Quindicesima Riunione annuale ASNP

Grand Hotel Tiziano e dei Congressi

Lecce, 12 - 14 giugno 2025

INSIGHTS INTO MORPHO-FUNCTIONAL FEATURES OF BRAIN NEURONS IN NEUROPATHIC PAIN ANIMAL MODELS

Lisa Fantoni* - Alessandro Sacchini** - Laura Cherchi* - Annalisa Canta* - Virginia Rodriguez Menendez* – Cristina Meregalli* - Silvia Fermi* - Paola Alberti* - Paola Marmiroli* - Antonio Zippo** - Valentina Alda Carozzi*

University of Milano-Bicocca, School of Medicine and Surgery, Monza, Italy.

** CNR Italian National Research Council, Institute of Neuroscience, Vedano al Lambro, Italy.*

Background

Neuropathic pain (NP) is a severe and disabling condition, which affects millions of people worldwide, and it is commonly characterized by allodynia, dysesthesia and hyperalgesia, symptoms poorly managed by current interventions especially in long-term contexts (Baron R. *et al*. 2010; Hanewinckel R. *et al*. 2016). NP is supported by central aberrant adaptations in response to peripheral injury or disease, a phenomenon taking place involving the cortical circuitry. Understanding its underlying mechanisms is essential for the development of effective treatments.

Aim of the study

In this study, we investigate the changes in some key brain regions, important for sensory processing and pain modulation, through electrophysiological analysis of neural activity and through Golgi-Cox morphological and morphometrical analysis of some cortical regions involved in nociception. We employed two different rat models of NP, one induced by a permanent **ligature of the sciatic nerve (SNL)** and one induced by chronic treatment with neurotoxic chemotherapy (**Paclitaxel, PTX**).

Materials and methods

Animals. A subset of Female Wistar rats were treated with PTX, or its vehicle, 10 mg/kg i.v. once a week for 4 weeks (end of treatment timepoint, ET), followed by 4 weeks of follow-up (FU). Another subset underwent permanent ligature of the sciatic nerve (SNL): following anesthesia, the sciatic nerve was exposed and partially ligated with a suture wire; sham animals underwent nerve exposure only.

Dynamic test. Mechanical withdrawal threshold was measured biweekly using a DPA device applying progressively increasing force to the hind paw (Vuralli D. *et al*. 2019).

Morphological studies. Brain samples were processed with Golgi staining and analyses of dendrites were executed. In the SNL group dendrites were characterized by their directionality by the related ImageJ plugin.

Electrophysiology. Multielectrode arrays (MEA) were surgically implanted in CNS areas involved in the emotional and sensorial modulation of pain: medial Prefrontal Cortex (**mPFC**), mediodorsal thalamic nucleus (**MD**) and periaqueductal gray (**PAG**). Spontaneous and pain-evoked (Dynamic test) neuronal activity was recorded. In the SNL group, the ventropostero lateral thalamus (**VPL**) was recorded instead of MD.

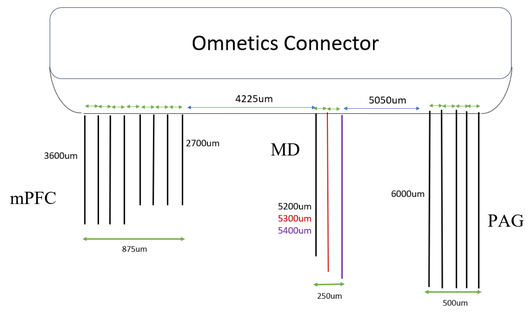

FIG 1. Schematic representation of MEA electrodes

Results

Neuropathic pain

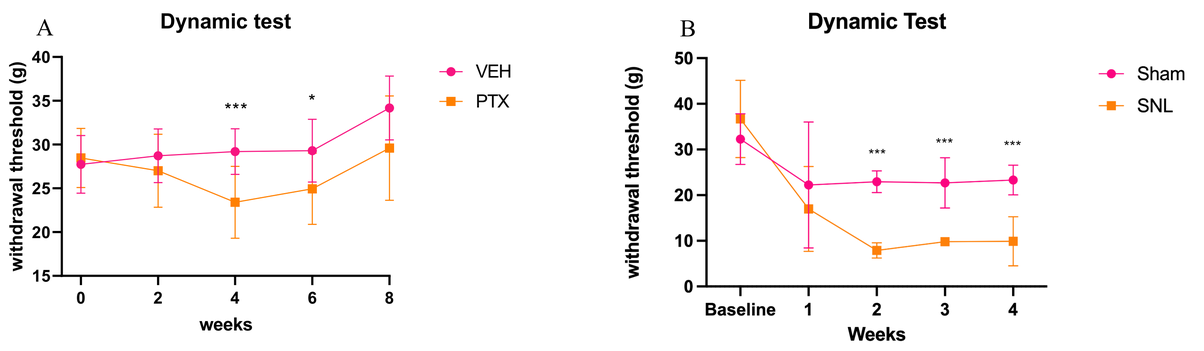

*FIG 2. Statistical analysis of mechanical allodynia with the Dynamic test. (A) PTX significantly decreased withdrawal threshold for mechanical allodynia at the ET and in the midpoint of the FU. (B) In another neuropathic model, SNL, mechanical allodynia and hyperalgesia resulted starting from week 2 and keep stable up to the fourth week. * p<0.01 vs VEH; ***p<0.0001 vs VEH, Mann-Whitney test).*

Cortical Golgi-Cox Staining

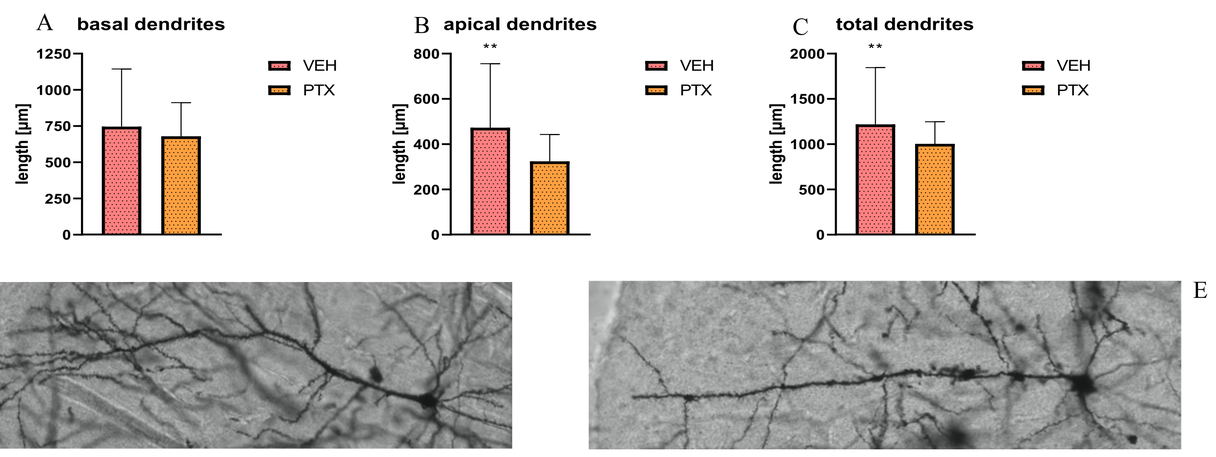

*FIG 3. Statistical analysis of basal and apical dendrites length in pyramidal neurons from the II/III layer of the mPFC at ET. Analysis of dendritic length shows no difference in basal dendrites (A) and a trend of decreasing length in apical and total dendrites (B and C) after 4 weeks of PTX treatment (ET). Images showing the dendritic extensions of neurons under VEH and PTX conditions (D and E). **p<0.001 vs VEH; Unpaired t test.*

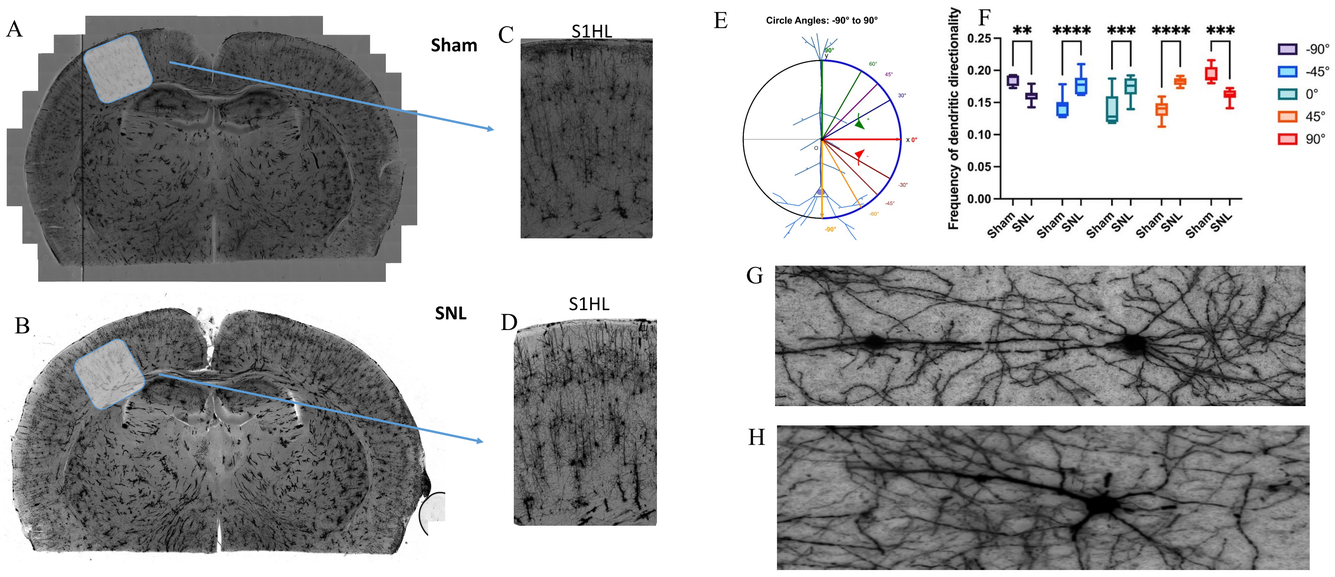

*FIG 4. (A, B) Histological characterization of Golgi-Cox staining of the primary somatosensory cortex (hindlimb projection, S1HL, B and C) in Sciatic Nerve Ligature (SNL) models of rat neuropathic pain. We characterized the preferential directionality of dendrites by circular statistics (E). Results show a significant decrease in upward neurites (90 and -90 degrees) in favor of lateral directions (-45, 0 and 45 degrees) by comparing sham animals to SNL group (N = 6). Images showing the dendritic direction of neurons under SNL and SHAM conditions (G and H). **p<0.001 vs sham; ***p<0.0001 vs sham; Two Ways Anova.*

MEA Electrophysiology

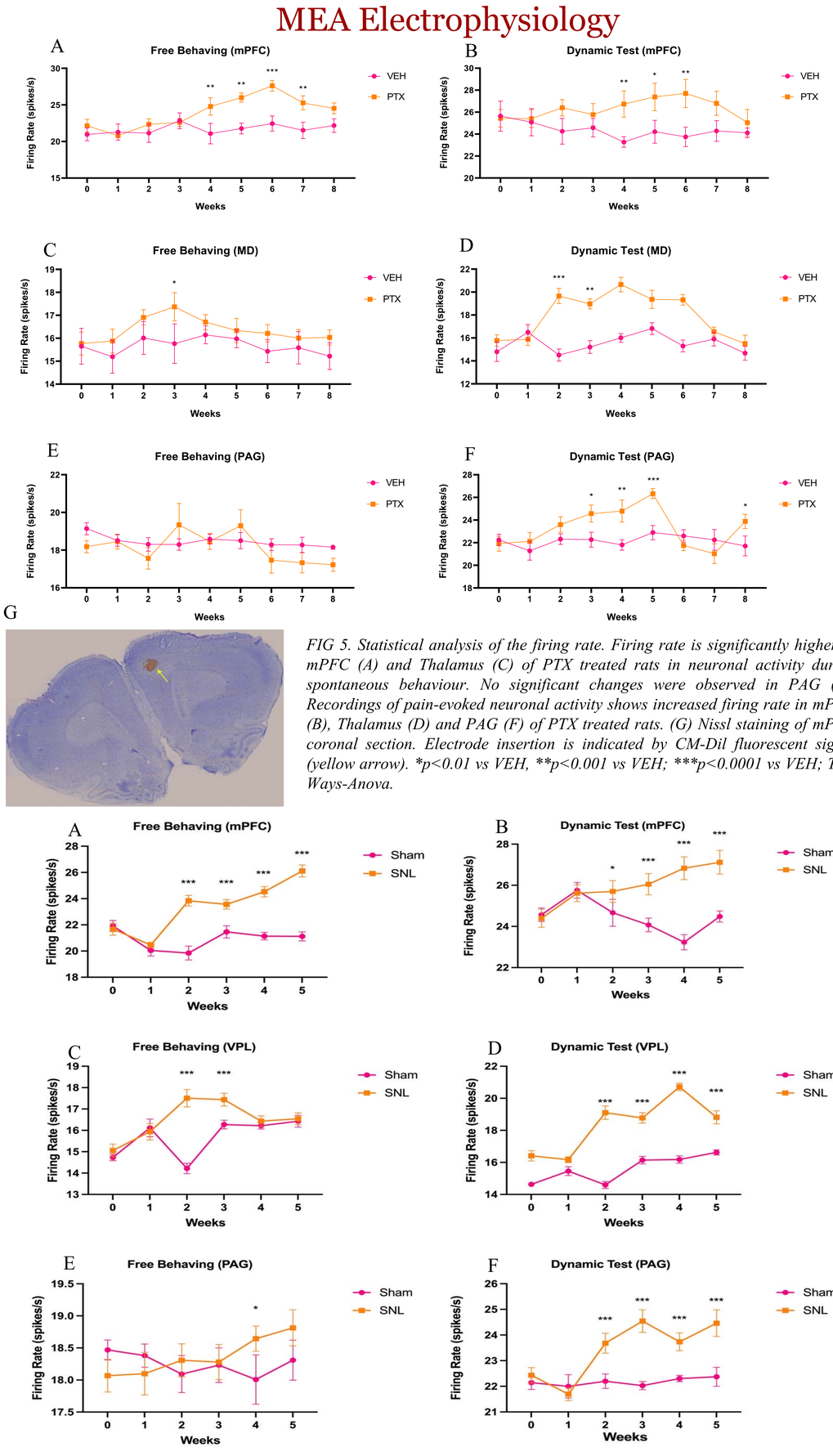

*FIG 5. Statistical analysis of the firing rate. Firing rate is significantly higher in mPFC (A) and Thalamus (C) of PTX treated rats in neuronal activity during spontaneous behaviour. No significant changes were observed in PAG (E). Recordings of pain-evoked neuronal activity shows increased firing rate in mPFC (B), Thalamus (D) and PAG (F) of PTX treated rats. (G) Nissl staining of mPFC coronal section. Electrode insertion is indicated by CM-Dil fluorescent signal (yellow arrow). *p<0.01 vs VEH, **p<0.001 vs VEH; ***p<0.0001 vs VEH; Two Ways-Anova.*

*Fig 6. Extracellular recording analysis shows significant increase of firing rate in mPFC and VPL in SNL models during freely moving recording sessions starting from the 2 weeks from the SNL induction, while during noxious stimulation during the dynamic test all regions show significant increase of firing rate in SNL models starting from the 2 weeks. *p<0.01 vs VEH,; ***p<0.0001 vs VEH; Two Ways-Anova.*

Conclusions

- ✓ PTX induced mechanical allodynia, as demonstrated by the Dynamic test.
- ✓ PTX-treated rats showed increased neuronal firing in pain-related brain regions (mPFC, MD, PAG) compared to controls, both at rest and during tactile stimulation.
- ✓ PTX treatment involved a reduction in the length of both apical and total dendrites in the anterior cingulate cortex.
- ✓ SNL models had severely impaired mechanical pain-threshold starting from the second to the fifth weeks from the induction.
- ✓ SNL models showed a significant rebranching of dendrites which preferred directionality included in the -45 to 45 degrees.
- ✓ Neuronal firing activity was increased in the SNL group compared to Sham group in crucial regions of nociception (mPFC, VPL, PAG), especially during noxious stimulations.

Next steps

- ✓ Deepen the investigation of neuronal activity during spontaneous and pain-evoked activity (burst activity, LFP, I/E characterization).
- ✓ Expand dendritic plasticity analysis with FU timepoint and spine density count.
- ✓ Including mPFC analysis of dendrite directionality for the SNL group.

References

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Email: lisa.fantoni@unimib.it; valentina.carozzi1@unimib.it;

This work was funded by «Ministero dell'Università e della Ricerca, PRIN PNRR ID 2022C5P3F, PI Antonio G. Zippo; PRIN 2022 ID 2022NBS28K, PI Valentina A. Carozzi