8370
$1x10^{-4}$	8499	3429
$1x10^{-5}$	4093	1771
$1x10^{-6}$	2307	985
$1x10^{-7}$	1414	617



Chromosomal Plot

Carvan et al., PLoS ONE 2017

RNAseq Analysis F2 Adults

Dysregulated KEGG pathways

Brain

Retina

FDR < 0.05

unpublished

Linking DOHaD, Epigenetics, Transcriptomics

Relationship between the germline epigenome and that of somatic cells associated with neurobehavioral defects (anatomy, physiology, MeDIPseq, RNAseq)

unpublished

Linking Epigenetics and Transcriptomics

We can do gene expression and DNAmet in the same cell prep

unpublished

Powerful System for DOHaD, Generational Effects

Strengths

- Genome resources
- Most cell/molecular pathways similar to humans
  - Complex behaviors
- NOT inbred
- Few limitations on replication
  - Large clutch size
- GFP-labeled lines for most cell types
- Can complement (limit) the use of mammals in research

Weaknesses

- They are not mammals
  - No mammary tissue
  - No lungs
- Small tissues can be limiting
  - Analytical chemistry
  - Biochemistry
  - Physiology

guardians of the future

The Bemidji Statement on Seventh Generation Guardianship

"The first mandate...is to ensure that our decision-making is guided by consideration of the welfare and well being of the seventh generation to come.“

http://www.sehn.org/bemidjistatement.html

Methylmercury Treatments

Total Hg Analysis and Evaluation of Dosimetry			
Media, Nominal (nM)	Media, Measured (nM)	Embryo, Measured (ppb)	Similar values in human cord blood
0	0.15 $\pm$ 0.02	5.5 $\pm$ 0.5	
1	1.5 $\pm$ 0.4	19.4 $\pm$ 1.0	
3	2.9 $\pm$ 0.2	51 $\pm$ 3.5	1% Lake Superior
10	10 $\pm$ 0.05	257 $\pm$ 10	Highest Lake Superior
30	32 $\pm$ 0.6	836 $\pm$ 68	Minamata, Japan
100	104 $\pm$ 0.4	2819 $\pm$ 152	

Carvan et al., PLoS ONE 2017