Investigating GxE neurotoxicant vulnerabilities across life stage and populations using iPSCs

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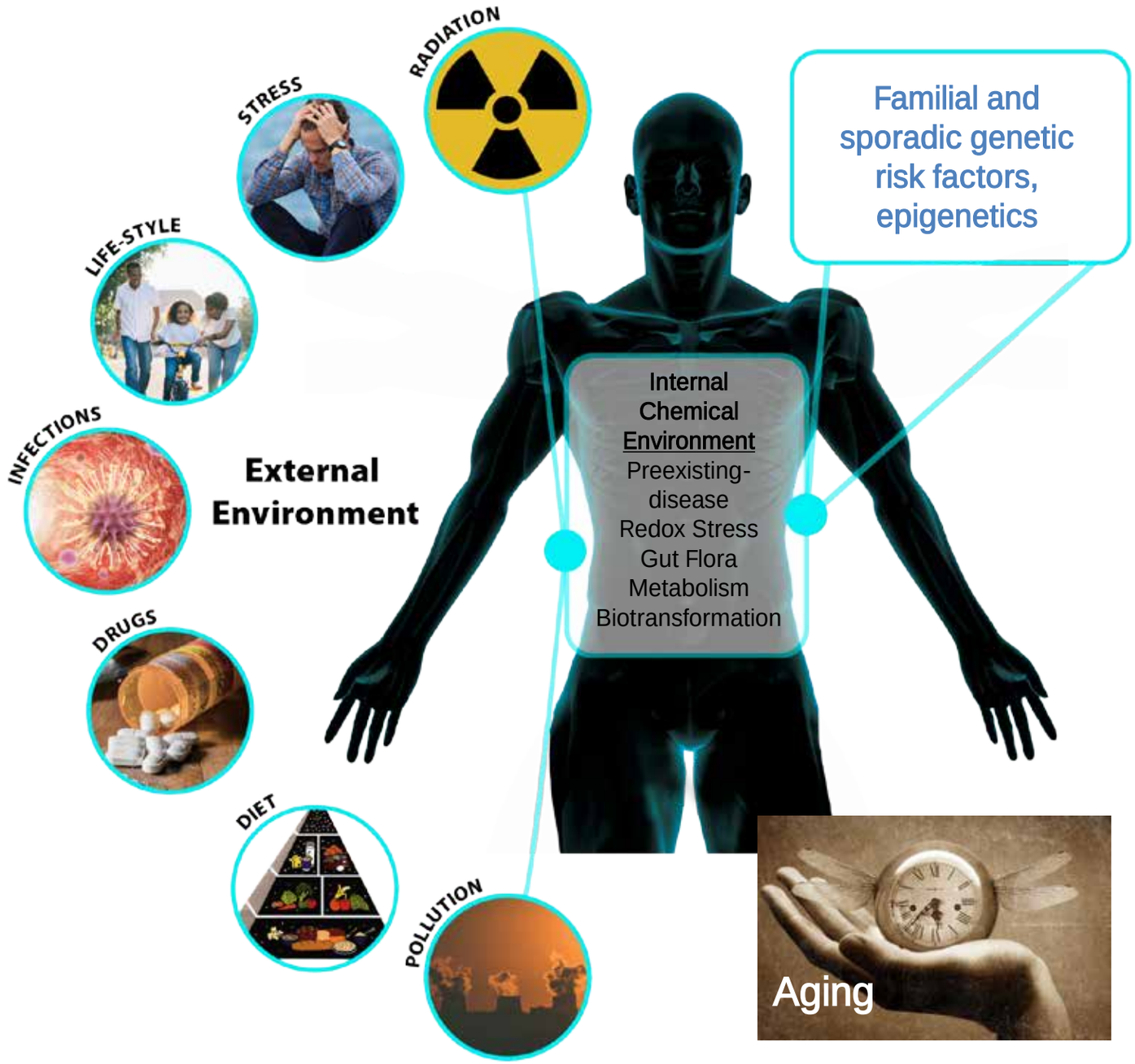
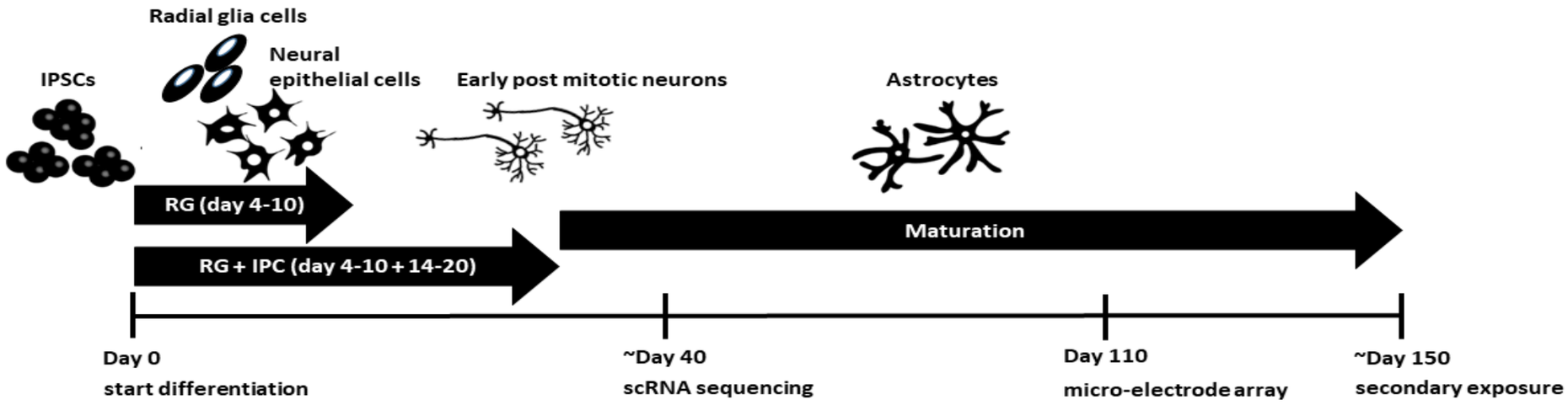
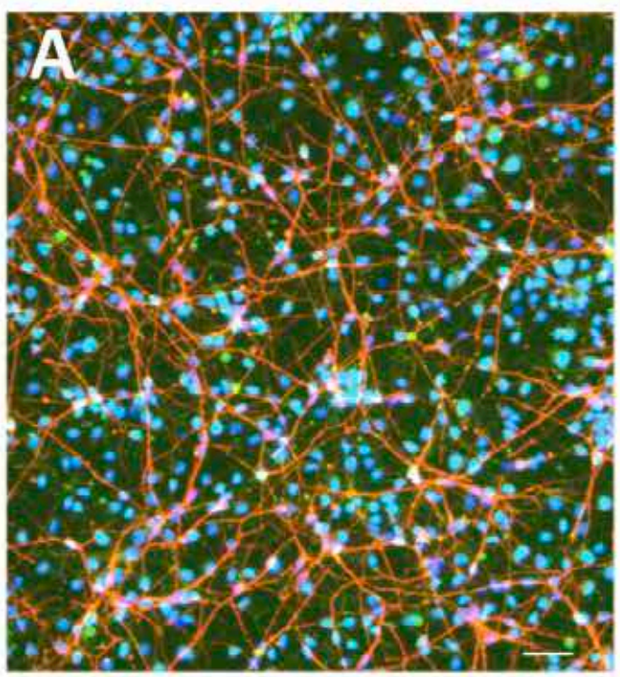
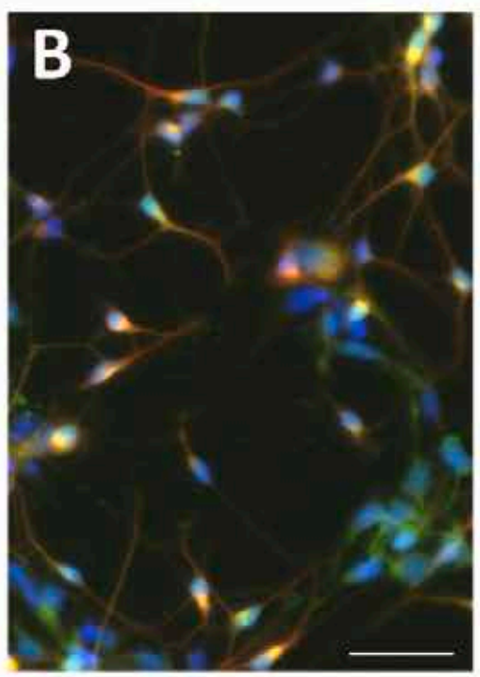
Genes, environment and aging effects, and their interactions, culminate in many human diseases including:

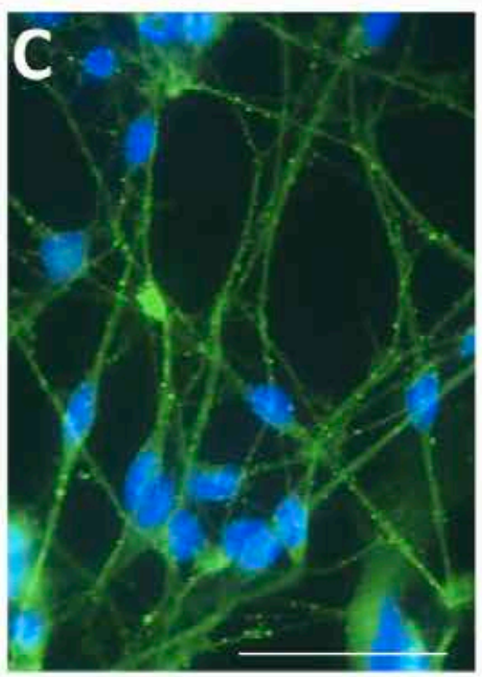


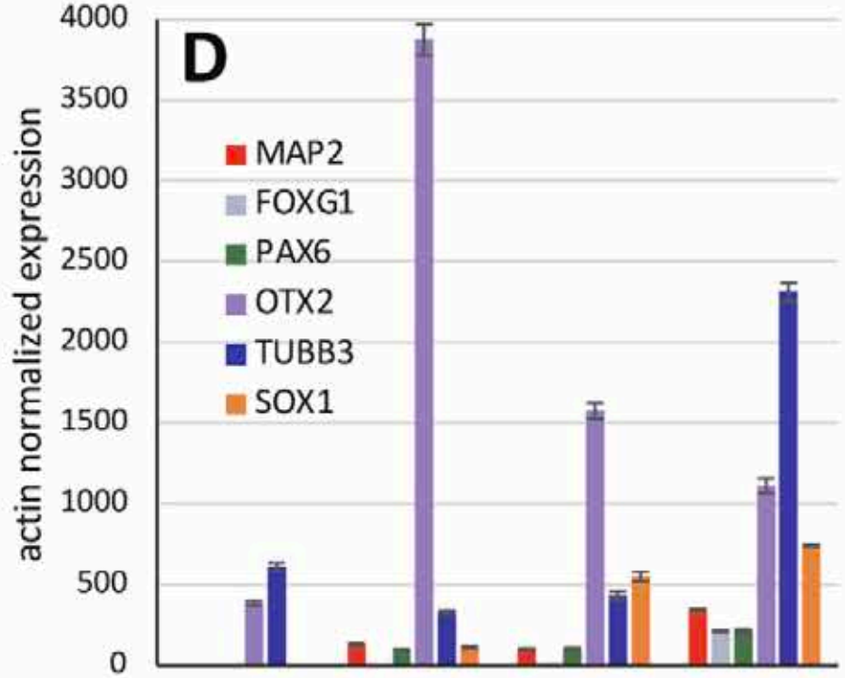
Differentiation of human induced pluripotent stem cells to cortical lineage neurons





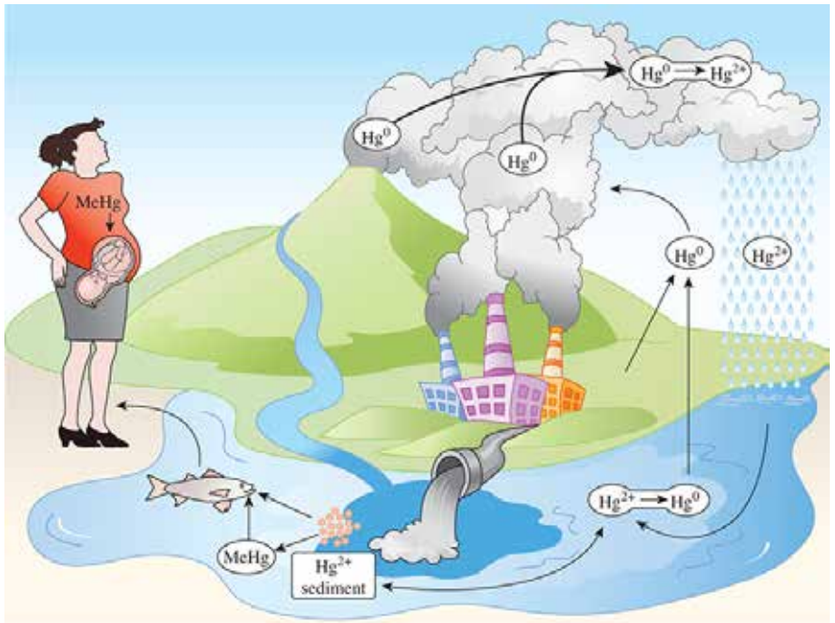






Prince *et al* Food and Chemical Toxicology, 2021
Neely *et al* Food and Chemical Toxicology, 2021
Anke Tukker - graphic

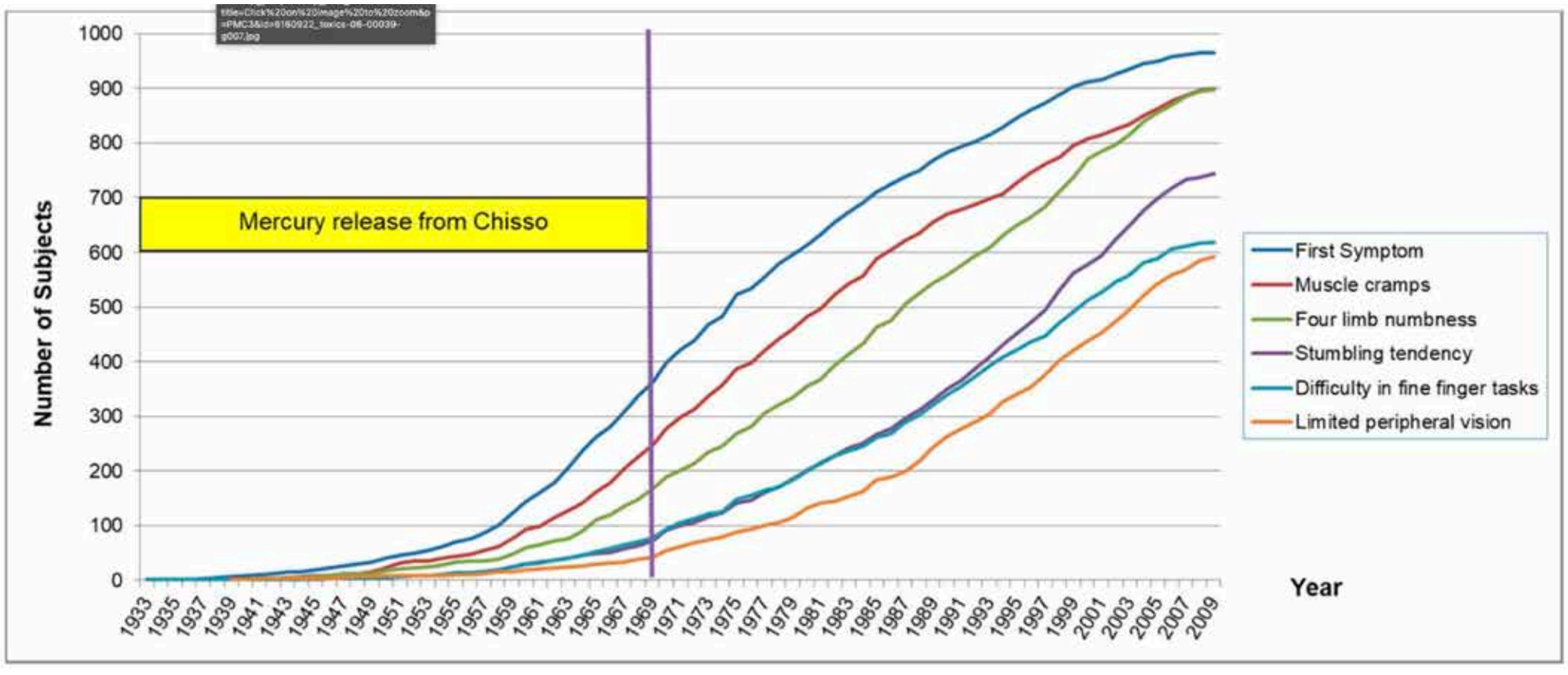
MeHg neurotoxicity



Chapter 23 Toxic Effects of Metals, Klaassen CD. *Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition*; 2019.

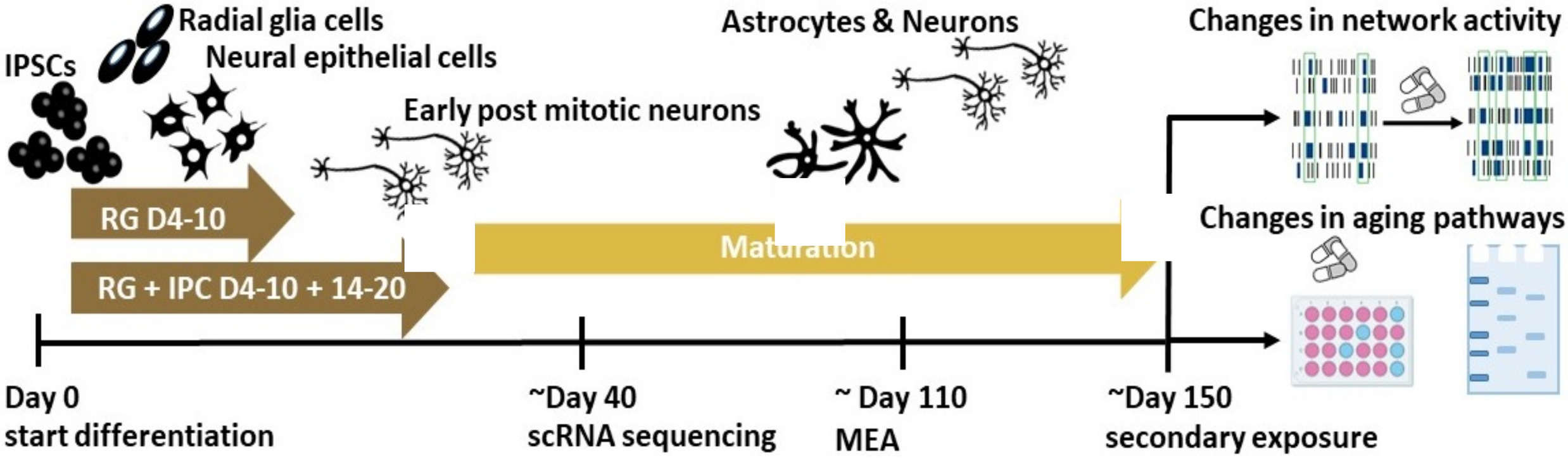
MeHg is associated with both latent and persistent neurotoxic effects

MeHg poisoning at Minamata Bay led to persistent toxicity up to at least 50 years later



Shigeru Takaoka *et al* Toxics. 2018

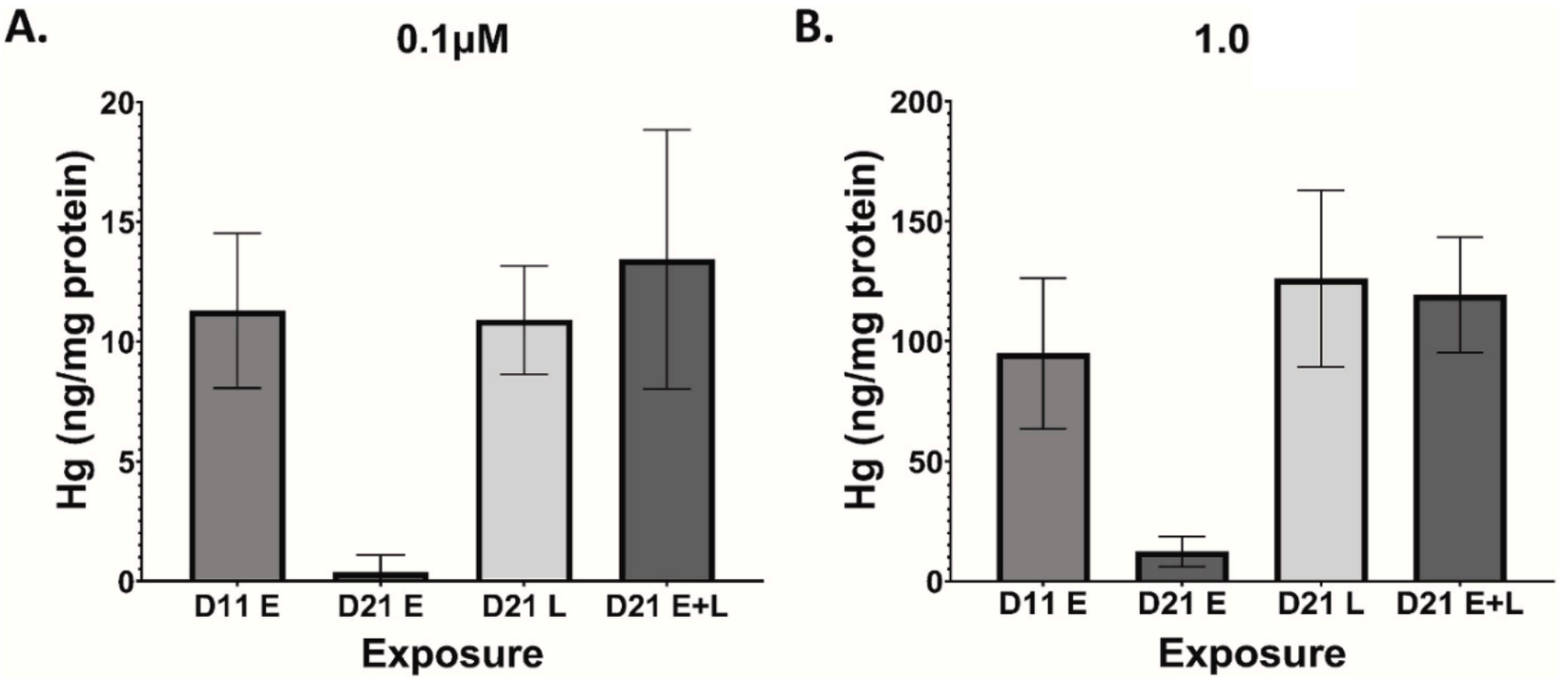
Modeling latent and persistent neurotoxicity of early life exposures in human stem cell based neuronal model system



Prince *et al* Food and Chemical Toxicology, 2021
Neely *et al* Food and Chemical Toxicology, 2021
Anke Tukker - graphic

MeHg exposure during cortical neurodevelopment phases leads to toxicologically relevant levels

Total Hg levels post-exposure; Day 11 or Day 21



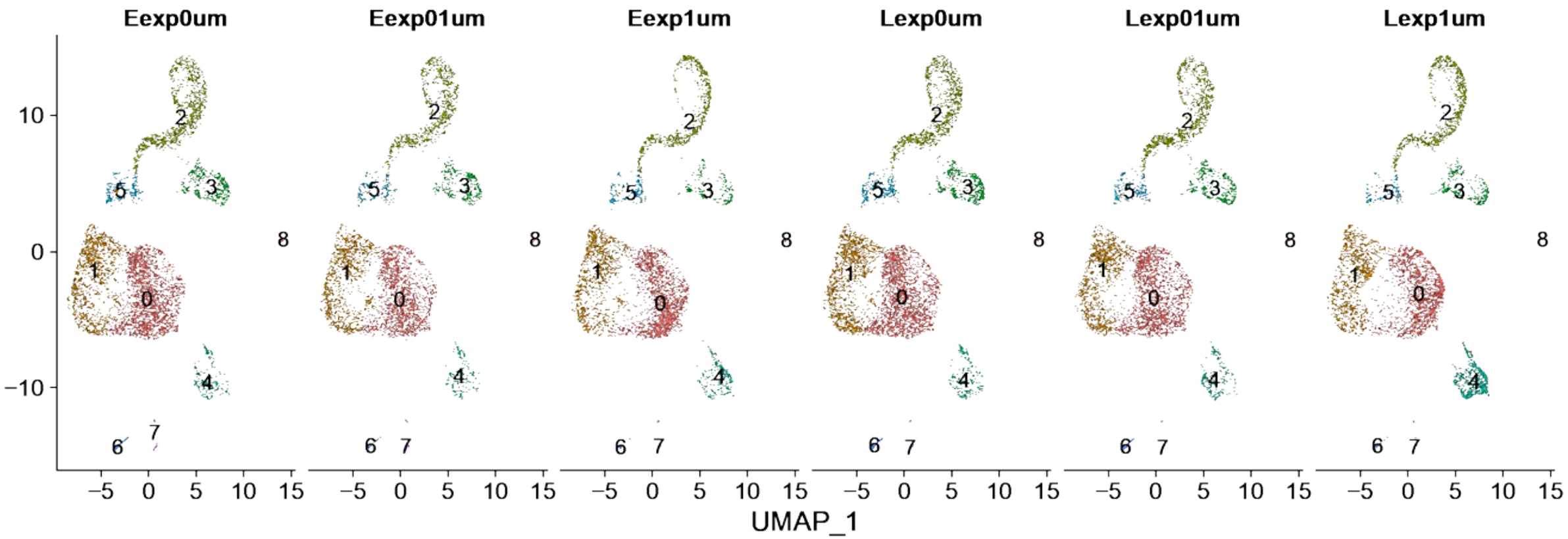
Levels reach ~1.5-3.0 PPM

Levels reach ~12-25 PPM

Threshold of human neurotoxicity 0.1-0.4 PPM; human fetal lethality brain levels 14 PPM

Prince *et al* Food and Chemical Toxicology, 2021
Neely *et al* Food and Chemical Toxicology, 2021
Anke Tukker - graphic

Single-cell RNA sequencing of Day 38 neurodevelopmental MeHg exposures shows minimal global impact on differentiation



Methylmercury exposure: E = Day 4-10 of differentiation; L = Day 4-10 and Day 14-20 of differentiation

scRNA-seq at Day 38 of differentiation

Cluster 0 and 1 are cortical radial glia (RG) cells, with cluster 1 being the more actively proliferating subpopulation.
Cluster 5 corresponds to these **intermediate progenitor cells** (**IPC),** EOMES/TBR2 and NeuroD1/D4/G1/G2 positive)
Cluster 2 cells are VGlut1 and VGLut2 positive postmitotic immature GLUergic excitatory cortical neurons (CNs), expressing markers of dorsal forebrain/telencephalon (e.g. FoxG1 and TBR1); consistent with deep layer V/VI cortical identities.
Cluster 3 cells are VGlut2 positive postmitotic immature GLUergic excitatory neurons, expressing dorsal forebrain markers (Dlx2, FoxP2) consistent with a thalamic GLUergic identity (e.g. Gbx2) (TNs).
Both **Clusters 2/3** GLUergic forebrain markers: DCX, Calb2, NCAM1/2, TUBB3, MAPT, Syn1, Homer1, and DLG4.
Cluster 4 cells are presumptive anterior telencephalon choroid plexus progenitors (Lun et al., 2015; Sivitilli et al., 2020).
Combined, **clusters 6, 7 and 8 represent less than 2% of all cells and are predicted to be other progenitor and mural cell types**.

Neely *et al* Food and Chemical Toxicology, 2021

Part I Conclusions - MeHg Neurotoxicity

1. **Methylmercury exposure associated latent and persistent toxicity phenotypes can be recapitulated in human induced pluripotent stem cells models**

2. **Persistent toxicity associated with alteration in healthy aging signaling pathways**

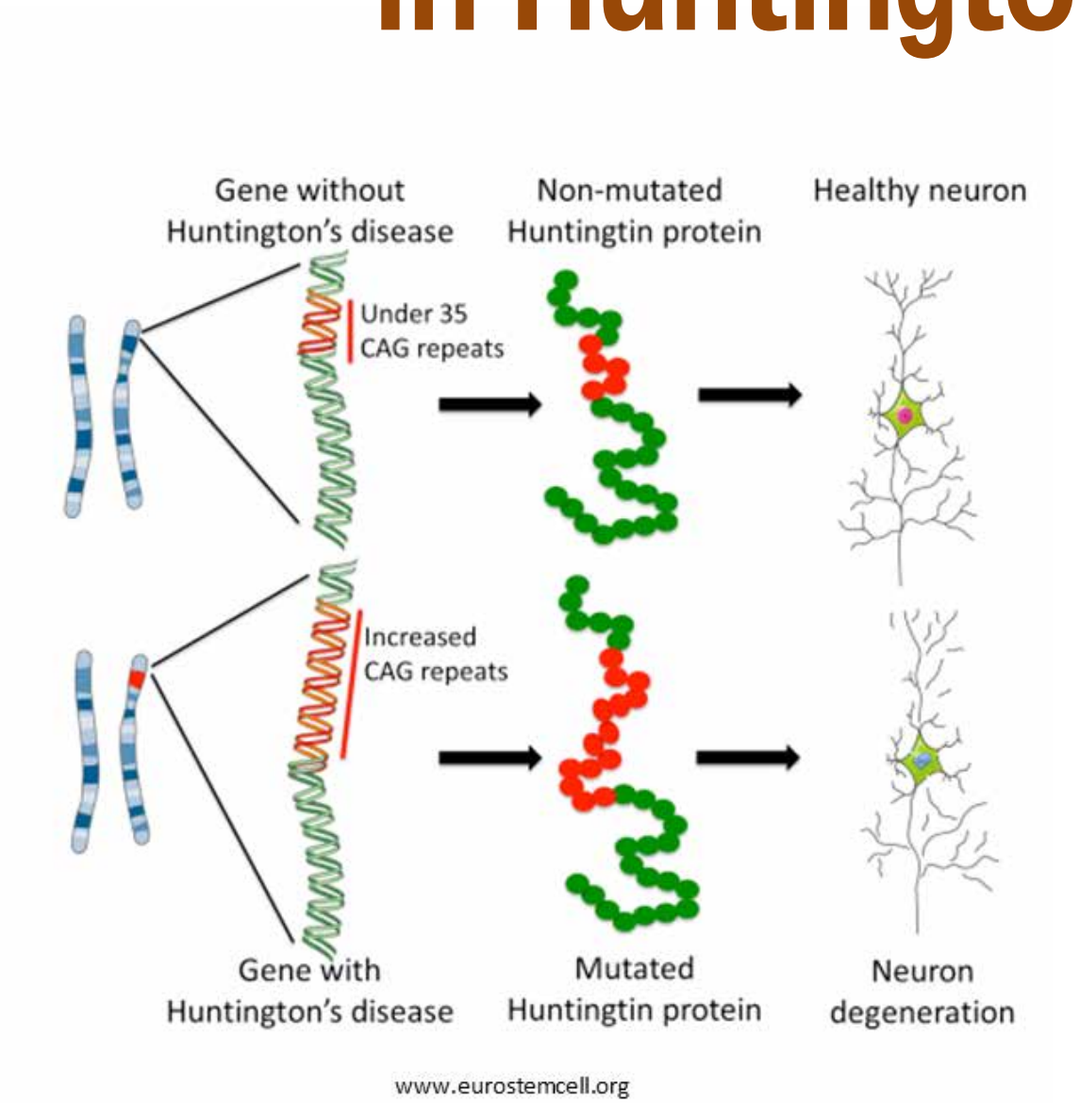
3. **Immediate effects of exposures are relatively mild, with latent functional and genetic effects evident**

4. ***What is the basis of MeHg latent and persistent effects?***
    - ***Epigenetic – chromatin or extrachromatin***
    - ***Developmental windows of susceptibility***
    - ***Duration and can it be reset?***

Part 2

Mn toxicity and GxE interactions

The evidence for loss of bioavailable Mn in Huntington's Disease



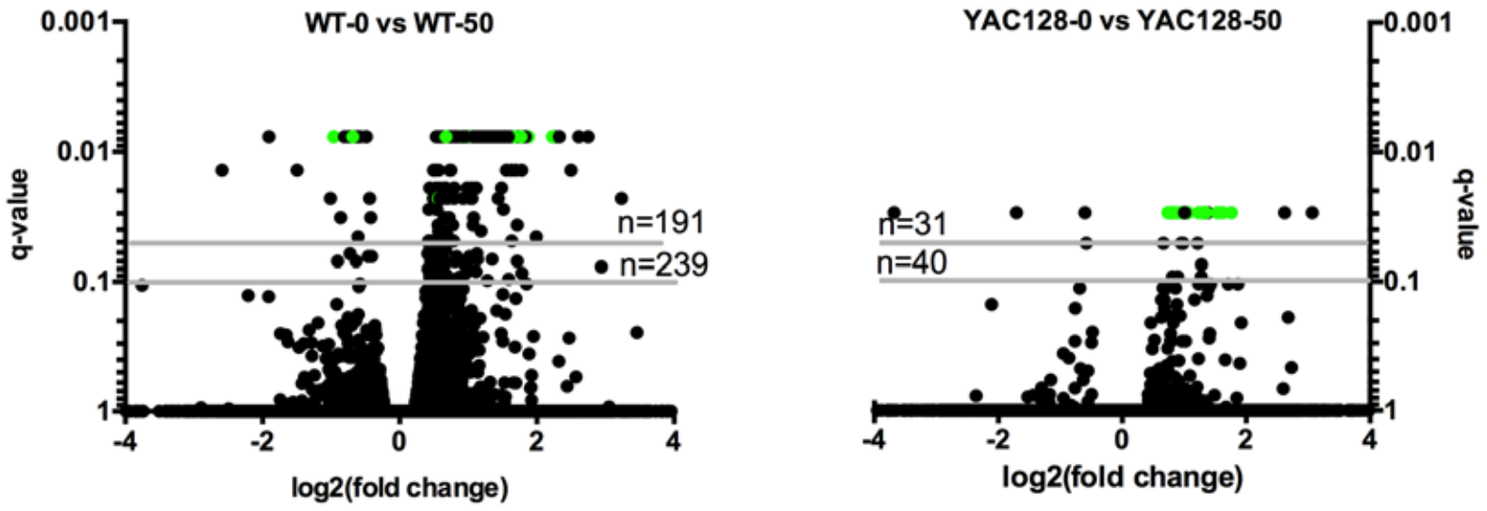
- Normally, among the highest concentrations of Mn in the body and brain are found in the basal ganglia, which degenerate in HD
- HD phenotypes and symptoms are consistent with decrease in neuronal Mn bioavailability (i.e. loss of Mn-dependent enzyme activities)
- **HD cell lines and mouse model consistently take up less Mn (unknown GOF mechanism)**
- **Decreased Mn uptake is causal in various Mn-responsive enzymatic defects in HD model systems**

Williams *et al* Tox Sci, 2010

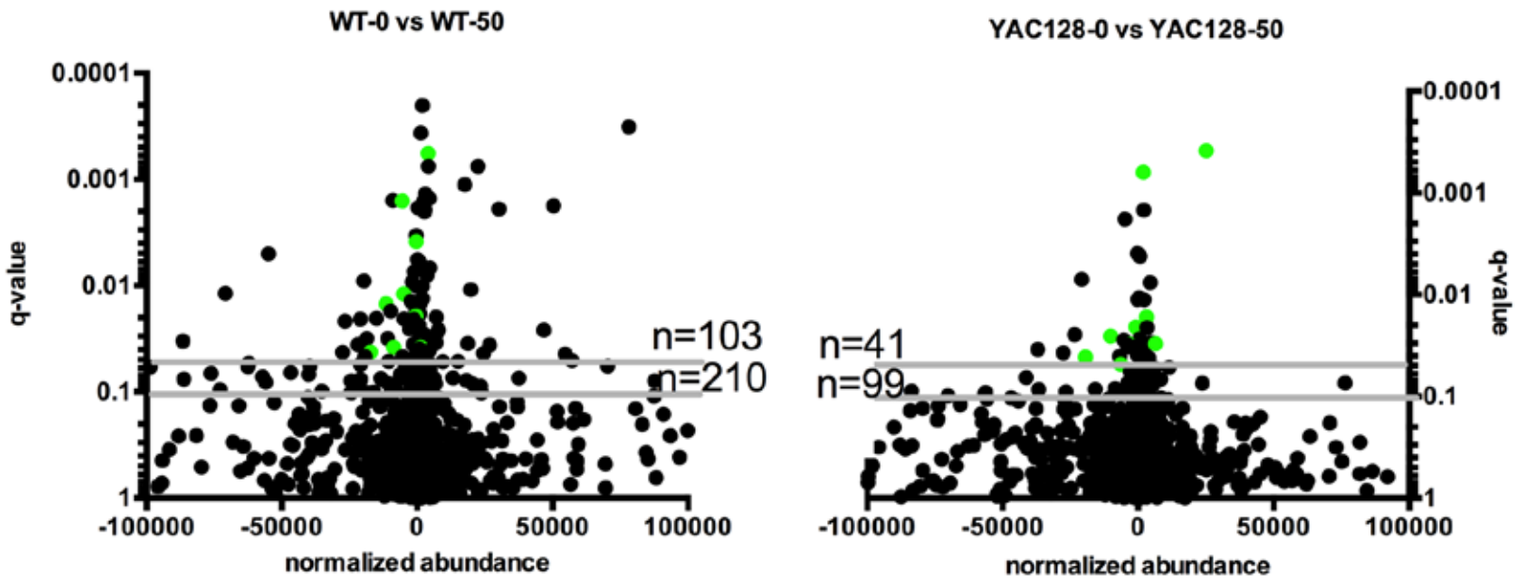
Andrew Tidbal *et al* Hum Mol Genet, 2015

Global analysis of HD mouse models support link between manganese biology and HD

RNASeq of Mouse Striatum following 1 week Mn exposure paradigm (50mg/kg MnCl2-tetrahydrate) in WT versus HD mice pre-manifest



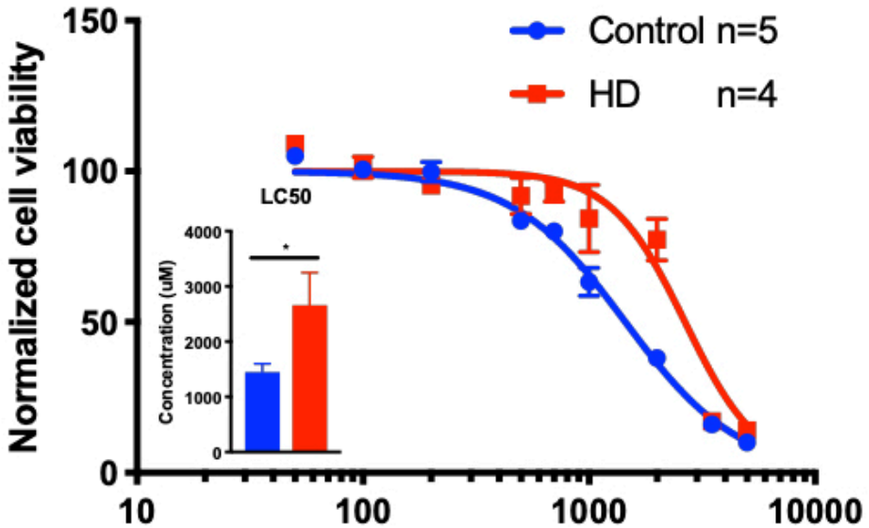
Metabolomics of Mouse Striatum following 1 week Mn exposure paradigm (50mg/kg MnCl2-tetrahydrate) in WT versus HD mice post-manifest (32 weeks)



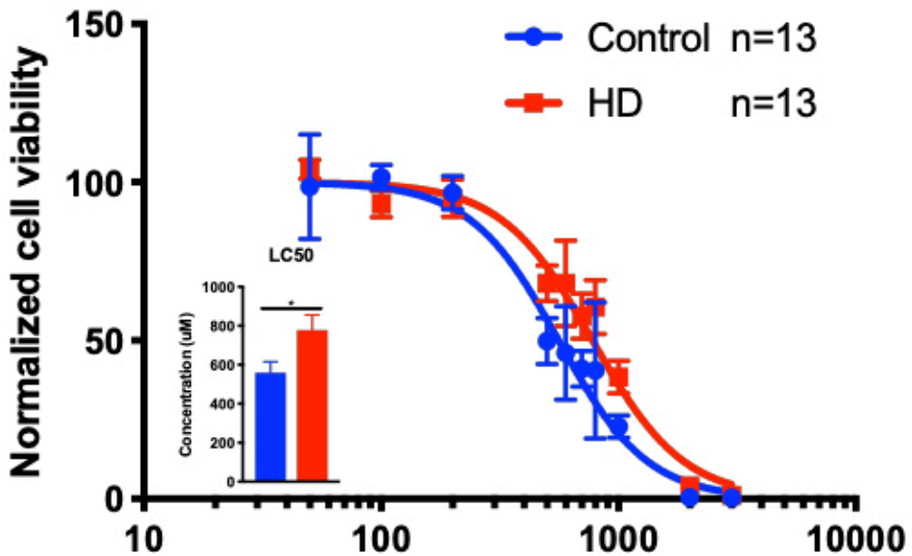
Anna Pfalzer *et al* Metallomics. 2020

HD neuroprogenitor cells exhibit lineage-specific and stage-specific differences in Mn cytotoxicity

Day 11 Cortical Glutamatergic NPCs

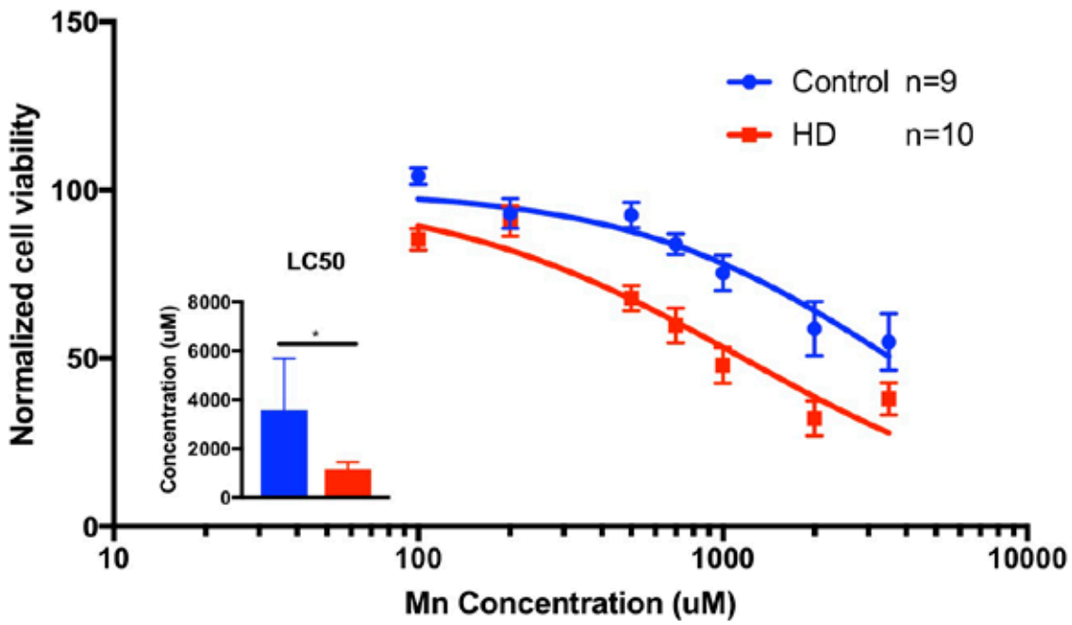


Day 11 Striatal NPCs



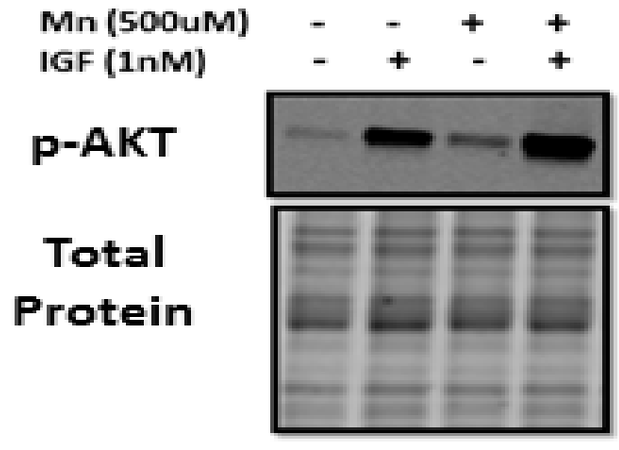
Day 50-125 Early Cortical Neurons

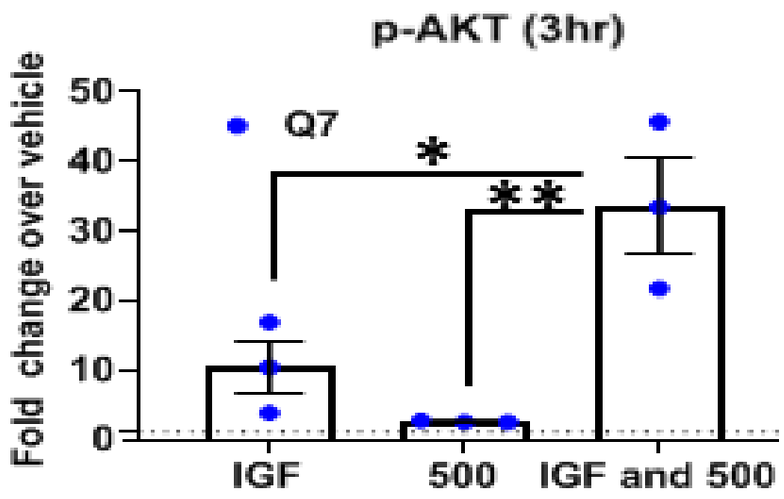
Day 25 Early Midbrain Neurons



Piyush Joshi *et al* Neurotoxicology. 2019

Mn+IGF exhibit synergistic activation of AKT and S6 signaling proteins





Near physiological concentration of IGF 1 nM;
500 µM Mn

Miles Bryan *et al* Mol. Neurobiology. 2020

Relationship of Mn biology to IGF/mTOR signaling

- **IGF-1 rescues HD phenotypes** via AKT signaling, consistent with a Mn and IGF-1 phenocopy
  - P-HTT (ser421), ROS, mitochondrial function, motor deficits, mutHTT aggregates (*Rego et al*)
- Mn can modulate **insulin/glucose tolerance** *in vivo (Baly et al, 1980)*
- Mn increases **IGFR/IR phosphorylation** (*in vitro*) more than Mg *(Xu et al, 1995)*
- **Mn neurotoxicity** has been associated with increased AKT phosphorylation but the mechanism and consequences are unknown
- Mn-induced p-AKT is **PI3K-dependent** *(Bryan et al, 2017)*
- **Mn^{2+} is a physiologically relevant cofactor for mTOR** (TORC1) – substantially more efficient than Mg^{2+} (*Nicastro et al, 2022*)

Conclusions - Part 2: Mn Toxicity Conclusions

Manganese toxicity is altered by the HD genotype

- ***– global gene expression and metabolomics***
- ***– Mn cytotoxicity***

Manganese synergistically alters insulin/IGF signaling (IIS)

- ***– to activate AKT and S6 pathways, S6 more so***

Acute effects of Mn on IIS signaling become a homeostatic response under chronic low-level Mn exposures.

Chronic Mn exposures nonetheless show evidence of persistent changes in IIS and related signaling pathways, and functional effects on neuronal activity

Overall Conclusions and Future Directions

Neurotoxicants associated with chronic/persistent toxicity exhibit changes in cell signaling pathways associated with healthy aging

Current hypothesis: **While homeostasis for acute signaling responses that regulate metabolism may be restored under chronic toxicant exposure conditions, long term changes in gene expression changes could underlie aging and genetic stress related disease susceptibility.**

Future directions: **Evaluate the potential of other early life toxicant exposures to influence healthy aging and disease susceptibility in elderly sibling pairs and across different genetic risk factors associated with chronic neurological disease**

Key Team Members for these projects

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Fiona Harrison (VUMC)

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