Role of the Receptor like Protein Tyrosine Phosphatase zeta (RPTPz): Initial *in vivo* characterisation.

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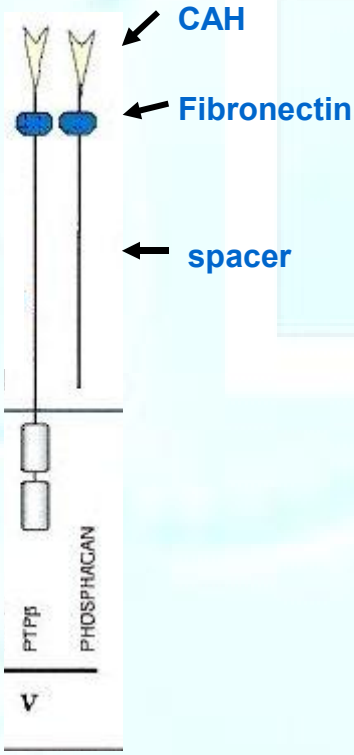
R-PTPz.

Structure :

- Intracytoplasmic portion with 2 tyrosine phosphatase domains: through β-cathenin intracellular signaling cascade.
- Single transmembrane domain.
- Extracellular domain with sequence homology to Carbonic Anhydrase (CAH), fibronectin domain type III, and a spacer.
- CAH+Fibronectin: bind to contactin

Zone of expression of R-PTPz :

Throughout the nervous system (CNS and PNS), including in glial cells (oligo, schwann, astrocyte but not in microglia).



Protein tyrosine phosphatases and neural development, Stoker and Dutta, Bioessays 1997

Role of the protein tyrosine phosphatase.

1. **In neural development:**

- **Axon formation and guidance (regulation and modulation of nerve cells adhesion, dynamic state of the cytoskeleton).**
- **Glial cells development: migration.**

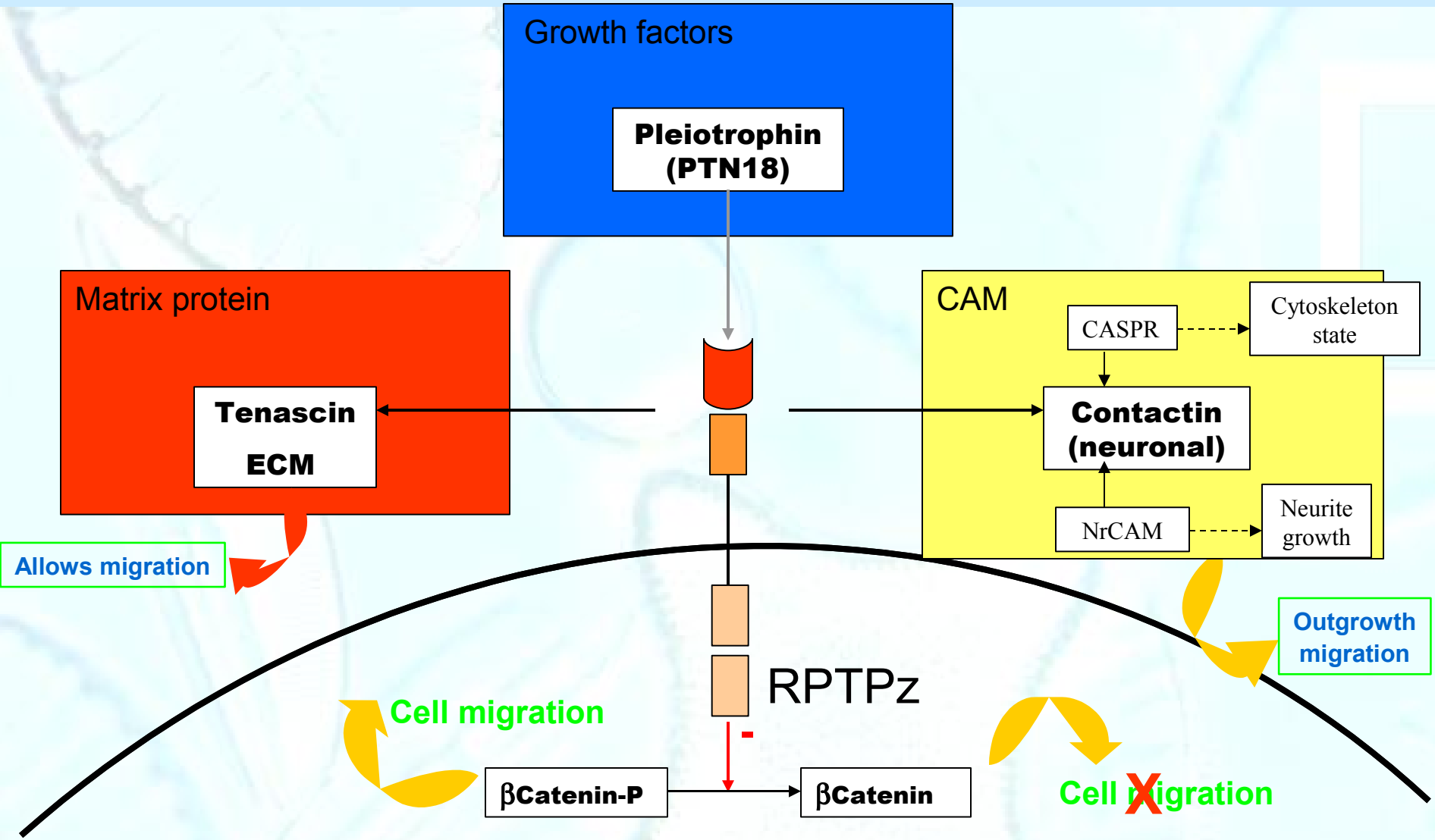
5. **In adult nervous system:**

- **Neuron/glia interaction: contactin/CAH+Fibronectin.**
- **Gliogenesis, glia differentiation (oligodendrocytes and Shwann cells), and survival.**
- **Neural migration, regeneration, and neurite outgrowth.**
- **Myelin formation.**

Protein tyrosine phosphatases and neural development, *Stoker & Dutta, Bioessays, 1998*

No obvious abnormality in mice deficient in receptor protein tyrosine phosphatase beta, Harroch et al, *Molecular and Cellular Biology, 2000*

Ligands and substrates of R-PTPz.



Glial cell

Published R-PTPz $^{-/-}$ mice.

- The nerves ultrastructure seems less compact, the myelin lamellae tend to separate, to be fragmented, and deformed in RPTPz KO mice.

- Toluidine blue staining shows intensive degeneration of the spinal cord in RPTPz KO mice compared with control.

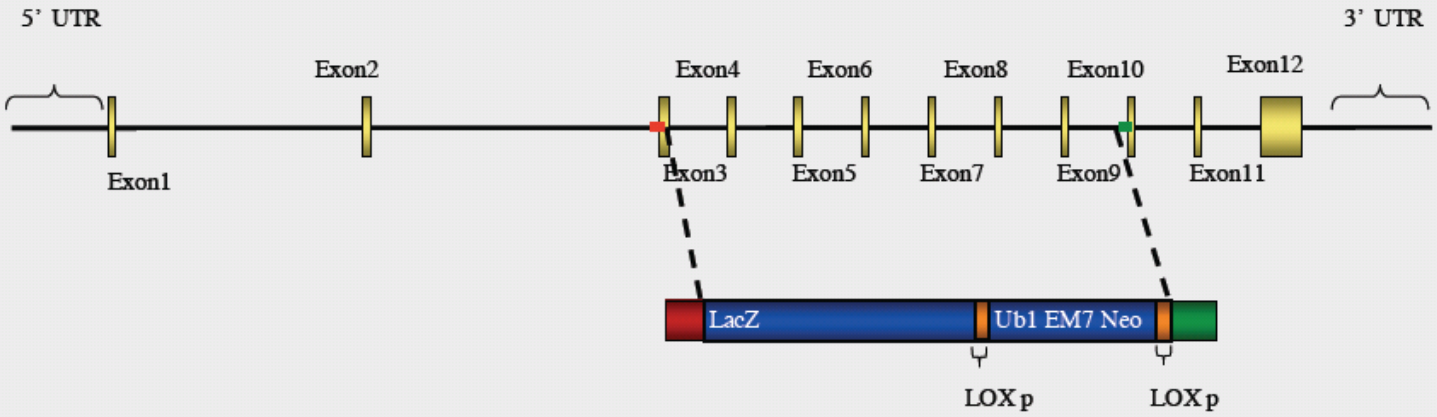
- KO mice are more susceptible to EAE, and show impaired recovery.

- Double staining (TUNEL/CNP) shows that a considerable proportion of TUNEL positives cells are oligodendrocytes in RPTPz KO mice.

*Harroch et al, Molecular and Cellular Biology, 2000; Harroch et al, Nature Genetics **32**:3, 2002*

KO construction.

Serono Identification: PTP zeta
Regeneron Identification: SRA 5022



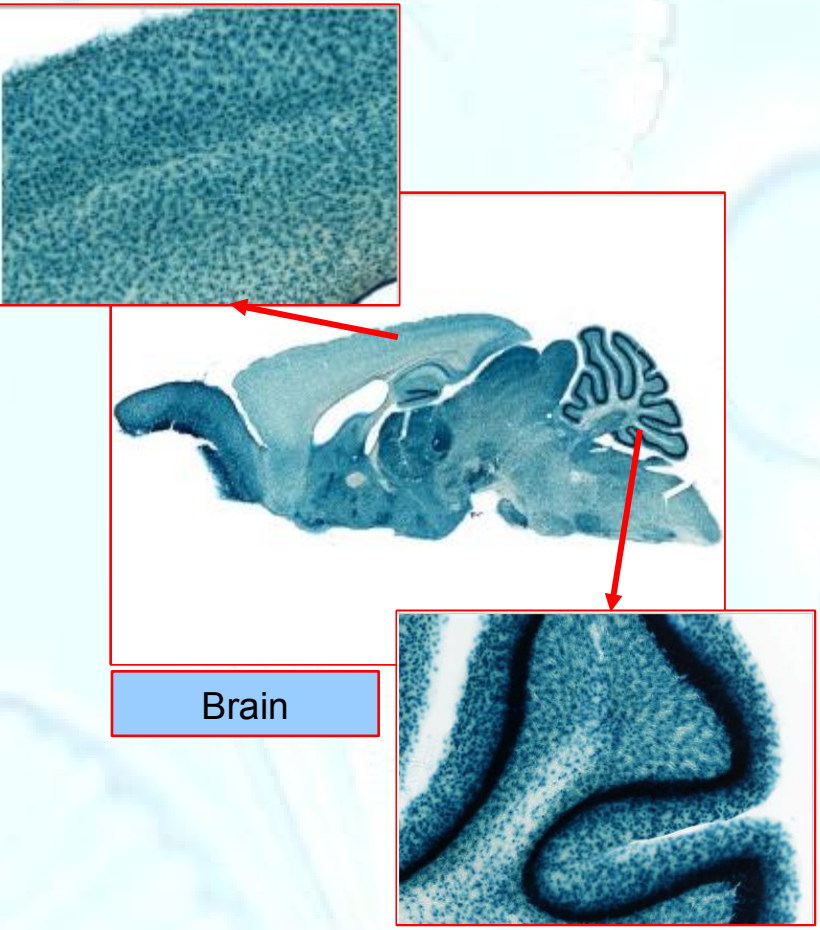
Deletion Type: Replacement of exon3 through exon9 with LacZ

Deletion of genomic sequence encoding CAH and FNIII domains.

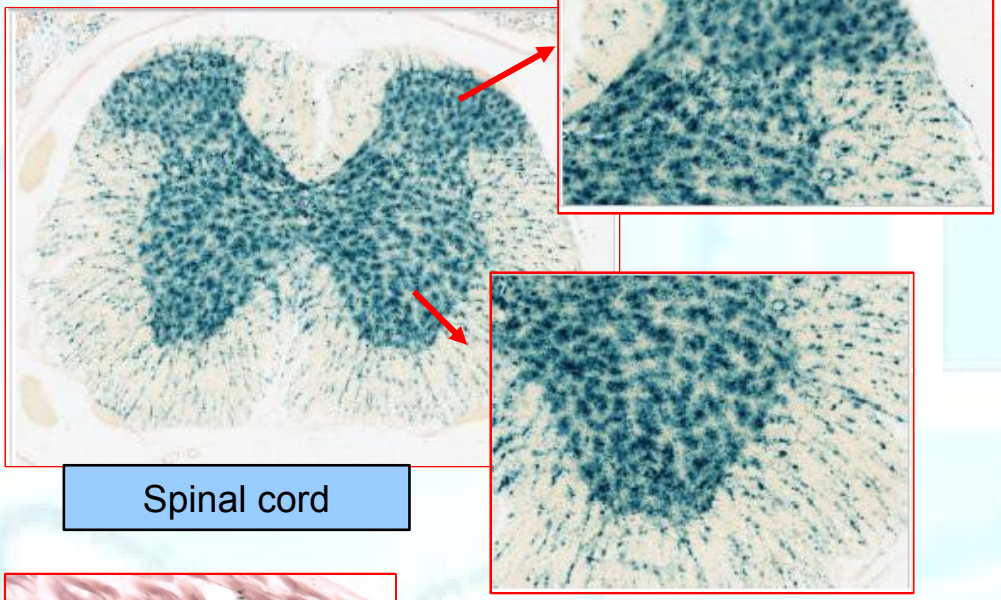
Deletion Size: Approximately 27 KB

Characterization of *in house* R-PTPz KO mice

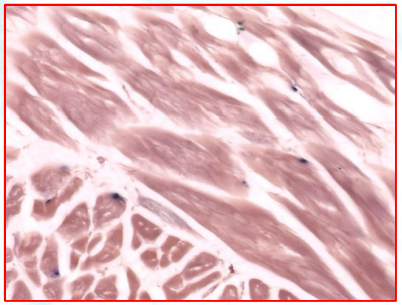
LacZ staining in adult +/-



Brain



Spinal cord



Peripheral Nervous System

- ongoing exploration of cell types in homozygotes

Working hypothesis: is RPTPz plaingy a key role in myelination/remyelination, and axon functions?

1- Behavioral phenotype:
to assess in vivo deficiencies due to RPTPz ko

2- Disease condition:
to assess the impact of RPTPz ko in disease condition related to de/remyelination

Behavioral phenotyping.

Test battery n= 10 -/-, n= 10 +/+ (littermates) male, F2 generation

- Locomotion: Grip strength *(fore-limb muscle strength)*
  Open field *(exploratory behavior and anxiety)*
  Accelerated rotarod *(motor coordination/learning & balance)*
- Memory: Y maze *(exploratory behavior and working memory)*
- Pain: Tail clip *(reflexive spinally mediated response to noxious stimulation)*
  Hot plate *(CNS mediated response to pain,requires neurological processing)*
  Plantar Hargreaves *(Thermal sensitivity)*
  Von Frey *(Tactile sensitivity)*

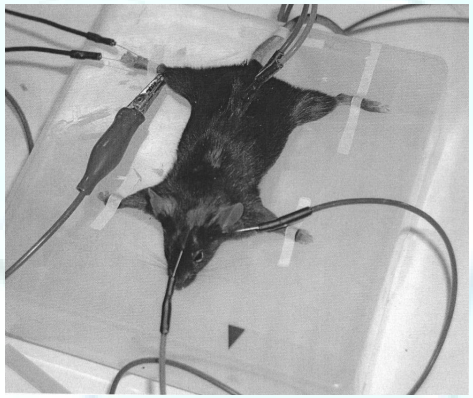
Behavioral Result: Summary.

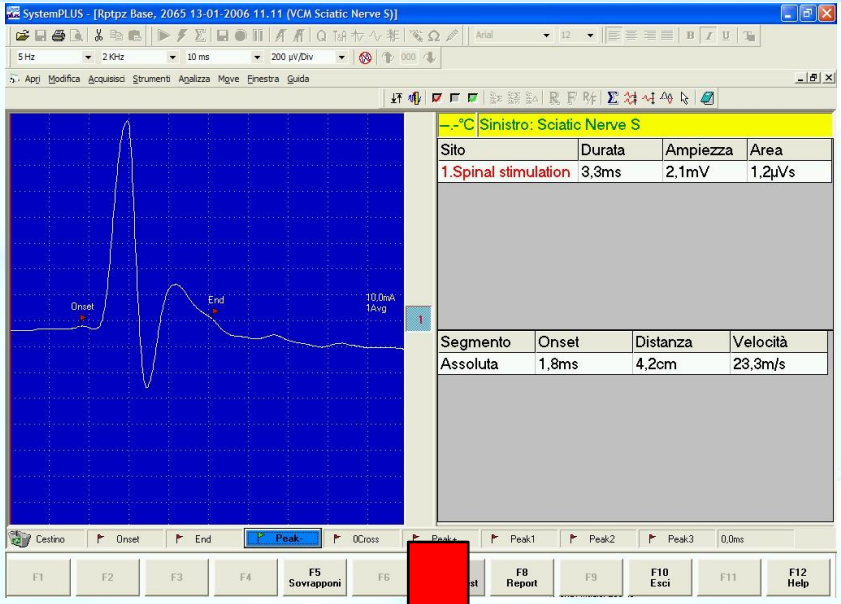
Test battery repeated in adult age

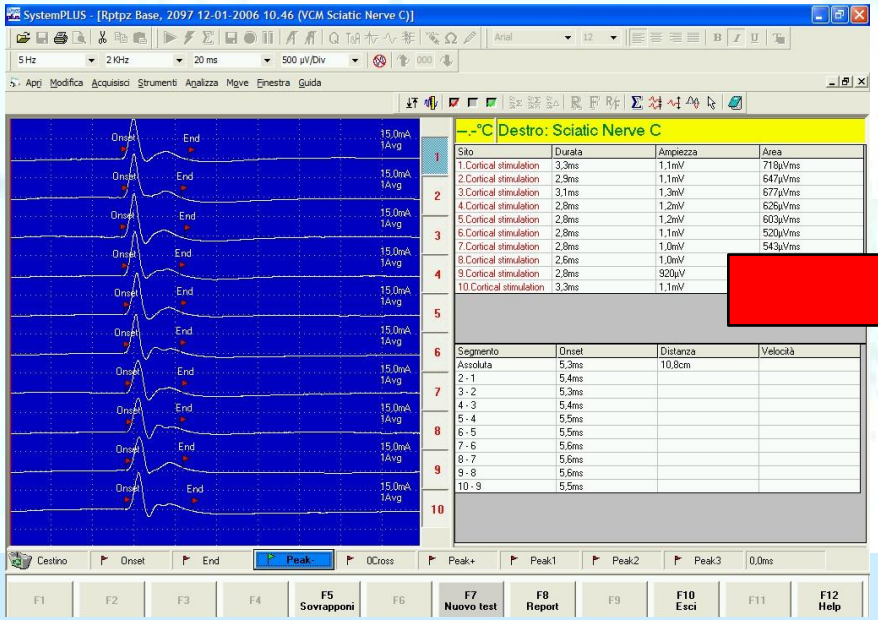
Test	Young (10-18 weeks)	Adult (40-48 weeks)
Grip strength	ns	ns
Openfield	ns	ns
Acc. Rotarod	** (T90)	** (T60); * (T90)
Y Maze	*	ns
Tailclip	ns	ns
Hotplate	*	**
Hargreaves	**	***
Von Frey	***	***

KO mice maintain behavioral phenotypes over time

Functional measure: Electrophysiology







1) Electrical stimulation at different places of the body (Ankle,Spinal cord, Head).

2) Recording at the Paw.

3) Functional measurement of myelin and axon integrity:

- Total Conduction Time (TCT),
- Peripheral Conduction Time (PCT),
- Central conduction time (CCT)

No differences in nerve conduction velocity.

Conclusions

- No observable differences between the 2 genotypes in:
  - general locomotion (grip strenght)
  - exploration of a new environment, and anxiety (open field)
  - nerve impulse conduction

- The impaired working memory test (Y Maze) in the -/-, and the presence of lacZ staining in hippocampal neurons may suggest a role of RPTPz in memory processes.

- The decreased ability to maintain balance on the rod in -/- mice and the presence of LacZ staining in cerebellar neurons may suggest the involvment of RPTPz in motor coordination.

- The hyposensitivity to thermal (hotplate, Hargreaves) and tactile (Von Frey), but not to mechanical pain (tail clip) in -/- mice suggest a potential role of RPTPz in nociception.

Disease Condition: Experimental Autoimmune Encephalomyelitis (EAE) Induced in RPTPz ko Mice

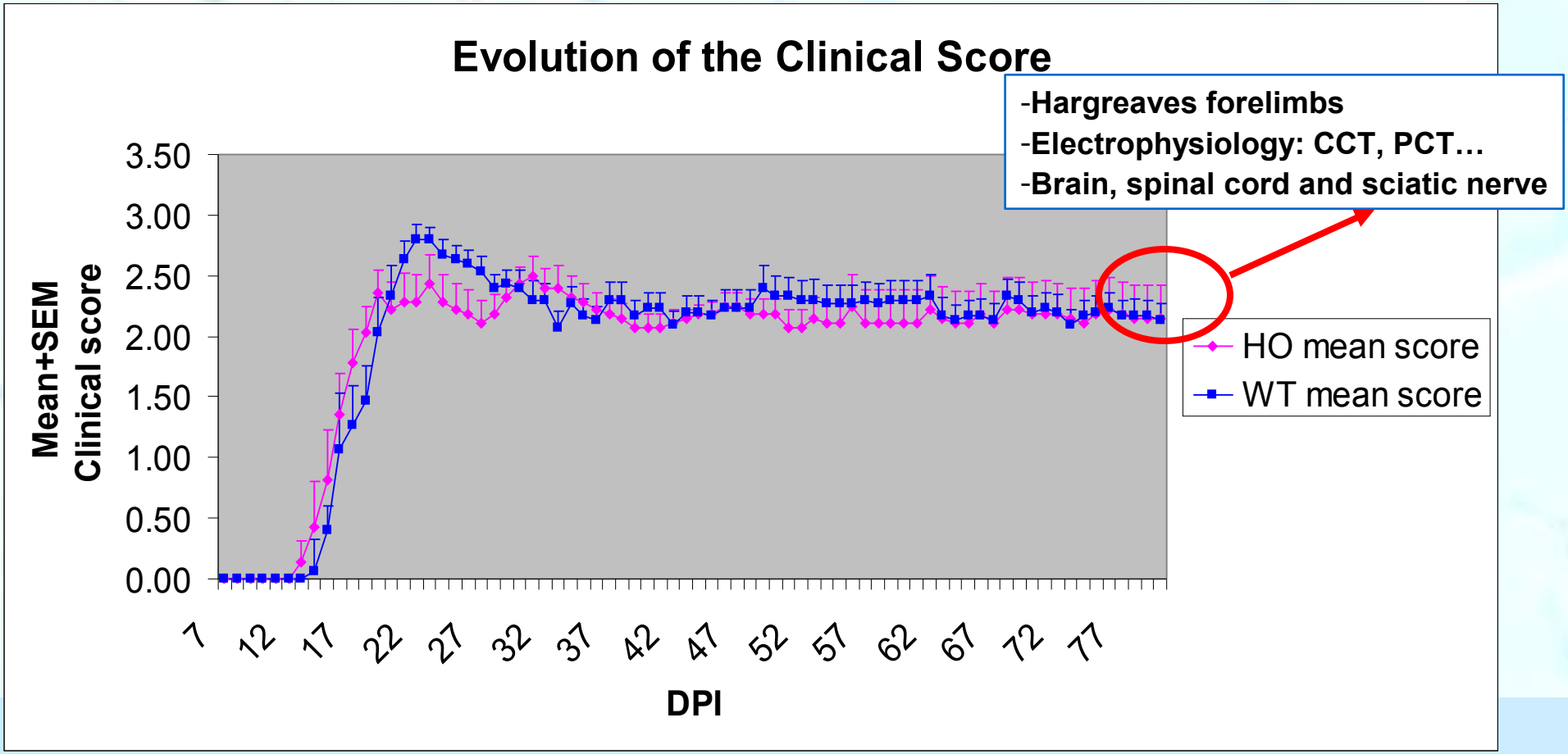
Long term EAE study (=80 days)

Protocol: 17+/+ & 14 -/- female mice, Day 0: immunization (MOG 200µg+PTX 400µL), DAY 2:2nd injection of PTX.

CS: 0: no clinical sign; 0.5: tail partially paralyzed, 1:tail completely paralyzed, 1,5: 1+ 1 hindlimb partial paralysis, 2: 1,5+ hindlimbs partial paralysis, 2,5: 1 hindlimb partial paralysis+1 hindlimb total paralysis, 3: hindlimbs complete paralysis, 3,5: 3+forelimbs partial paralysis, 4: 4 limbs total paralysis, 5: dead animal.

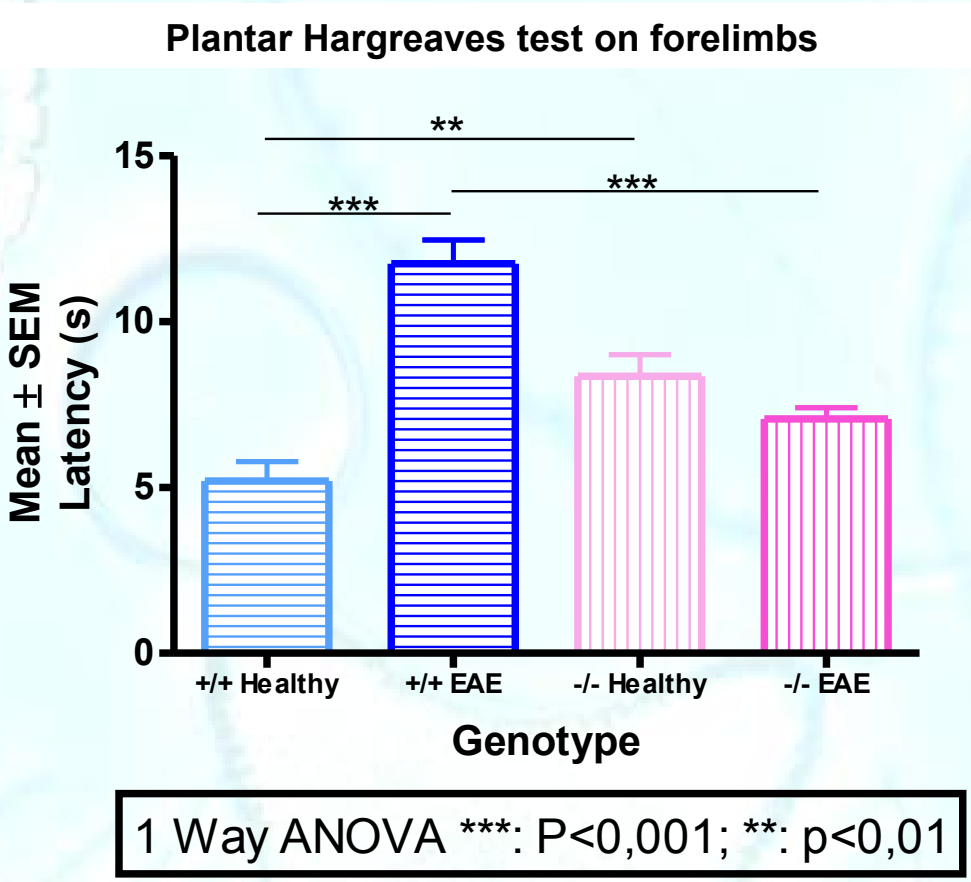
Time onset: 16,2±0,6 for +/+mice, 14,8±0,4 for -/- mice

Stat: Day by Day comparison



Pain readouts on EAE mice

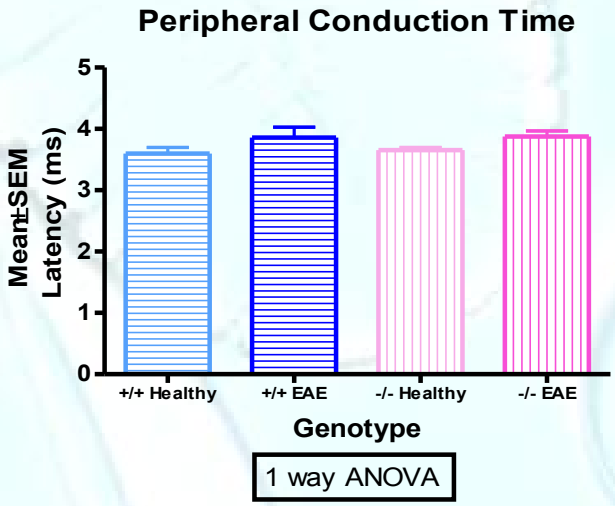
n= 10 Healthy +/+, 10 Healthy -/-,
15 EAE +/+, 13 EAE -/-



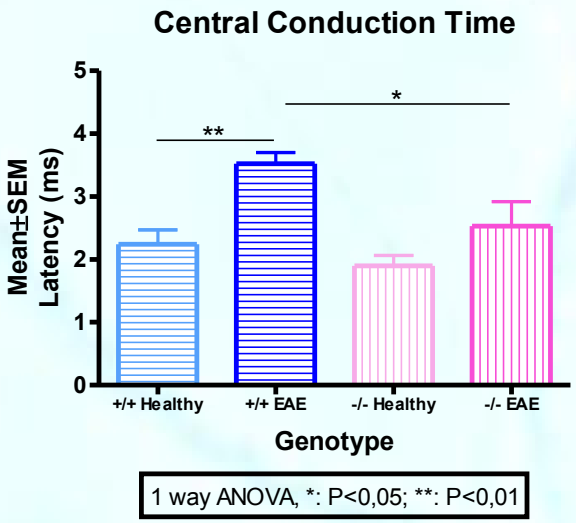
- Healthy mice: -/- mice are significantly less sensitive to a painful stimulus than +/+: confirmed hyposensitivity in female
- EAE induce hypoalgesia (increase in latency) in +/+ mice but not in -/- mice
- EAE +/+ have longer latency than EAE-/- mice.

Electrophysiology in EAE mice.

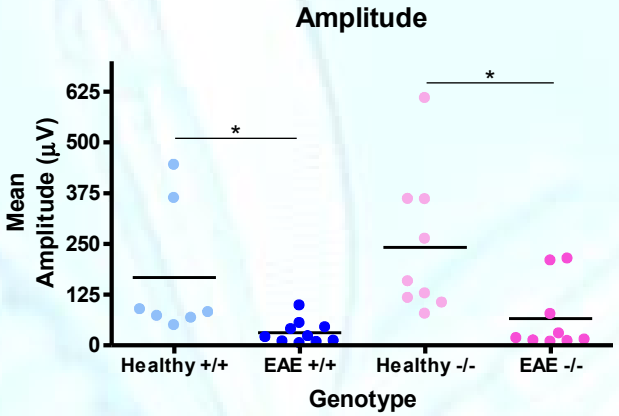
N= 10 Healthy +/+, 9 Healthy -/-, 9 EAE +/+, 9 EAE -/-



1 way ANOVA



1 way ANOVA, *: P<0,05; **: P<0,01



1 Way ANOVA, *: P<0.05

- Peripheral conduction time:
  - no changes in healthy vs disease mice.
- Central conduction time as an index of demyelination:
  - ***Impaired in EAE +/+***
  - ***no difference between healthy and EAE -/-***
- Amplitude as an index of axonal loss:
  - ***EAE induces a worsening of amplitude in +/+ and -/- mice.***

•EAE DEFICITS IN RPTPz KO MICE MAY BE DUE TO AXONAL PATHOLOGY RATHER THAN DEMYELINATING DYSFUNCTION

EAE Results Summary.

- No significant difference in the course of the disease between -/- and +/+ mice.
- EAE doesn't induce significant difference, in terms of pain and nerve central conduction, in -/- mice, like it does in +/+ mice.
- The worsening in the Amplitude but not in the Central Conduction Time in -/- mice shows that RPTPz may be involved in axonal pathology.

What's next: immunohistochemistry on spinal cord to detect axonal loss.

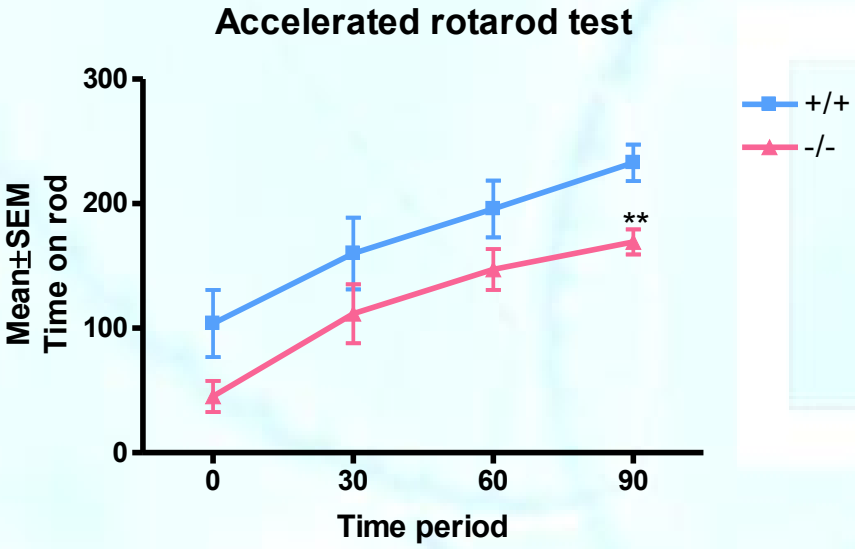
Thank you...

Preclinical Research Pharmacology:
Neuropharmacology Unit

Università degli Studi del Piemonte Orientale
"Amedeo Avogadro"

Accelerated Rotarod

Age : 14 weeks



T-test, ** : p<0,01,+/+ Vs. -/-

Rotarod: no habituation, max 5min test for each time point (0, 30, 60, 90 min)-

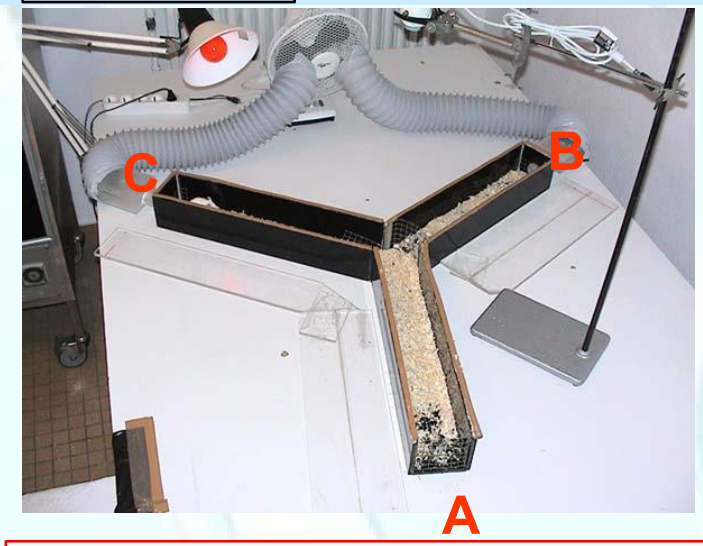
Increasing speed: 4 to 40 rpm

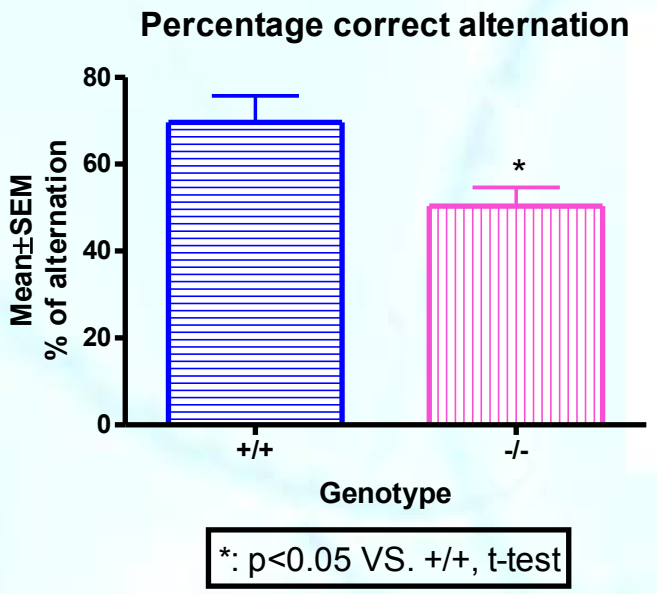
Measure: locomotion, coordination, learning

- No difference in the learning curve
- Trend for -/- mice to be worst performers
- KO present deficit in motor activity/coordination at 90 minutes

Y Maze

Age : 15 weeks





*: p<0.05 VS. +/+, t-test

Y maze: working memory, sensitive to hippocampal damage- 5min testing

1- exploration: nr of entries

2- working memory: nr of correct alternation defined as successive entries into the three arms (no re-entry in already explored arm)

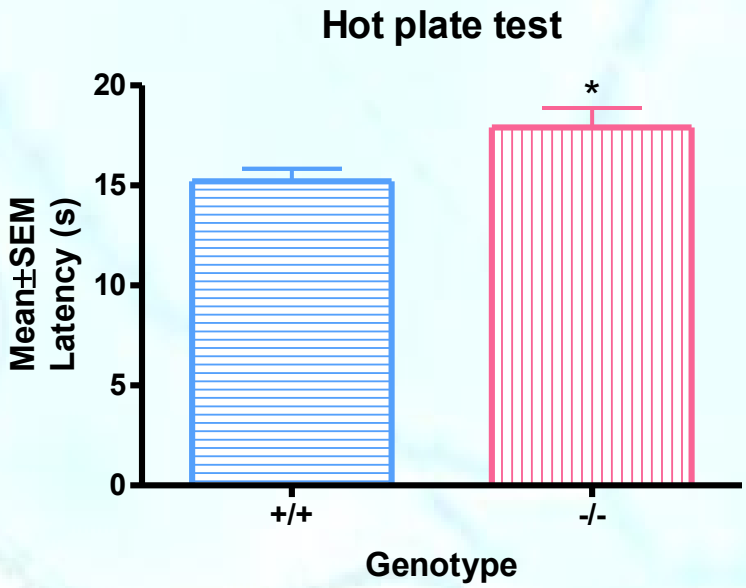
% correct alternation:

{(number of alternation)/(Total arm entries – 2)} x 100

•KO present impaired working memory

Hot Plate

Age : 10 weeks



Hot Plate: 52,5°C.

Measure reaction time of mice to show behavioral sign (licking or shaking paws), max: 45sec.

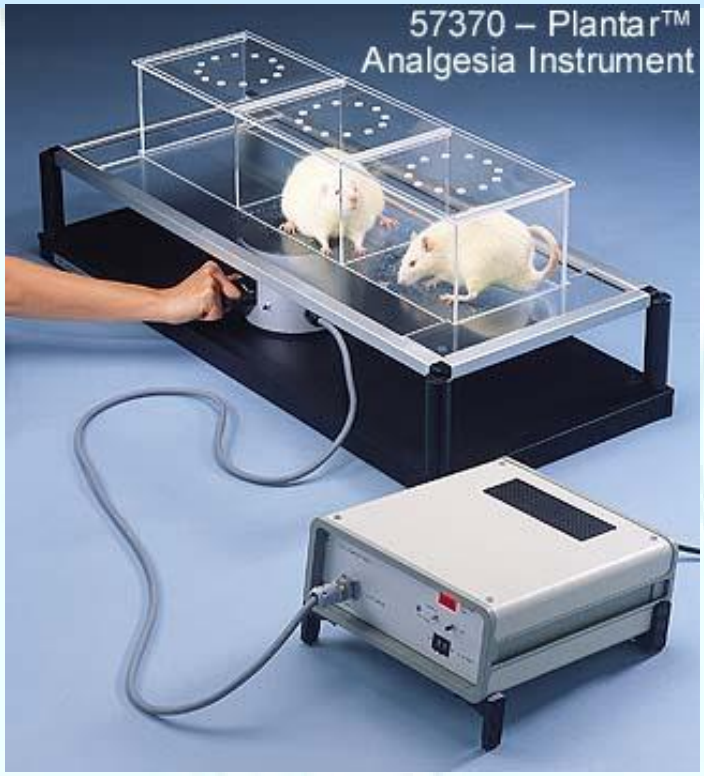
Centrally mediated response, C- Aδ fibers

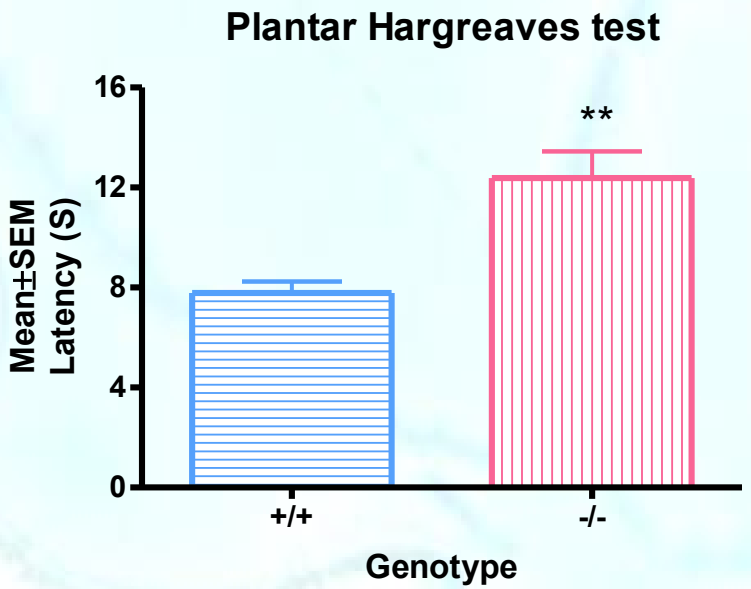
* : T-test, VS. +/+, P<0,05

KO present hyposensitivity to thermal pain.

Plantar Hargreaves

Age : 16 weeks





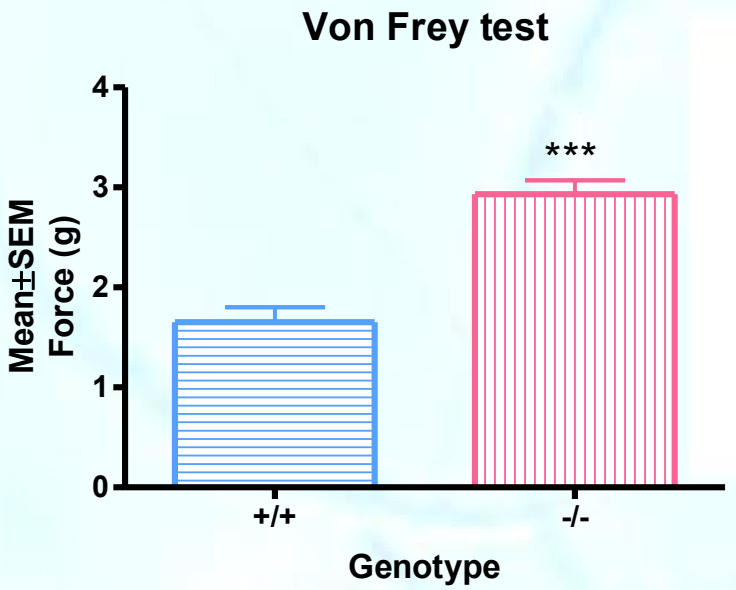
** : T-test, VS. +/+, p<0,01

HG: Heat beam source on paws.
Measure time withdrawal paw from beam.
Mainly C-fibers

KO present hyposensitivity to focal thermal stimulation.

Plantar Von Frey

Age : 16 weeks

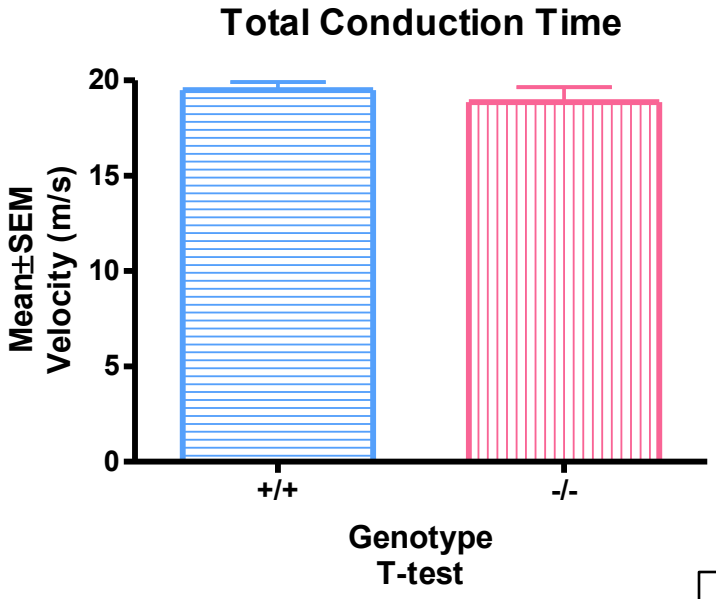


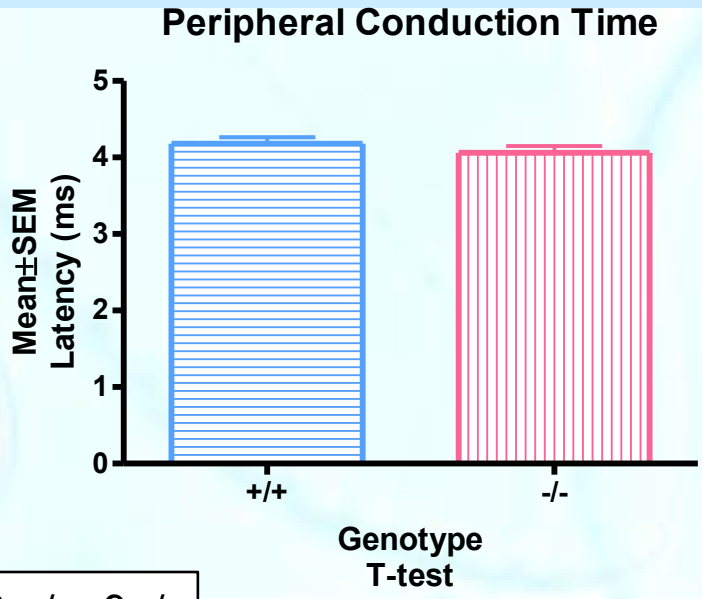
*** : T-test, VS. +/+, p<0,01

Increse strength of probe pressure on paw-
max: 5 g strength. Measure force at withdrawal of paw.
Tactile sensitivity.
Mainly Aδ fibers

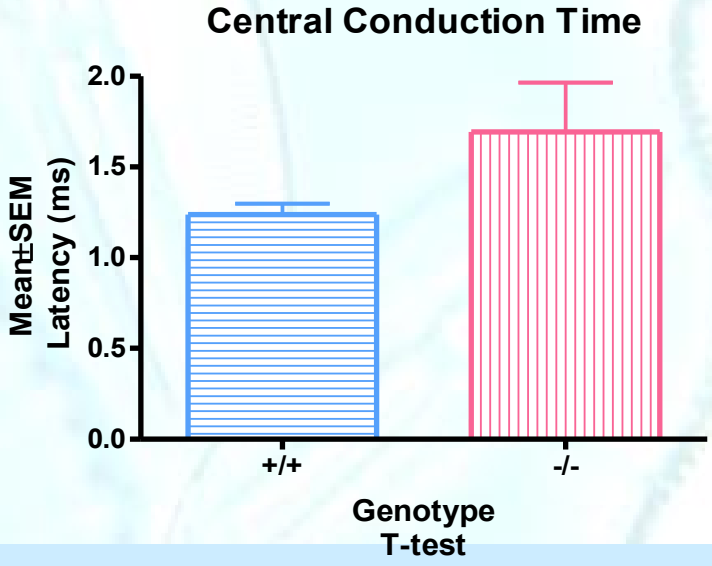
KO present hyposensitivity to mechanical stimulation.

Electrophysiology.



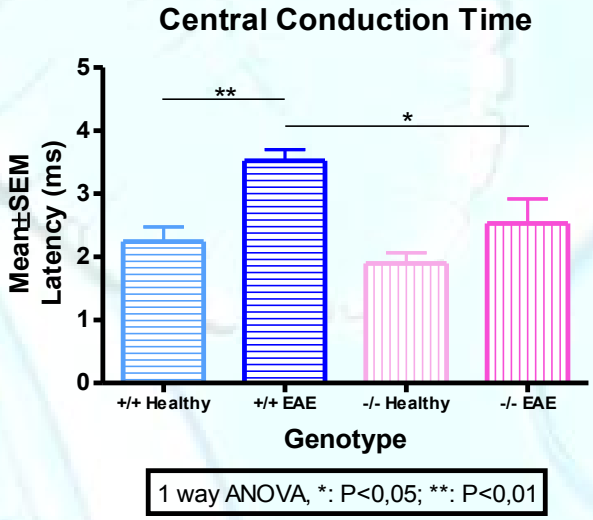


N=10 +/+, 9 -/-



No differences in nerve conduction velocity.

Demyelinating dysfunction on EAE mice



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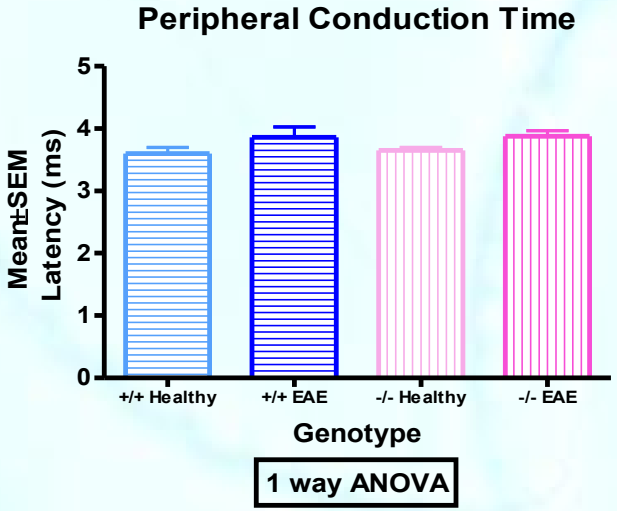
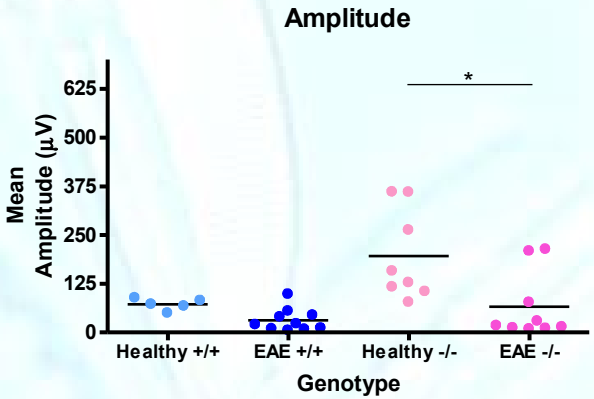


1 way ANOVA



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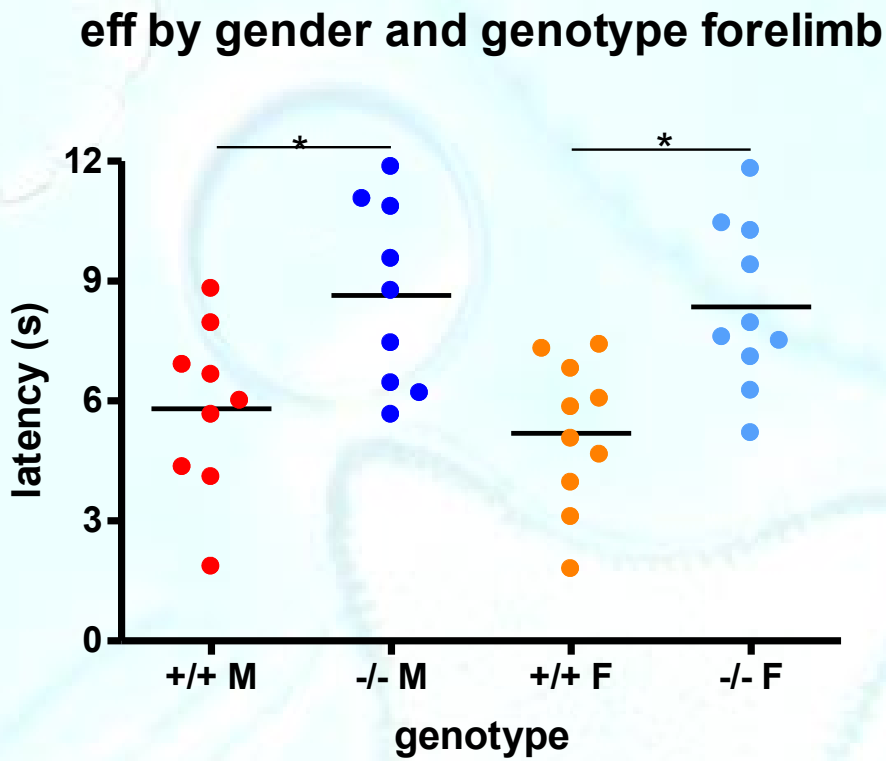
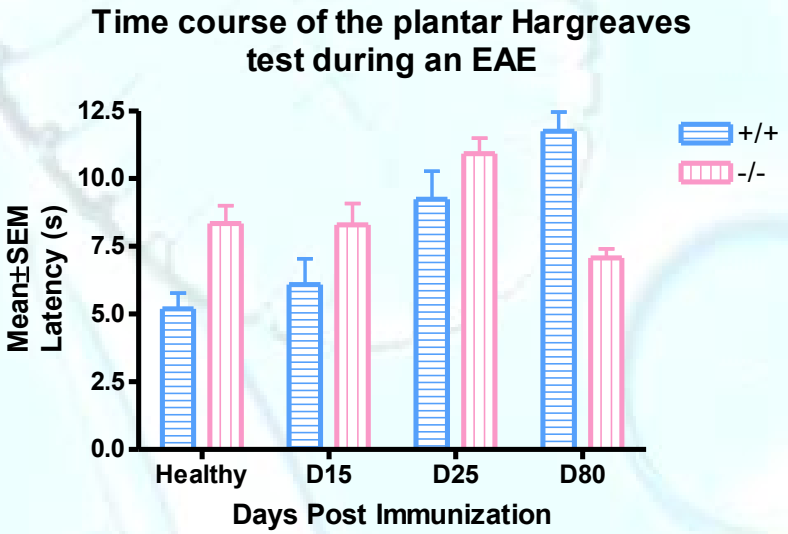
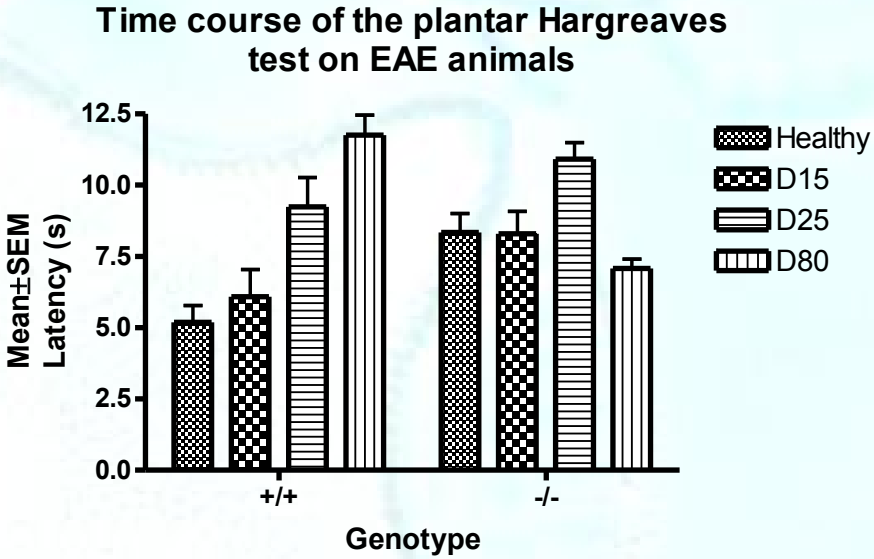
EAE DEFICITS IN PTPRz KO MICE MAY BE DUE TO AXONAL PATHOLOGY RATHER THAN DEMYELINATING DYSFUNCTION

No difference between genders for the Hargreaves test




Time course of the plantar Hargreaves test during an EAE
Mean±SEM Latency (s)
12.5
10.0
7.5
5.0
2.5
0.0
Healthy
D15
D25
D80
Days Post Immunization
+/+
-/-


Time course of the plantar Hargreaves test on EAE animals
Mean±SEM Latency (s)
12.5
10.0
7.5
5.0
2.5
0.0
+/+
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Genotype
Healthy
D15
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D80