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The function of pain associated fibers in MOG induced EAE:

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Introduction:

Neuropathic pain may affect patients with multiple sclerosis (MS) even in early disease. Most of the pain assessment methods in animals are relay on withdrawal reaction from a specific stimuli. Evaluation of pain level using withdrawal reaction in EAE mice is challenging for obvious reasons. Therefore, an alternative method for assessing the functionality of the sensory nerve fibers is required. In this study we used the Neurometer ® for that purpose.

Methods:

The EAE-MOG murine model consists of a sensitization period, induced by the single subcutaneous (SC) injection of MOG emulsified in complete Freund's adjuvant (CFA) on study day 0, followed by intraperitoneal (IP) supplemental immunostimulation with pertussis toxin (PT) carried out once at the time of EAE induction and once again 48 hours later.

Clinical score assessment:
All animals are examined for signs of any neurological responses and symptoms prior to EAE induction (study day 0) and thereafter, examined on a daily basis throughout the entire 35-day observation period. EAE reactions are scored and recorded according to the EAE clinical signs scoring:
EAE reactions are scored and recorded according to a 0-15 scale. The clinical score is determined by summing the score of each section (Weaver at al, 2005):

Signs / Symptoms		Grade
Tail	No Signs	0
	Half paralyzed tail	1
	Fully paralyzed tail	2
For each of the hind or forelimbs	No Signs	0
	Weak or altered gait	1
	Paresis	2
	Fully paralyzed limb	3
Mortality	---	15

Peripheral pain associated nerve recording:
The peripheral sensory system function was assessed using the neurometer system which evaluates more than 90% of sensory nerve fibers (types Aβ, Aδ and C).

Conclusion

1. This study propose a non-withdrawal dependent method for evaluating the functionality of the sensory nerves in EAE mice.
2. The data suggest that the functionality of the sensory nerve system decreased in a later stages of the disease.

Results:

Disease score

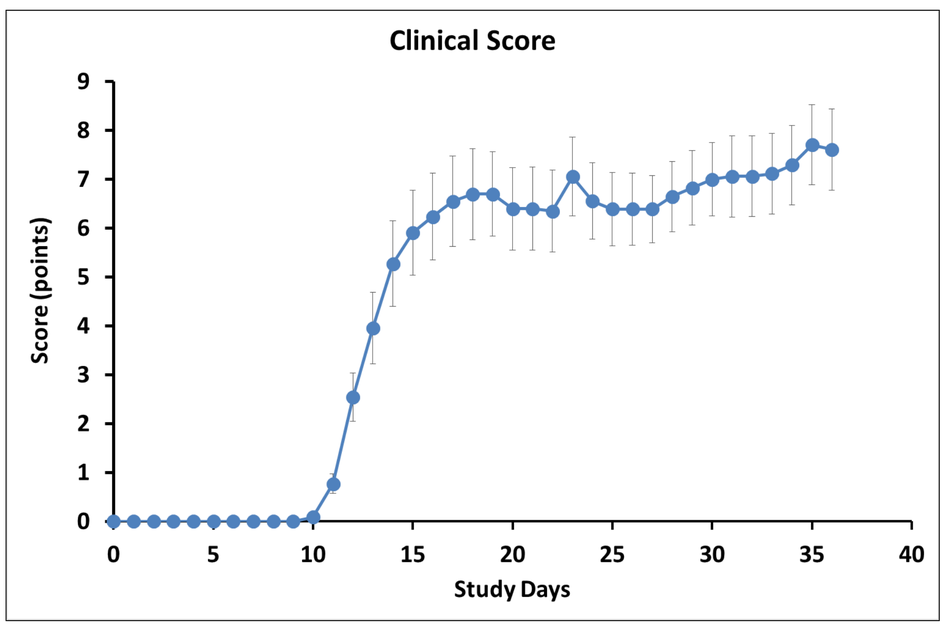

Figure 1: Disease associated clinical score in MOG –EAE mice

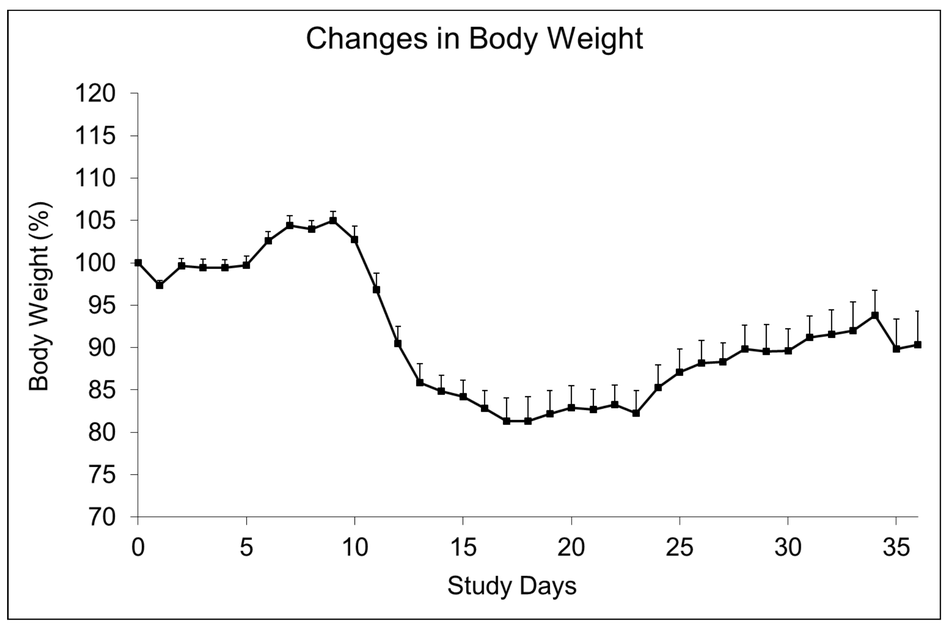

Figure 2: Changes in body weight that are typical to MOG-EAE model in mice

Demyelination on spinal cord

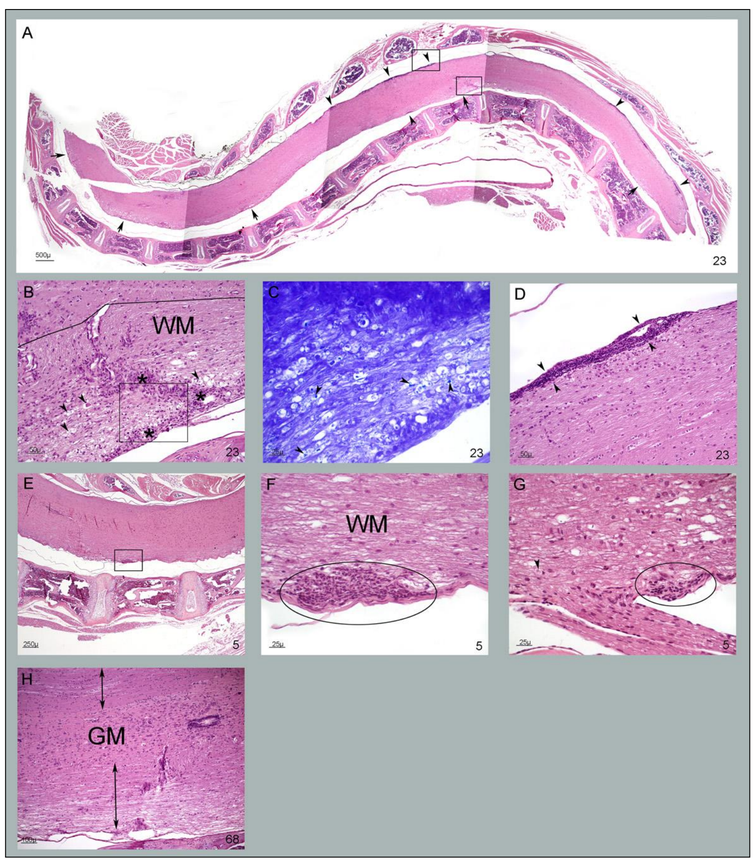

Figure 3: EAE legend

A, B and D-H: H&E ; C-LFB ; A-D: mouse 23 ; E-G: mouse 5 ; H: mouse 68.

A A composite image taken at low magnification and showing a longitudinal section of the spinal cord in a diffusely affected mouse. Clusters of infiltrating inflammatory cells, visible as superficially located dark areas are identified (arrowheads). Regions with white matter damage are seen as vacuoles (arrows). These two alterations, either singly or in combination affected the white matter diffusely. The boxed areas are shown in B (ventral) and D (dorsal). The cranial aspect is to the left.
B High magnification of the ventral boxed area in A. Inflammatory cells (asterisks) are present below the meninx (pia mater) and in the white matter (WM). In the white matter there are vacuoles, some of which contain debris (arrowheads). The boundary between the gray and white matter is identified with a black line. The boxed area is shown in C.
C In the white matter there are many vacuoles, some of which contain myelin debris (arrowheads) which stains light blue with Luxol fast blue stain.
D High magnification of the dorsal boxed area in A. Infiltrating lymphocytes are arranged as a dark band below the meninx (between arrowheads).
E Medium magnification of the spinal cord of a mouse with mild multifocal EAE. The boxed area is shown in F. There are no additional histologic changes in this field.
F A small focus of inflammatory cells (circled) is located in the superficial white matter (WM).
G Another small focus of superficially located inflammatory cells (circled) from this mouse. Altogether there were only a few foci of inflammation in this slide and minimal evidence of white matter damage (arrowhead).
H Medium magnification of the spinal cord of a normal mouse. The white matter (spanned by double headed arrows) and the gray matter (GM) are identified.

Peripheral recording of nerve function

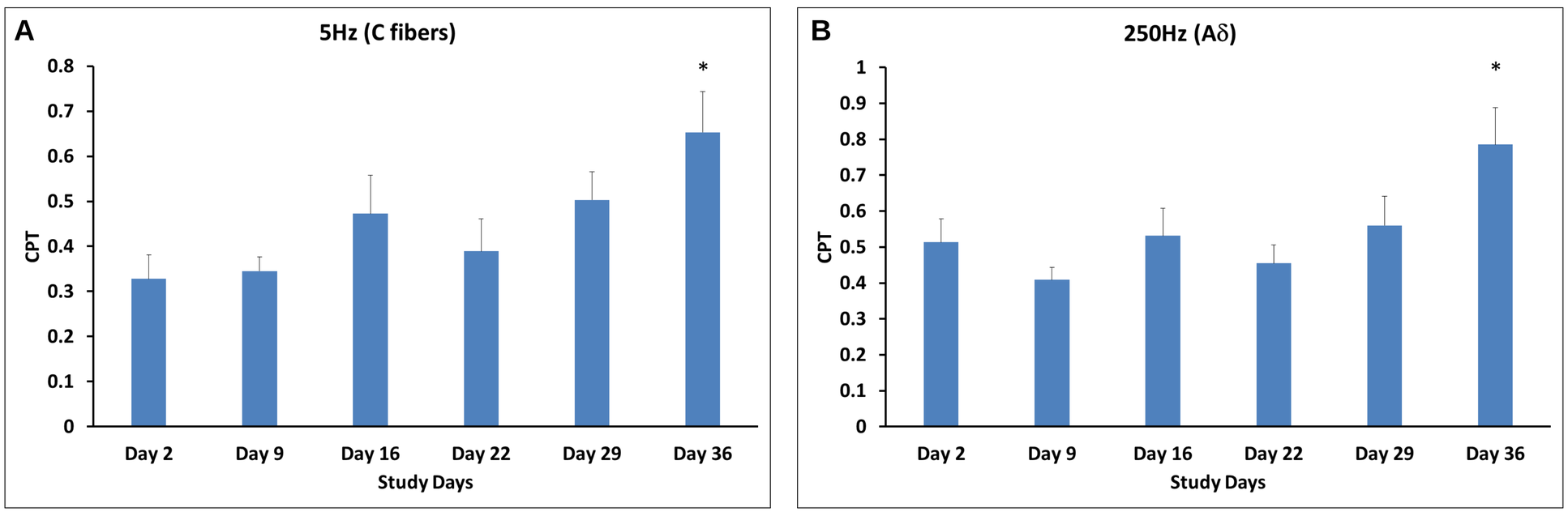

Figure 4: Changes in current perception threshold (CPT) in MOG induced EAE in mice.

A- CPT as a response to 5Hz stimulation, reflecting the functionality of non myelinated C fibers.
B- CPT as a response to 250 Hz stimulation, reflecting the functionality of small myelinated Aδ fibers
C- recording following 2000Hz stimulation, reflecting the functionality of Aβ fibers.

*p<0.05 vs. CPT on study day 2.