Intraspinal injection of human mesenchymal stromal cells in SOD1G93A ALS mice

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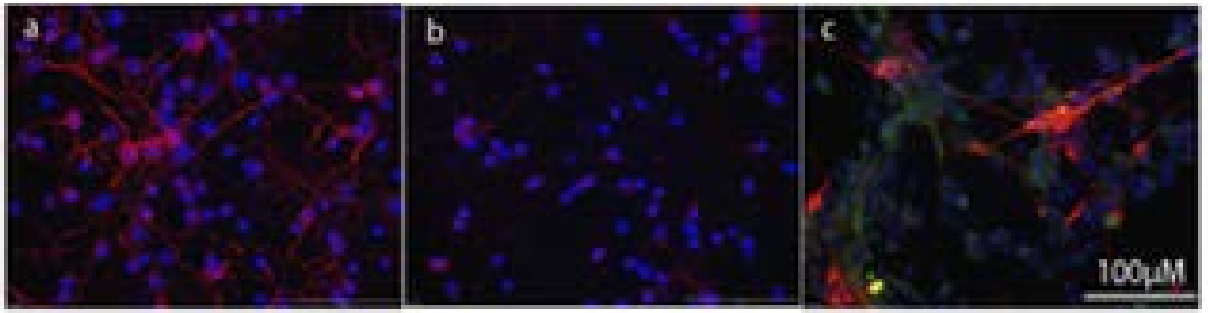
Cellular therapy

- Cellular therapy in ALS: neuroprotection by non-neuronal cells
- Non-neuronal cells are crucial for survival of motor neurons (Clement et al, 2003; Sun et al, 2013; Ferraiuolo et al, 2016;)
- Stem cell studies in human patients (Mazzini et al, 2015; Glass and Hertzberg et al, 2016; Chen et al, 2016)
- Mesenchymal stromal cells (MSCs)

  →Wide proliferation and differentiation potential

  →Good availability

  →Release of neuroprotective factors and cytokines



Sun et al, 2013

SMI 32 (red), DAPI (blue), CD44 (green)

Criteria of the International Society for Cellular Therapy to define MSCs

- Adherence to plastic
- Expression of CD105, CD90 and CD73
- No Expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II
- Differentiation into adipocytes, chondroblasts and osteoblasts

Dominici et al 2006

Isolation of human MSCs

- Goal: Good manufacturing practice (GMP) protocol
- Cooperation with GMP development unit (Prof. Köhl, MHH)
- hMSCs from bone marrow (healthy donors)
- Selection via plastic adherence

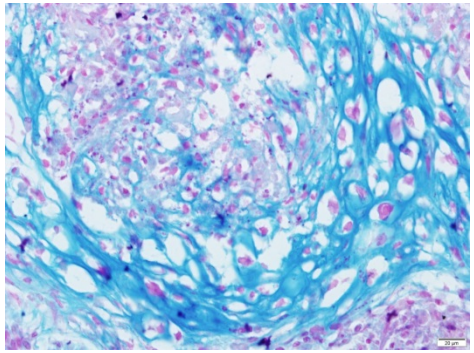
Quality control of MSCs

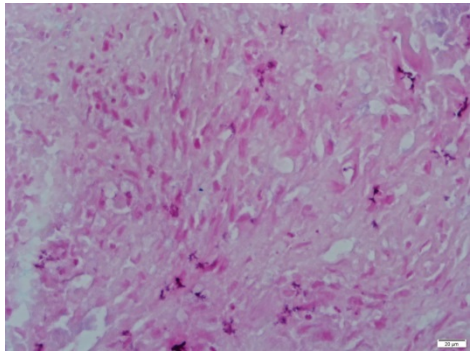
Chondrocytes

Differentiation medium

Negative control

Day 24





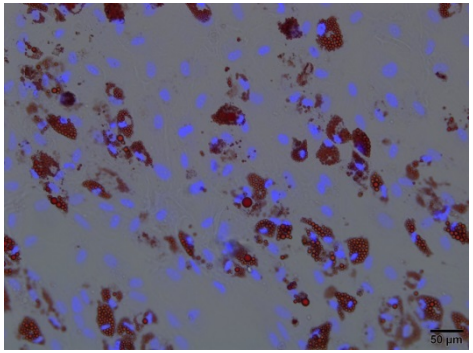
Alcian blue staining

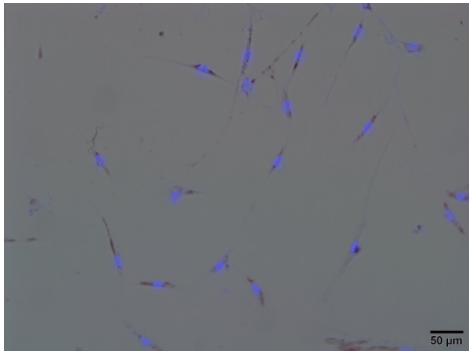
Adipocytes

Differentiation medium

Negative control

Day 14



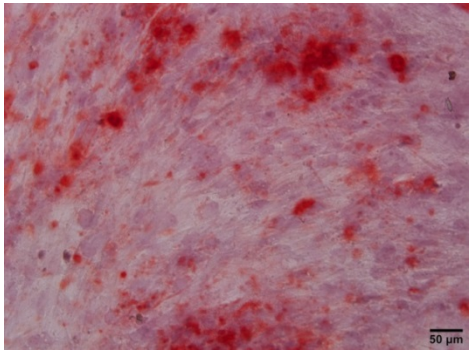
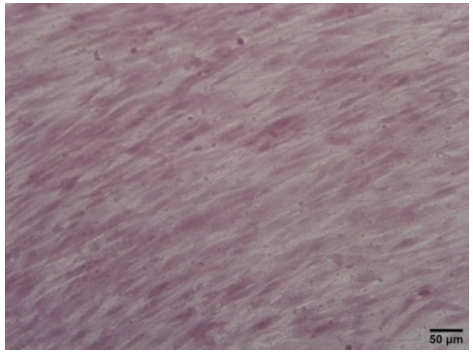
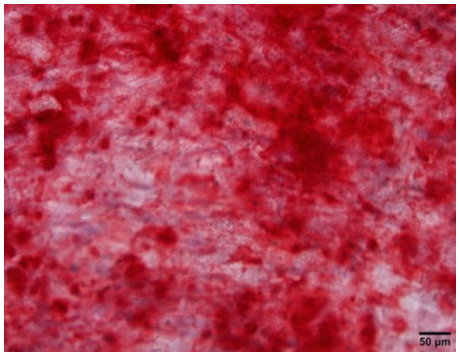
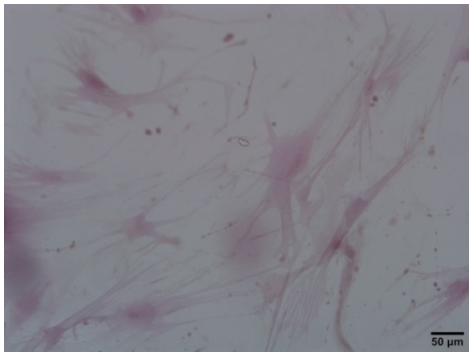


Oil Red O staining

Done by A. Sarikidi

Quality control of MSCs

Osteocytes

	Differentiation medium	Negative control
Day 14		
Day 21		

Alizarin Red S staining

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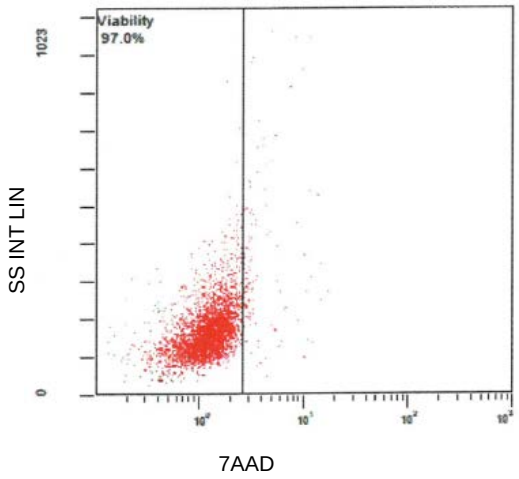
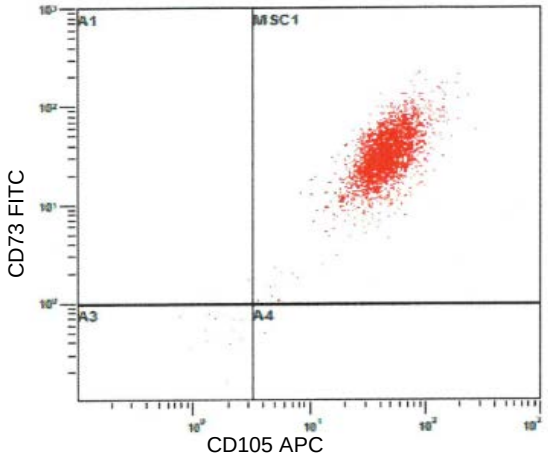
Quality control of MSCs

FACS analysis of MSCs

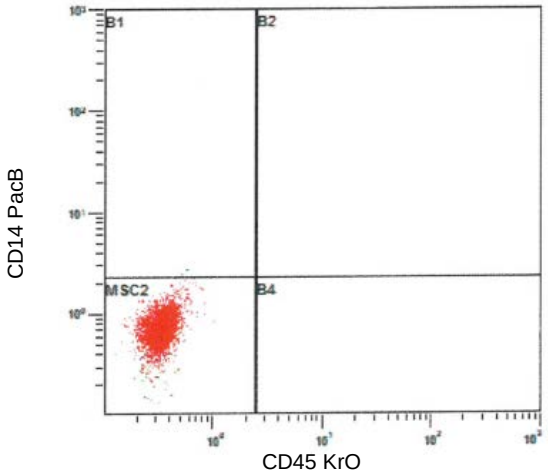
[CD45 neg] FL4 INT LOG/SS INT LIN



[Viability and CD45 neg] FL6 INT LOG/FL1 INT LIN



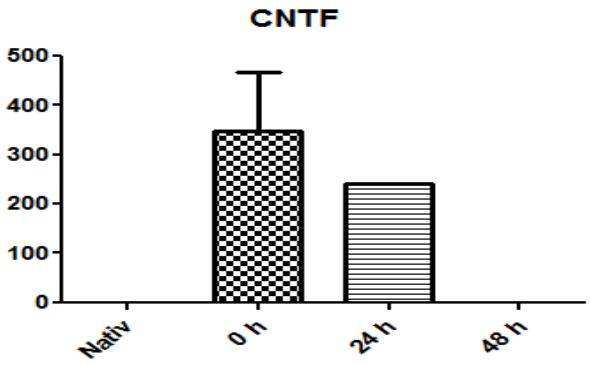
[Viability and CD45 neg] FL10 INT LOG/FL9 INT LOG

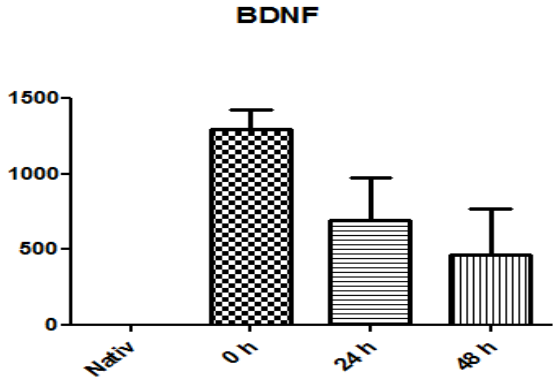


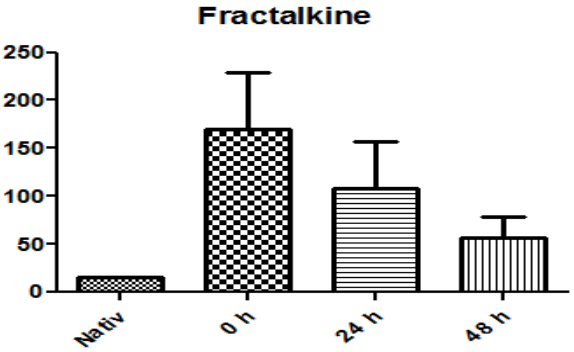
→ The isolated MSCs fulfill criteria of International Society for Cellular Therapy

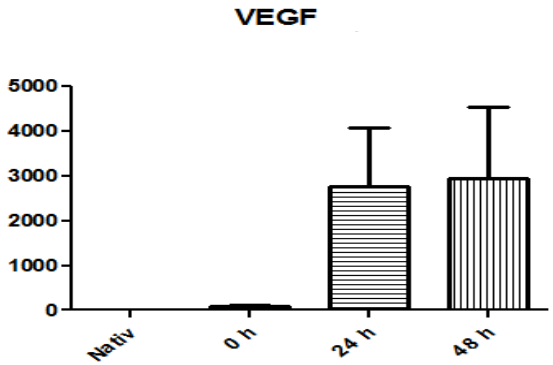
Done by A. Sarikidi

In vitro characterisation of MSCs









Cooperation with Institute of Transplant Immunology, MHH

MSCs secrete growth factors and immune modulating factors (measured in conditioned medium using Bio-Plex® Multiplex Immunoassays).

In vivo studies of the MSCs

Aims of the *in vivo* studies

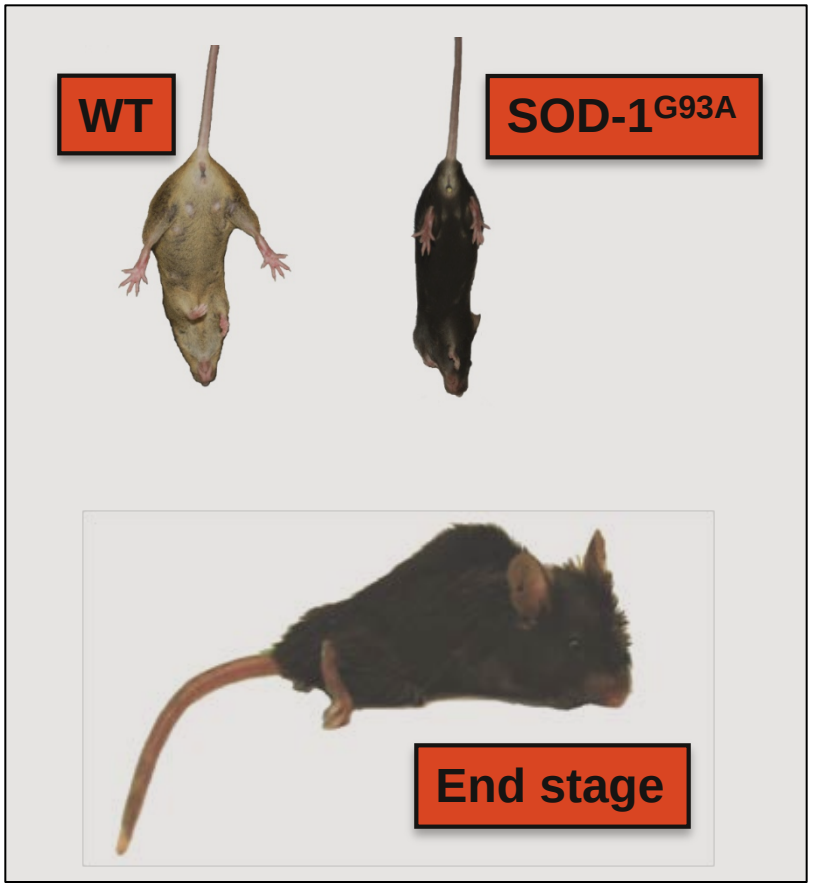
- Preclinical evaluation of administration of human MSCs

  → compare frequency of intraspinal injections (single vs. double)

- *In vivo* imaging of the MSCs by radioactive tracing

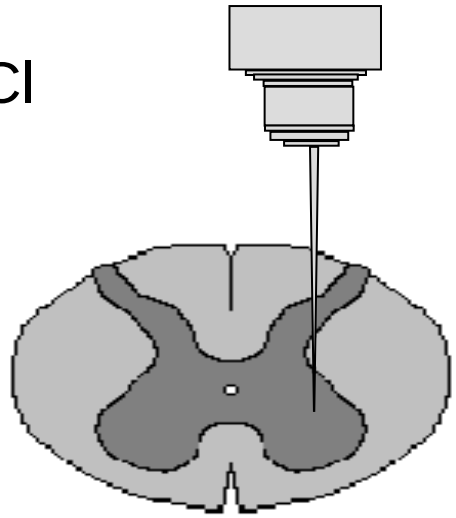
Transgenic ALS mouse model

- B6.SJL-Tg(SOD1*G93A)1Gur/J
- Disease onset at around 90 days
- Death at the age of 130±11 days



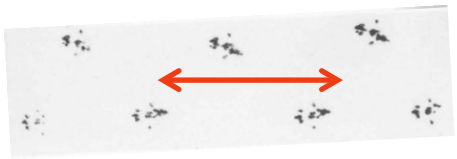
Intraspinal injection

- Surgery at the age of day 40 or at day 40 and day 90
- Injection at T13-L1 bilaterally into left and right ventral horn
- Injection of 100,000 cells/site in 1µl NaCl
- Control: injection of NaCl



Behavioural assessments

- Survival time
- General condition score
- Weight
- Rotarod test
- Steplength
- Runtime

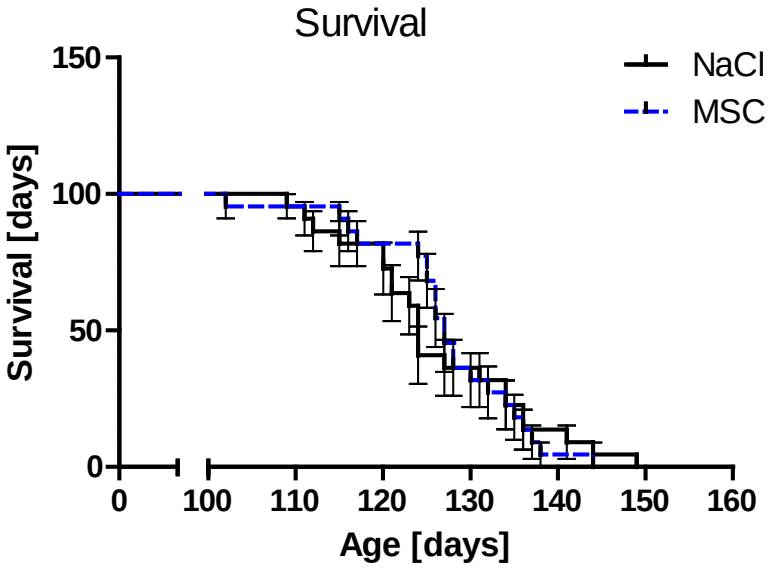


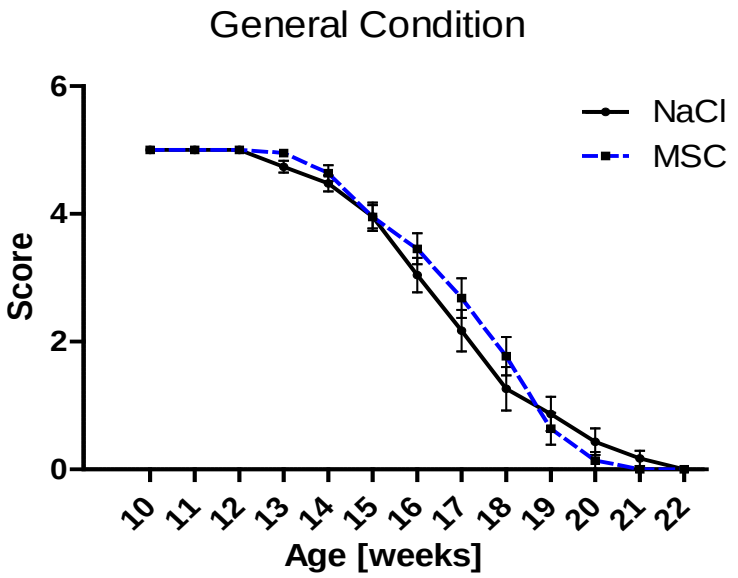
Score	Description
5	Healthy
4	First signs of paralysis and destabilized gait
3	Paralysis is obvious
2	Hind limbs are completely paralysed
1	Animals are not able to right themselves within 5 seconds or lost more than 20 % of original weight (week 10)

Knippenberg et al, 2010

Intraspinal injection at day 40

Intraspinal injection at day 40



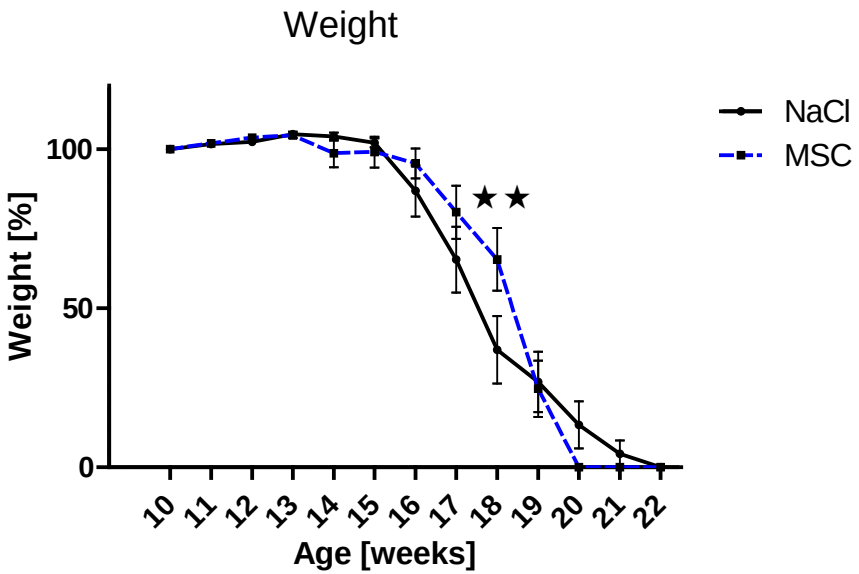


NaCl n=23
MSC n=22

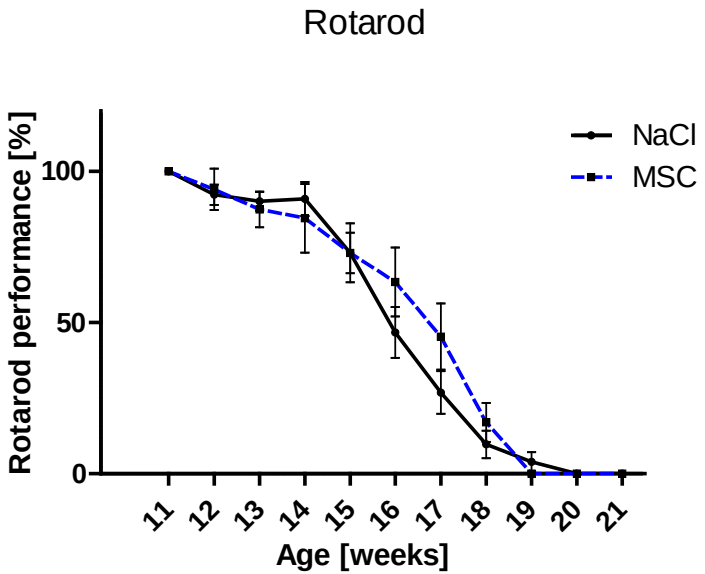
Statistical analysis: Survival :Kaplan-Meyer-Curve, followed by Gehan-Breslow-Wilcoxon test; General Condition: 2 way Anova followed by Bonferroni post-test ★p≤0.05, ★★p≤0.01

Done together with K.J. Rath

Intraspinal injection at day 40



NaCl n=22
MSC n=22



Statistical analysis: 2 way Anova followed by Bonferroni post-test ★ p≤0.05, ★★ p≤0.01

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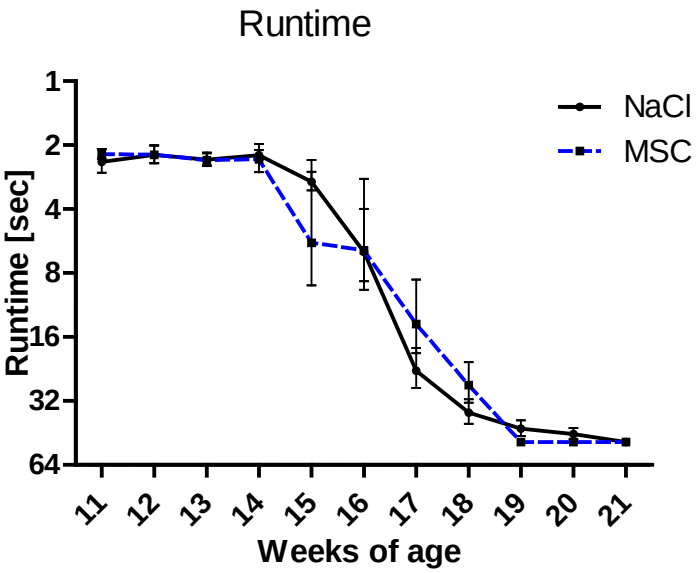
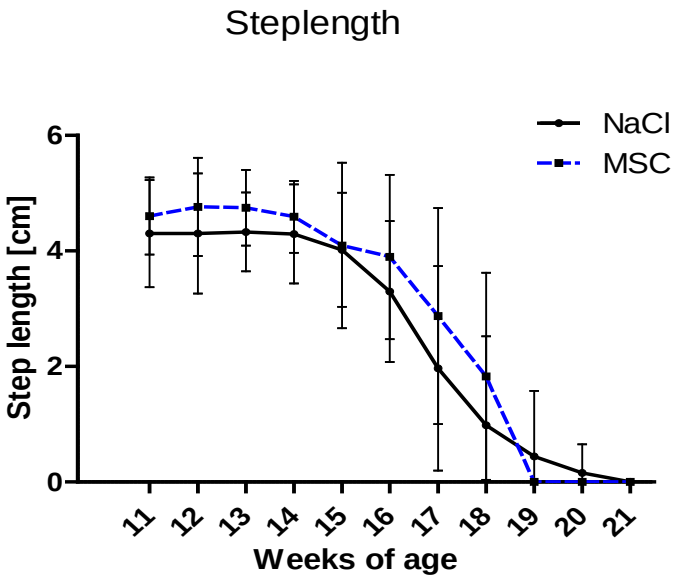
Intraspinal injection at day 40





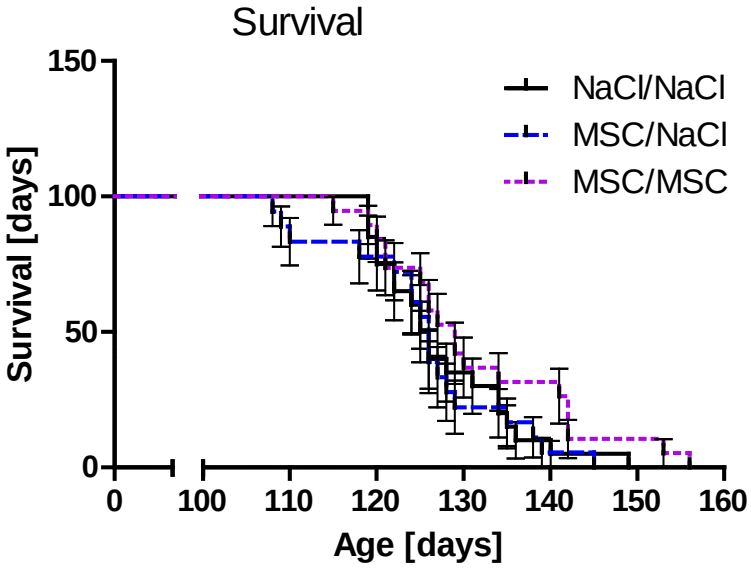
NaCl n=20
MSC n=14

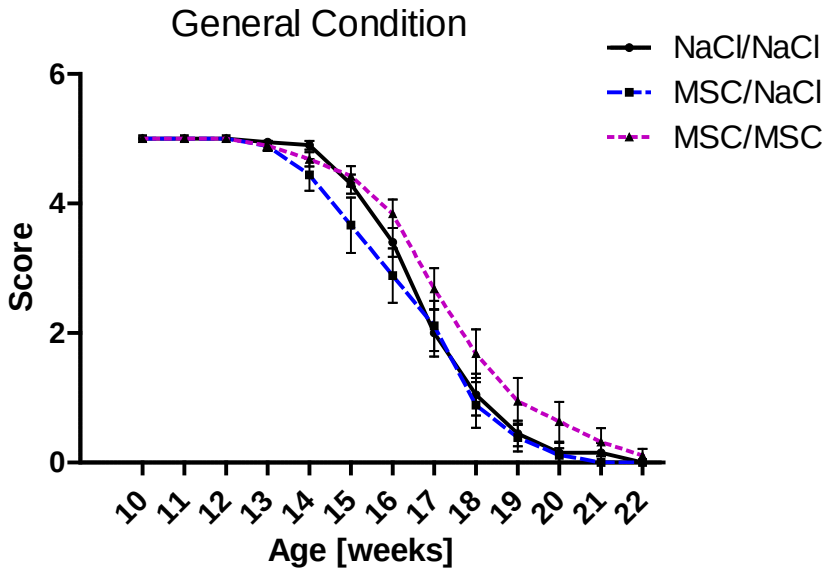
Statistical analysis: 2 way Anova followed by Bonferroni post-test ★ p≤0.05, ★★ p≤0.01

Done together with K.J. Rath

Intraspinal injection at day 40 and at day 90

Intraspinal injection at day 40 and at day 90

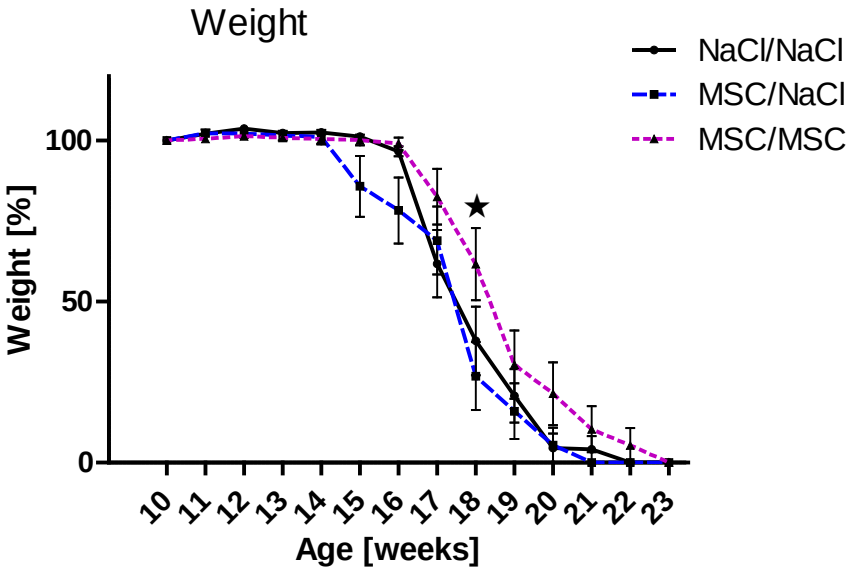




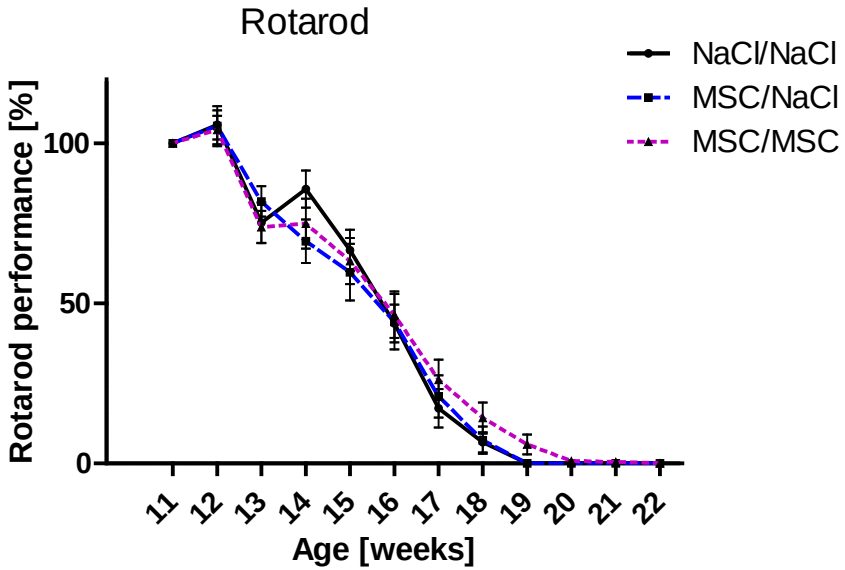
NaCl/NaCl n=20
MSC/NaCl n=18
MSC/MSC n=19

Statistical analysis: Survival :Kaplan-Meyer-Curve, followed by Gehan-Breslow-Wilcoxon test; General Condition: 2 way Anova followed by Bonferroni post-test ★p≤0.05, ★★p≤0.01

Intraspinal injection at day 40 and at day 90

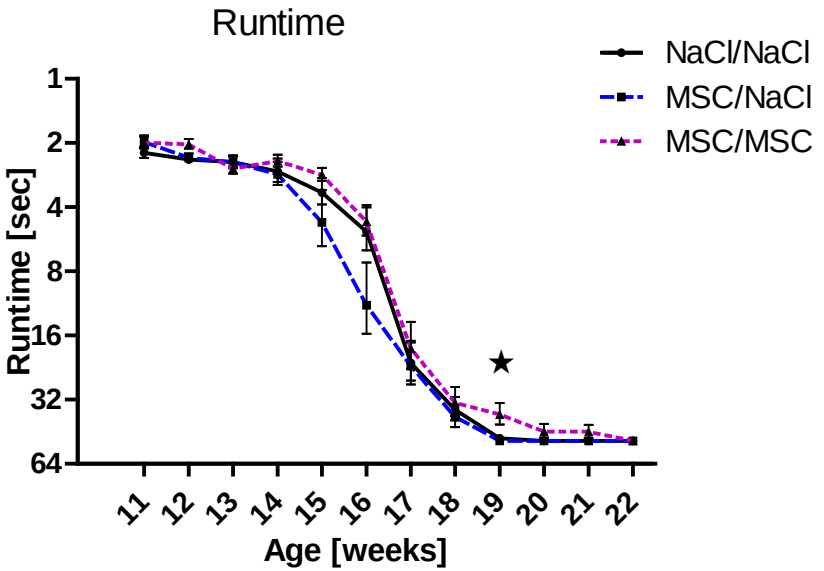


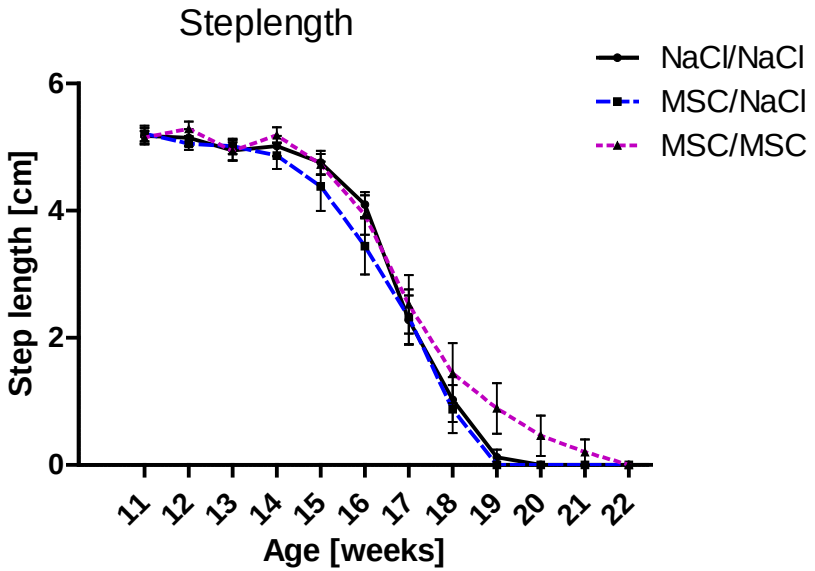
NaCl/NaCl n=20
MSC/NaCl n=18
MSC/MSC n=19



Statistical analysis: 2 way Anova followed by Bonferroni post-test ★ p≤0.05, ★★ p≤0.01

Intraspinal injection at day 40 and at day 90





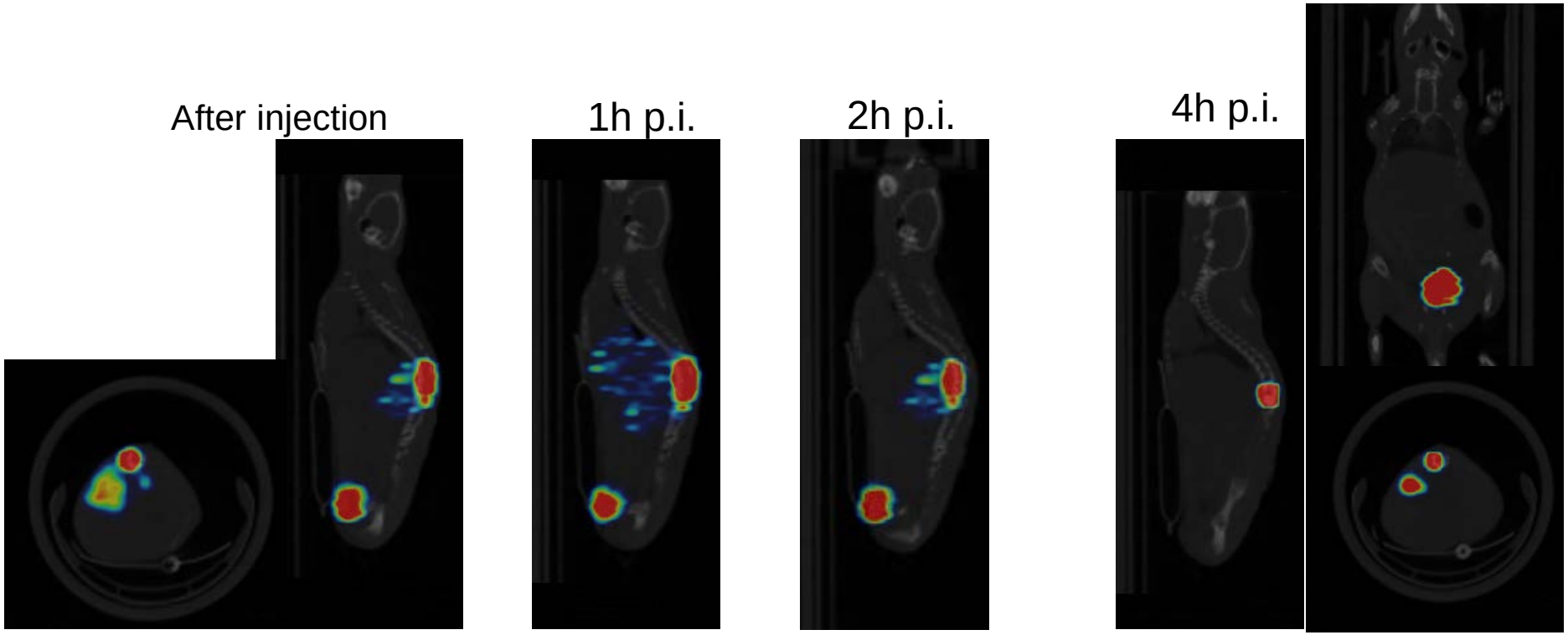
NaCl/NaCl n=20
MSC/NaCl n=18
MSC/MSC n=19

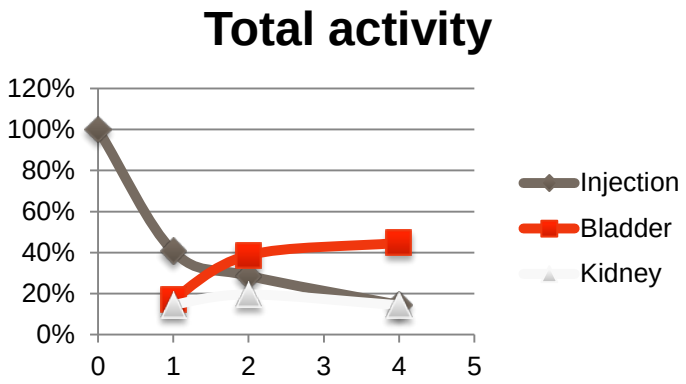
Statistical analysis: 2 way Anova followed by Bonferroni post-test ★ p≤0.05, ★★ p≤0.01

Radioactive tracing of MSCs

- Cells incubated with 400 MBq of fluordesoxyglucose (FDG) for 60 minutes
- Injection of 2x100,000 MSCs in the spinal cord
- Scans after surgery and 1, 2 and 4 h after injection (20 min static scans)

Cooperation with Department of Nuclear Medicine, MHH





Scans and analysis done by Dr. Bascunana Almarcha, Department of Nuclear Medicine, MHH

Summary

Single MSCs injection (day 40)

- significant effect in weight loss

Repeated MSCs injection (day 40 and day 90)

- significant difference in weight loss and runtime
- trend towards prolonged survival

In vivo imaging with radioactive tracer

- MSCs can be detected in the spinal cord

Outlook

- Compare intraspinal injection with intrathecal injection
- Immunohistochemical analysis of motorneuron loss and glial activation
- Analysis of survival of MSCs after injection over time

  → use of more stable radioactive markers

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Thank you for your attention !