# Mesenchymal stem cells secretome-induced axonal outgrowth is mediated by BDNF

Martins LF, Costa RO, Pedro JR, Aguiar P, Serra SC, Teixeira FG, Sousa N, Salgado AJ, Almeida RD. Scientific Reports | 7: 4153 | DOI:10.1038/s41598-017-03592-1

# Secretomes of apoptotic mononuclear cells ameliorate neurological damage in rats with focal ischemia.

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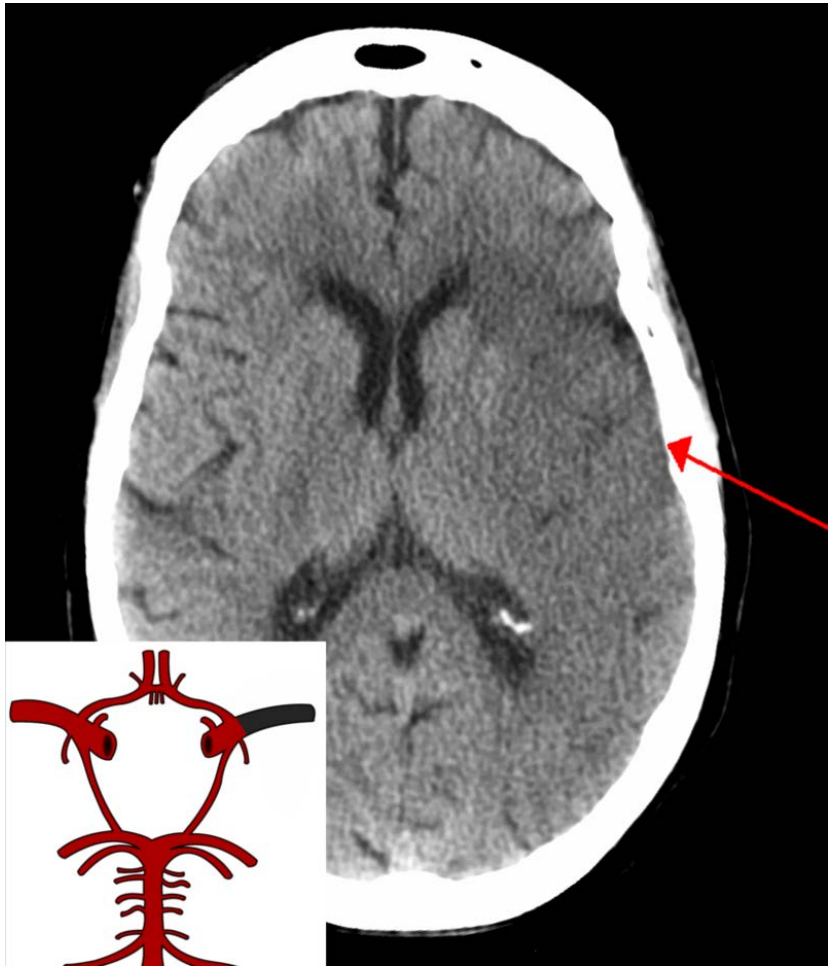
# Introduction
# Stroke

- Pathophysiology of stroke:
  - Loss of blood supply → Ischemia → failure of ATP-production → failure of ion pumps → reduction of transmembrane gradient → release of glutamate → calcium influx → enzyme/signal activation, failure of mitochondria → further energy depletion → Apoptosis
  - Ischemia → production of oxygen free radicals, reactive oxygen species

# Introduction
# Stroke

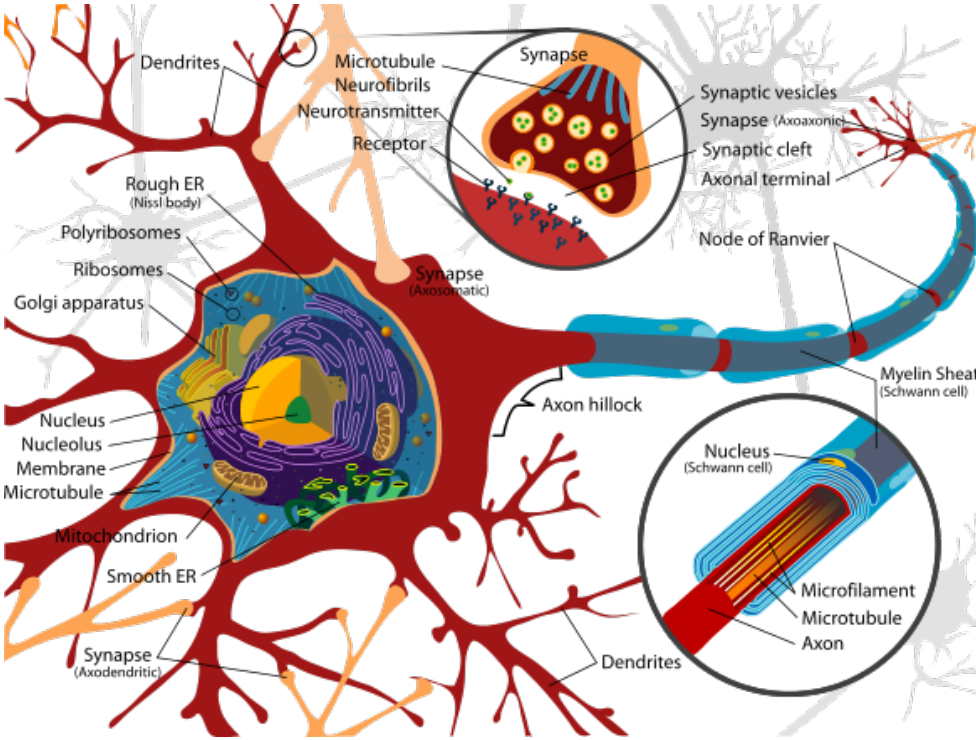


- Etiology: Ischemic vs hemorrhagic
  - Ischemic: thrombosis, embolism, systemic hypoperfusion, cerebral venous thrombosis
  - Hemorrhagic: cerebral or subarachnoidal hemorrhage

https://en.wikipedia.org/wiki/Stroke#/media/File:StrokeMCA_overlay.png

# Introduction
# Neurons

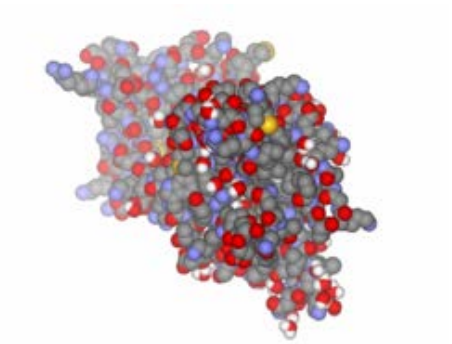
- = electrically excitable cell, receives, processes and transmits information
- Sensory vs motor neurons
- Neurite = dendrite or Axon
- Dendrite = multiple, receiving information
- Axon = only one; signal transduction from axon to dendrite of another neuron




https://en.wikipedia.org/wiki/Neuron#/media/File:Complete_neuron_cell_diagram_en.svg

# Introduction
# Neurotrophins

- Neurotrophins = family of proteins that induce survival, development, function of neurons
- NGF = Nerve growth factor
- Neurotrophin 3&4
- BDNF= brain deriverd neurotrophic factor
  - support the survival of existing neurons,
  - growth and differentiation of new neurons and synapses
  - active in the hippocampus, cortex, and basal forebrain
  - Receptors:
    - TrkB
    - LNGFR (low affinity nerve growth receptor)



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Brain-derived_neurotrophic_factor

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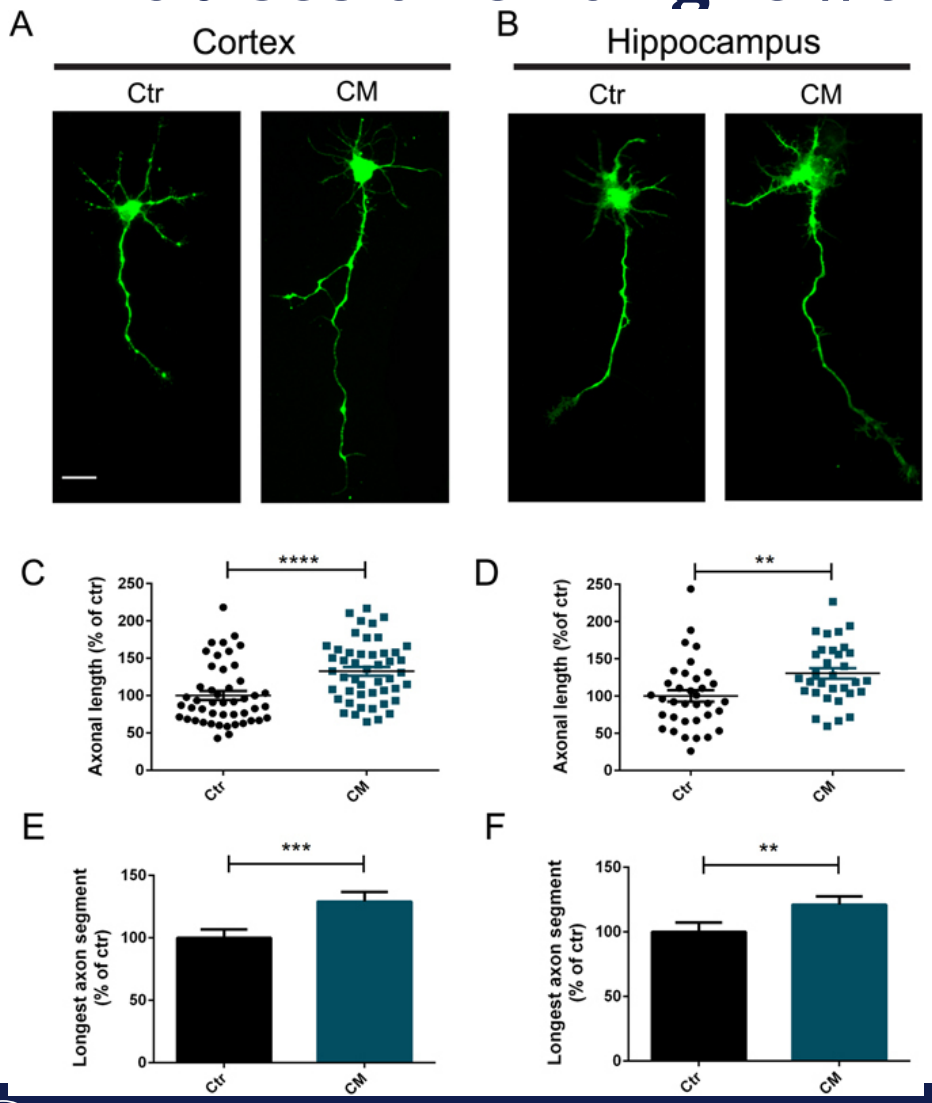
- Abbreviations
  - MSC = mesenchymal stem calls
  - HUCPVC = human umbilical cord perivascular cells
  - NBM = neurobasal medium
  - BDNF = brain derived neurotrophic factor
  - CM = conditioned medium
  - DIV = days in vitro
  - EGFP = enhanced-green fluorescence protein

# Methods

- Neuronal culture: 17 Wistar-han rats, embryonic day 17-18; hippocampi and cortices gained
- Microfluidic devices described as follows
- Conditioned medium:
  - HUCPVCs isolated; resuspended in alpha-MEM medium + 10% FBS;
  - For CM, HUCPVCs in Neurobasal-A medium; 4000cells/$cm^2$ grown for 3 days, medium renewed, cultivated for 24h, 100x concentrated
- BDNF-depletion: human recombinant TrkB Fc chimera protein

# HUCPVC Conditionated Media (CM) induces axonal growth in CNS neurons.



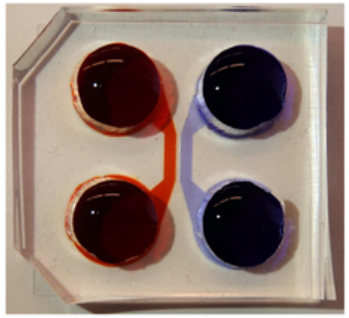

1a, b) rat embryonic cortical/hippocampal neurons, stained for Tau

1c,d) increased axonal length with CM

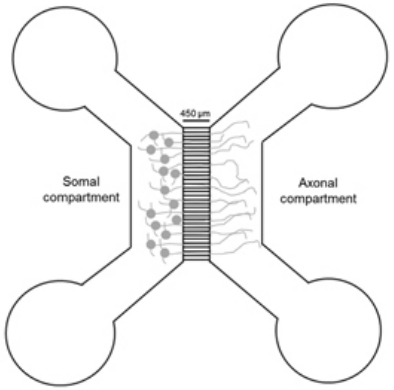
1e,f) longest segment of as % of ctrl

# Microfluidic chambers for culturing CNS neurons

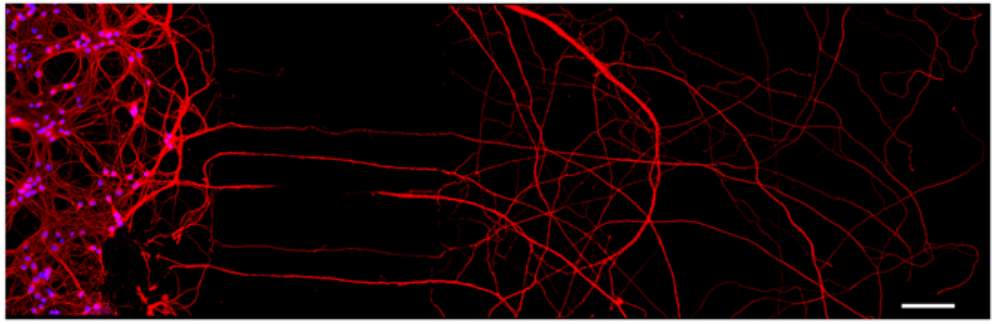
A



B




C



2a,b) composition of microfluidic chambers allow separation of axons from soma and dendrites

2c) tubulin-staining of neurons in microfluiic chamber DIV 5-6

# Axonal-specific stimulation with CM induces axonal growth of CNS neurons.

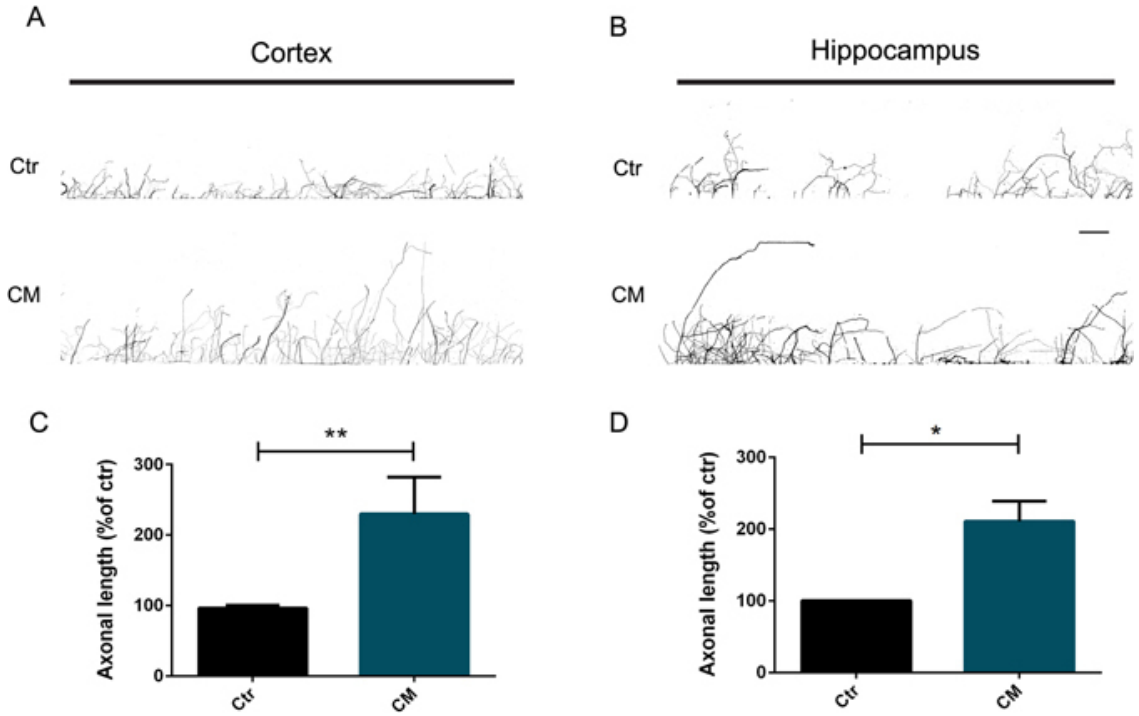
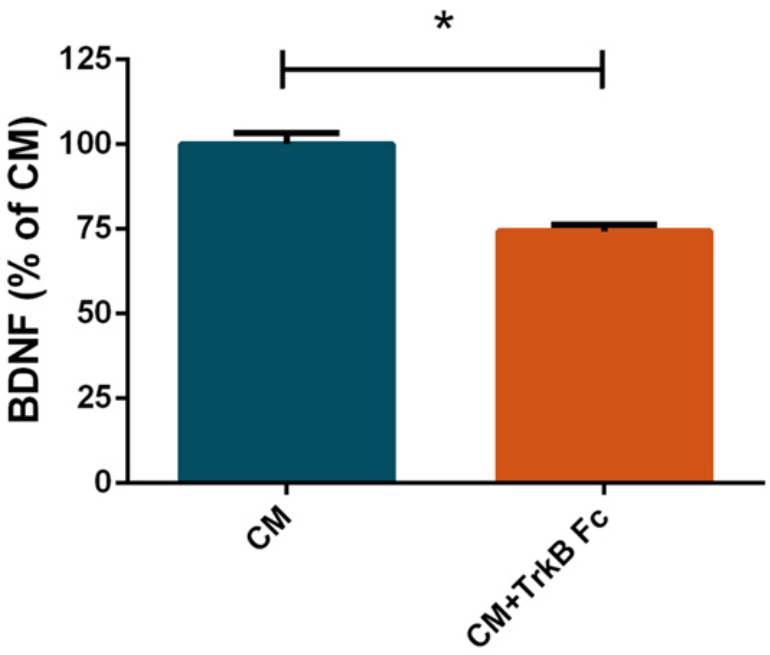



Fig. 3

- in CM-presence, axonal network is increased
- Axonal length is increased

# BDNF is an important molecule for CM-induced axonal outgrowth in cortical neurons.

A



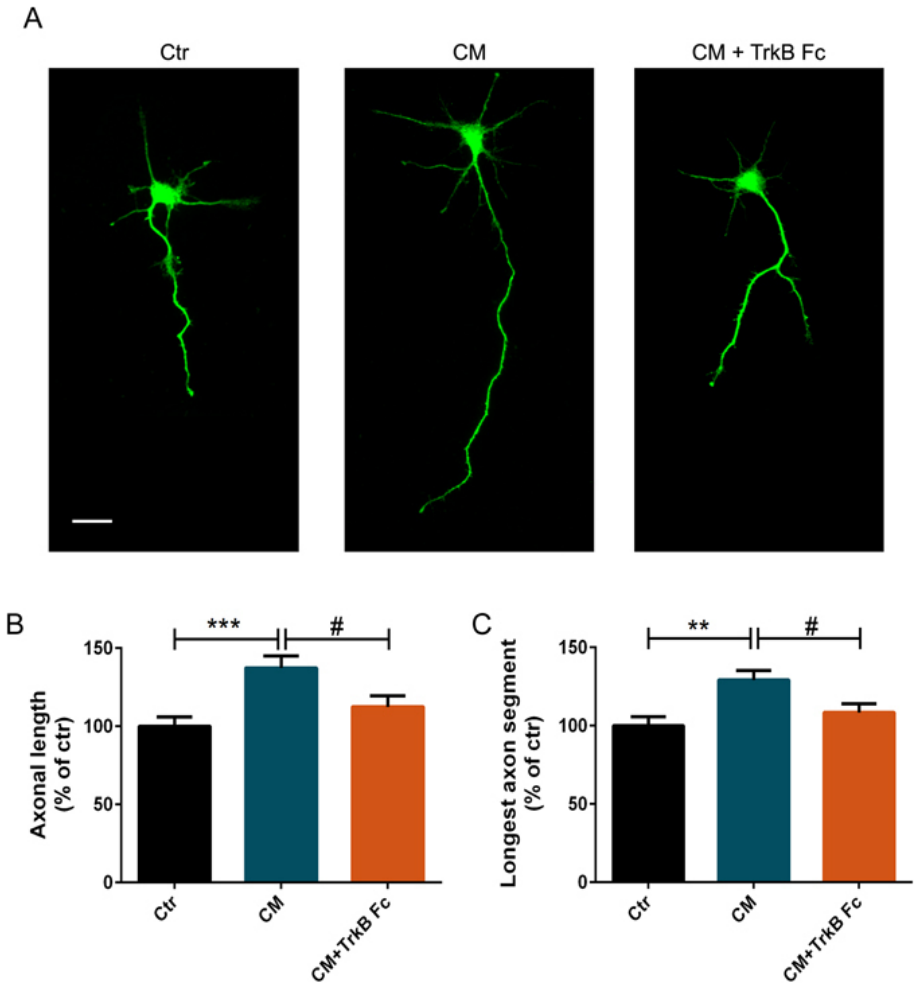

B

| | CM | CM+TrkB Fc | Statistic |
|---|---|---|---|
| BDNF (pg/ml) | 37.03 ± 1.240 | 27.54 ± 0.690 | *p*=0.0216 |

Fig 4)
TrkB Fc neutralizes BDNF in CM

TrkB Fc = BDNF binding/neutralizing molecule

# BDNF is the main component of CM-induced axonal outgrowth in cortical neurons




5a) BDNF-depletion from CM reduced CM-mediated axonal outgrowth

5b) axon outgrowth with TrkB Fc treated CM was similar to basal levels

# BDNF is the molecule responsible for CM-induced axonal elongation in distal cortical axons

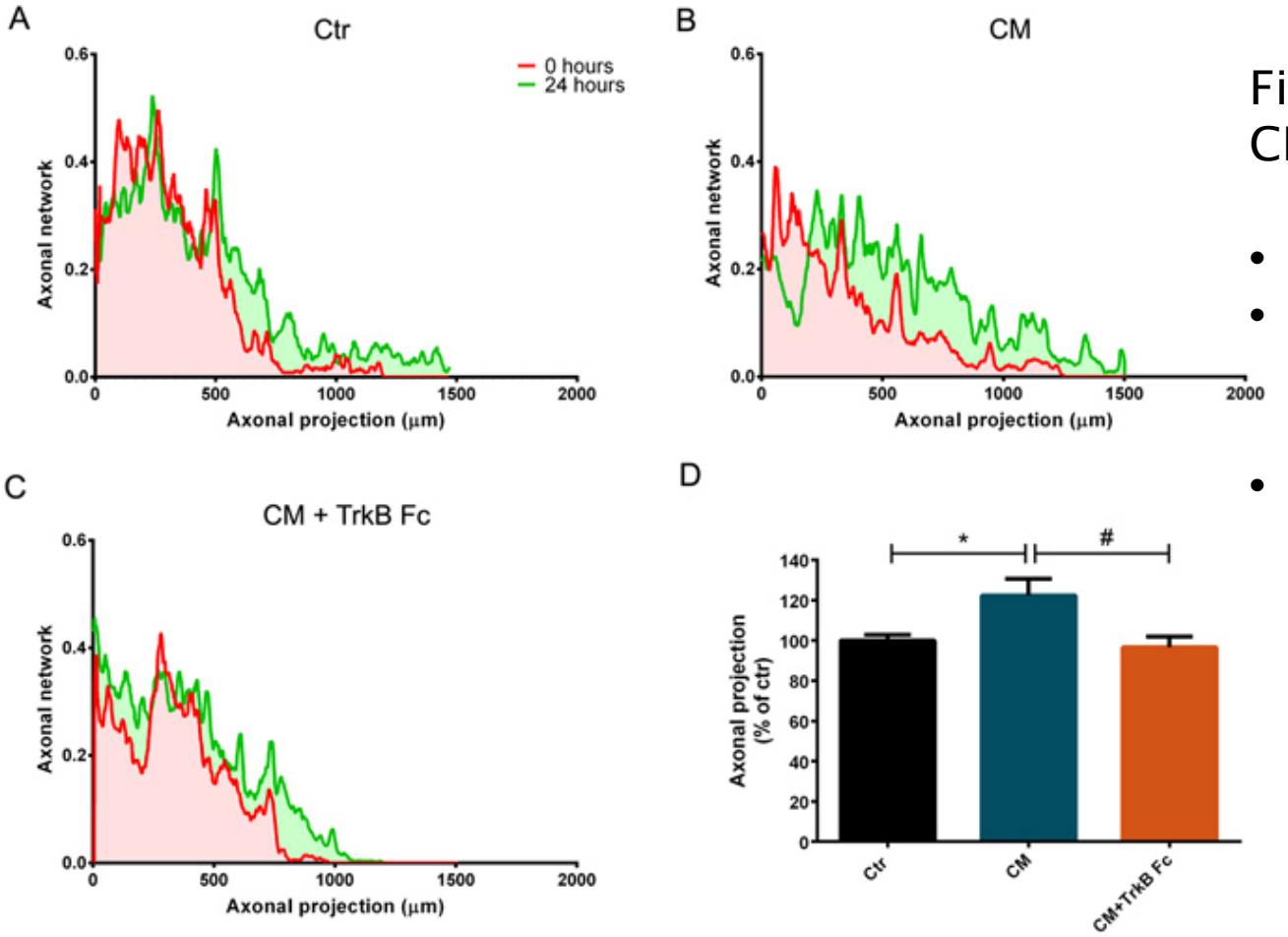



Fig. 6: Local effect of CM/CM+TrkB Fc/control

- on axonal length
- Before and after 24h of stimulation

- BDNF acts locally witout contribution from the cell body

# BDNF works as a localized signal in CM-induced axonal outgrowth

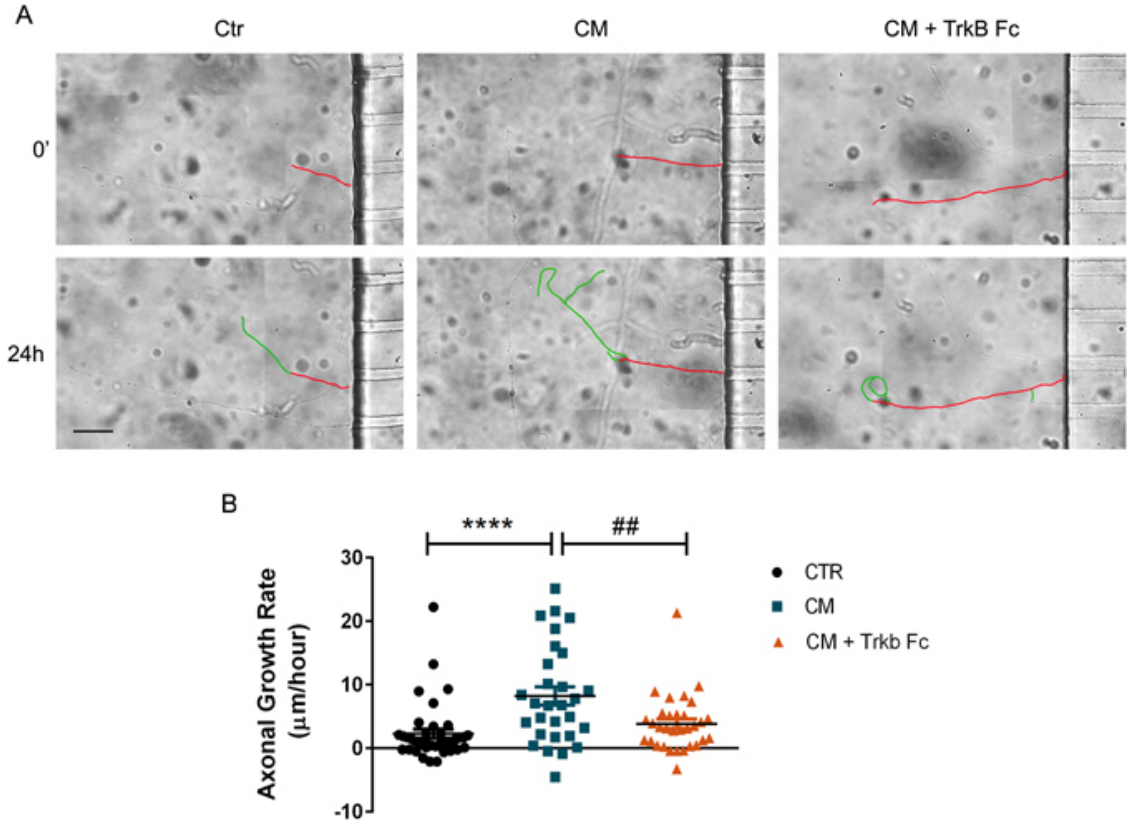



Fig. 7
Calculation of growth rate

- CM-treated axons had 4-fold outgrowth rate
- TrkB Fc-mediated BDNF-depletion attenuates outgrowth rate

# Proposed model for secretome-induced axonal outgrowth

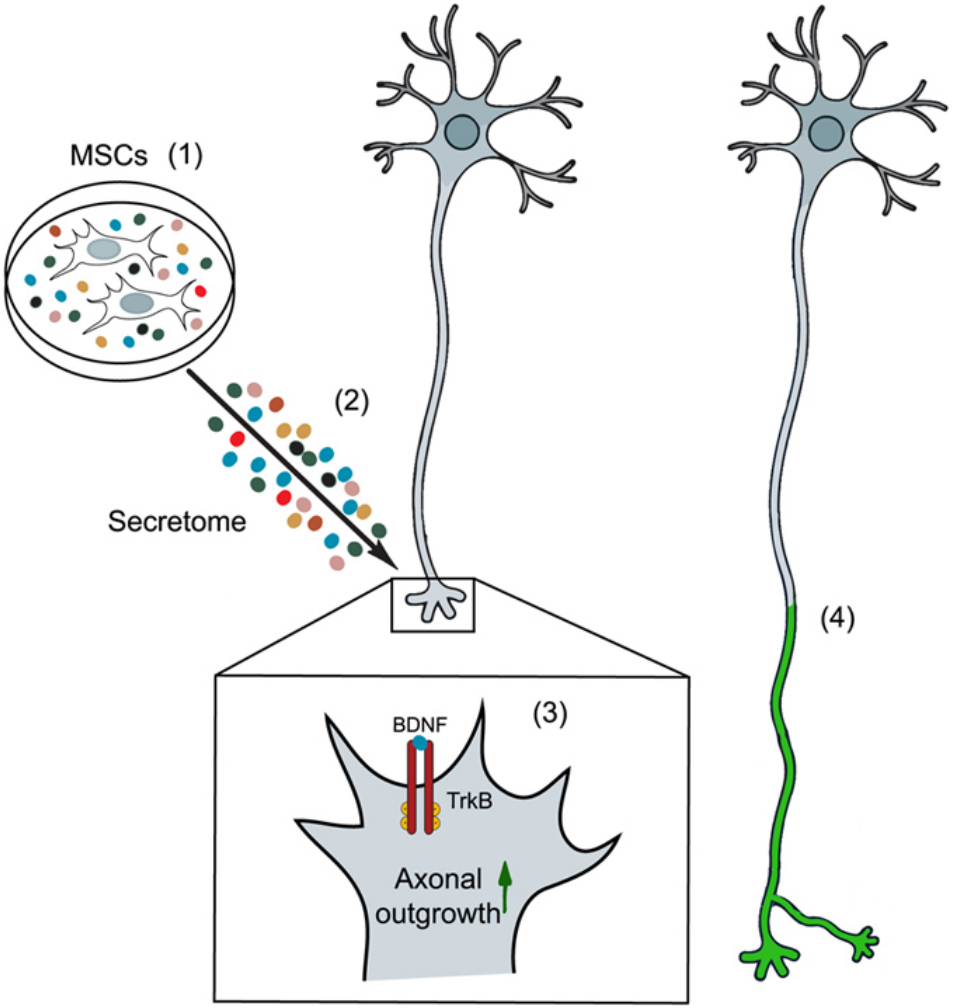



Fig. 8:
BDNF (from MSC-secretome) binds to TrkB in the membrane of growth cones, activates signalling pathways responsible for axonal outgrowth

# Discussion

- **Pros:**
- Easily understandable paper, conclusive figures
- Microfuidic-chamber model
- **Cons:**
- TrkB Fc relevance in vitro? → only inconclusive in vivo data
- Exact amount of BDNF in their CM? → easy, just do an ELISA!
- What about other neurotrophic/growth factors?
- Valid control?

  → Compare BDNF only (e.g. recombinant) to CM
- Signalling cascade/downstream molecules of BDNF after CM-/vs. BDNF treatment?
- Relevance for human use/translational science?

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Abbreviations

- rMNC $^{apo\ sec}$/hMNC $^{apo\ sec}$ = rat/human apoptotic mononuclear cells
- MCAO = middle cerebral artery occlusion
- HLV = hemispheric lesion volume

# Methods

- Animals: 84 adult male Wistar rats
- Production of rat MNC-secretome: harvesting of spleens, lysing of red blood cells, irradiation (45 Gy), resuspension in serum-free medium, cultivated for 18h, cells removed (centrifugation), lyophilisation
- Production of human MNC-secretome: GMP-according; venous blood samples; Ficoll-separation, irradiation, concentration: 25x10^6 cells/ml; methylene blue and light treatment, gamma irradiation for pathogen removal
- Verification of apoptosis via flow cytometry
- Animal experiment for focal ischemia: MCAO via suture model as prescribed
- Postoperative MNC-administration as described in Fig.1)
- Neurological evaluation (blinded investigator): 7 point-scale: left forepaw extension, instability to lateral push from right, tail hanging, walking on ground, whisker movement on the left, hearing, and vision
- Determination of BDNF in rat plasma: injection intraperitoneally; Euthanization; measurement of BDNF with ELISA in rat plasma

# Experimental study setting

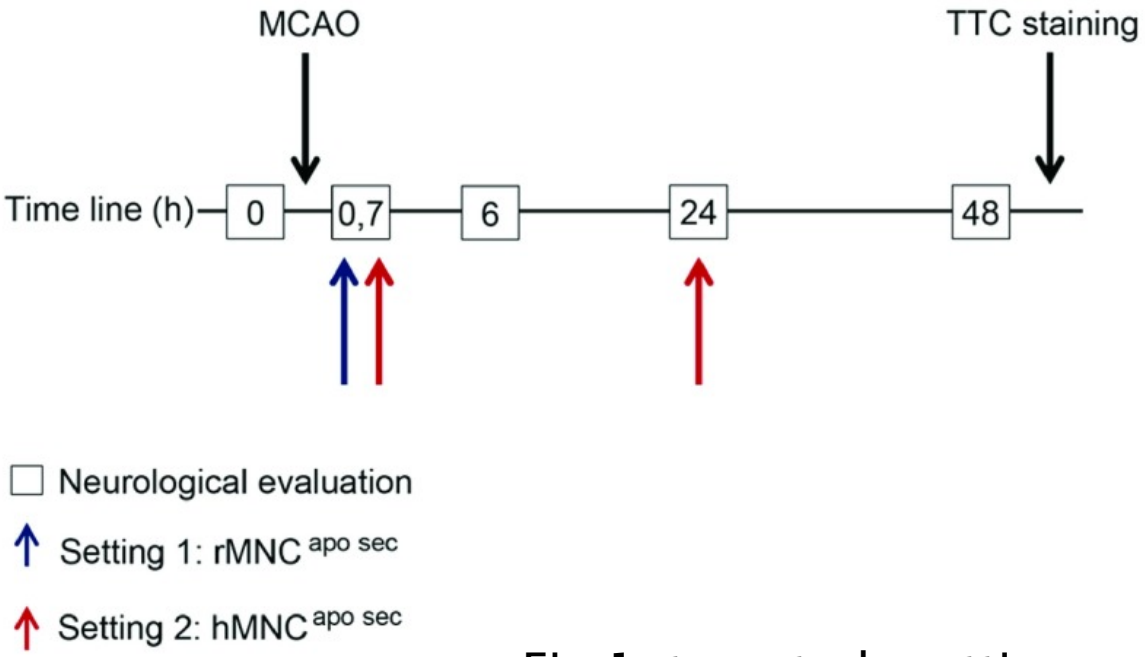


Fig 1: two study settings using different time points for rMNC $^{apo\ sec}$/hMNC $^{apo\ sec}$ administration

# Apoptotic MNC-secretomes reduce the infarction volume in an experimental MCAO model

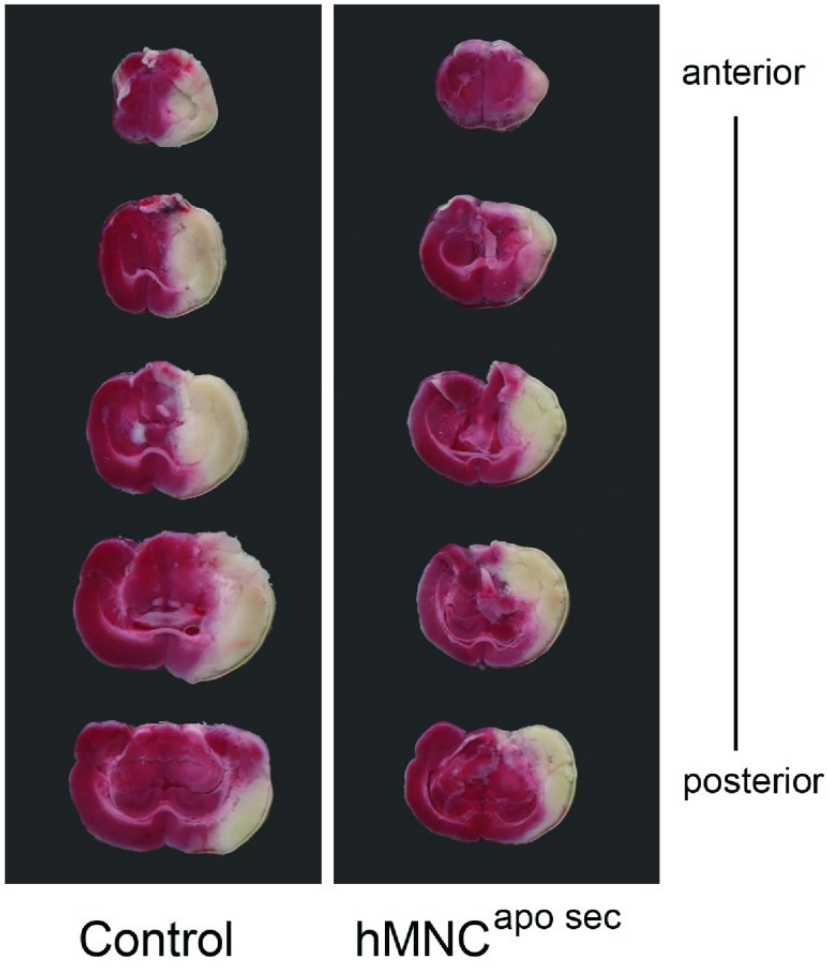


Figure 2

Represantative brain slices of rats with MCAO, treated with hMNC and control

(48h after MCAO)

# Apoptotic MNC-secretomes improve neurological outcome in an experimental MCAO model

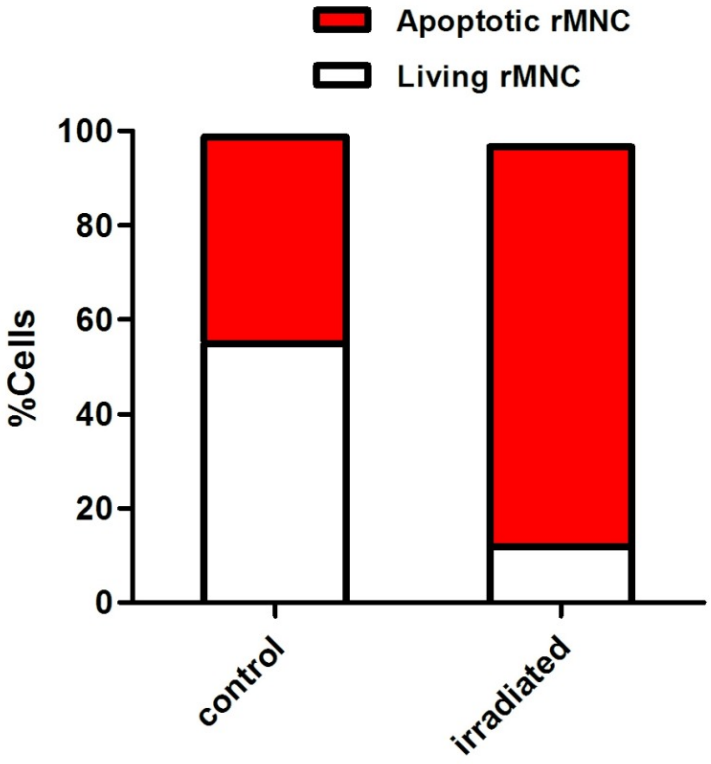


Fig. 3: Quantification of viable cells in irradiated vs non irradiated cultured MNCs

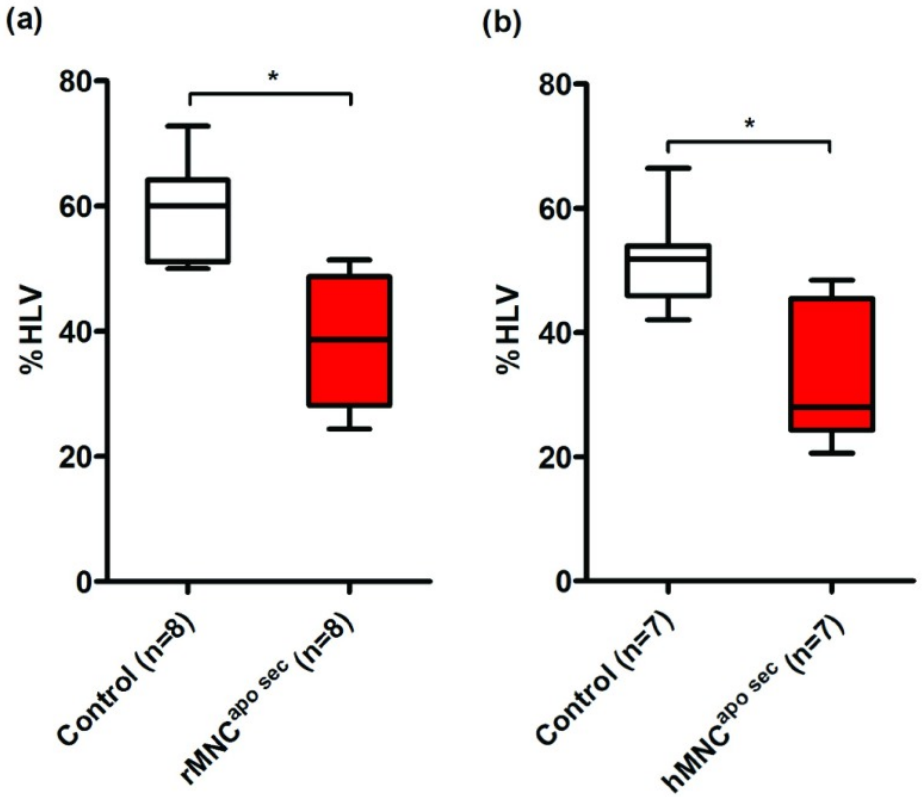


Fig. 4: rMNC $^{apo\ sec}$/hMNC $^{apo\ sec}$ decreased HLV after MCAO in two experiment settings

# Apoptotic MNC-secretomes improve neurological outcome in an experimental MCAO model

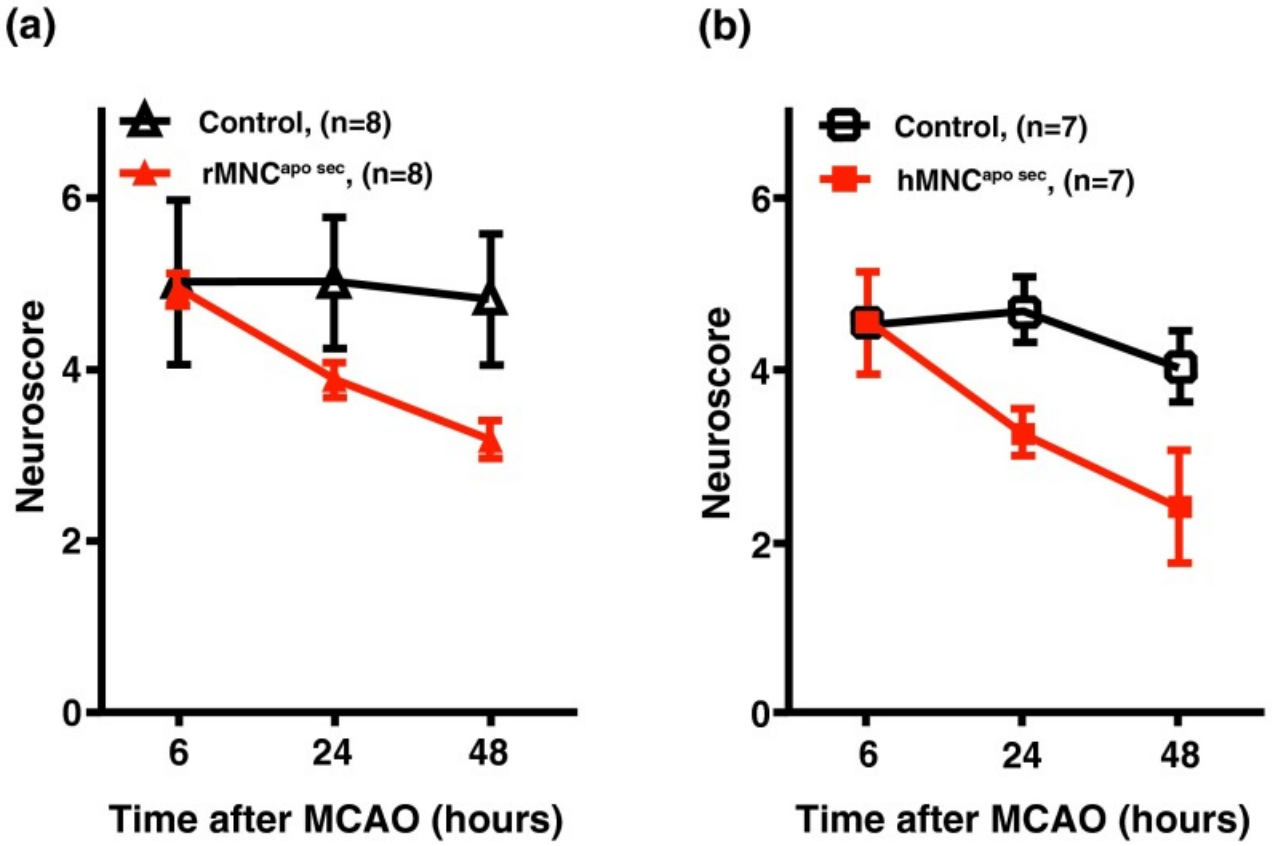


Fig 5: Neurological outcome of rats after MCAO with rMNC $^{apo\ sec}$/hMNC $^{apo\ sec}$ or control in two experiment settings

- Neurological eximanation on 4 time points
- Significant neuroscore decrease over time in treatment group

# Apoptotic MNC-secretomes activate signaling cascades involved in cytoprotection in glia cells

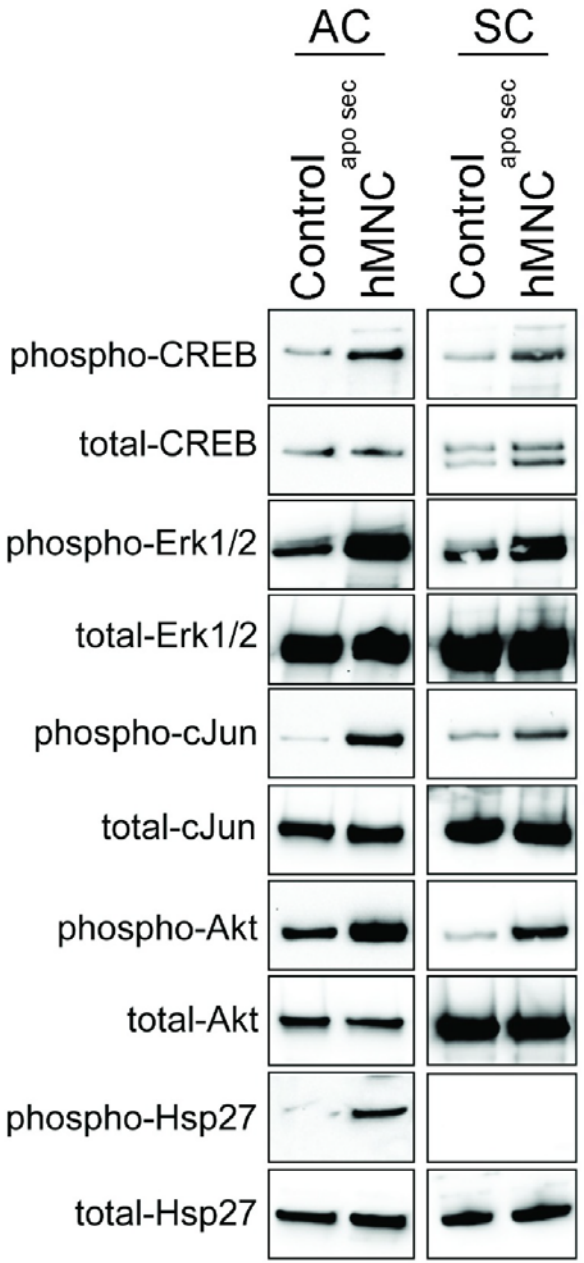


Fig. 6: hMNC $^{apo\ sec}$-administration activates/phosphorylates signaling molecules in human Schwann cells (SC) and astrocytes (AC)

- Increased phosphorylation of CREB, Erk1/2, c-Jun, Akt

# Apoptotic MNC-secretomes induce CREB phosphorylation and neuronal sprouting in human primary neurons and contain BDNF

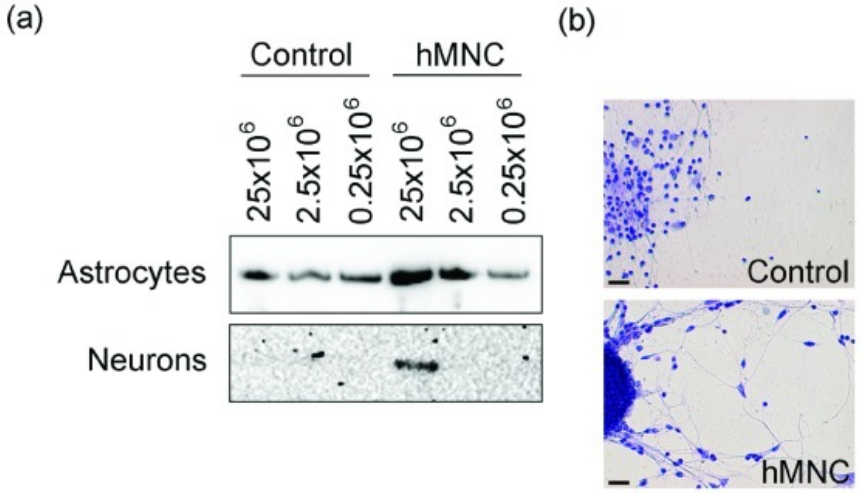


Fig. 7
a) dose-dependent activation of CREB in hMNC $^{apo\ sec}$–treated human primary neuron cultures

b, c) hMNC $^{apo\ sec}$ – treatment leads to increase in neuron length

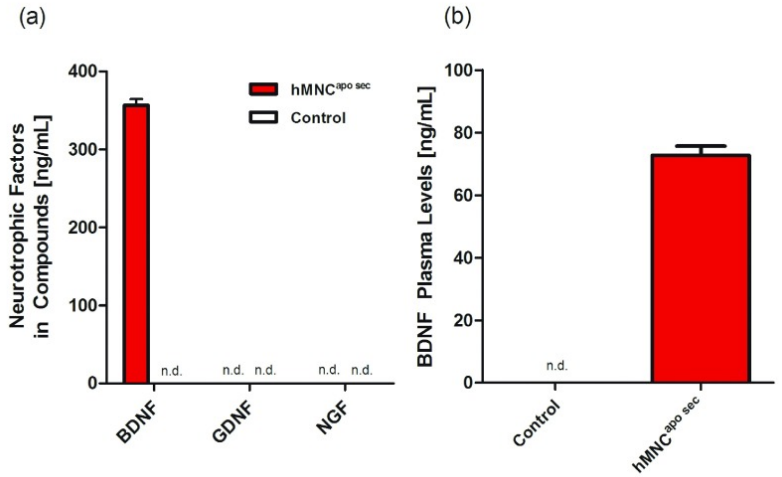


Fig. 8:
8a) BDNF is the only neurotophic factor in hMNC $^{apo\ sec}$

8b) BDNF-levels are higher in hMNC $^{apo\ sec}$ –treated rats after

# Discussion

- „(i) hMNC $^{apo\ sec}$ activate several mechanisms ultimately leading to the expression of protective proteins in cultured primary human glial cells, such as astrocytes, Schwann cells and human neurons, and
- (ii) induce notable sprouting of neurites in primary neuron cultures”
- “ … Apoptotic MNCsecretomes derived from human blood can aid in the development of new treatment strategies in ischemic stroke.”
- Rat and human secretome hard to compare but similar effects

# Discussion

- **Pros**
- Conclusive, thoroughly argued study
- Easliy understandable
- Two experiment settings, precisely described
- Large number of animals for significant results
- Effective and easy neurological examination
- Relevance for human use (GMP-according hMNC $^{apo\ sec}$)
- **Cons**
- Limitations of a small animal study
- Exact anti-inflammatory action of hMNC $^{apo\ sec}$ to be studied

# Danke!

Noch Fragen?