

# *In Vitro* Lecture and Luncheon

Sponsored by the Colgate Palmolive Company

March 11, 2019



## *In Vitro* Lecture Goals

- Feature important research using *in vitro* and alternative techniques to study basic mechanisms
- Illustrate how these test methods benefit animal welfare by replacing animal use whenever it is feasible
- Encourage students and postdoctoral scholars to use alternative techniques in their research



**Patient-Based Cellular Model  
Systems to Assess Individual  
Risk to Neurotoxicants**

**2019 *In Vitro* Lecture**

**Aaron Bowman**

**Speaker**

# Patient-Based Cellular Model Systems to Assess Individual Risk to Neurotoxicants



**Aaron Bowman, PhD**

**Head and Professor of Health Sciences**

**PURDUE**  
UNIVERSITY

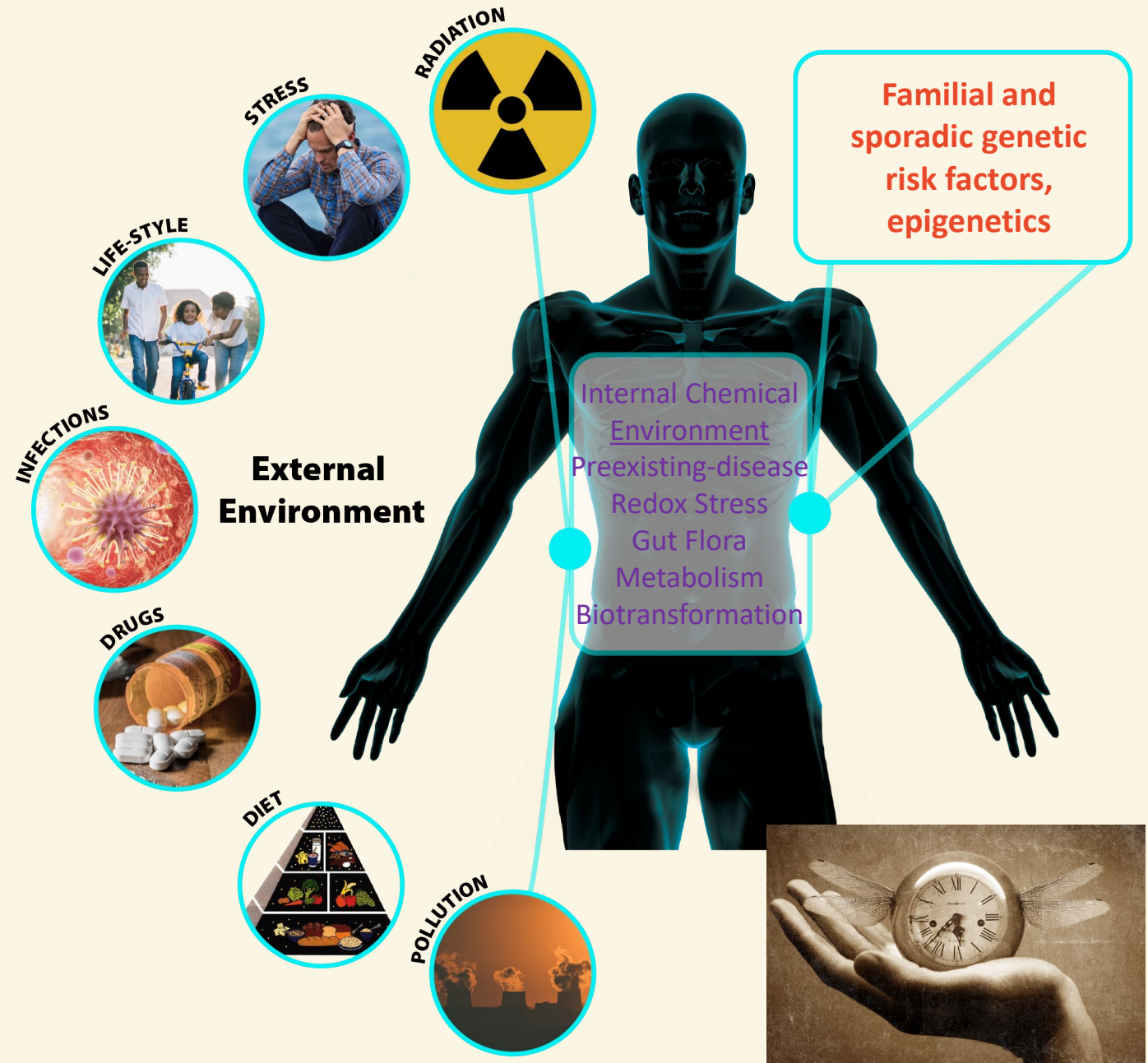
School of Health Sciences

**PURDUE**  
UNIVERSITY

Purdue Institute for Integrative Neuroscience  
DISCOVERY PARK



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



# Parkinson's Disease

## **Cardinal Symptoms:**

Tremor (resting)

Muscular rigidity

Bradykinesia (slow movement)

Disturbances in gait and posture

## **Etiology:**

Pesticides, metals (e.g. manganese and copper) and other environmental risk factors impinging upon backdrop of genetic risk factors, sex-effects and aging

## **Pathology:**

Loss of substantial nigral dopamine neurons

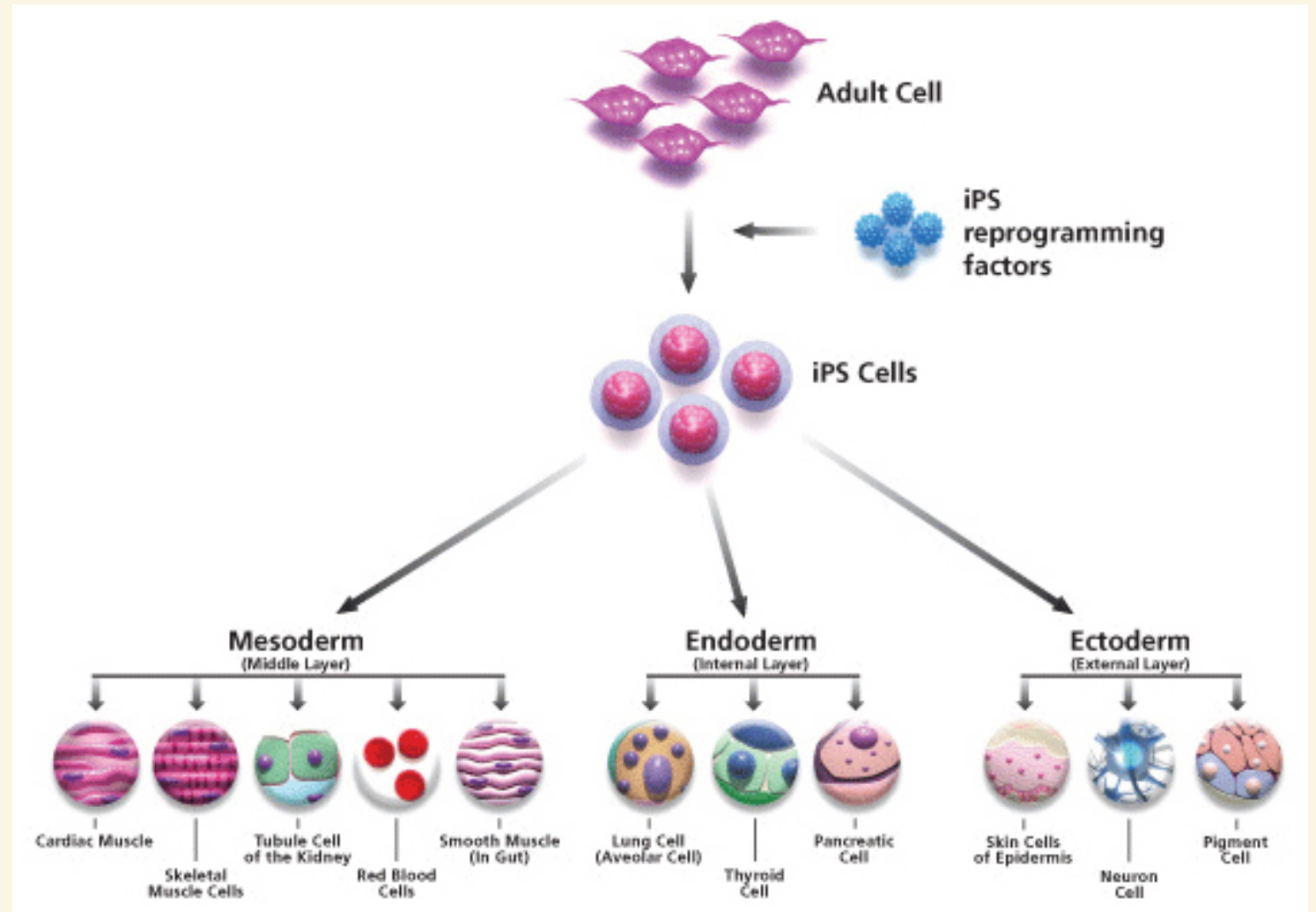
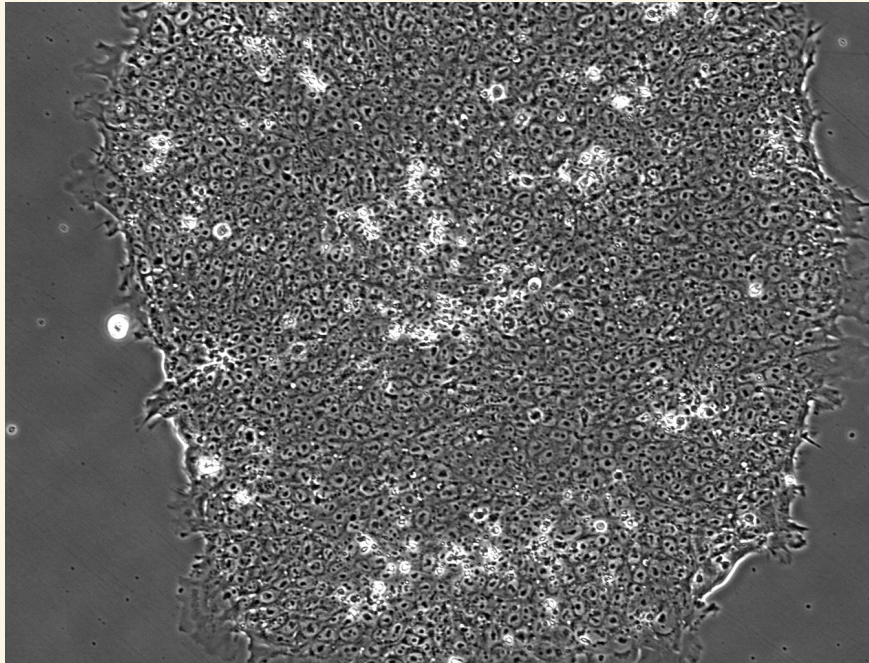
Accumulation of alpha-synuclein in Lewy bodies



Sir William Richard Gowers, 1886

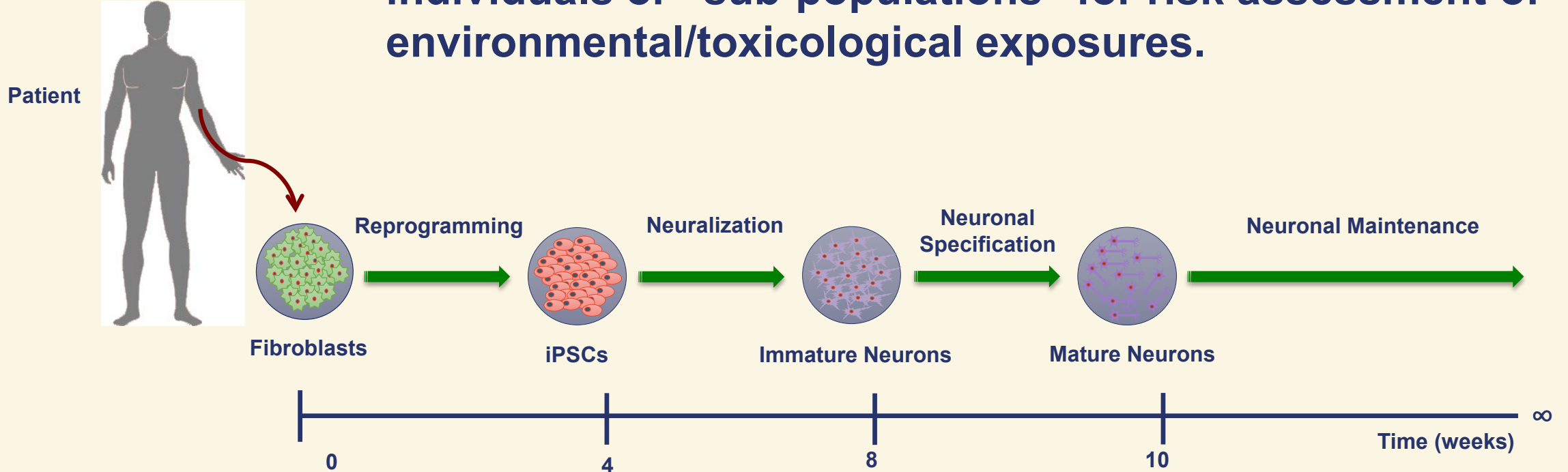
*A Manual of Diseases of the Nervous System*

# Human Pluripotent Stem Cells Made by “Reprogramming” Adult Skin Cells



## Key Concept:

Human induced pluripotent stem cells may model individuals or “sub-populations” for risk assessment of environmental/toxicological exposures.



### Selection of Human Subjects

Susceptibility Differences  
Genetic Risk Factors  
Sex Differences  
Disease-State

### Types of Exposure Models

Acute Toxicity  
Chronic Toxicity  
Multi-hit Toxicity  
Delay-effect Toxicity

**Key Concept:** Environmental risk factors for neurological disease have been difficult to detect in epidemiological cohorts, potentially due to human variability of risk.

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## Occupational copper and manganese exposure are putative risk factors for Parkinson's Disease

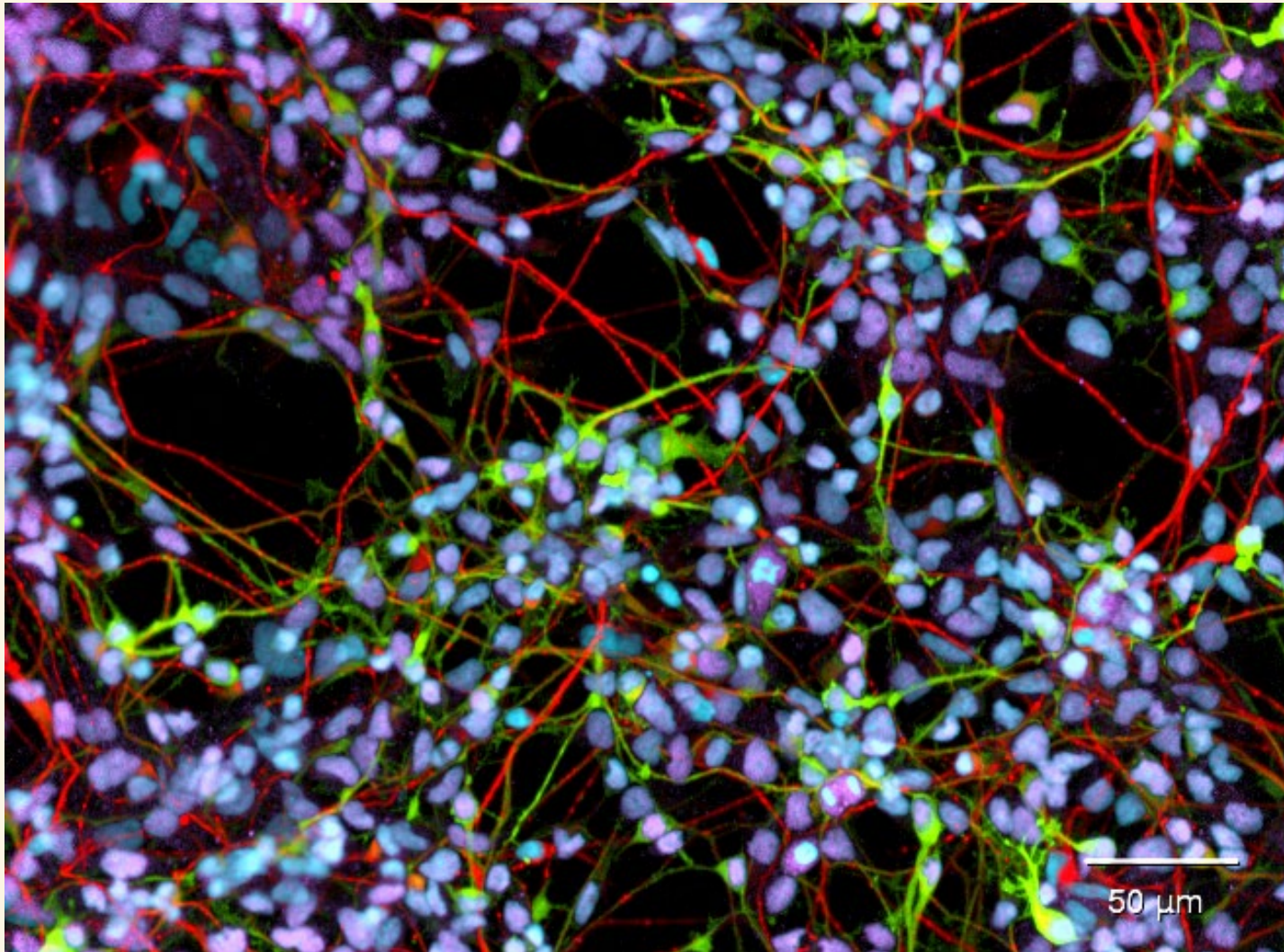
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- Adjusting for sex, race, age and smoking status, 20 years of occupational exposure to copper or manganese was associated with Parkinson's Disease, but not iron, zinc, or mercury alone.
- Occupational exposure for > 20 years to combinations of lead-copper, lead-iron, or iron-copper was also associated with disease.

Neurotoxicology. 1999 Apr-Jun;20(2-3):239-47.

**Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease.** Gorell JM<sup>1</sup>, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, Richardson RJ.

# Developing Human Mesencephalic (Nigral/Midbrain) Dopamine Neurons



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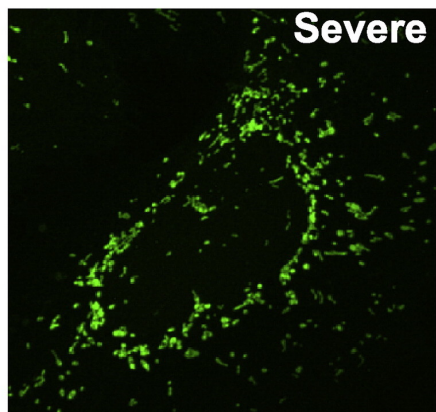
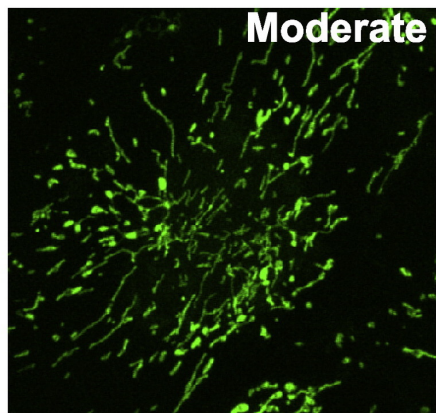
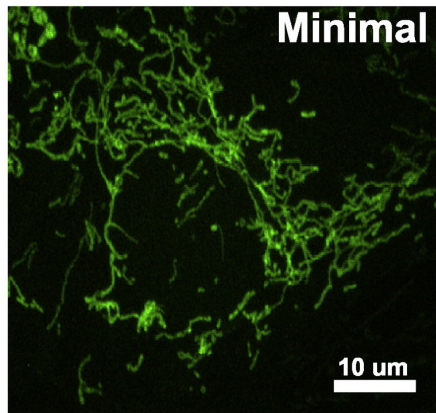
**Floor plate lineage  
differentiation at 25 days**

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Spontaneous and evoked release of dopamine (neurotransmitter) seen by ~45 days of differentiation, reaching steady state levels by ~55 days differentiation

**Tyrosine hydroxylase** **b3-tubulin** **Lmx1A** **Hoechst**

## Key Concept:



hiPSC derived neurons can be used to evaluate clinically-relevant and toxicant-relevant outcomes measures

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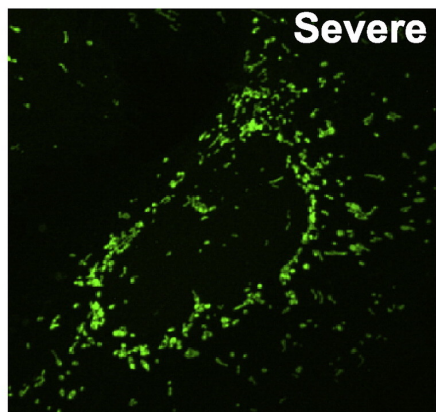
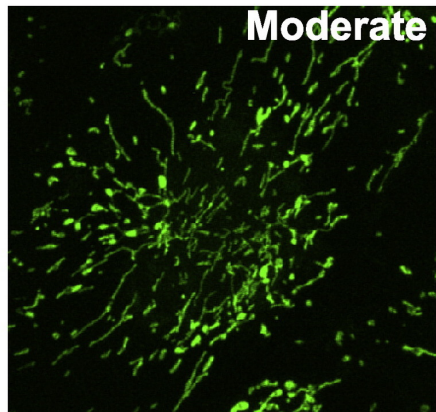
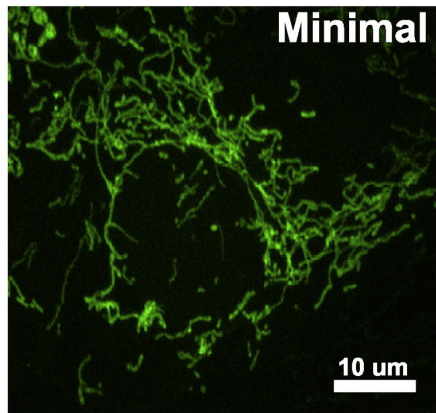
Examples of clinically relevant outcomes to copper or manganese exposures in hiPSC neurons

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1. Reductions of spontaneous and evoked dopamine release.
2. Increased mitochondrial fragmentation
3. Increased measures of oxidative stress such as glutathione levels, isoprostane levels and changes to redox-sensitive dyes
4. Reductions in neurite length
5. Changes in mitochondrial membrane potential

Aboud AA, et al, Neurotoxicology, 2012  
Aboud AA, et al, Neurobiology of Disease, 2015  
Neely ND, et al, Toxicological Sciences, 2017

## Key Concept:



**Genetic risk factors can increase vulnerability of neuroprogenitor cells from individuals to disease-relevant environmental risk factors.**

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**PARK2 patient neurons have elevated sensitivity to copper**

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- 1. Increased levels of mitochondrial fragmentation in familial PD patient neurons vs control subjects at 25 $\mu$ M Cu exposure.**
- 2. PARK2 patient neurons show decreased 'lowest observed adverse effect concentration' (LOAEC) for Cu exposure induced mitochondrial fragmentation (10 $\mu$ M extracellular).**

About AA, et al, Neurobiology of Disease, 2015


# Conclusion and Open Questions

**Strong genetic risk factors associated with neurological disease impact susceptibility to toxicant exposures relevant to disease-associated environmental risks in individual patient-derived stem cell- based models.**

**Challenges to be faced for personalized toxicological risk assessment:**

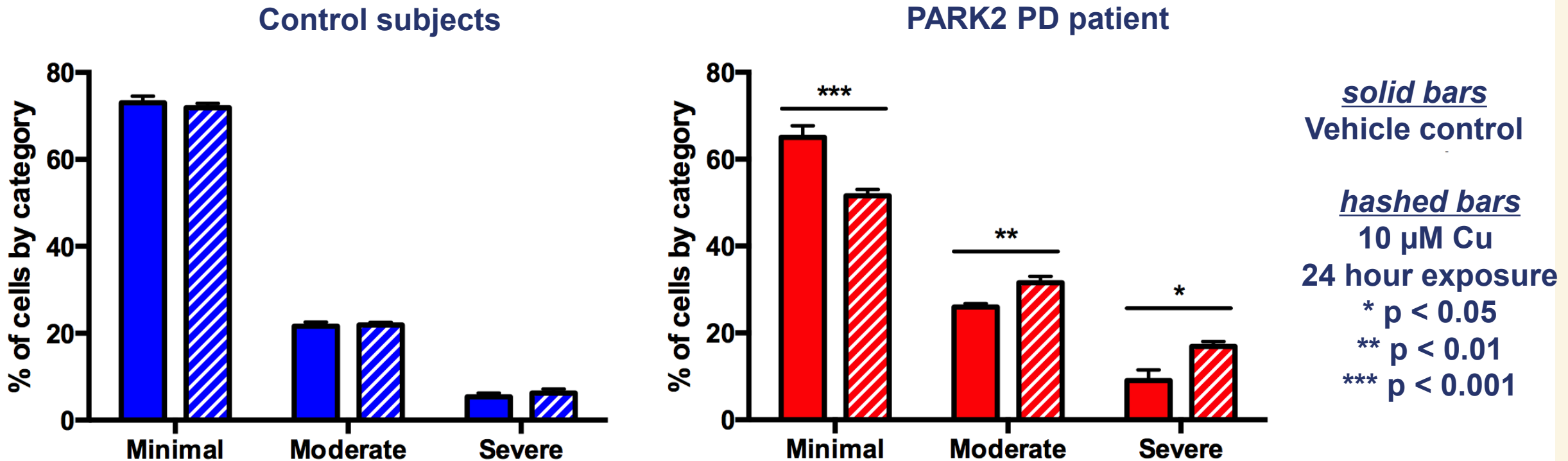
- **Undefined modifiers of toxicity**
- **Chronic exposures**
- **Multicellular/tissue-based modeling**
- **Disease validation at cellular level**

# Developing an effective iPSC model requires knowledge about the disease. Discuss the features of an appropriate iPSC disease model by considering the following:

1. Are there characteristics of individuals, populations, sub-populations, or risk groups that would be critical in the identifying whom to select for generation of a stem cell-based model system of the disease?
  2. Is there a target cell type primarily impacted or contributing to pathogenesis?
  3. Are there known or suspected toxicants that contribute to disease risk?
  4. Are there particular developmental stages or sequential time points that are important in the pathogenesis?
  5. Are there genetic-based animal models that recapitulate human pathogenesis?
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# Supporting Slides

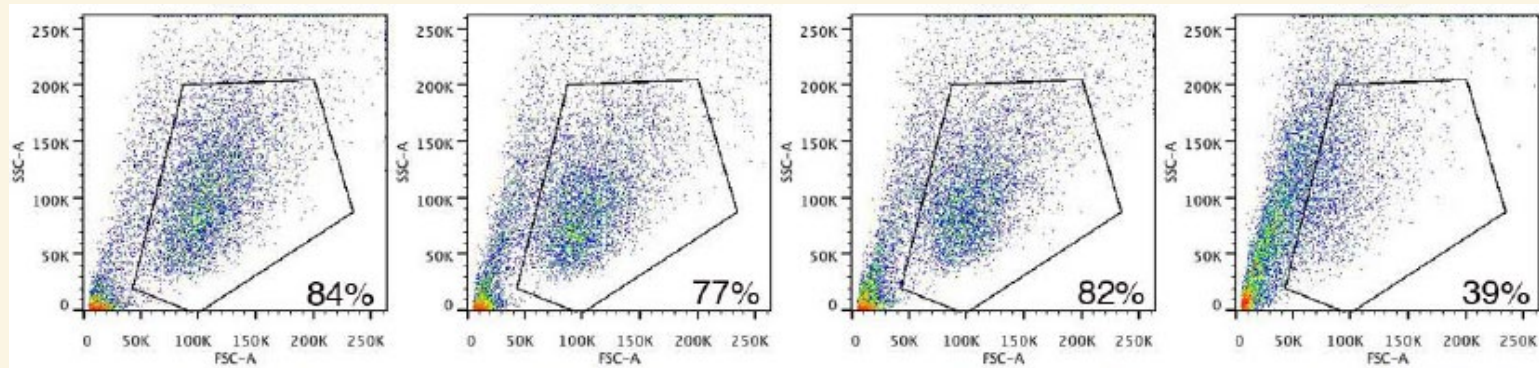
**Key Concept:** Genetic risk factors can increase vulnerability of neuroprogenitor cells from individuals to disease-relevant environmental risk factors.



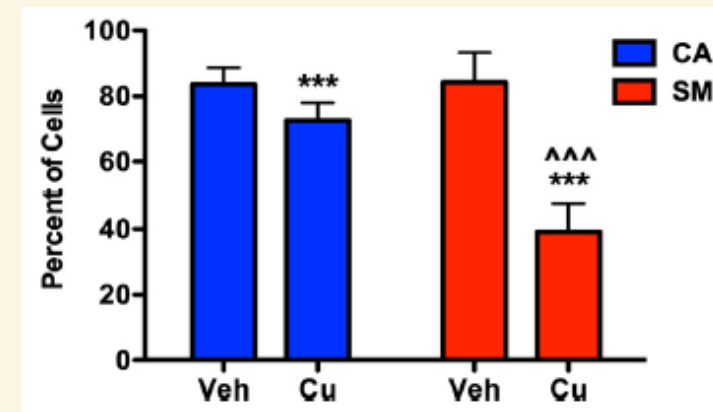
Two-way ANOVA Copper x genotype interaction (p<0.001)

**Key Concept:** Genetic risk factors can predispose individuals to disease-relevant environmental risk factors.

Subject **SM** derived neuroprogenitor cells with known **Parkinson's Disease** genetic risk factor show elevated sensitivity to copper cytotoxicity vs control subject **CA** derived cells

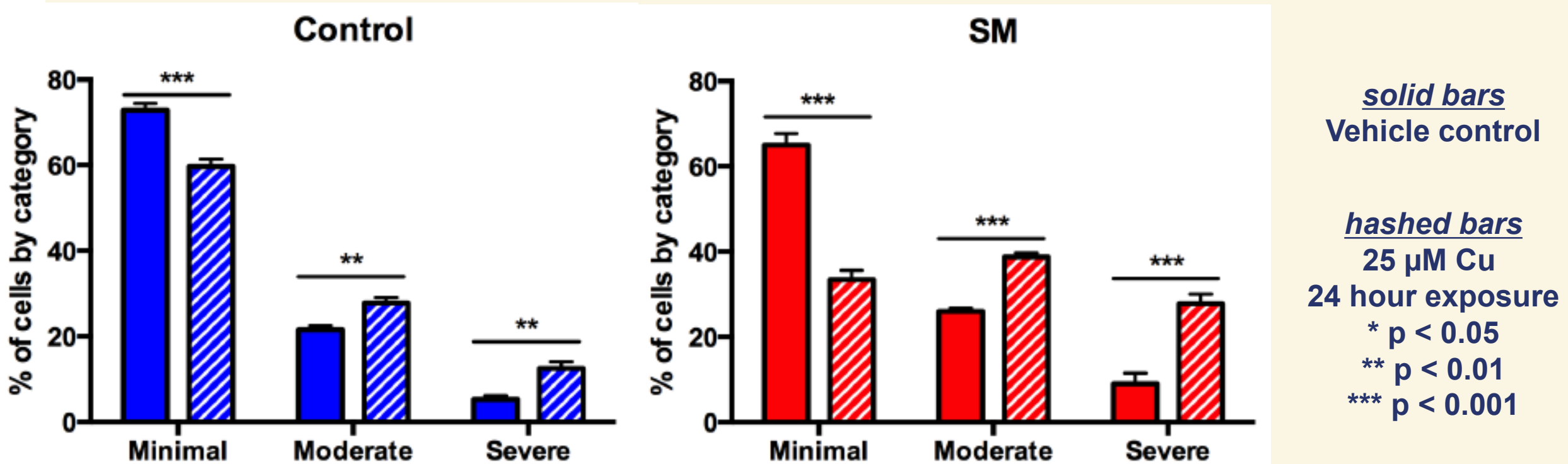


50 $\mu$ M Cu, 48 hours exposure –  
Flow cytometry analysis for viable  
cells (% viable indicated)



**Key Concept:** Environmental risk factors can elicit disease-relevant pathogenic effects that are more severe in presence of known genetic risk factors.

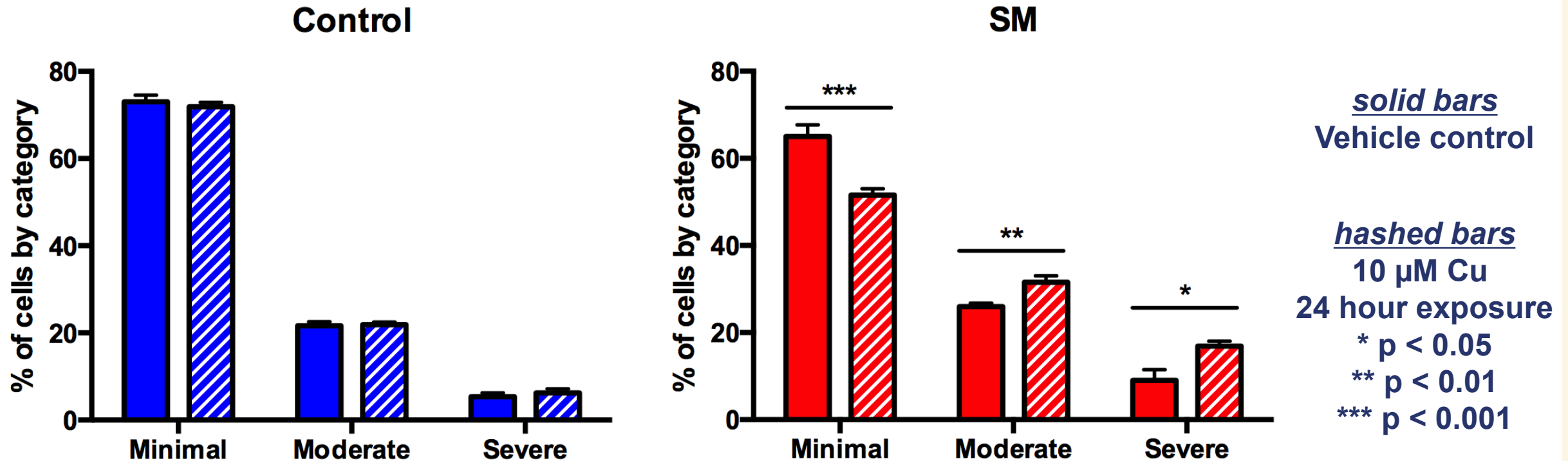
Subject **SM** derived neuroprogenitor cells with known **Parkinson's Disease** genetic risk factor show increased mitochondrial fragmentation vs **control** subjects neuroprogenitor cells



Two-way ANOVA Copper x genotype interaction (p<0.001)

**Key Concept:** Genetic risk factors can increase vulnerability of neuroprogenitor cells from individuals to disease-relevant environmental risk factors.

Subject **SM** derived neuroprogenitor cells with known **Parkinson's Disease** genetic risk factor show decreased lowest observed adverse effect concentration (LOAEC) for copper-dependent mitochondrial fragmentation vs **control** subjects neuroprogenitor cells



Two-way ANOVA Copper x genotype interaction (p<0.001)

# Key Message

With testing advances, we continue to improve research and reduce reliance on animal models.



**Thank you for your  
participation.**