USING LUHMES CELLS AS A MODEL SYSTEM TO STUDY DOPAMINERGIC NEURON CELL BIOLOGY

Tigwa H. Davis, Ph.D.

Senior Scientist
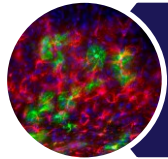
October 16, 2014

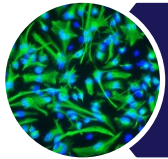ATCC | THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA

- World's premiere biological materials resource and standards development organization

- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions

- Broad range of biomaterials

  - Cell lines

  - Microorganisms

  - Native & synthetic nucleic acids
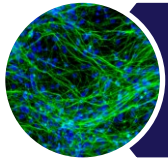
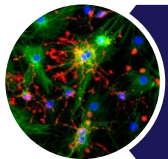 - Reagents

Outline

Current Neuronal Models

Basics of Dopaminergic Biology
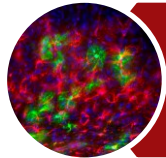
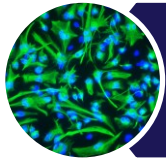Generation of LUHMES Cell Line

Neuronal Phenotype
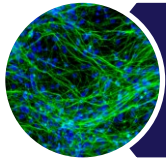
Two Studies Using LUHMES Cells
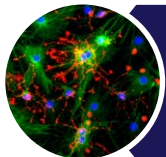
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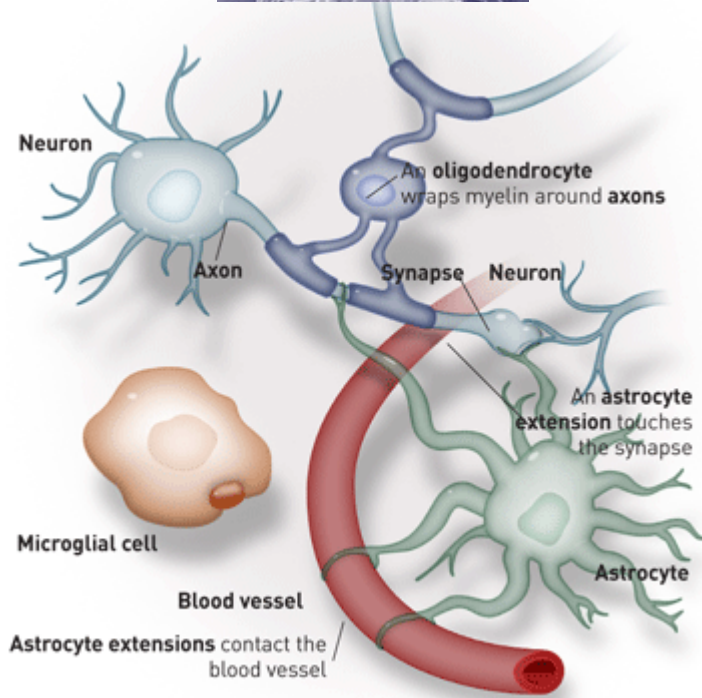Basics of Dopaminergic Biology

Generation of LUHMES Cell Line

Neuronal Phenotype

Two Studies Using LUHMES Cells

ATCC brain cell lines

Stanford medicine, 2009

Major brain tumors	ATCC cell lines
Glioblastoma	✓
Neuroblastoma	✓
Astrocytoma	✓
Medulloblastoma	✓
Oligodendroglioma	✓

Special focus	ATCC cell lines
Pediatric brain tumor	✓
Primary tumor	✓
Metastatic tumor	✓

Tools for neurobiology studies

Brain cell type	ATCC cell lines
Astrocyte	✓
Oligodendrocyte	✓
Microglia	✓
Neural progenitors	✓

PC12 and SHSY5Y - commonly used cell lines in neuroscience
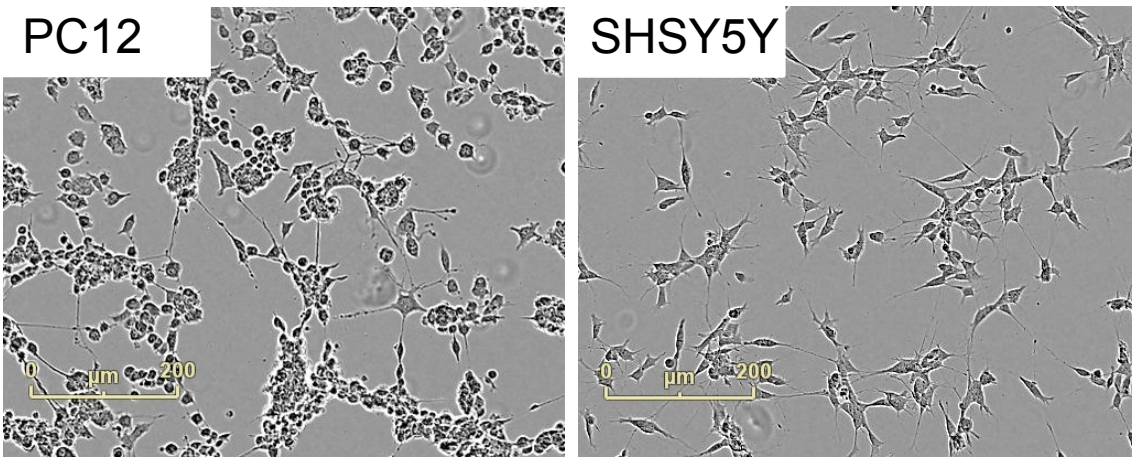
PC12

SHSY5Y

PC12 – Pheochromacytoma (Rat) adrenal medulla

SHSY5Y – Neuroblastoma (Human) extracted from metastasis of human bone marrow

ATCC offers many neuronal and glial cell lines that are ideal for your research

- > 100 neuronal, glial and endothelial cell lines for neuroscience research
- Many organisms and specific cell types.
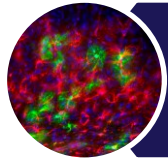- > 40,000 peer reviewed publications associated these cell lines.

ATCC® No.	Species	Cell Line	Cell Type	Genes Expressed
CRL-1721™	Rat	PC-12	Neuroendocrine	NGF receptor, Dopamine
CRL-2266™	Human	SHSY5Y	Neuroblastoma	Tyrosine Hydroxylase
CRL-2302™	Human	ARPE-19	Retinal Pig Epithelium	CRAL-BP, RPE-65
CRL-10742™	Human	HCN-2	Cortical neuron	BIII Tubulin, Glutamate, GABA
CRL-2927™	Human	LUHMES	Ventral Mesencephalon precursor neuron	TH, Dopamine, BIII tubulin
CCL-107™	Rat	C6 glioma	Glial cell	S100, GPDH
CCL-82™	Rat	GH1	Pituitary tumor	GH, Prolactin, Somatotropin
CCL-131™	Mouse	Neuro-2a	Neuroblast	AchE, BIII tubulin
CCL-147™	Mouse	NB41A3	Neuroblast	TH, AchE, ChAT
CRL-11179™	Mouse	CATH.a	Neuron	Dopamine, NE
CRL-2925™	Mouse	NE-4C	Neural stem cells	Sox-2, Otx-2, En-1
CRL-2299™	Mouse	bEnd.3	Cortex,endothelioma	ICAM-1, V-CAM-1

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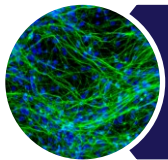| ATCC® No.	Species	Cell Line	Cell Type	Genes Expressed
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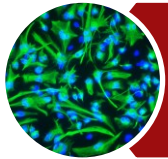
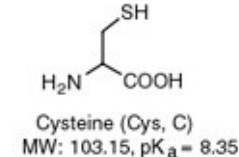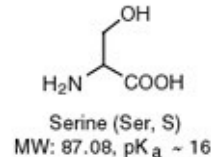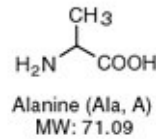Basics of Dopaminergic Biology

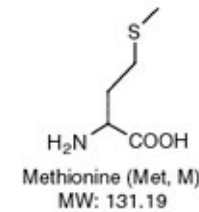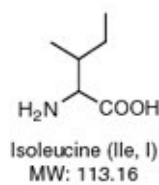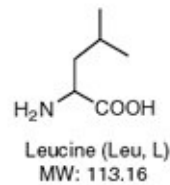Generation of LUHMES Cell Line
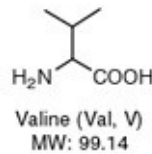
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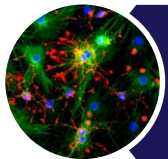
Two Studies Using LUHMES Cells

The amino acid, tyrosine, is the cornerstone of dopaminergic biology
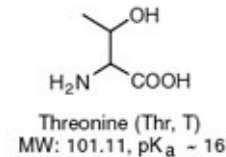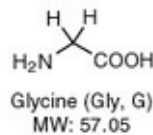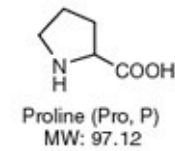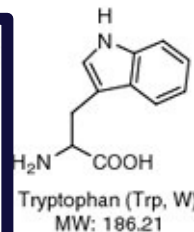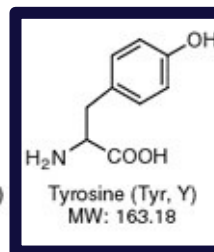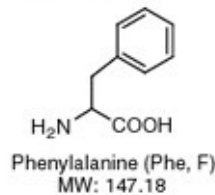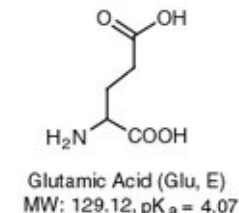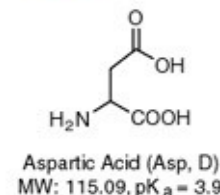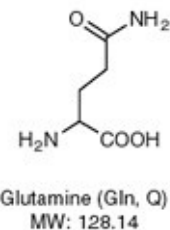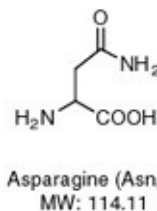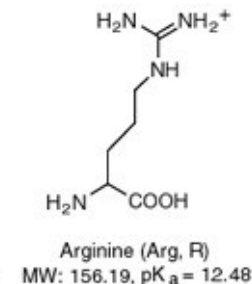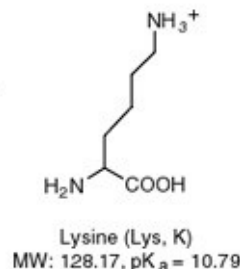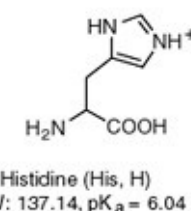
Enzymatic generation of the neurotransmitter dopamine

http://www.newworldencyclopedia.org/entry/Tyrosine
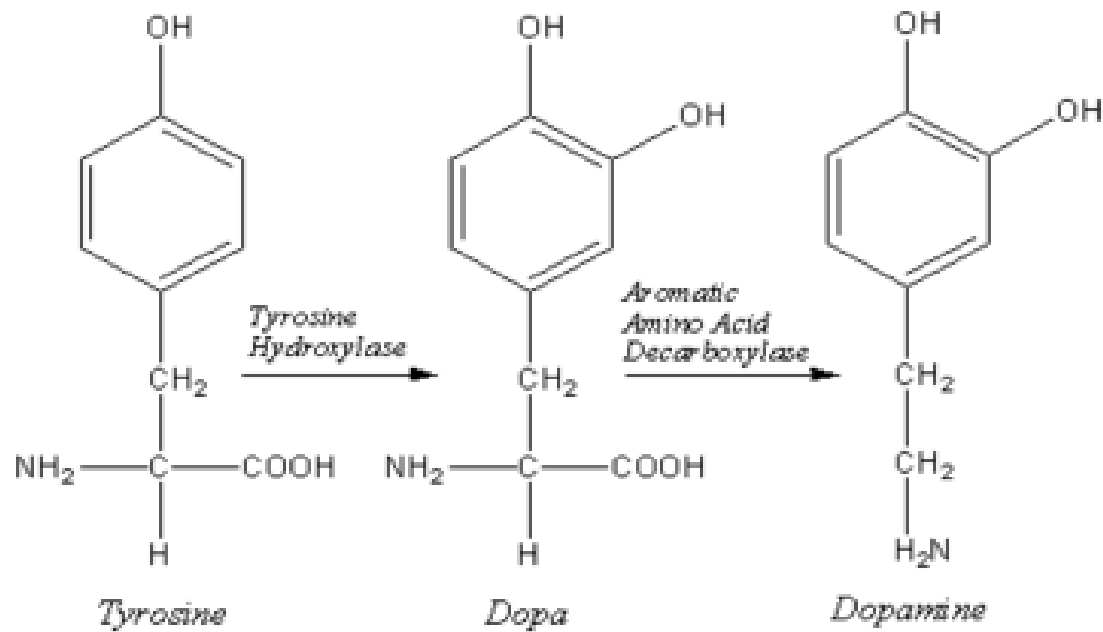
Dopaminergic biology is involved in many facets of life

Functions of Dopaminergic System

- Sensation of pleasure
- Motivation and reward
- Motor function
- Compulsion

http://neuro-science.blogspot.com/2011/10/dopamine-and-adult-neurogenesis.html
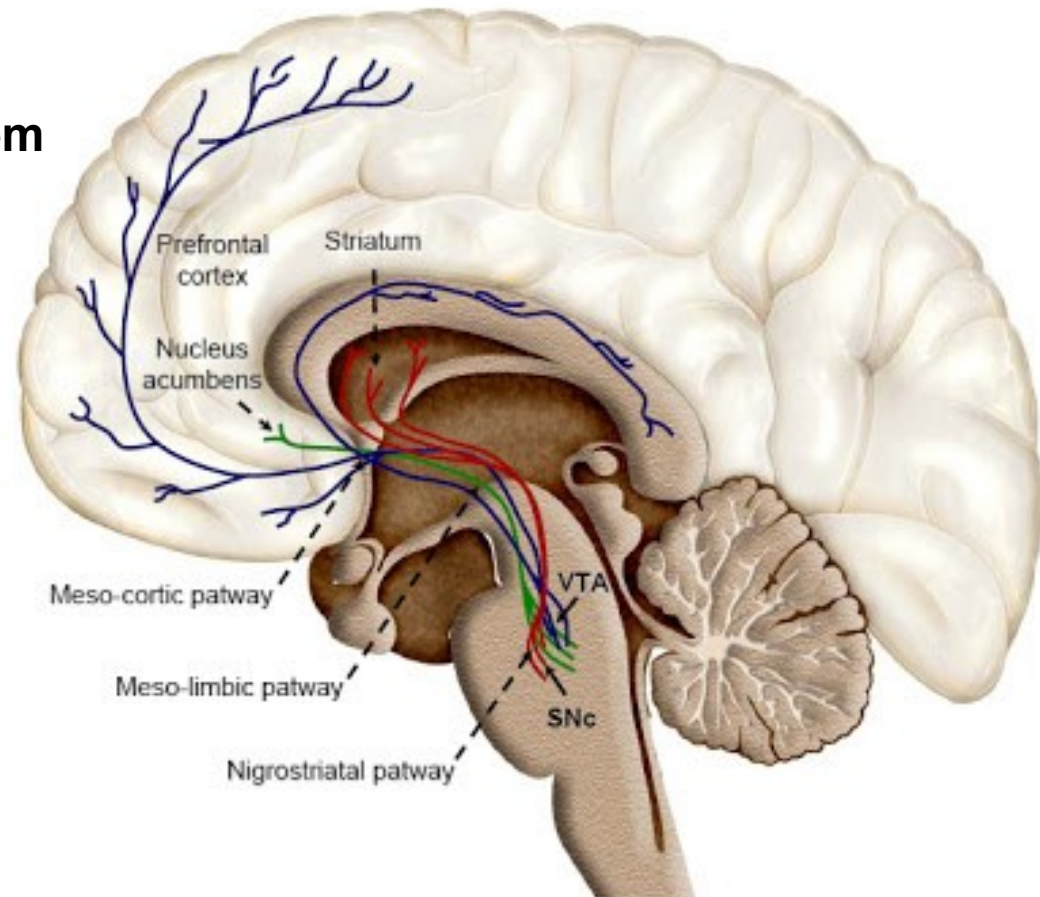
Dopaminergic biology is involved in many facets of life

Dysfunction of Dopaminergic System

- Parkinson's disease
- Schizophrenia
- Drug abuse and addiction

http://neuro-science.blogspot.com/2011/10/dopamine-and-adult-neurogenesis.html
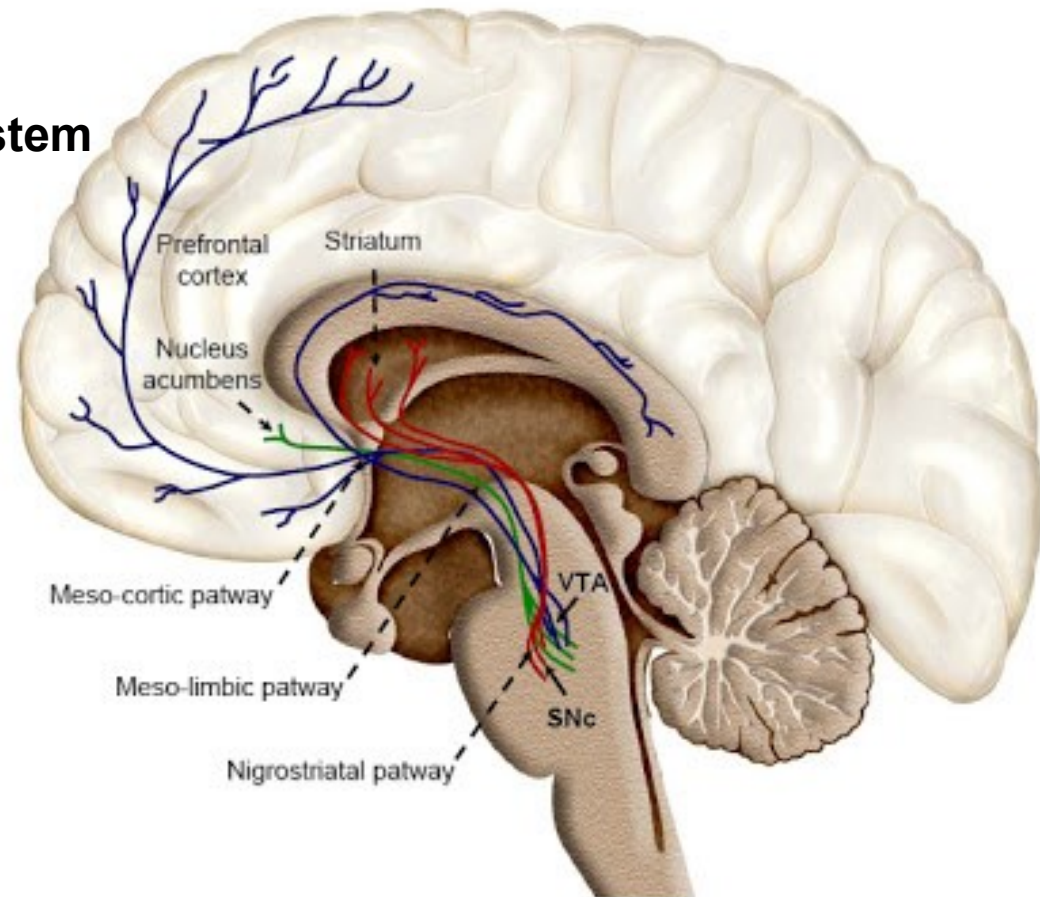
Studying dopaminergic biology *in vitro - a* difficult and expensive proposition

Relevant Models

1. **Primary neurons (Human, Mouse, Rat etc)**
   - Isolate at early embryonic timepoints
   - Low yield from Ventral Mesencephalon

2. **Neurons derived from Induced Pluripotent Stem Cells or Neural Progenitors**
   - Great Models
   - More Expensive
   - Time Consuming

An ideal model would capitulate multiple aspects of the desired *in vivo* system, but be quick and easy to generate as well as cost efficient.

Substantia nigra, dopamine - producing cells
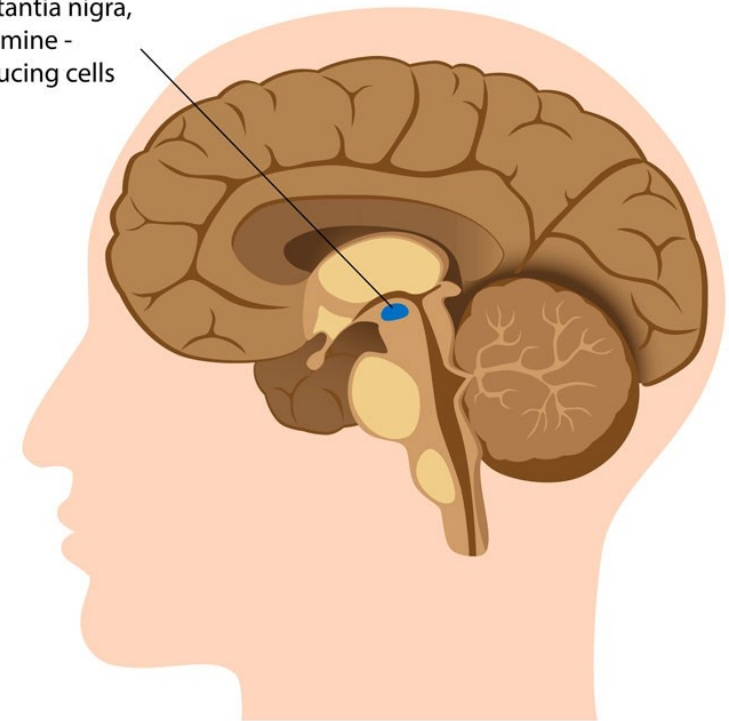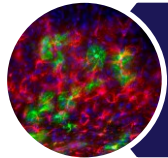
http://www.parkinsoninfo.org/wp-content/uploads/2012/10/pd-brain-pic.jpg

Outline

Current Neuronal Models

Basics of Dopaminergic Biology

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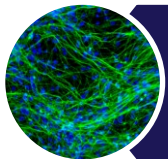
Neuronal Phenotype
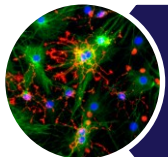
Two Studies Using LUHMES Cells

Effect of Mutant α-Synuclein on Dopamine Homeostasis in a New Human Mesencephalic Cell Line*

Julie Lotharius‡, Sebastian Barg§, Pia Wiekop¶, Cecilia Lundberg‡, Heather K. Raymon‖, and Patrik Brundin‡**

From the ‡Section for Neuronal Survival, Wallenberg Neuroscience Center, §Department of Physiological Sciences, Lund University, Lund 221 84, Sweden, the ¶Section for Microdialysis, Neurosearch A/S, Ballerup DK-2750, Denmark, and the ‖Signal Research Division, Celgene Corporation, San Diego, California 92121

Isolation of LUHMES Cells

- Eight week embryonic mesencephalon tissue was isolated and dissociated.

- Cells were immortalized with a linx v-myc retrovirus.

Termination of V-myc expression with tetracycline results in robust differentiation of LUHMES cells

Lotharius J et al. J. Biol. Chem. 2002;277:38884-38894

Termination of V-myc expression with tetracycline results in robust differentiation of LUHMES cells

96 Hrs Differentiation

Lotharius J et al. J. Biol. Chem. 2002;277:38884-38894
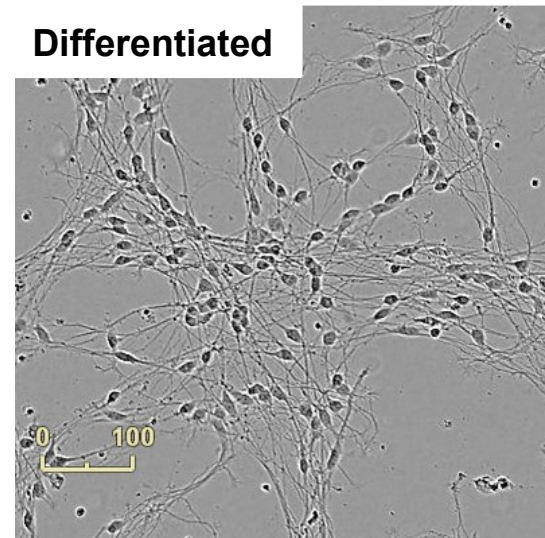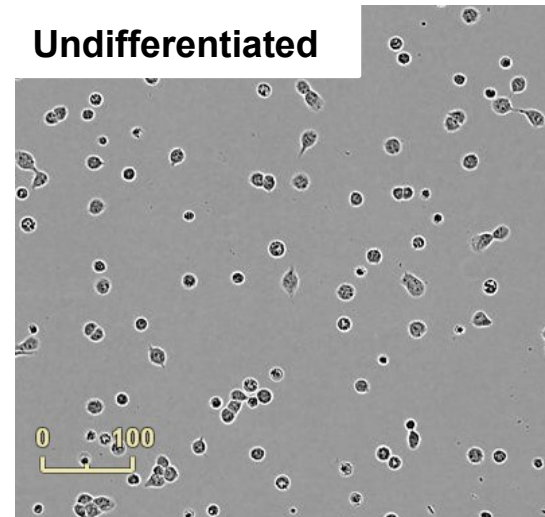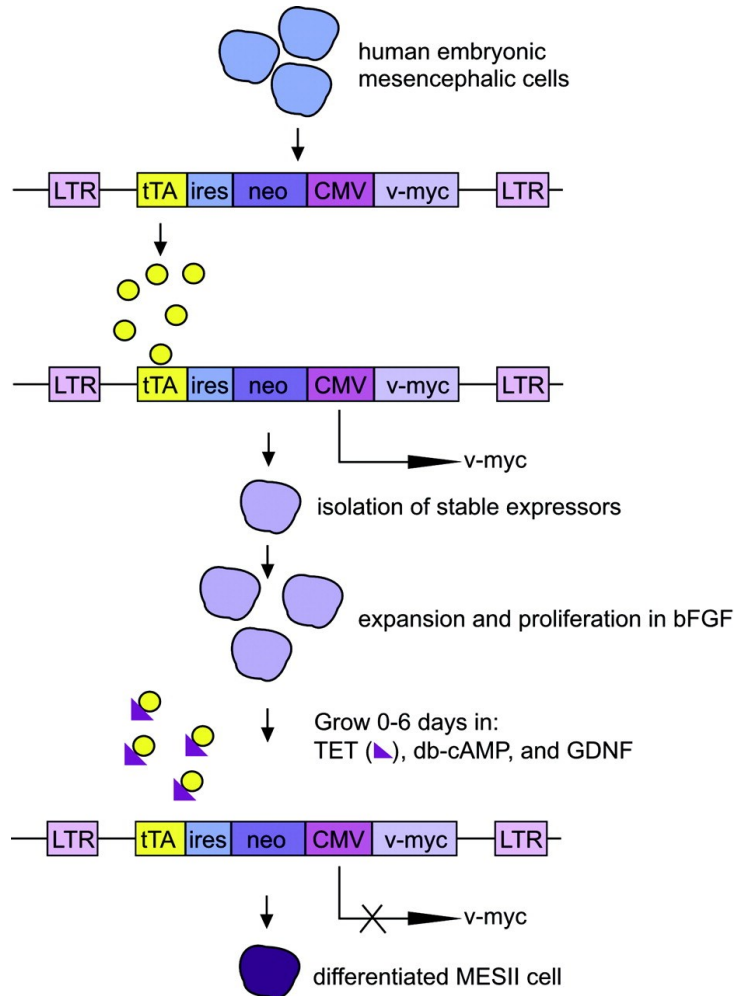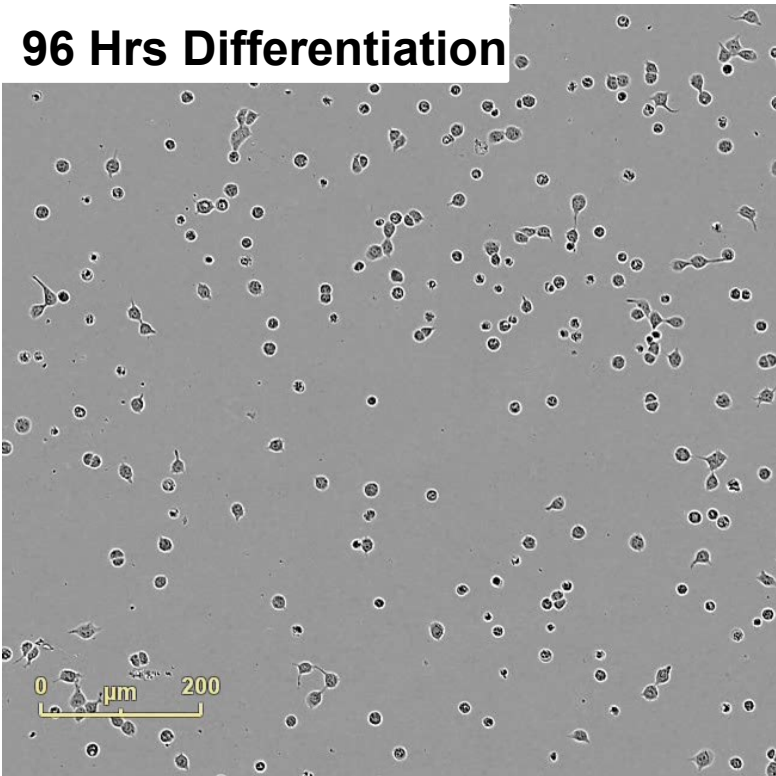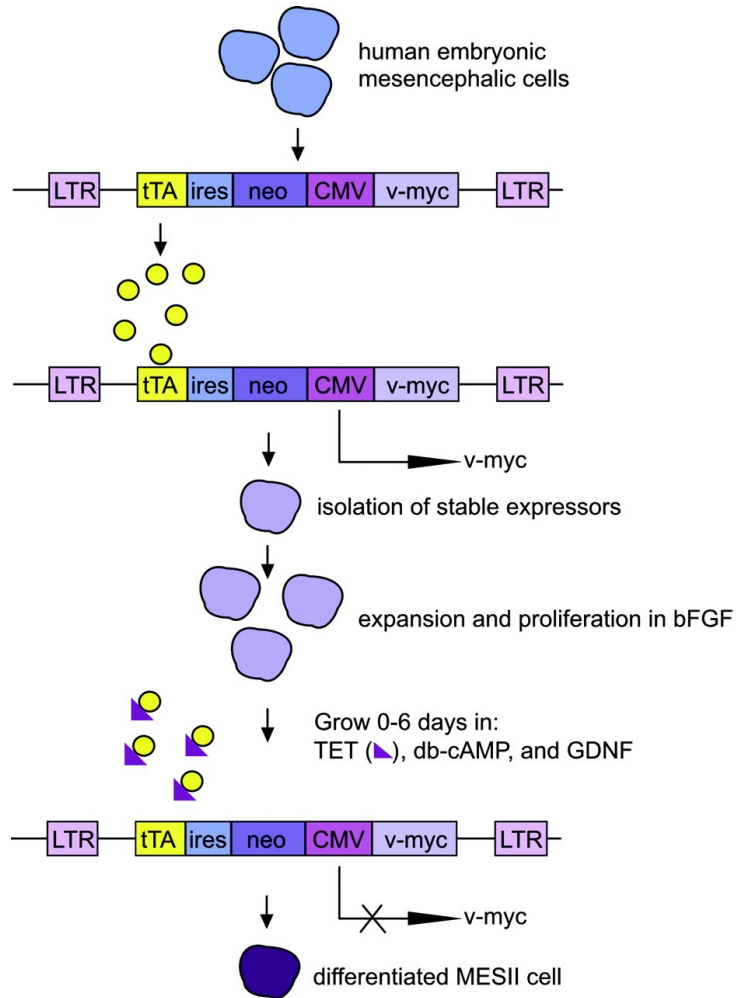
LUHMES are easy to grow and maintain

To Grow	DMEM: F12	N2	bFGF	cAMP	GDNF	Tetracycline
Proliferating LUHMES	X	X	X			
Differentiated LUHMES	X	X		X	X	X

Undifferentiated

Differentiated

Black: Luhmes Undifferentiated
Gray: SHSY5Y Undifferentiated
Red: Luhmes Differentiated
Green: SHSY5Y Differentiated
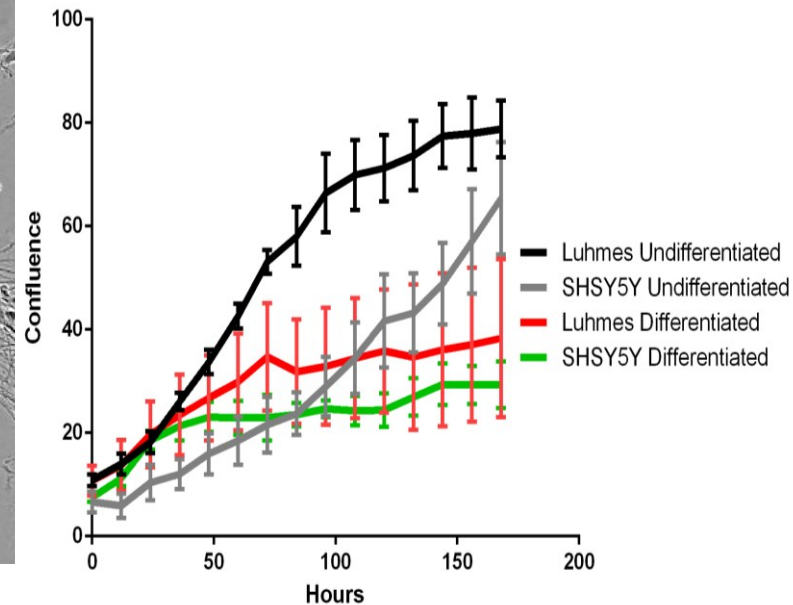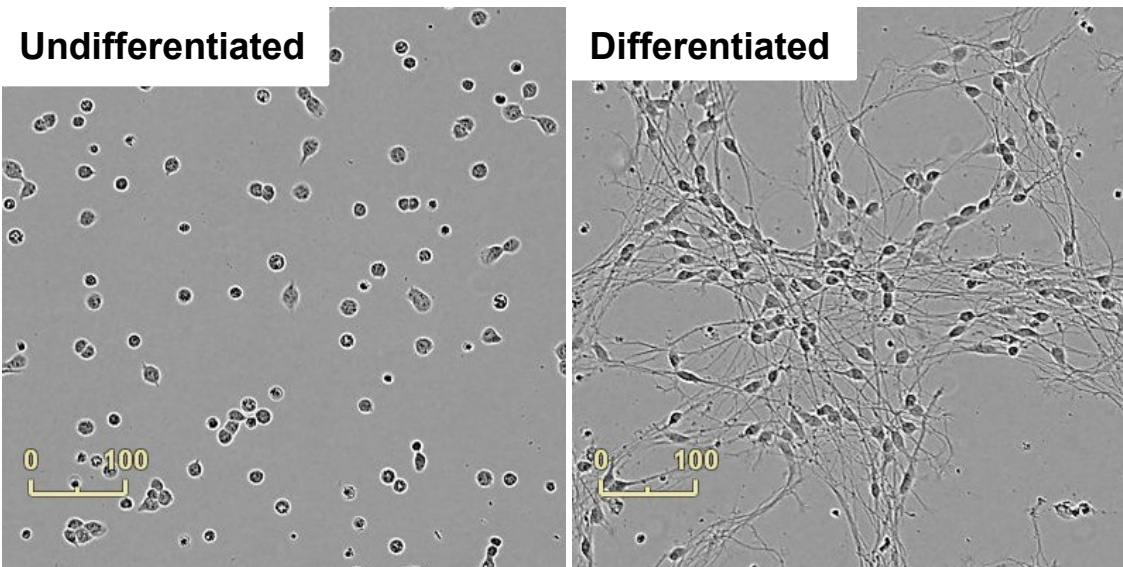
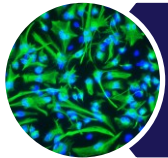# DO LUHMES CELLS EXPRESS MARKERS ASSOCIATE WITH DOPAMINERGIC BIOLOGY?

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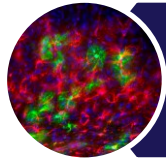
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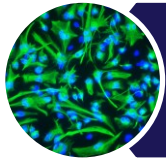
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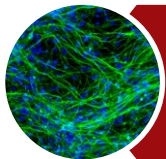
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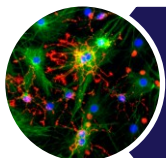
Two Studies Using LUHMES Cells

Undifferentiated LUHMES cells express floorplate markers that identify dopaminergic cells

B LMX1A

C FOXA2
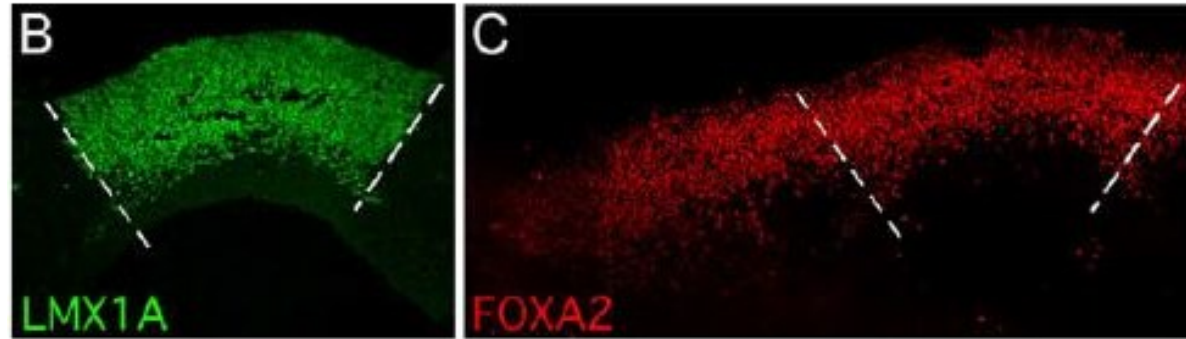
J. Nelander et al. / Gene Expression Patterns 9 (2009) 555–561

Undifferentiated LUHMES

- FoxA2
- Lmx1A

Fold Δ mRNA (vs. control)

Human WB   (4DIV)   (8DIV)
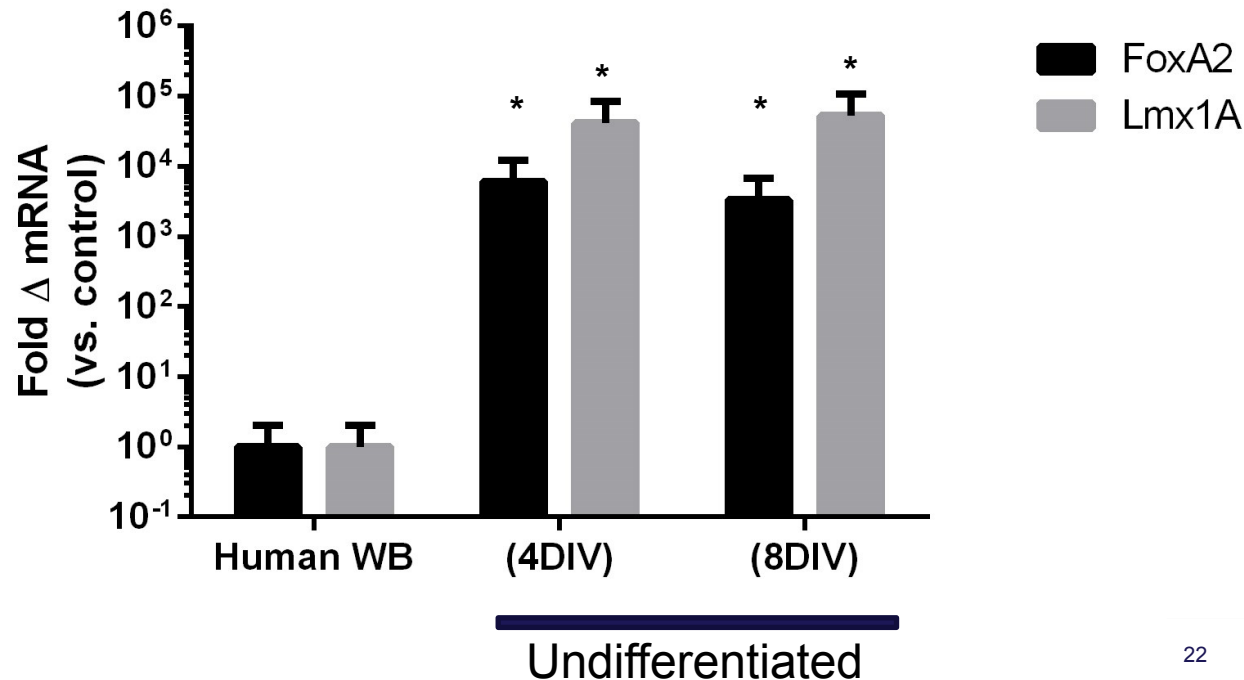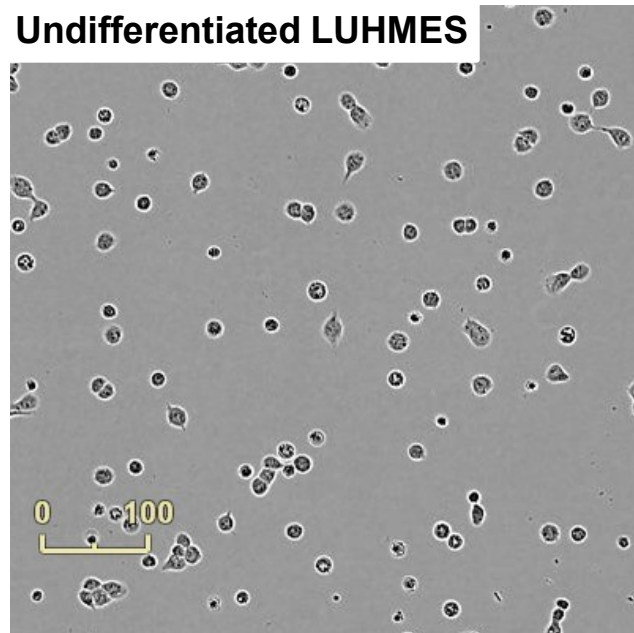
Undifferentiated

LUHMES cells express neuronal markers following differentiation

LUHMES   Tubulin   1° Neurons   Tubulin

8 days *in vitro*
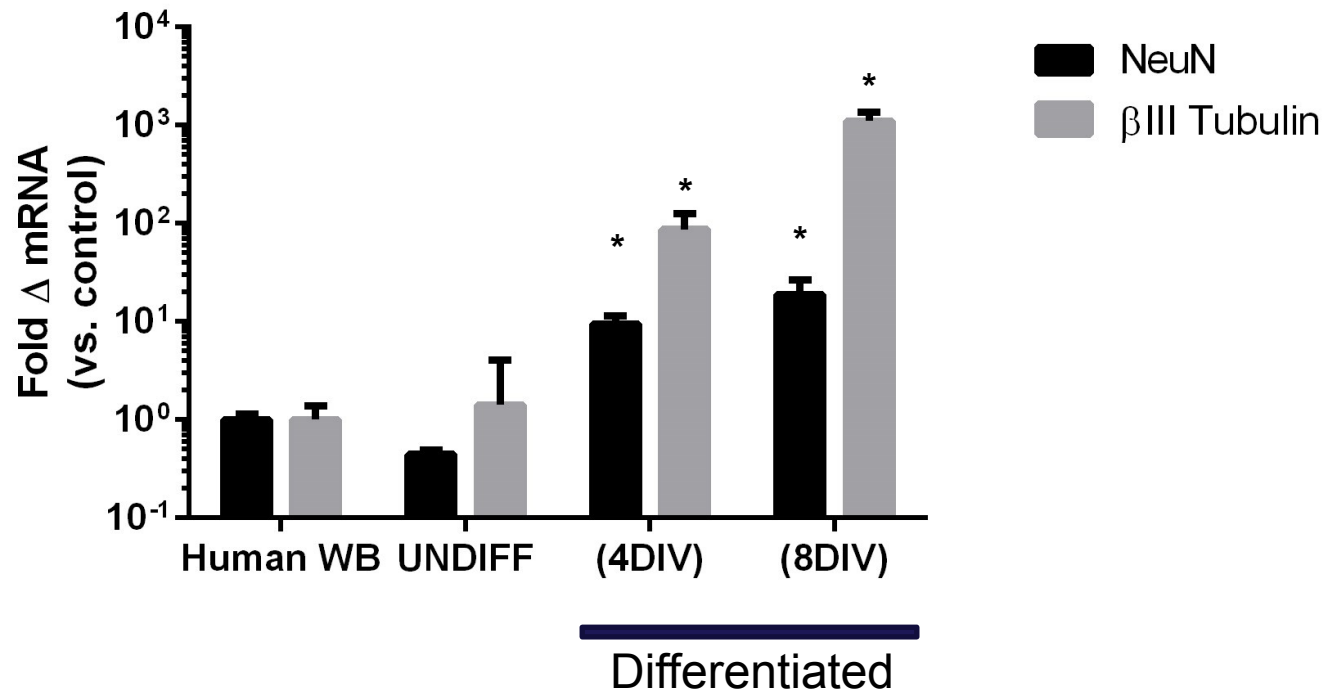
Differentiated

LUHMES cells express growth cones containing lamellopodia and filopodia during differentiation

LUHMES   Tubulin   1° Neurons   Tubulin

8 days *in vitro*

* Growth Cone
* Filopodia
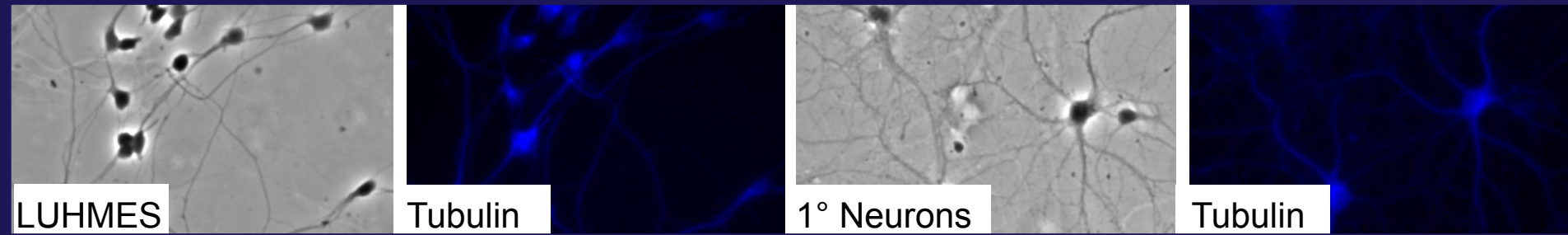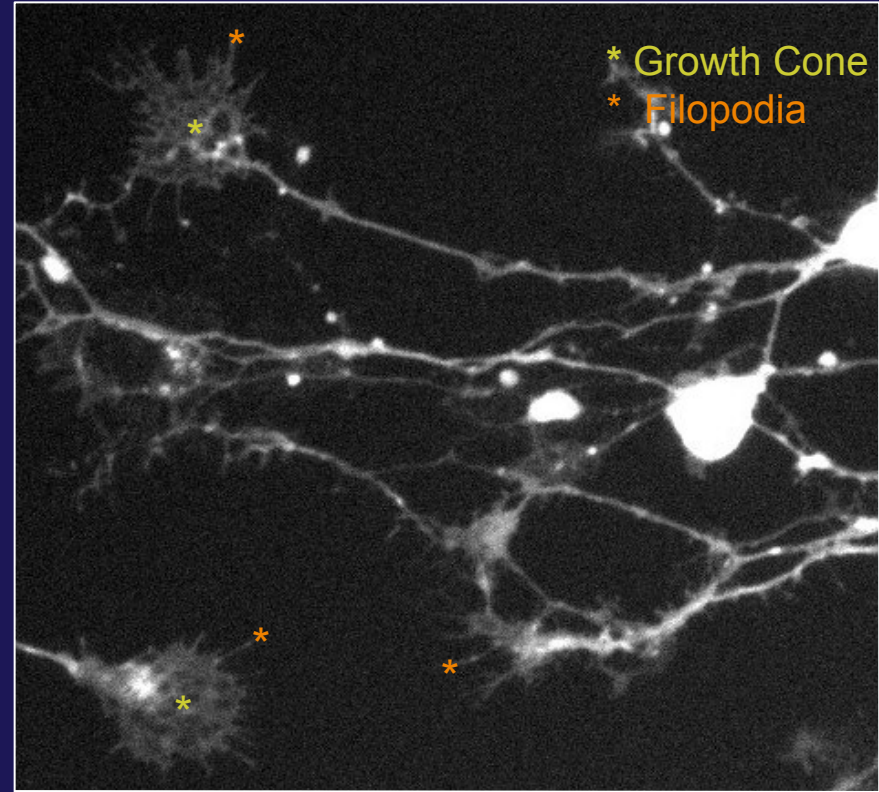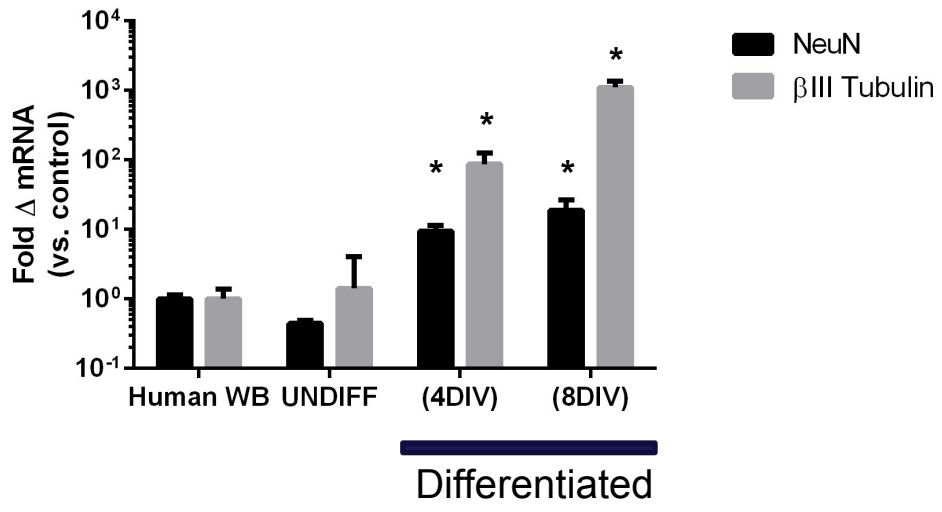
Dopaminergic markers are expressed following differentiation of LUHMES cells

Synaptic Terminal

Tyrosine → DOPA → Dopamine

TH | DDC

LUHMES | TH | 1°Neurons | TH
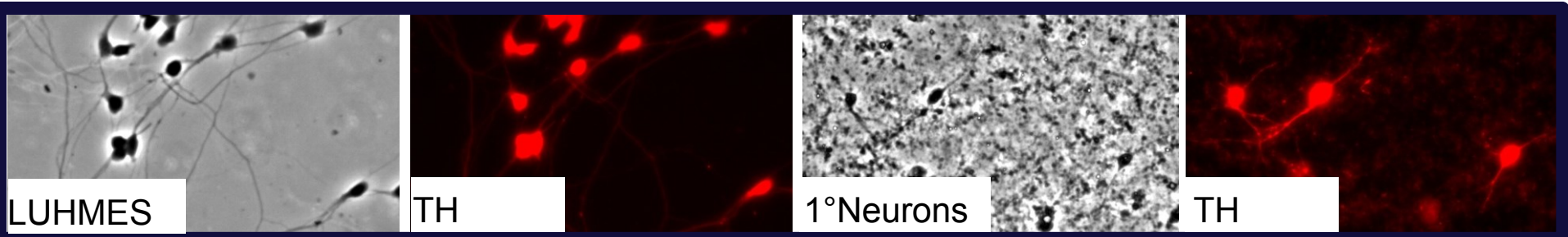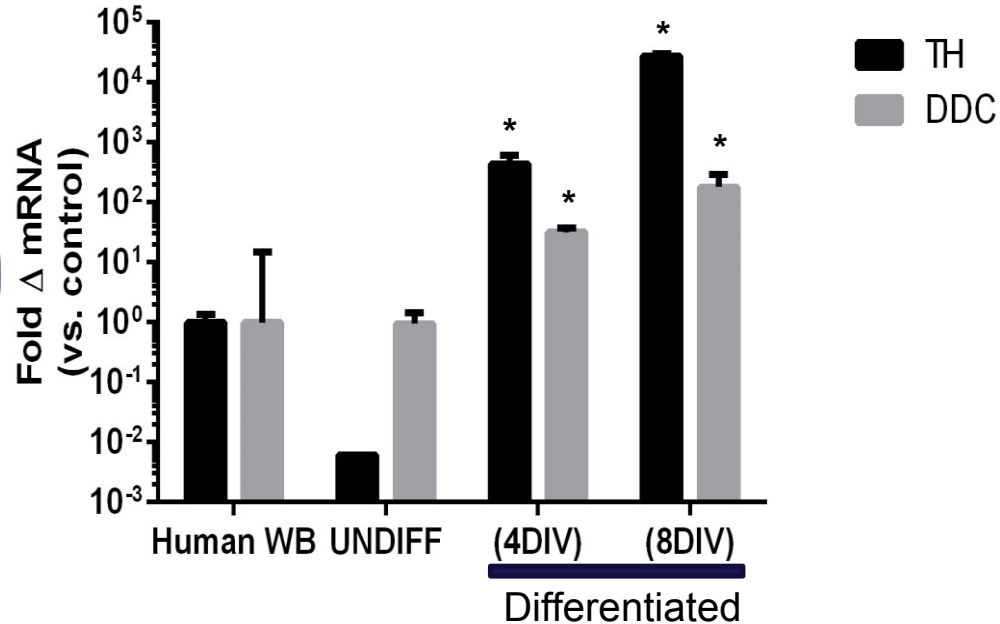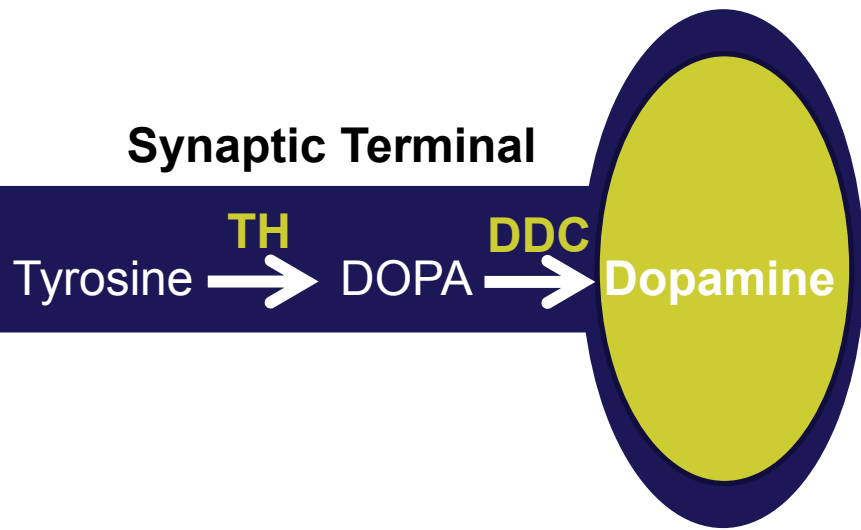
8 days *in vitro*

LUHMES cells express RNA for many proteins important in dopaminergic biology

- Profiled undifferentiated, 4 and 8 days *in vitro* differentiated LUHMES cells for 84 dopaminergic and serotonergic markers.

LUHMES cells express RNA for many proteins important in dopaminergic biology

- Profiled undifferentiated, 4 and 8 days *in vitro* differentiated LUHMES cells for 84 dopaminergic and serotonergic markers.

GENE	LUHMES – P4	LUHMES – P8
DRD1	N.E	N.E
DRD2	↑	↑
DRD3	N.E	N.E
DRD4	↓	↑
DRD5	N.E	N.E

N.E - No expression
(-) - No change relative to undifferentiated LUHMES
↓↑ - Up/down regulation > 2 fold

Pre-synaptic

Synaptic Terminal

Tyrosine
↓
Dopa
↓
Dopamine

Mitochondria

Dopamine Auto-receptor

Synaptic Vesicle

VMAT

MAO

DAT

Dopamine Receptors

Post-synaptic

D1   D2

Adenylate Cyclase

COMT

ATCC®

LUHMES cells express RNA for many proteins important in dopaminergic biology

- Profiled undifferentiated, 4 and 8 days *in vitro* differentiated LUHMES cells for 84 dopaminergic and serotonergic markers.

GENE	LUHMES – P4	LUHMES – P8
DRD1	N.E	N.E
DRD2	↑	↑
DRD3	N.E	N.E
DRD4	↓	↑
DRD5	N.E	N.E

N.E - No expression
(-) - No change relative to undifferentiated LUHMES
↓↑ - Up/down regulation > 2 fold

Pre-synaptic

Synaptic Terminal

Tyrosine → Dopa → Dopamine

Dopamine Auto-receptor

Synaptic Vesicle

VMAT

Mitochondria

MAO

DAT

Dopamine Receptors

Post-synaptic

D1   D2

Adenylate Cyclase

COMT

LUHMES cells express RNA for many proteins important in dopaminergic biology

- Profiled undifferentiated, 4 and 8 days *in vitro* differentiated LUHMES cells for 84 dopaminergic and serotonergic markers.

GENE	LUHMES – P4	LUHMES – P8
ADCY1	↑	↑
ADCY2	N.E	N.E
ADCY3	(-)	↑
ADCY5	↑	↑
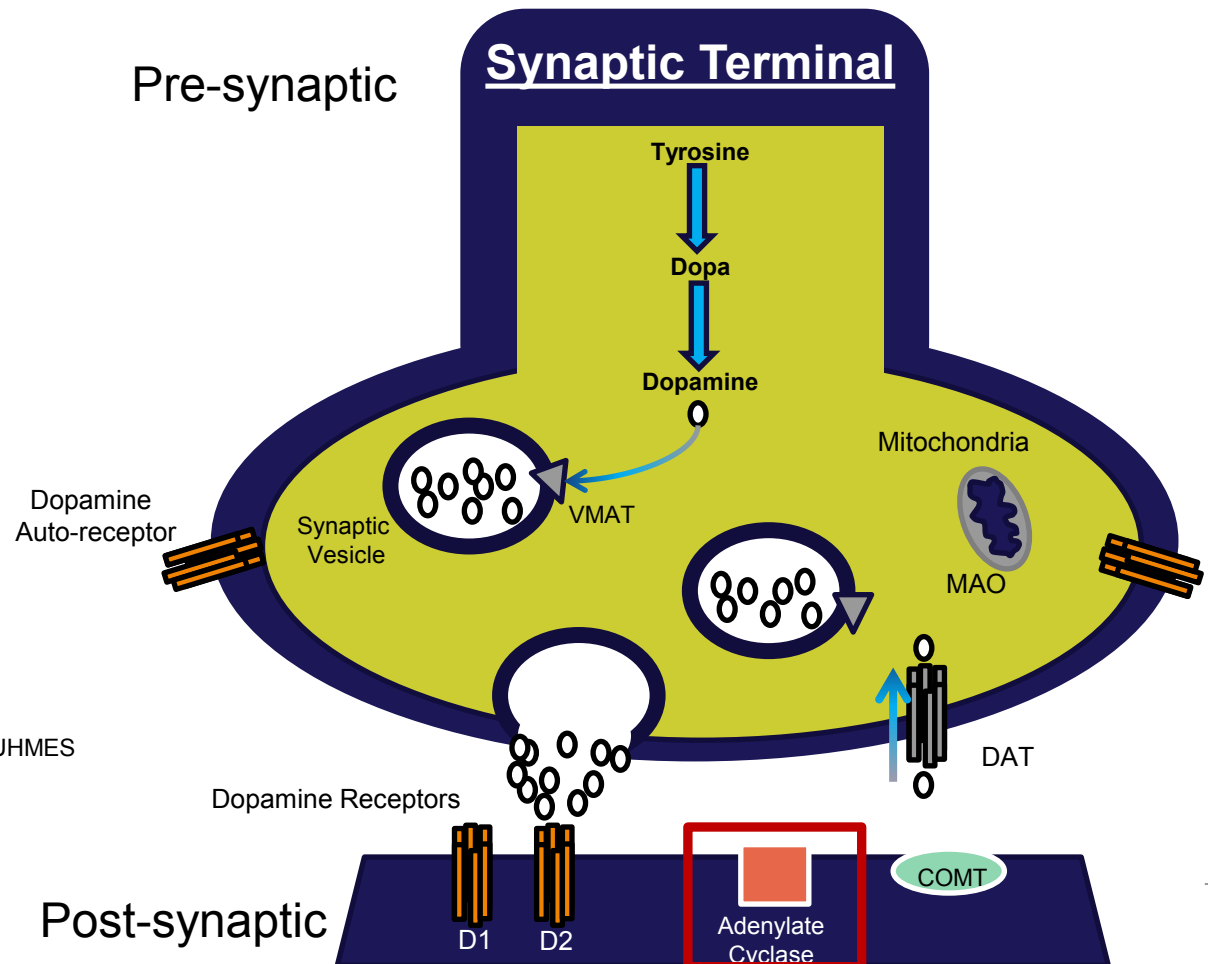
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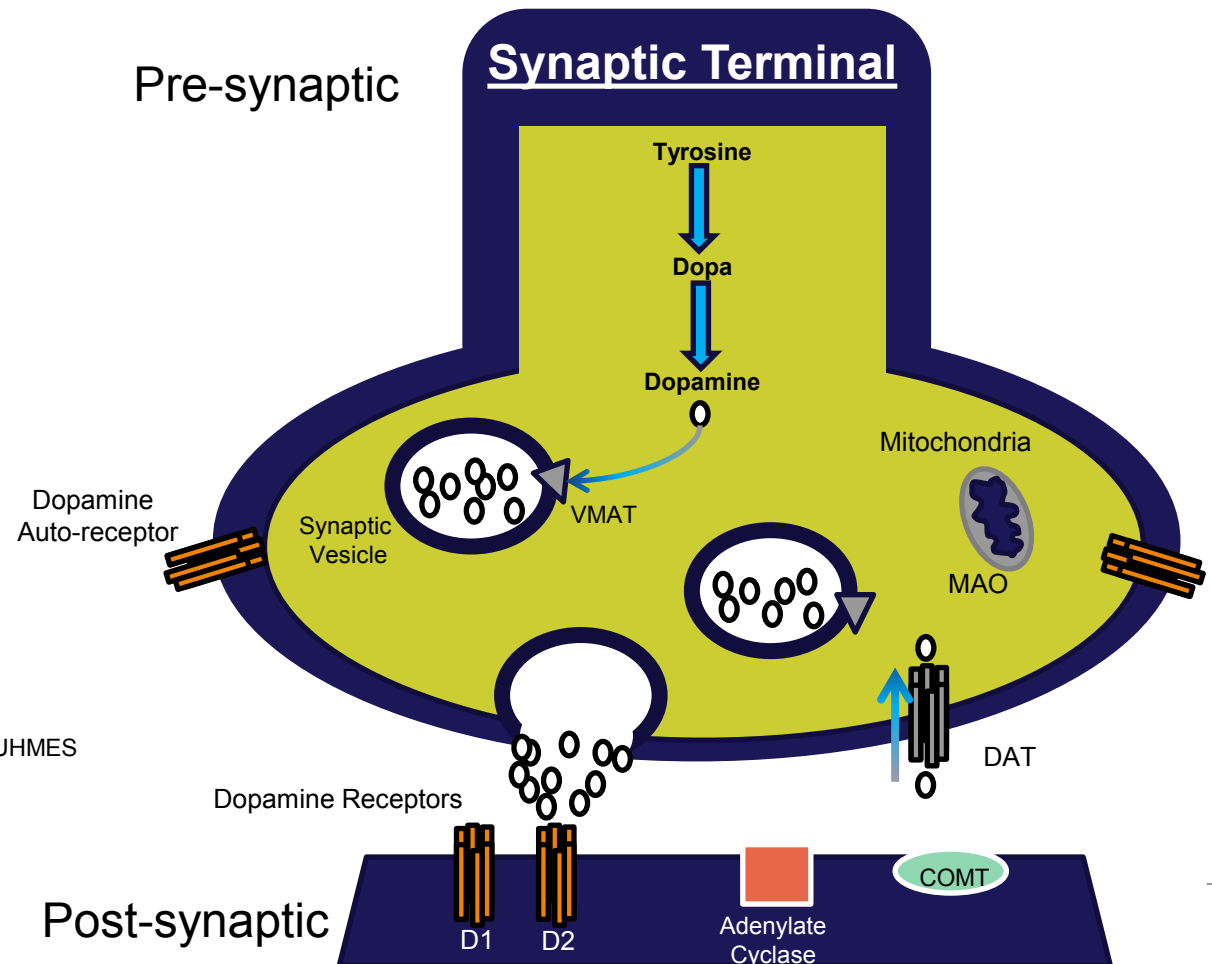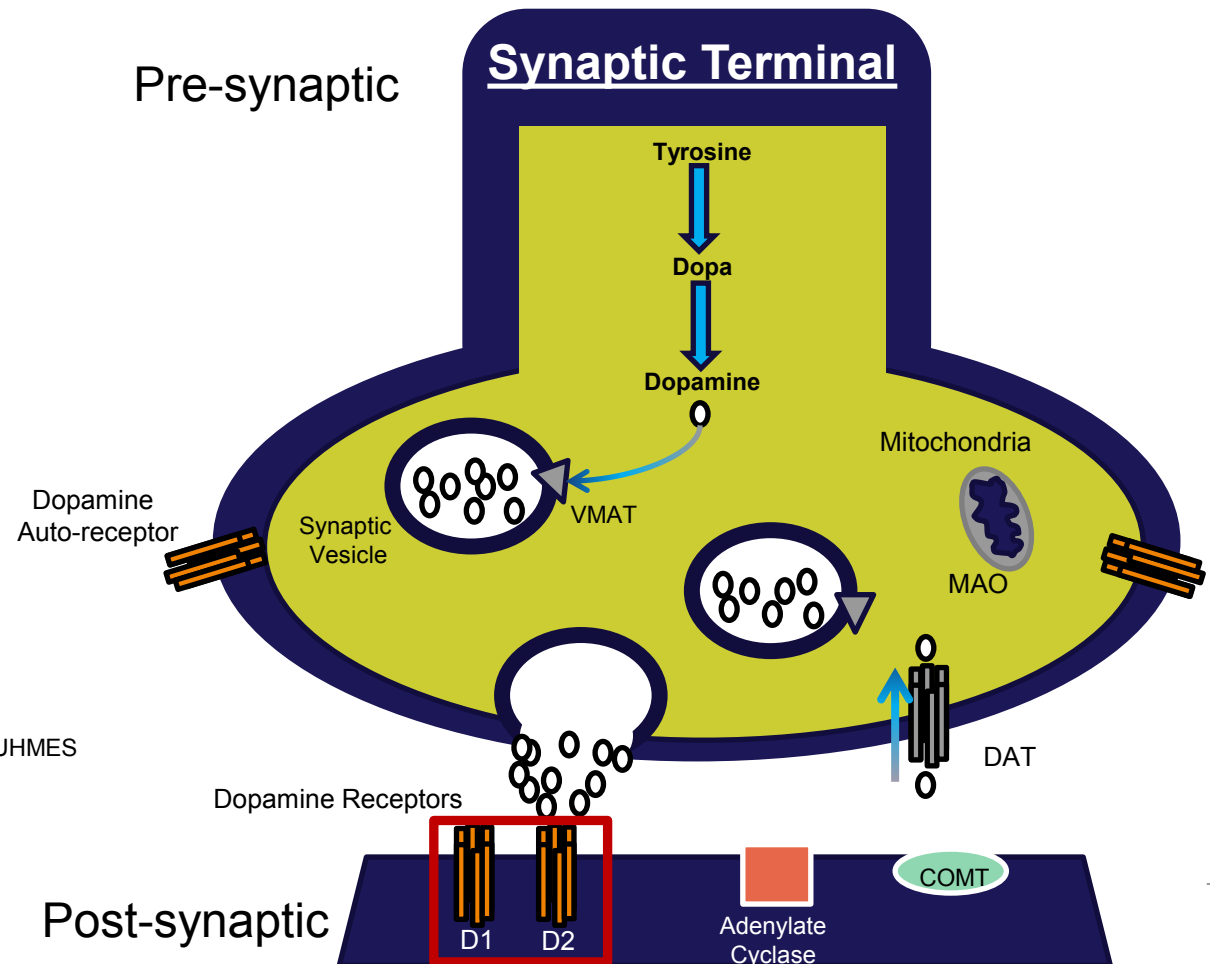
D1 D2

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GENE	LUHMES – P4	LUHMES – P8
SLC18A1	(-)	(-)
SLC18A2	(-)	⬆
SLC6A3	⬆	⬆
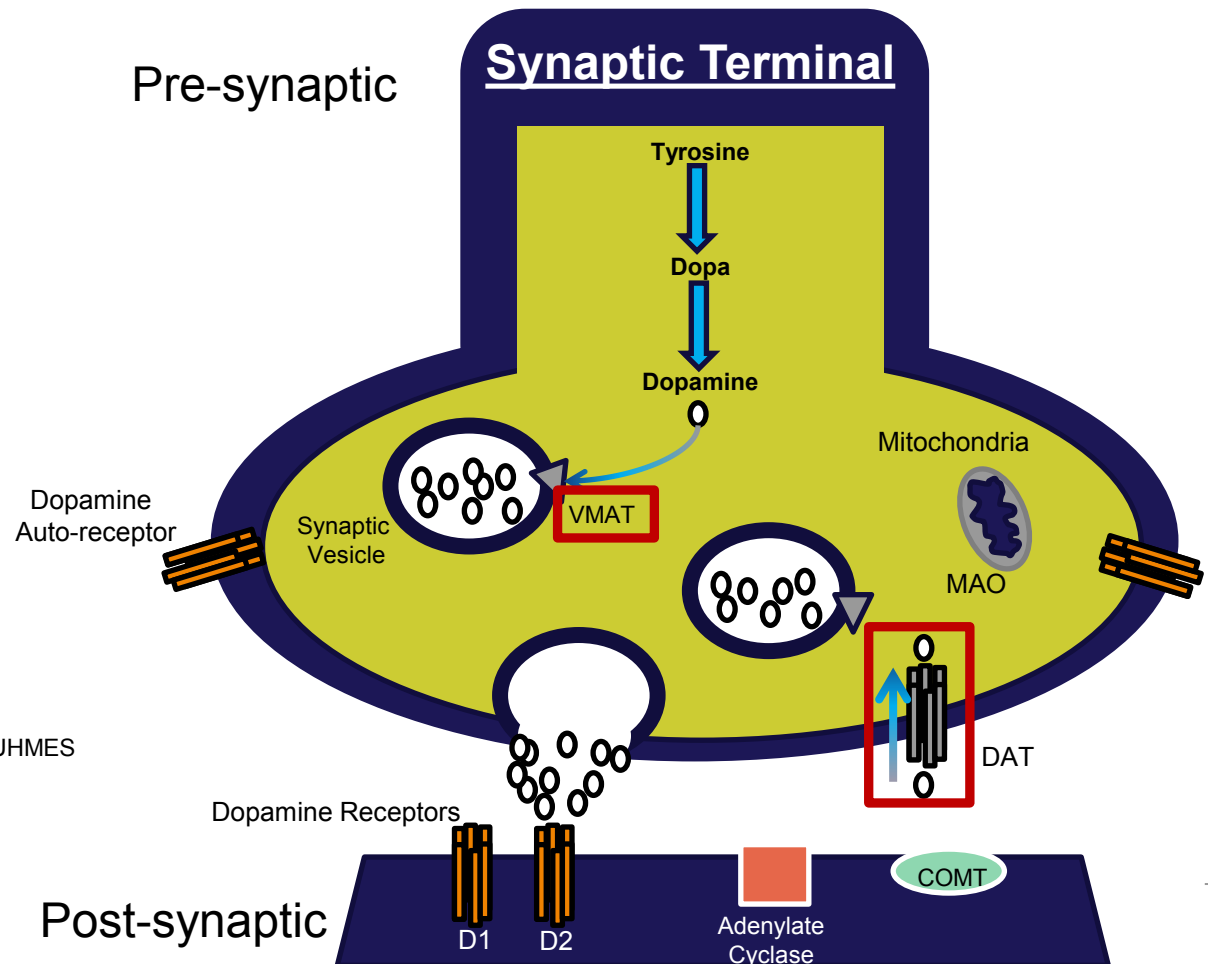
N.E   -   No expression
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⬇⬆   -   Up/down regulation > 2 fold

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D1   D2

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COMT

ATCC®
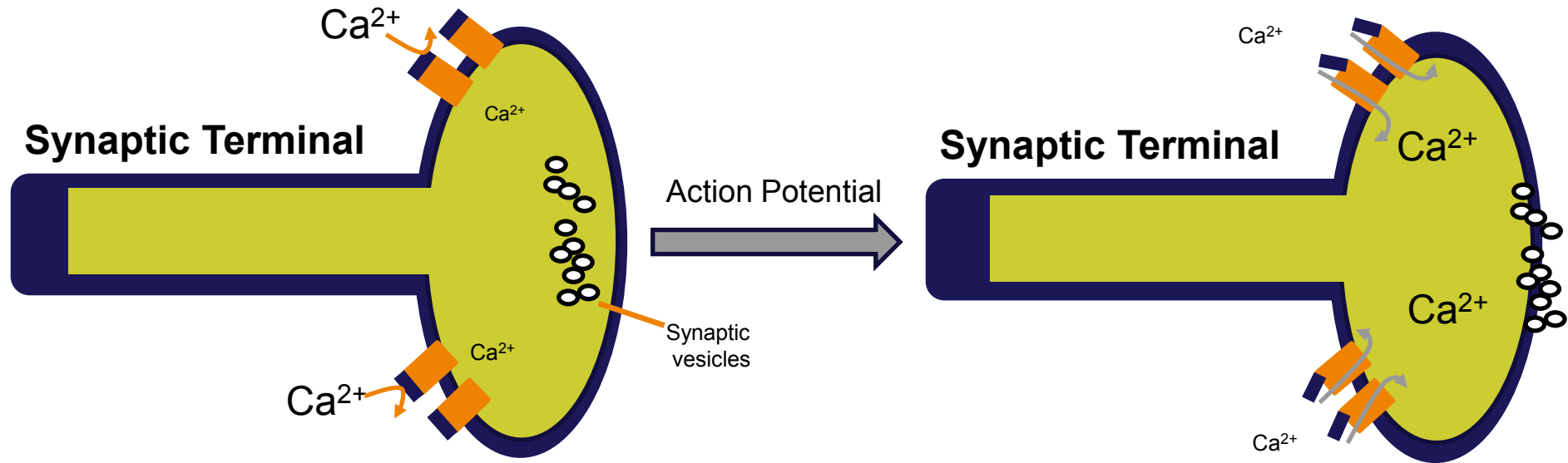
Differentiated LUHMES cells are electrically active

Differentiated LUHMES cells are electrically active

Synaptic Terminal

Ca^{2+}

Ca^{2+}

Ca^{2+}

Ca^{2+}

Synaptic vesicles

Action Potential

Synaptic Terminal

Ca^{2+}

Ca^{2+}

Ca^{2+}

Ca^{2+}

Fluorescent voltage sensitive Calcium Detector Dye

Primary Rodent Neurons

Differentiated LUHMES cells are electrically active

Synaptic Terminal

Ca^{2+}

Ca^{2+}

Ca^{2+}

Ca^{2+}

Action Potential

Synaptic vesicles

Synaptic Terminal

Ca^{2+}

Ca^{2+}

Ca^{2+}

Ca^{2+}

Ca^{2+}
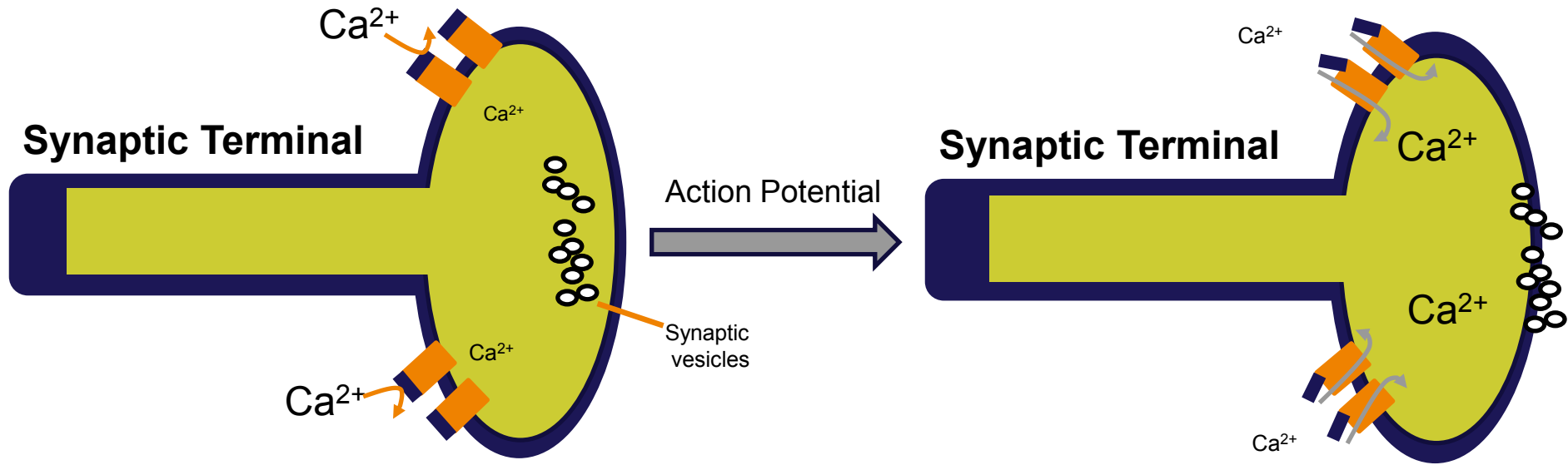
Differentiated Luhmes Cells

Summary Slide – Part I

- LUHMES cells are precursor cells isolated from 8 week embryonic ventral mesencephalon.

- LUHMES cells are easily differentiated into neurons upon the addition of minimal growth factors including, GDNF, cAMP, and tetracycline.

- LUHMES cells express mature neuronal markers including NeuN and bIII tubulin.

- Expression of various dopaminergic genes including tyrosine hydroxylase, dopamine decarboxylase, the dopamine transporter, and dopaminergic receptors are observed by qPCR.

- Experiments using a calcium sensitive fluorescent dye indicate the presence of neuronal activity, as well as the presence of functional glutamate receptors.

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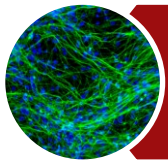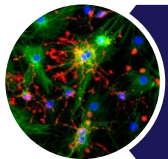# Parkinson's disease – A heavy emotional and economic burden
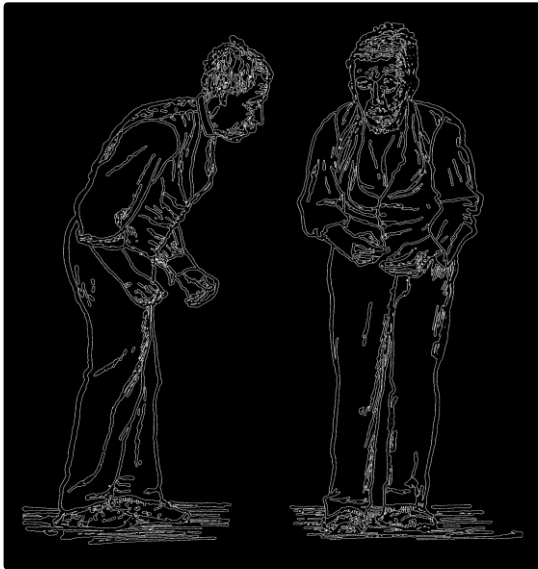
Gowers W R. A Manual of Diseases of the Nervous System, J & A Churchill. 591, 1886.

- Neurological Disorders affect more than 50 million Americans each year, costing more than $500 billion to treat.

- According to a recent study from the Center for Disease Control, complications from **Parkinson's disease (PD)** is the **14th** leading cause of death in the United States.

- In the US, 50,000 – 60,000 new cases of **PD** are diagnosed each year. PD cost the US ~ **$25 billion** per year.

- There are approximately 1 million individuals with PD in the US and 4-6 million worldwide.

PD involves the malfunction and death of vital nerve cells in an area of the brain called the substantia nigra. Some of these dying neurons produce dopamine, a chemical that sends messages to the part of the brain that controls movement and coordination. As PD progresses, the amount of dopamine produced in the brain decreases, leaving a person unable to control movement normally.

Pre-synaptic

Substantia Nigra
-Normal-

http://chen2820.pbworks.com/w/page/11951480/Stem%20cells%20in%20Parkinson%27s%20Disease

Normal

Tyrosine

Dopa

Dopamine

Mitochondria

Dopamine Auto-receptor

Synaptic Vesicle

VMAT

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Dopamine Receptors

Post-synaptic

D1   D2

ATCC

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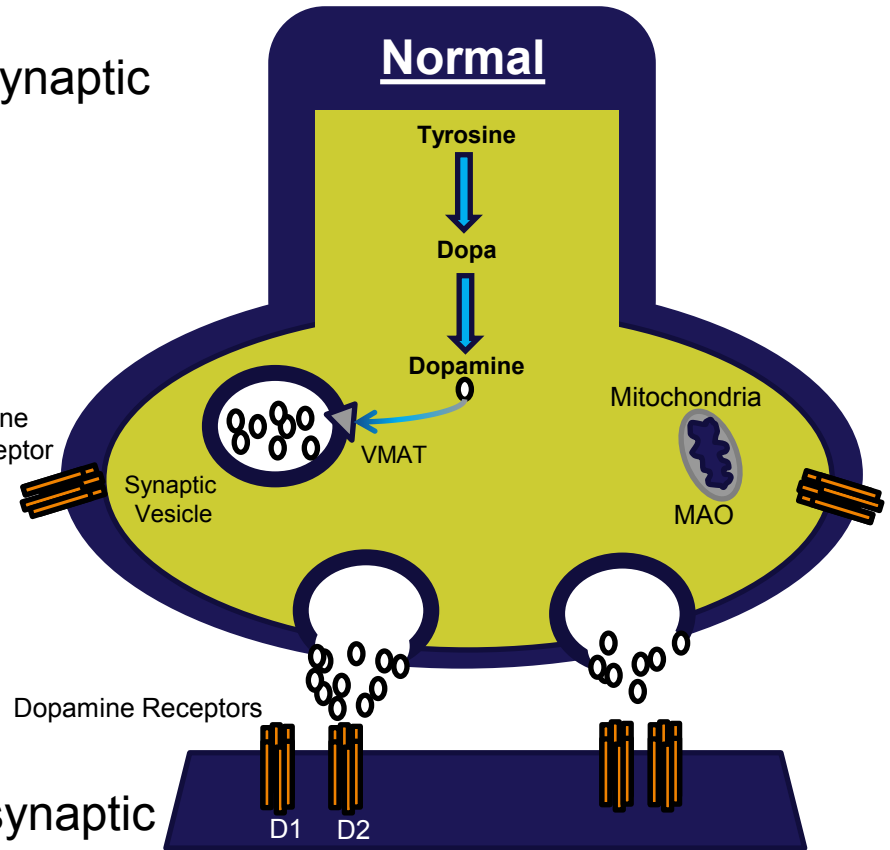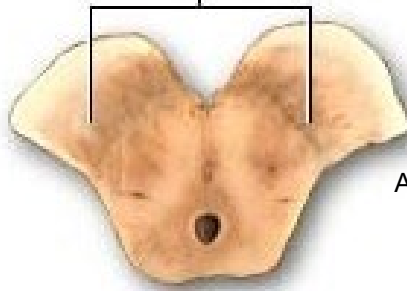
Pre-synaptic

Substantia Nigra
-PD-

Dopamine
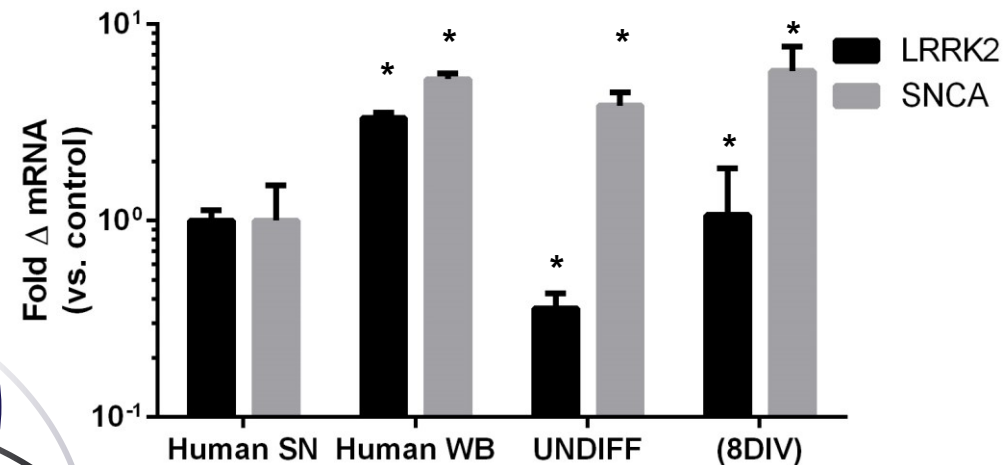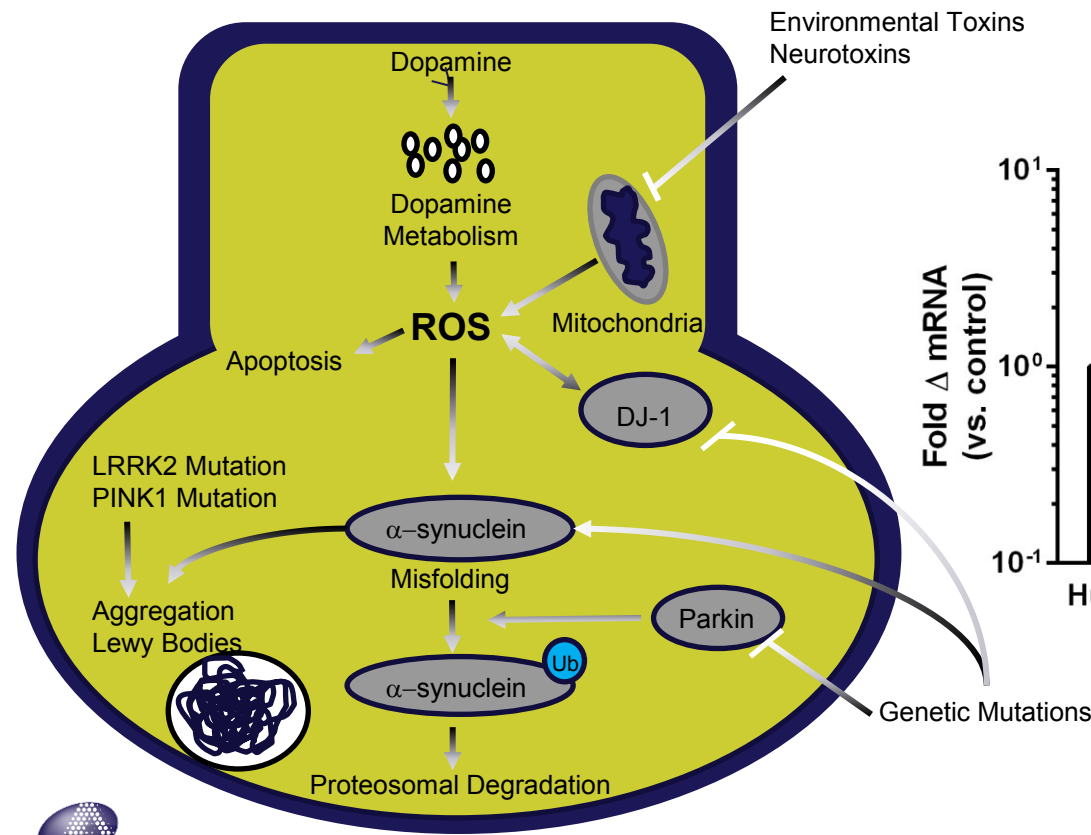Auto-receptor

Dopamine Receptors

Post-synaptic

D1 D2   D2 D4

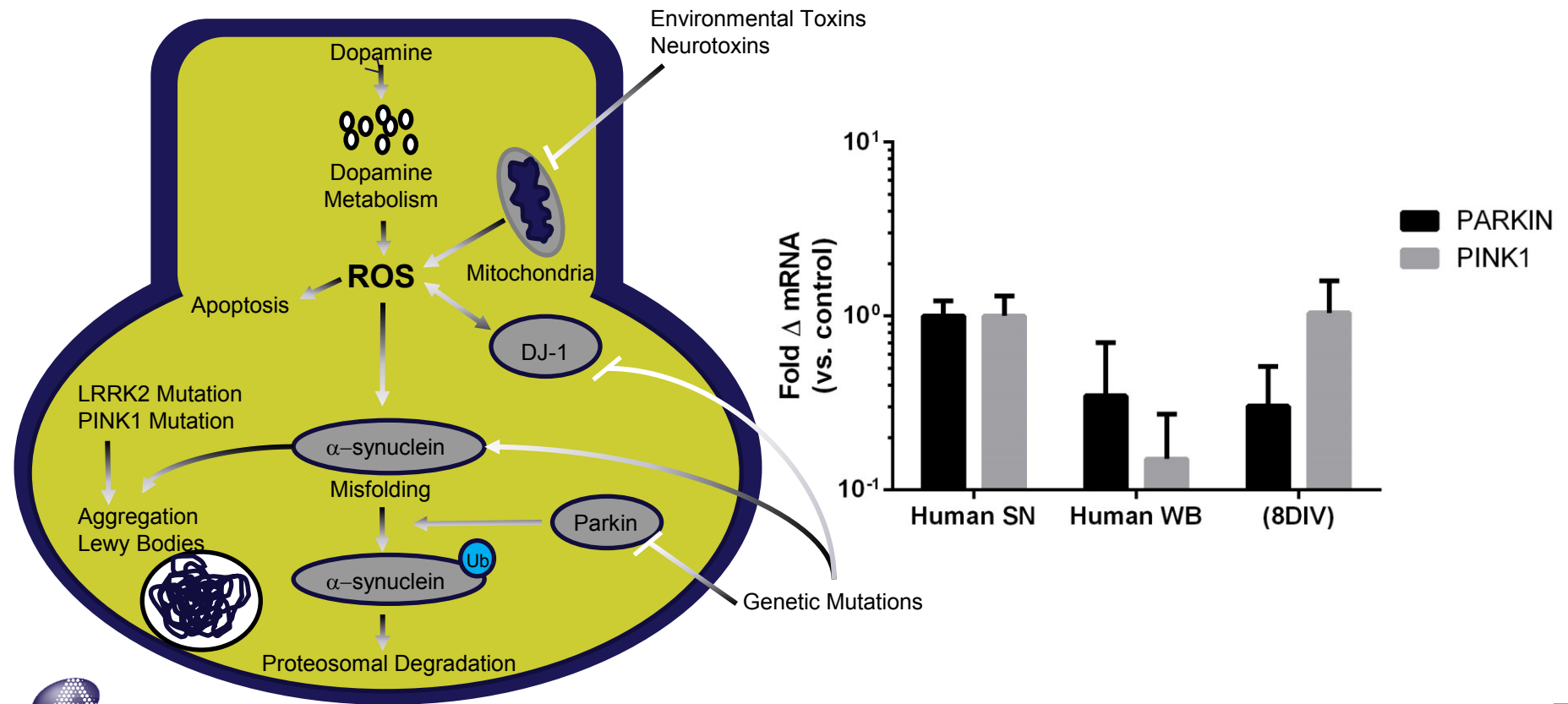# LUHMES cells express markers important for the study of Parkinson's disease

- **α- synuclein** – Major component of pathogenic Lewy bodies
- **LRRK2** – Associated mutations are the most common in PD
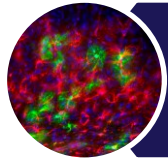
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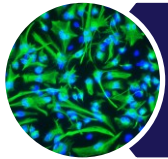
- **Parkin** – Protein that tags and targets damaged mitochondria for mitophagy
- **PINK1** – Mediates the activation and translocation of parkin
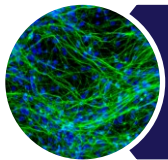
Outline

Current Neuronal Models

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Two Studies Using LUHMES Cells

OPEN

Transcriptional and metabolic adaptation of human neurons to the mitochondrial toxicant MPP+

AK Krug[*,1], S Gutbier[1], L Zhao[2], D Pöltl[1,3], C Kullmann[1], V Ivanova[3,4], S Förster[1], S Jagtap[5], J Meiser[6], G Leparc[7], S Schildknecht[1], M Adam[1], K Hiller[6], H Farhan[8], T Brunner[9], T Hartung[2], A Sachinidis[5] and M Leist[1]

Nature Reviews | Neuroscience

- Attempting to re-evaluate the transcriptional and metabolic changes in response to MPP+ a classical toxicant.

Nat Rev Neurosci. 2003 May;4(5):365-75.

OPEN

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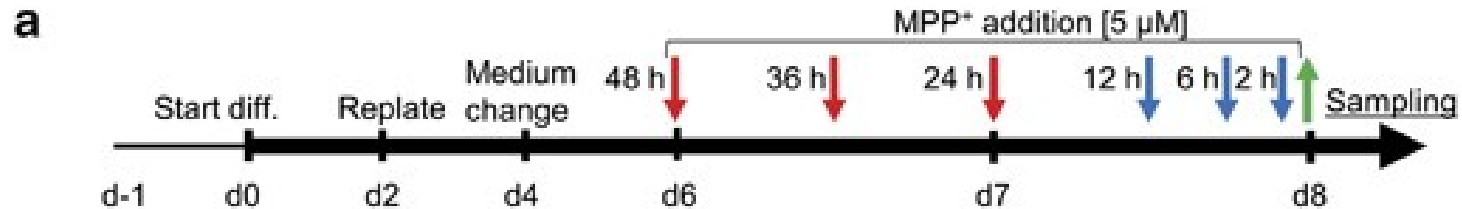
← LUHMES Cells

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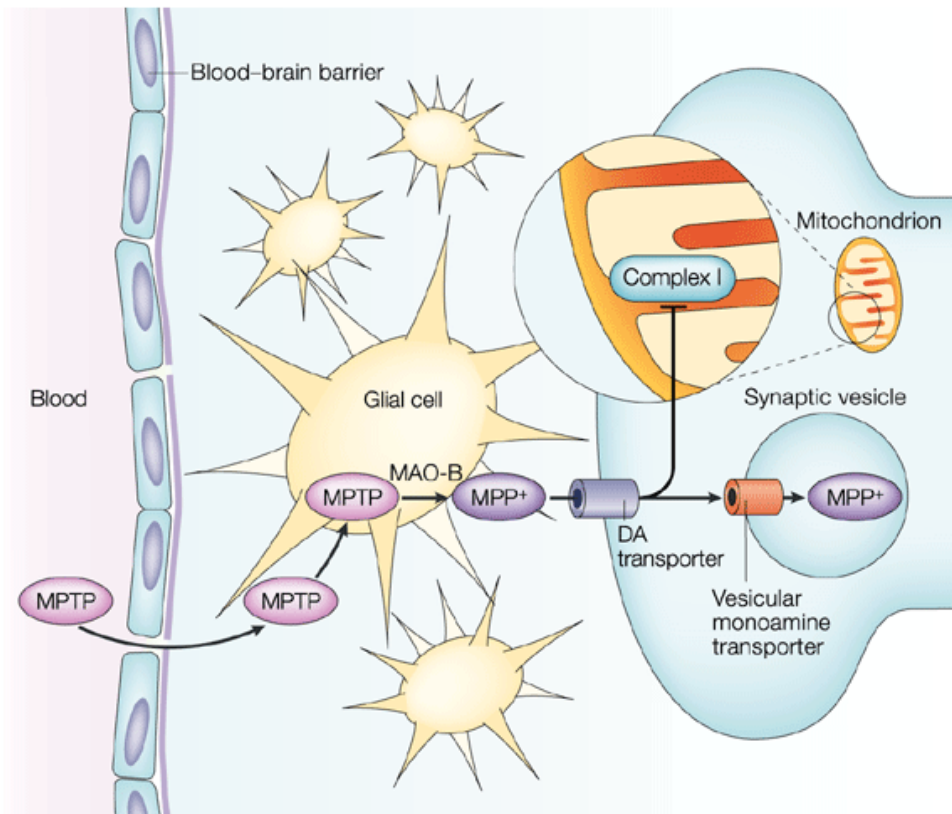
- Attempting to re-evaluate the transcriptional and metabolic changes in response to MPP+ a classical toxicant.

- MPP+ inhibits complex I in mitochondria and is assumed to cause death due by energy failure.

- Desired a homogeneous population of fully postmitotic dopaminergic neurons

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Nature Reviews | Neuroscience

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OPEN

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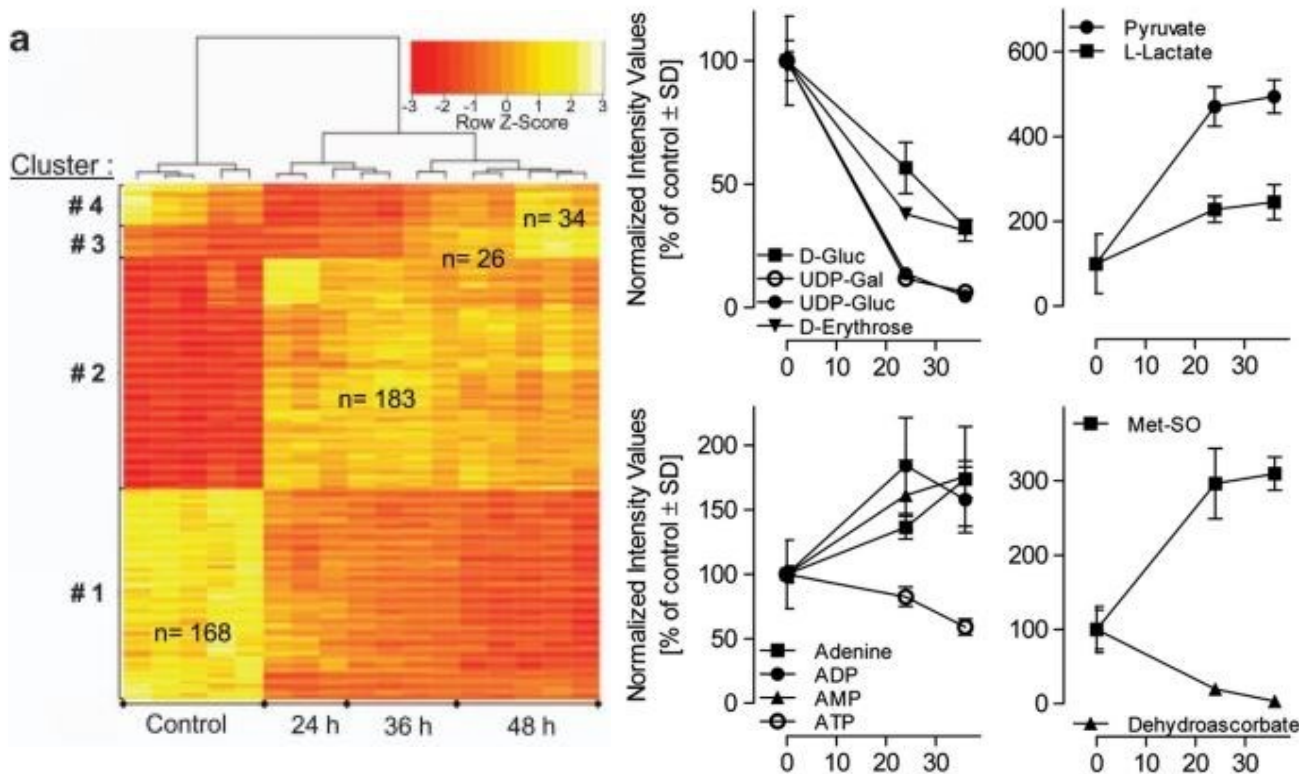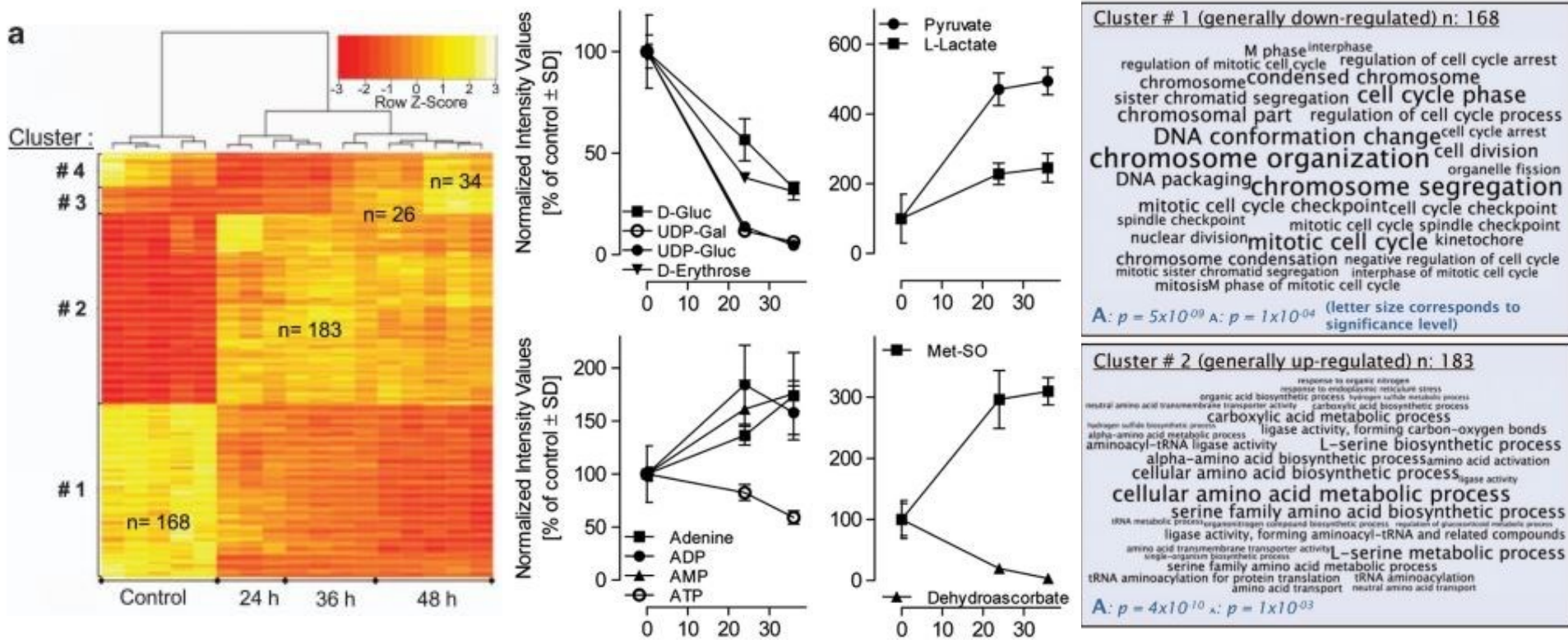
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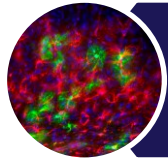
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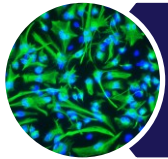# Transcriptional and metabolic adaptation of human neurons to the mitochondrial toxicant MPP+

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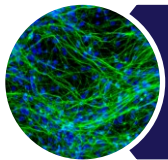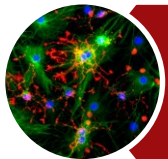
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Cell-based assays for Parkinson's disease using differentiated human LUHMES cells
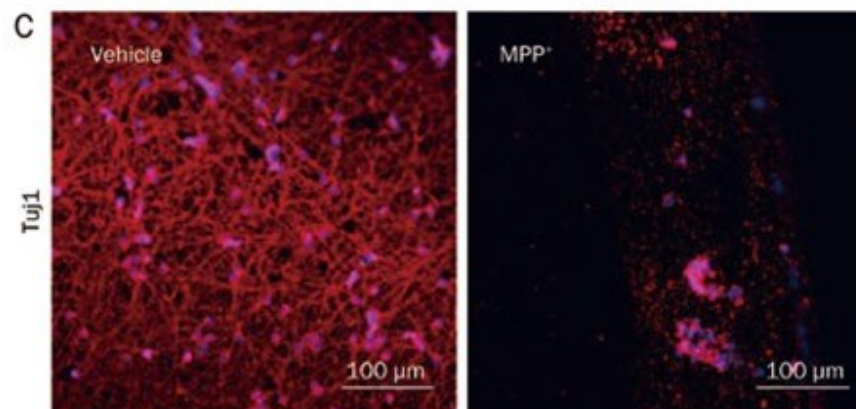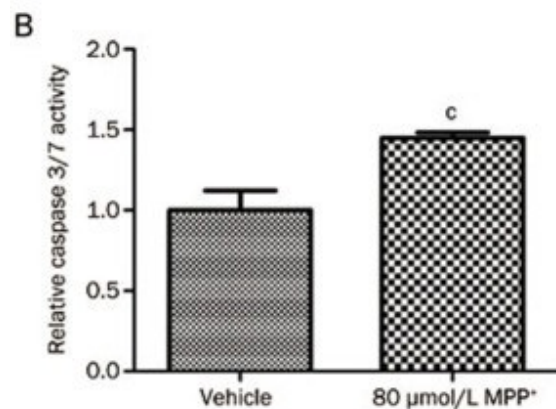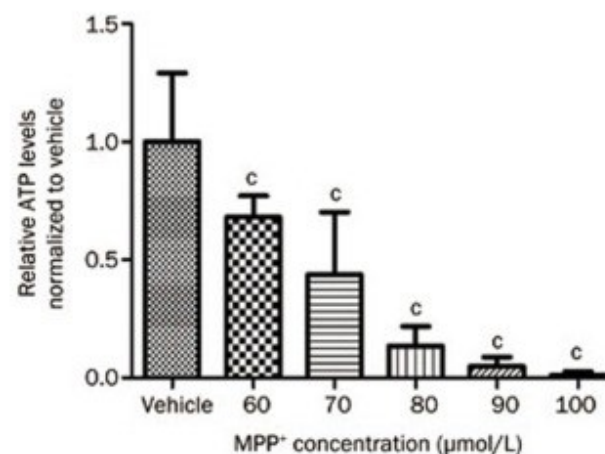
Xiao-min ZHANG[1, 2], Ming YIN[1], Min-hua ZHANG[2, *]

- Zhang *et al.* wanted to develop a cell based assay/High-throughput screen to evaluate drugs for the treatment of PD

- Criteria for a cellular model included:
    - Highly physiologically relevant model of dopaminergic biology
    - Consistent model
    - Scalable

Method:
- Cells differentiated for 6 days *in vitro*, treated with MPP+ for 2 days
    - Assayed for ATP levels
    - Assayed for caspase 3/7 activity

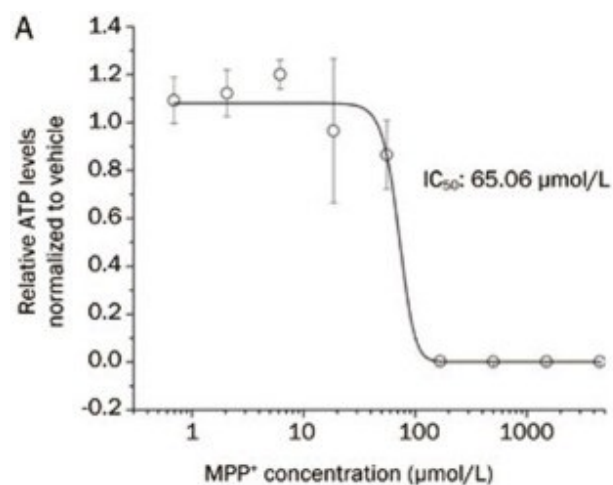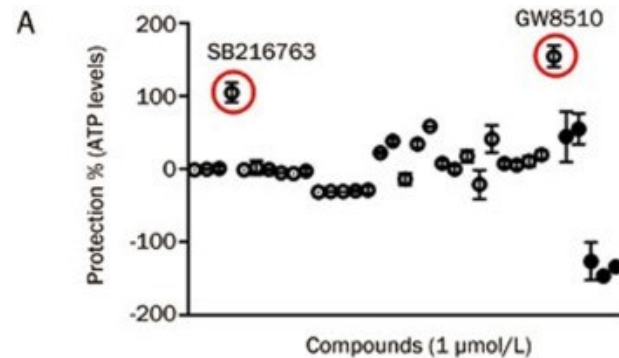# Cell-based assays for Parkinson's disease using differentiated human LUHMES cells

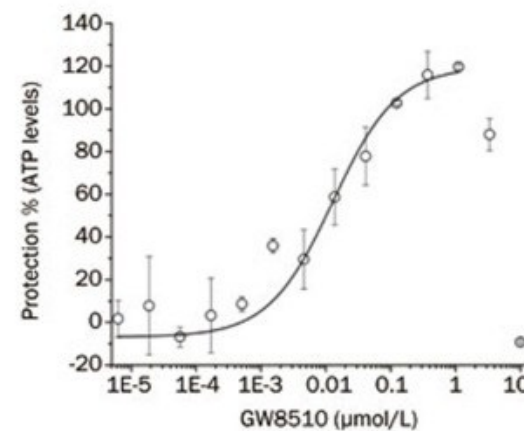Xiao-min ZHANG[1,2], Ming YIN[1], Min-hua ZHANG[2,] *

Original Article

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Assayed 35 commercially available compounds

Original Article

npg
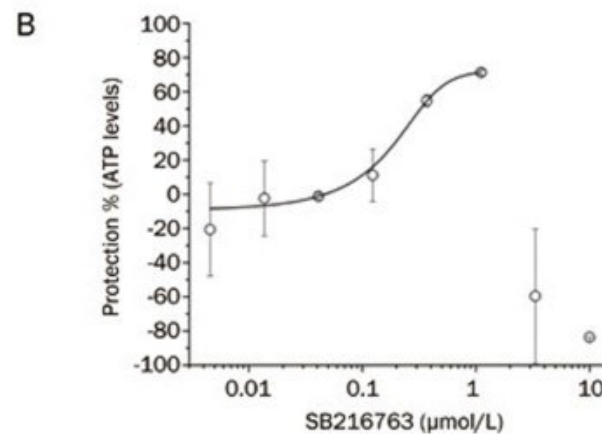
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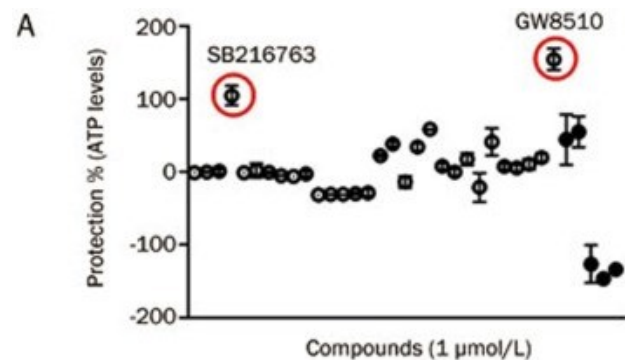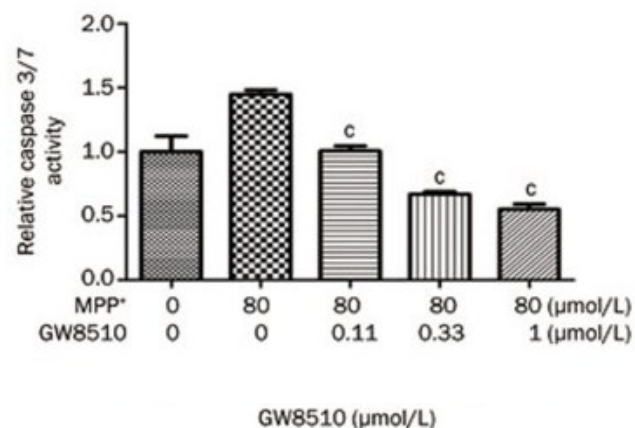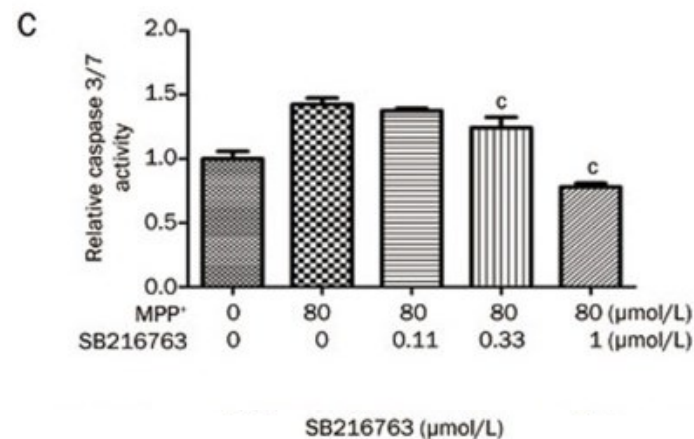Assayed 35 commercially available compounds

Summary

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- LUHMES cells express mature neuronal markers including NeuN and βIII tubulin.

- Expression of various dopaminergic genes including tyrosine hydroxylase, dopamine decarboxylase, the dopamine transporter, and dopaminergic receptors are observed by qPCR.

- Experiments using a calcium sensitive fluorescent dye indicate the presence of neuronal activity, as well as the presence of functional glutamate receptors.

Summary

- Expression of various markers including α-synuclein, LRRK2, PINK1, and Parkin makes LUHMES cells a useful cellular model for studying neurodegenerative diseases including PD.

- Expression of the dopamine 2 receptor subtype, make this line useful for studies related to various neuropsychiatric disorders, including Schizophrenia.

- LUHMES cells can be differentiated into a homogenous population of dopaminergic neurons that can be used to study dopaminergic biology or develop/execute assays for Parkinson's related studies.

Thank you!

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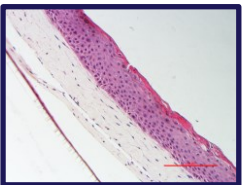
October 30th, 2014
10:00 AM, 3:00 PM ET
Dr. Francisco Bizouarn
Precise Counting Of Targeted Nucleic Acids Has Never Been Easier

November 13th, 2014
10:00 AM, 3:00 PM ET
Dr. John Pulliam
3-D Tissue Modeling

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