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for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

Impact of the Secretome of Human Mesenchymal Stem Cells on Brain Structure and Animal Behavior in a Rat Model of Parkinson's Disease

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CONTENTS

- Parkinsons Disease
- Materials & Methods
- Results
- Discussion
- Outlook

Parkinsons Disease (PD)

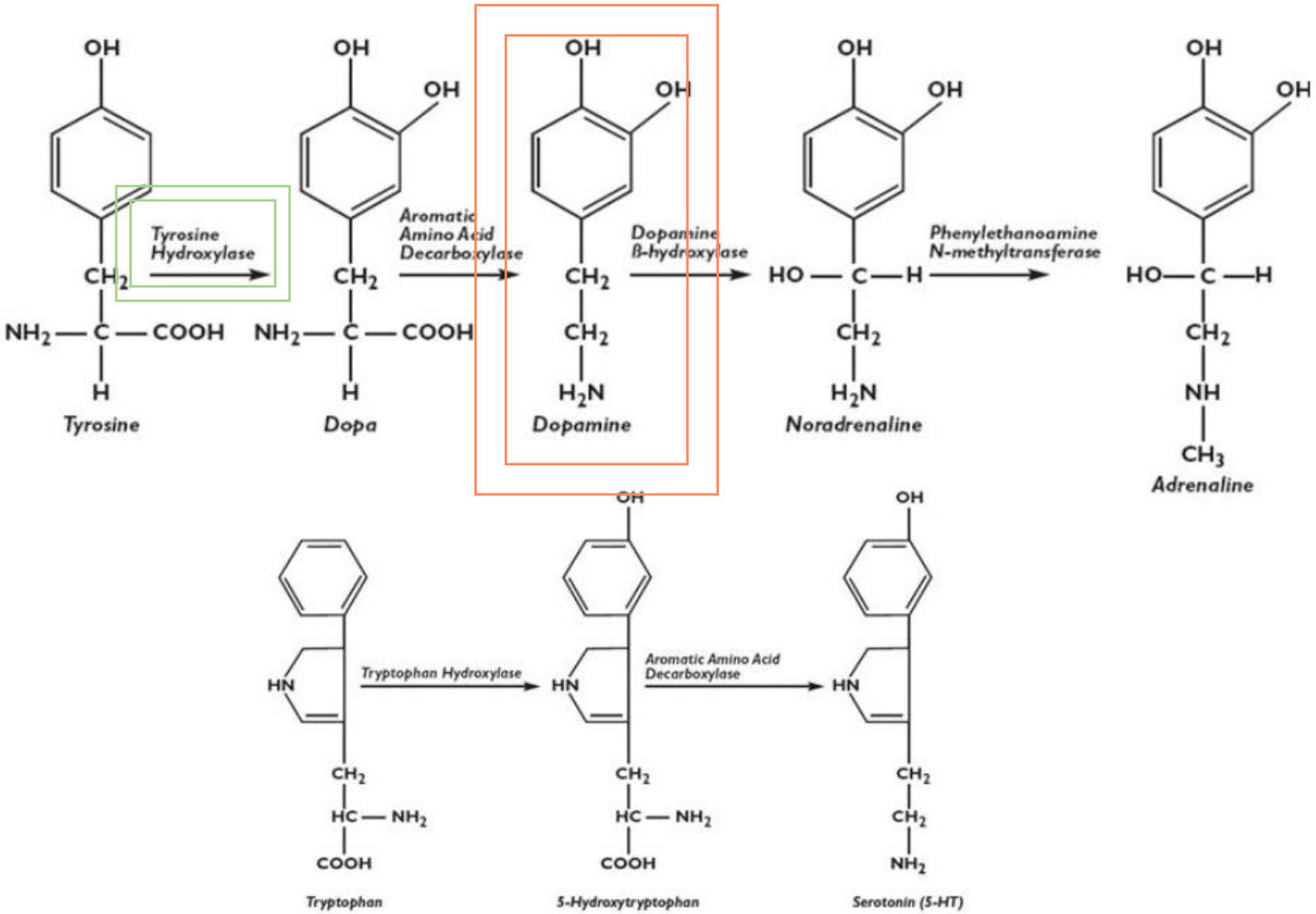
- PD is a chronic, progressive, neurodegenerative disease

- Four cardinal motor manifestations - Parkinsonism
  - Tremor at rest -rigidity
  - postural instability - bradykinesia

- Freeze phenomenon → "motor block" , "start hesitation"

- Cognitive and psychiatric impairments
  → dementia and depression

Dopamine Synthesis/Metabolism



http://amino-acid-therapy.com/wp-content/uploads/2012/08/neurotransmittermetabolism.png - last checked on 8th of October

PD Pathophysiology

Basal Ganglia – Thalamus – Cortex

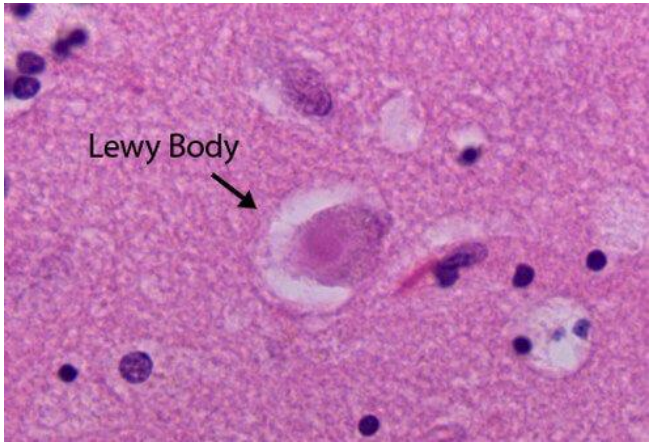
Loss of Dopaminergic Neurons SNc→ Basal Gangla dysfunction

Pathological Hallmark - aquisitition of LEWY- bodies

Dopamine depletion

alpha – synuclein accumulation

ROS, mitochondrial damage



https://www.medicinenet.com/lewy_body_dementia_dementia_with_lewy_bodies/article.htm - last checked 6th of Octobrer 2017

Basal Ganglia Network

- processing of information
  → execution of movement

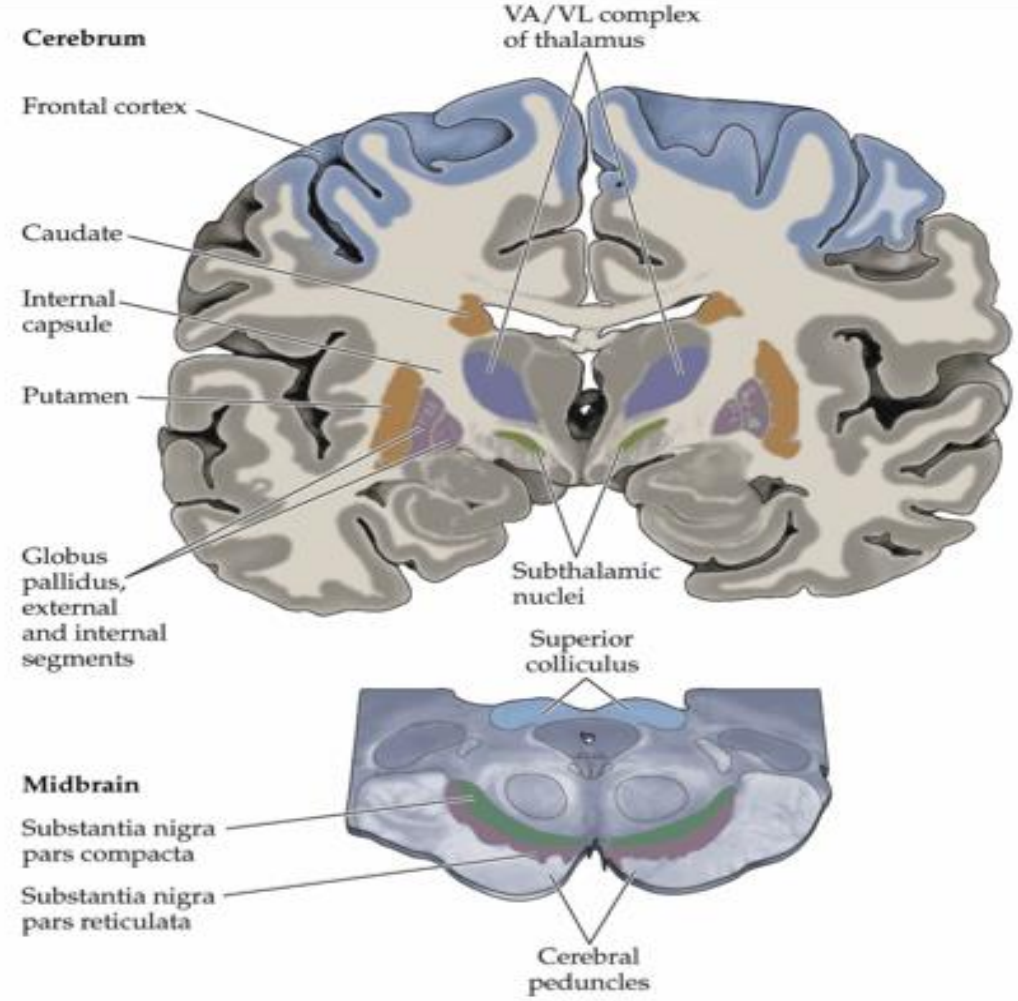
- Caudate Nucleus, Putamen, Globus Pallidus (intenus/externus), Subthalamic Nucleus and Substantia Nigra (pars compacta/reticularis)

  Neurotransmitters involved

  -Dopamine -Glutamate -GABA

  - Substance P -Acethylcholoin -Enkepahalin

Neurological Axis – Basal Ganglia



NEUROSCIENCE, Fourth Edition, Figure 18.1

https://kin450-neurophysiology.wikispaces.com/Parkinson%27s+Disease - last checked 6th of October 2017

Two Pathways – Physiological Funtion

Direct Pathway

- Striatum excitated by the Cortex
  → inhibits internal segment of the Globus Pallidus

- Disinhibition of the Thalamus allowing excitatory stimulation of the Cortex

  → facilitate Movement

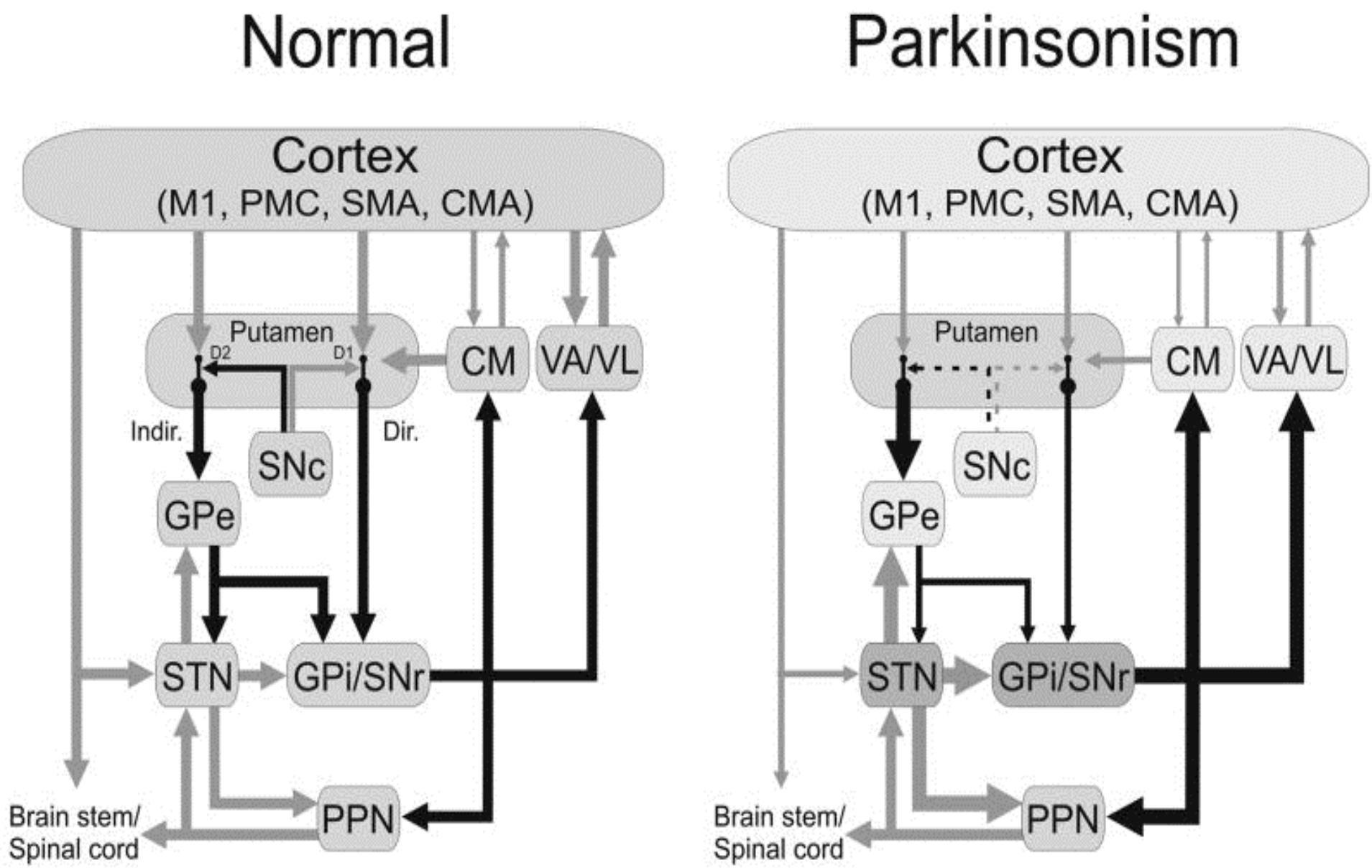
Indirect Pathway

- Striatum excitated by the cortex
  → inhibits external segment of the Globus Pallidus

- Disinhibitin of Subthalamic nucleus

  → excitatatory stimulation of GPi and SNr.

- Results in inhibition of the Thalamus
  → indirect inhibition of Cortices



Clin. Neurophysiol. 2008 Jul. 119(7):1459-1474

Causes for degeneration of Dopaminergic Neurons (DN)

- Oxidative Stress Hypothesis

  1) degradative pathway by MAO resutlts in formation of H2O2
  → reacts witch FE^{3+} to hydroxy radicals

  2) nonenzymatical reaction with O_2 formig quinones and semiquinones and subsequently super radicals, hydroxy radicals

- 6-Hydroxydopamine (6-OHDA), 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP)

PD

- Is a result of pathway imbalace due to disruption of dopamaminergic neurotransmission
- Disruption is caused by loss of function in the SNc
  Striatum disinhibited → indirect Pathway

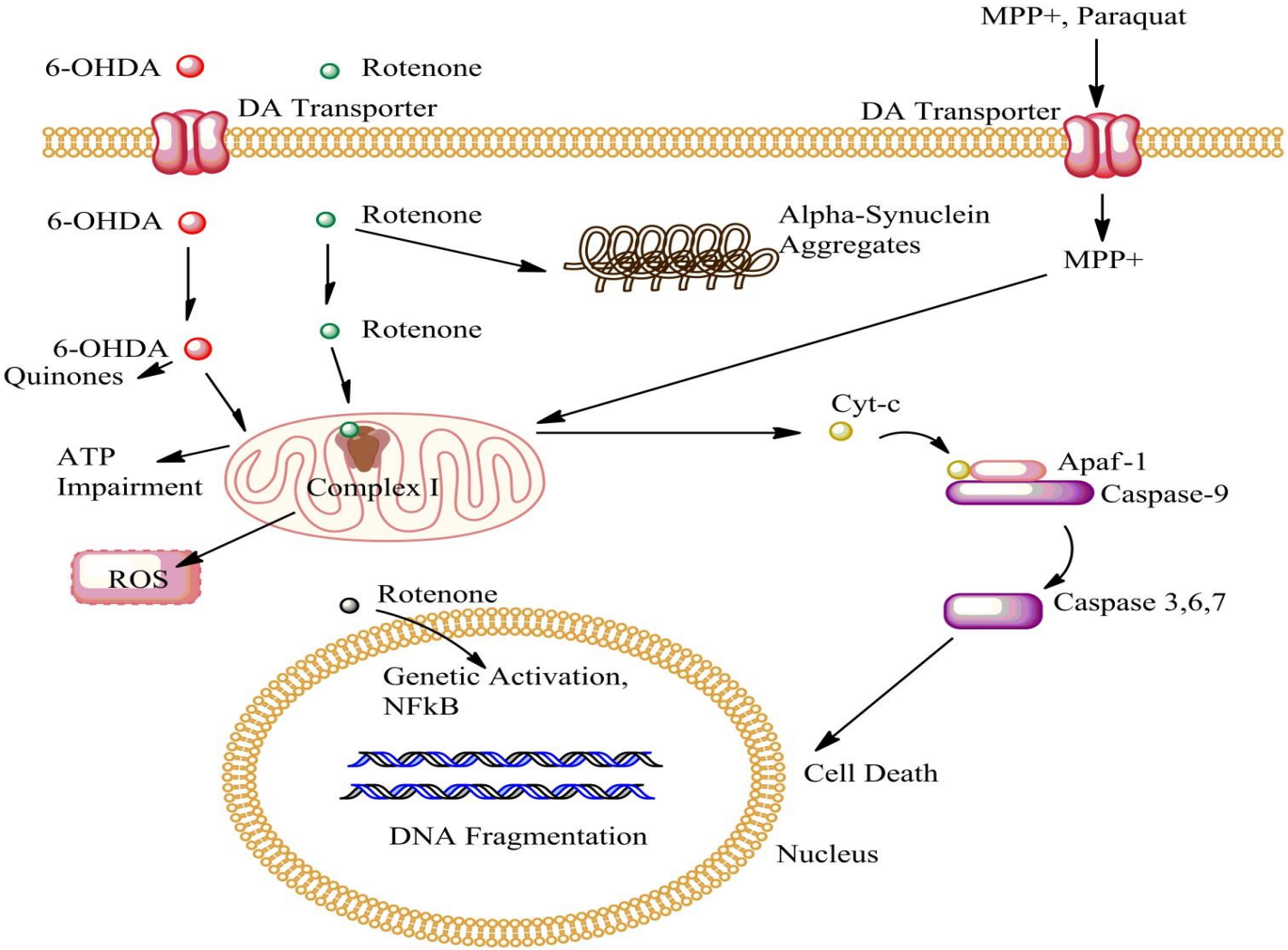
- Symptoms reflect the increase of movement inhibition and decrease of movement facilitation

Treatment

- Dopamin Substitution → Levodopa (Dopa – Decarboxylase!)
- Inhibitor of Dopamin Metabolism – MAO-B inhibitors
- Dopamin Agonists (Apomorphine)

- Surgical Intervention
  Deep-Brain-Stimulation

Experimental Models in Parkinson Disease



Astrocytes Role in Parkinson: A Double-Edged Sword 517 http://dx.doi.org/10.5772/54305

Secretome as a novel source for treatment ?

- Several studies with PD animal models and Human Mesenchymal stem cells (hMSC)
  → promote neuroprotection

  Secreted trophic factors such as neurothrophin.3, VEGF, GDNF and others
- Application of hMSC secretome into dentate gyrus in rat model → increase endogenous cell proliferation and cell density

Teixeira FG, Carvalho MM, Neves- Carvalho A et al. Secretome of mesenchymal progenitors from the umbilical cord acts as modulator of neural/glial proliferation and differentiation. Stem Cell Rev 2015;11:288–

Materials and Methods

- dynamic culture of hMSCs in computer-controlled suspension bioreactors

DASGIP system Microcarries

- hMSCs derived from bone marrow were expanded in static culture before inoculation in the bioreactor system
- Cells were cultured for 72 hours
- Supernatant (Condotioned Medium, CM) was removed
- Microcarrier washing step, SN collection 24 hous later

- Harvested SN centrifugated for 10 mins at 300g

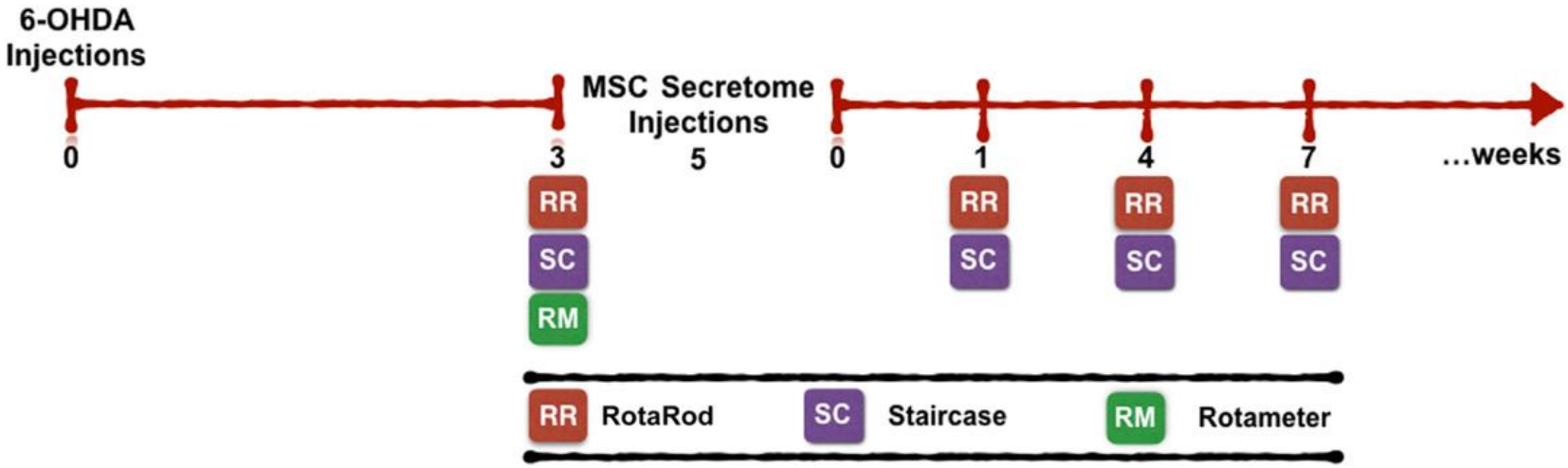
Stereotactical Surgery

- Ten week old Wister – Han male rats n=36
- 12-hour light/dark cycles and fed with regular rodents' chow and tap water ad libitum
- handled for 1 week before beginning injections to reduce the stress induced by the surgical procedures
- Sham group vs 6-OHDA group
- placed on a stereotaxic frame and unilaterally injected (Hamilton syringe with a 30-gauge) either vehicle (sham group, n = 11) or 6-hydroxidopamine (6-OHDA; n = 25; directly into the medial forebrain bundle (MFB)

- Sham animals received 2 ml of 0.2 mg/ml ascorbic acid in 0.9% of NaCl
- 6-OHDA animals were injected with 2 ml of 6-OHDA hydrochloride (4 mg/ml) with 0.2 mg/ml ascorbic acid in 0.9% of NaCl at a rate of 1.0 ml/minute.

- After each injection, the needle was left in place for 4 minutes to avoid any backflow up the needle tract.

- Behavioral assesment 3 weeks after surgery



Five weeks after the injection of 6-OHDA → hMSC secretome application. n=12

Vehicle/ Controle = Neurobasal A Medium + Kanamycin n=13

1, 4, and 7 weeks following surgery, behavioral assessment was performed

Behavioral Assesment

- RotaRod
  - motor coordination and balance, 3 days of training phase

  On the 4th day the latency to fall was observed

- Skilled-Paw Reaching test (Staircase Test)
  forelimb use and skilled motor function
  2 days to familiarize the animals with the test

- Apomorphine Turning Behavior (rotameter test)
  subcutaneously injected, contralateral rotations

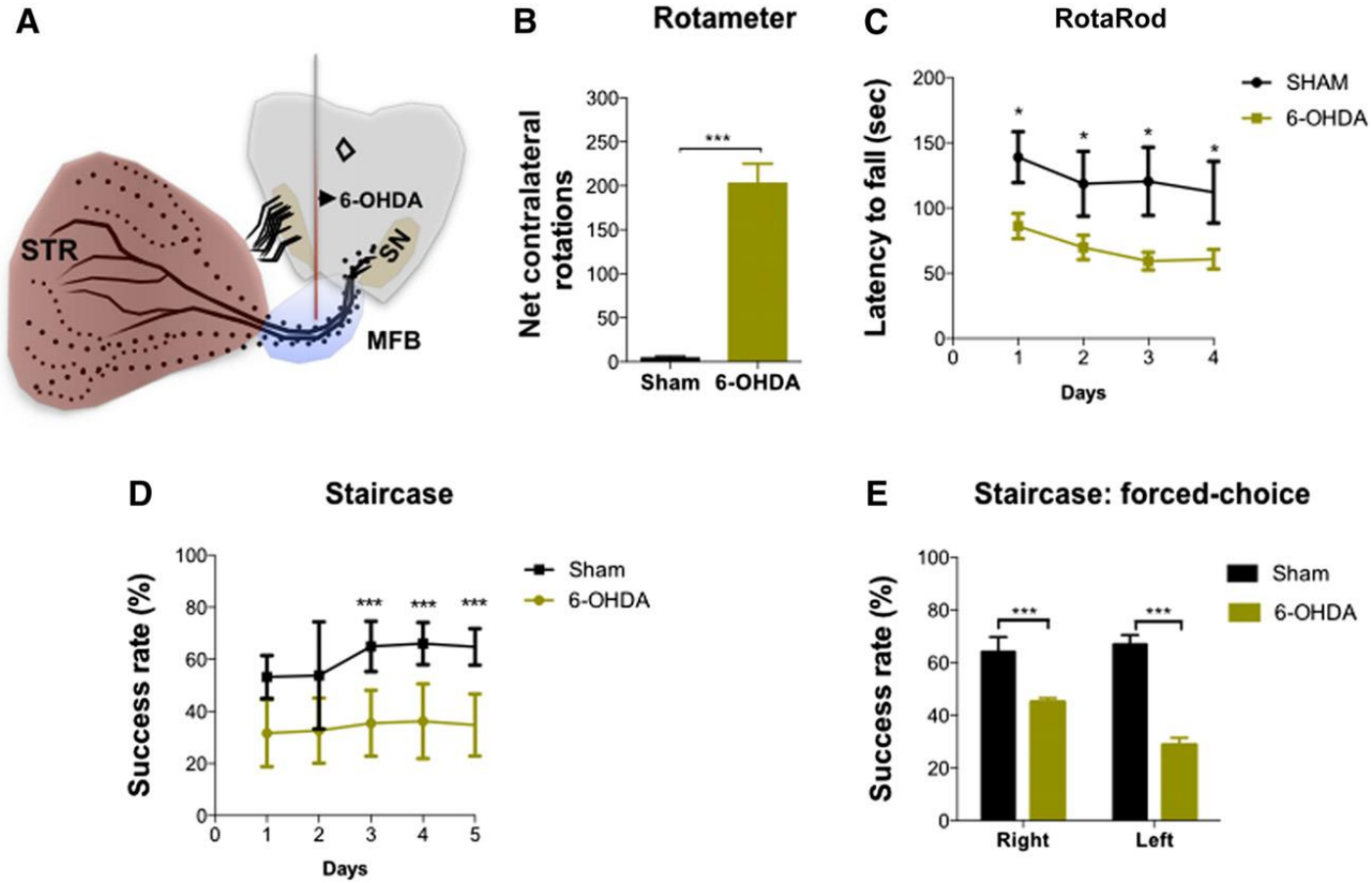
TH - Immunohistochemistry

- After 13 weeks the animals were killed with sodium pentobarbital
- Coronal sections of the Striatum and mesencephalon were obtained, four series were obtained
- One was processed as a free-floating tyrosine hydroxylase (TH) immunohistochemistry.
- total TH+ cells in the SNc area were counted in both hemispheres

RESULTS

- Phenotypic Characterication of 6-OHDA Lesions
- Transplantation of hMSC CM-Attenuated Motor Deficits of 6-OHDA-Injected Animals
- Transplantation of the hMSC Secretome Restored the Neuronal Structure

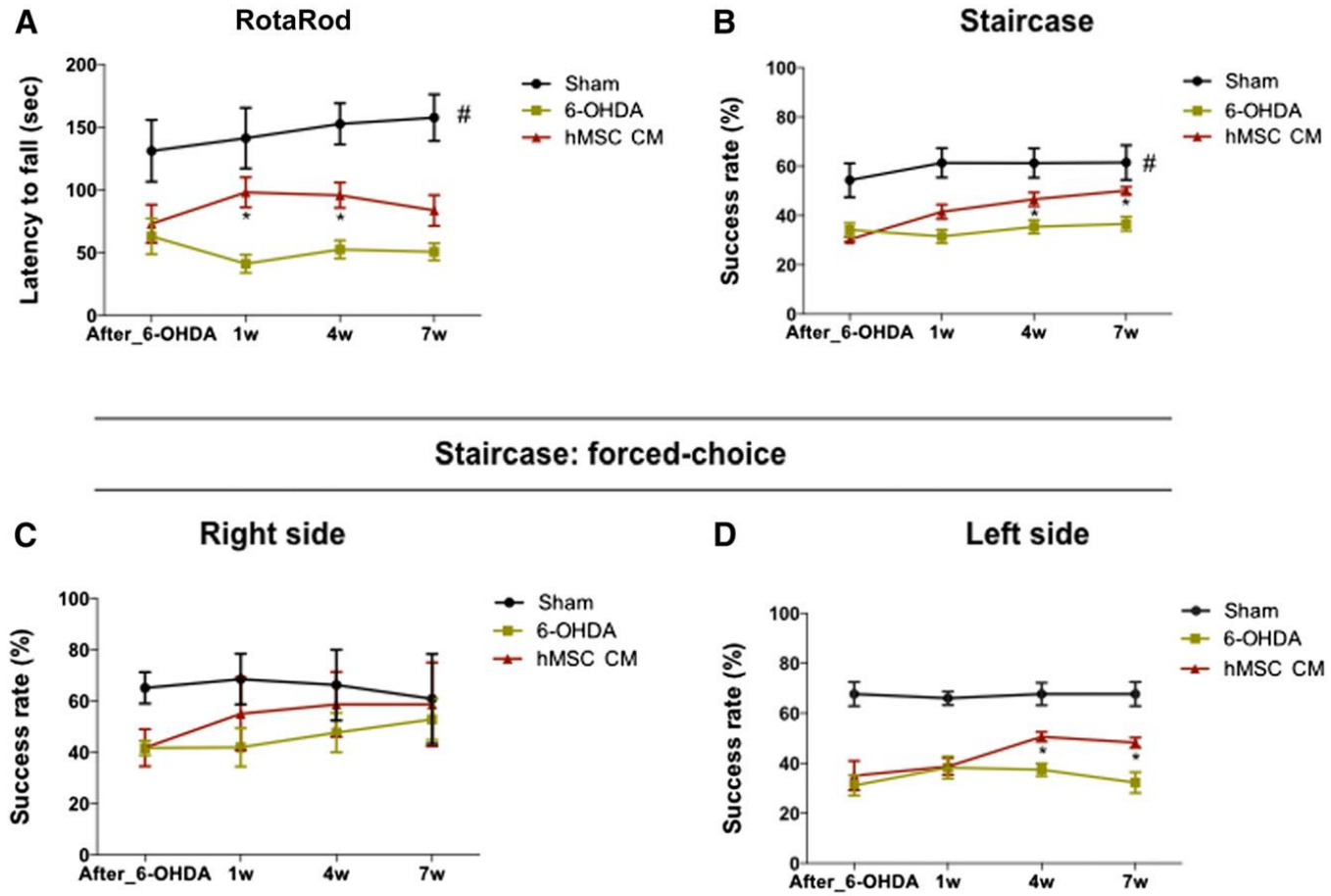
Phenotypic Characterication of 6-OHDA Lesions



- injection of 6-OHDA in the MFB (A) led to an intense turning behavior in the apomorphine-induced turning behavior when compared with sham group

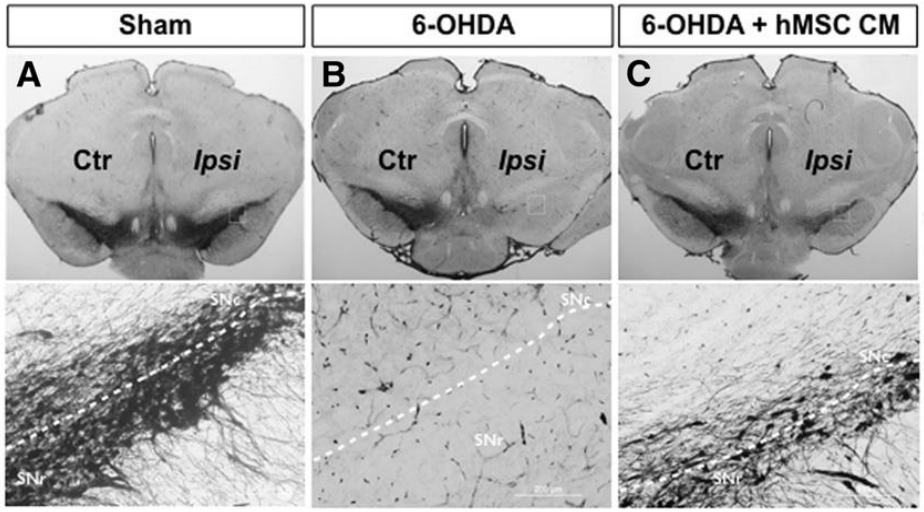
- 6-OHDA-injected animals also exhibited significant impairment in motor coordination on the RotaRod test as well as in paw - reaching - test performance

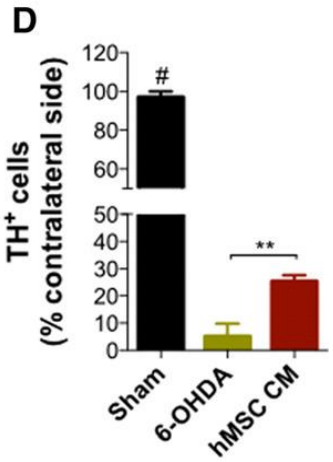
Transplantation of hMSC CM-Attenuated Motor Deficits of 6-OHDA-Injected Animals



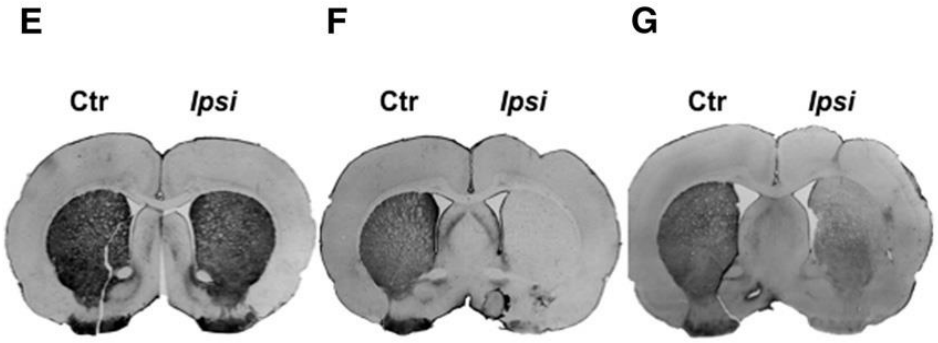
- hMSC CM-injected animals had a significant improvement in their motor coordination when compared with the 6-OHDA group
- Paw-reaching performance also demonstrated a significant improvement of the forelimb coordination of the hMSC CM-injected animals

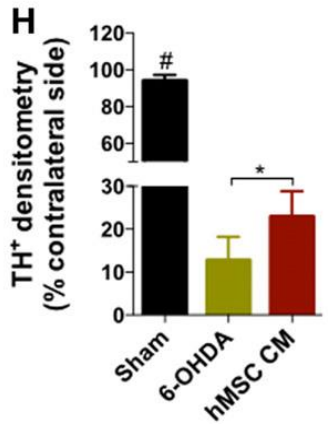
Transplantation of the hMSC Secretome Restored the Neuronal Structure





95% vs75%



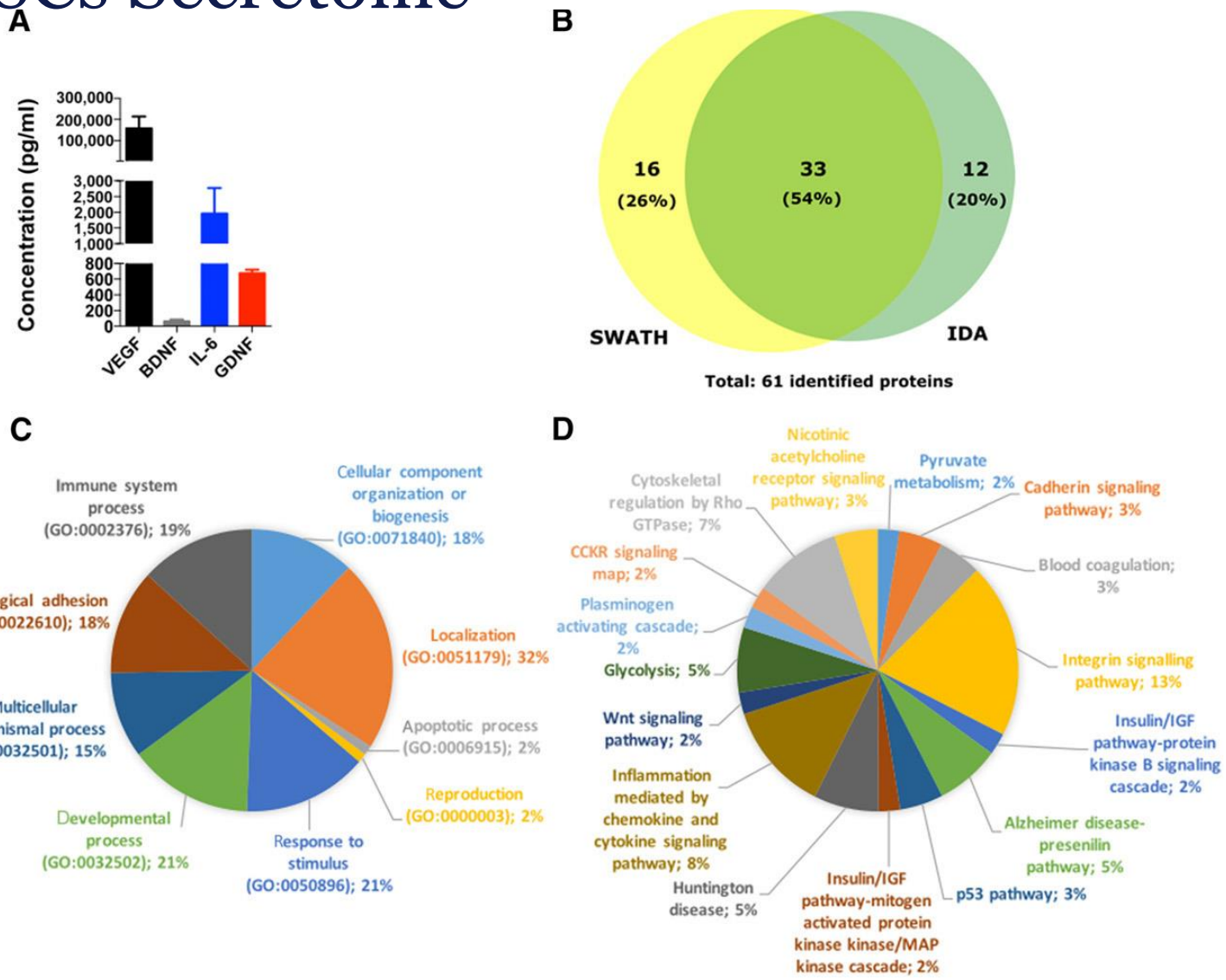


88% vs 77%

Representative photomicrographs of brain slices stained for TH

- there was a significant loss of DA neurons after injection of 6-OHDA into the SNc
- There was a significantly higher number of TH- positive cells observed in the SNc (CM: 25.36% 6 5.45%) when compared with the 6-OHDA group
- The same tendency was also observed in the striatum by assessing TH-positive fibers by densitometry analysis.

hMSCs Secretome



- characterized the secretome through targeted and nontargeted proteomic approach-based analyses
- Bioplex assay → hMSCs secreted important neurotrophic factors such as VEGF, BDNF, IL-6, and GDNF
- Through the combined MS analysis they found additional proteins with important actions

  CNS regulators such as Cys C glia-derived nexin (GDN); galectin-1 and pigment epithelium-derived factor (PEDF)
- Only PEDF was found to be an important neurotrophic and neuroprotective molecule in the context of PD

Discussion

- Which factors of the Secretome exert the beneficial effect ?

- molecules such as VEGF, BDNF, IL-6, and GDNF were present in the hMSC secretome – promoting neuroprotection in dopamingeric neurons
- when applied in vitro, BDNF induced the differentiation and neurite outgrowth in DA neurons
- In vivo, in nonhuman primates, BDNF has demonstrated the ability to reduce DA neuronal loss

- stimulation by the hMSC secretome is **not dependent** on the presence of **just one secreted factor but several**

Reflection on the work process

- Well structured paper, adequate language. Great figures
- Effects on Microglia, Astrocytes …?
- Insight into new methods