Activation of spinal dorsal horn inhibitory networks by the CLTMR derived chemokine TAFA4

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Introduction:

Pain is essential to protect animals from potentially harmful stimulus. Its elaboration results from the integration within dorsal spinal cord networks of sensory and nociceptive information conveyed by primary afferents. These information are then forwarded to the brain where the pain sensation elaborates. Among primary afferent neurons, C-Low threshold Mechano Receptors (C-LTMR) have been recently identified as modulators of pain transmission. These fibres whose soma are localised in dorsal root ganglia, specifically project toward the inner Rexed lamina II (IIi). Recent molecular characterisation revealed that C-LTMRs express a distinct set of molecular markers among which the antinociceptive chemokin TAFA4. **The aim of this work is to investigate the role of this chemokin in the modulation of synaptic transmission within spinal network using pharmacological approach.**

Methods:

***In vitro* electrophysiology:** C57Bl 6j mice (3-4weeks) were used for pharmacological experiment. After removal of the spinal cord from backbone, acute transversal slices (300µm) from the lumbar enlargement were obtained. Whole cell patch-clamp recording was used to record synaptic transmission within lamina IIi. To record excitatory post synaptic currents (EPSCs) the recording electrode was filled with K-gluconate solution. To study inhibitory post synaptic currents (IPSCs) a chloride enriched solution was used and slices were superfused with DL-AP5 (25µM), CNQX (10µM) to block ionotropic glutamatergic transmission. Miniature EPSCs (mEPSCs) and IPSCs (mIPSCs) were recorded in presence of tetrodotoxine TTX (1µM). A glass succion electrode was used to stimulate spinal dorsal root .

To block inhibitory transmission we used antagonist of $GABA_A$ receptor bicuculline (10 µM), of $GABA_B$ receptor CGP (1 µM) and antagonist of glycinergic receptor strychnine (50 µM).

Inflammatory model: Mice received a 10 µL subcutaneous injection of Complete Freund's Adjuvant (CFA, 1 mg/mL Mycobacterium Tuberculosis, 0.85 mL parafinn oil, 0.15 mL mannide monooleate) into the left hindpaw. Behavioral measurement were done before injection, 48h after the CFA injection and 1h after the intrathecal injection of TAFA4 (200µg/ml). To test the mechanical sensitivity of inflammated mice, we used Von Frey test consisting in a stimulation of plantar surface of the left hindpaw with calibrated filaments of increasing force (0.07,0 .16, 0.4,1 , 1.4 g). Mice were sacrified 48h after the injection of CFA. In this experiment slices were then incubated either in artificial cerebro-spinal fluid (aCSF) or in TAFA4 (20nM) during 30 min before recordings.

***In vivo* electrophysiology:** C57Bl 6j mice (8 weeks) were used for these experiments 4 days after CFA injection. VonFrey filaments were used to stimulate the hindpaw and thus induce the neuronal discharge recorded with a glass electrode.

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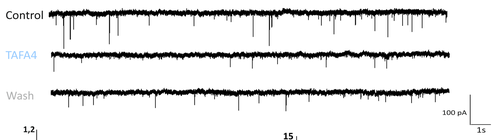
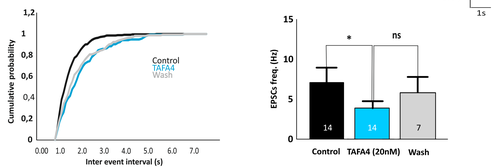
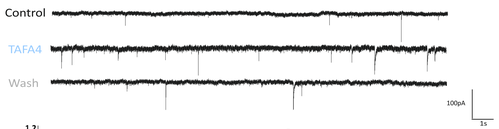
1- TAFA4 decreases spontaneous excitatory activity in dorsal horn

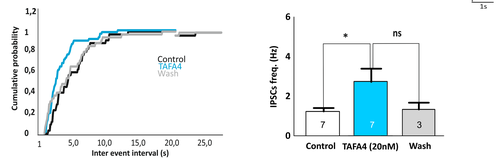




We measured the occurrence of EPSCs over 3 min recordings in control condition after 40 min of superfusion of TAFA4 (20nM) and after wash. We show that a bath application of TAFA4 induces a significant decrease (reversible in 3 cells upon 7) of the EPSCs frequency.

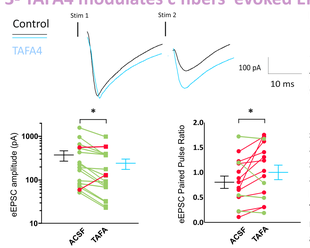
2- TAFA4 increases spontaneous inhibitory activity in dorsal horn





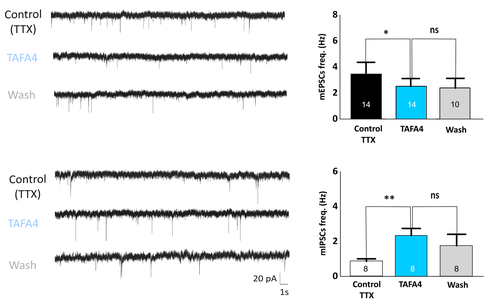
Regarding inhibitory synaptic activity in control condition there is a significant increase of IPSC frequency after 40 min of application of TAFA . This increase is reversible after 30 min of wash.

3- TAFA4 modulates c fibers evoked EPSCs



In attempt to characterise if TAFA4 modulates synaptic transmission between C fibers and lamina IIi interneurons, we recruited nociceptive fibers through high intensity stimulation (500 µA, 500 ms) of dorsal root and measured the amplitude and the paired pulse ratio (PPR) of evoked excitatory synaptic responses in control condition and after application of TAFA4. There is a significant decrease of eEPSCs amplitude as well as an increase of the PPR following a bath application of TAFA4 which thus appears to **directly modulate synapses between primary afferences and neurons of lamina IIi.**

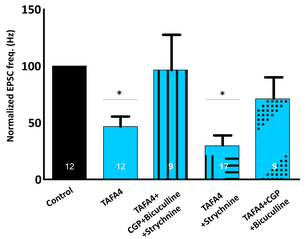
4- Presynaptic action of TAFA4 on synaptic activity



To determine if the modulation of synaptic activity by TAFA4 is of presynaptic origin, we recorded m EPSCs (top) and mIPSCs (bottom). We found that the TAFA4 induced decrease of EPSC and increase of IPSCs frequency are preserved in presence of TTX.

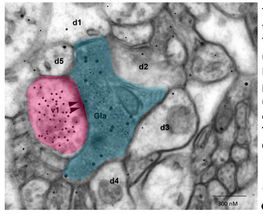
These results demonstrate that TAFA4 acts on neuronal networks through a presynaptic mecanisms.

5- Effect of TAFA4 mediated though inibitory transmission



We hypothesized that the decrease of EPSCs fequency could be a consequence of the increase of the IPSCs frequency. Thus we recorded EPSCs with antagonists of GABA receptors (bicuculline and CGP) and antagonist of glycinergic receptor (strychnine). We demonstrate that TAFA4 acts though inhibitory transmission. By blocking either GABAergic or glycinergic transmission, we also demonstrate **the effect of TAFA4 is mediated though the modulation of the GABAergic transmsission.**

6- Direct synaptic contact between C-LTMRs and GABAergic terminals

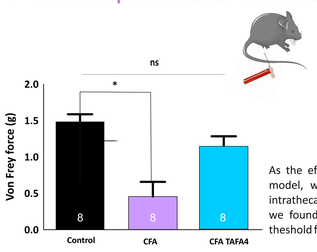


To assess if there is morphological correlation to this functional effect of TAFA4, we took advantage of TAFA4Venus knock-in mice and performed ultrastructural studies. Quantitative analysis of 295 IR-profiles showed that there was a net predominance of contacts between Venus+ fibers (blue) and GABA-IR presynaptic vesicle-containing dendrites (red) (97.6%).

This result reinforce the action of TAFA4 on GABAergic interneurons.

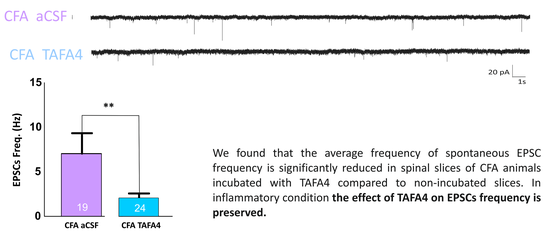
- GFP (10nM)
- GABA (20nM)
- VGlut3 (30nM)

7- Antinociceptive action of TAFA4 in CFA inflammatory pain model



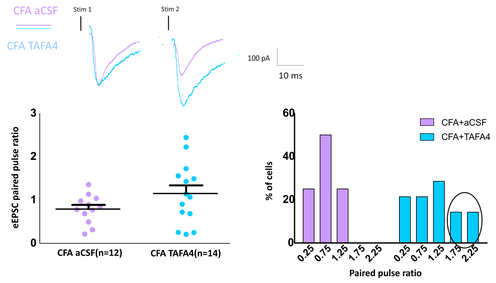
As the effect of TAFA4 has not been tested in CFA model, we assessed the antinociceptive action of intrathecally injected TAFA4. Using Von Frey filaments, we found a significant increase in paw withdrawal theshold following TAFA4 injection in inflammed mice.

8- TAFA4 decreases spontaneous EPSC frequency in CFA model



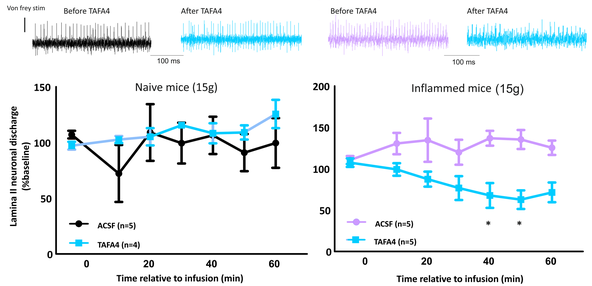
We found that the average frequency of spontaneous EPSC frequency is significantly reduced in spinal slices of CFA animals incubated with TAFA4 compared to non-incubated slices. In inflammatory condition **the effect of TAFA4 on EPSCs frequency is preserved.**

9- Effect of TAFA4 on C-fibers evoked EPSCs in CFA condition



To determine if TAFA4 modulates the nociceptive transmission between primary afferences and the interneurons of lamina II in inflammatory condition, the PPR of evoked responses of interneurons were measured in slices incubated or not with TAFA4. In incubated slices, the average PPR was higher than in non incubated slices (p=0.1), with a clear shift of the population toward higher PPR values. **This result indicate that the effect of TAFA4 on synaptic transmission between C fbers and lamina II interneurons is preserved in inflammatory condition.**

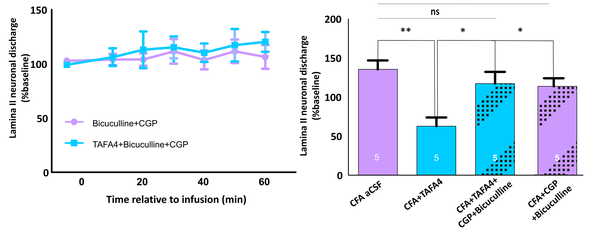
10- TAFA4 decreases neuronal discharge in CFA animals



In attempt to caracterise the effect of TAFA4 on sensory input integration within the lamina II in the spinal cord the neuronal discharge was first measured in naive animal (left) by *in vivo* electrophysiology. Following a local infusion of TAFA4 within lamina II TAFA4 does not alter the neuronal discharge. However in inflammed animals (right), there is a significant decrease of the neuronal discharge 40 min after the infusion of TAFA4 .

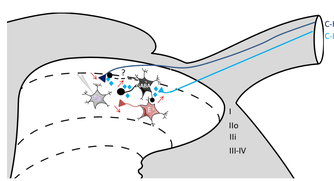
These results demonstrate that TAFA4 could modulate the integration of nociceptive input in inflammatory condition.

11- Effect of TAFA4 mediated though GABAergic transmission



In attempt to caracterise if TAFA4 acts in inflammatory condition though GABAergic transmission, we assessed the effects of TAFA4 on neuronal discharge in the presence of GABA receptors antagonists. With bicuculine and CGP, TAFA4 is no longer able to decrease the discharge evoked by Von Frey stimulation of the paw, indicating that the effect of TAFA4 on nociceptive transmission is mediated though GABAergic transmission.

Conclusion:



The objective of this project was to identify the functionnal consequences of TAFA4 on lamina Iii neuronal networks using a pharmacological approach.

Our results demonstrate that TAFA4 interacts with inhibitory interneurons. TAFA4 modulates presynaptically GABAergic transmission. As a consequence there is a decrease of evoked and spontaneous excitatory synaptic activity.

TAFA4 induces a shift of the excitation /inhibition balance toward increased inhibition. Thus GABAergic interneurons are the first relay for the modulation of painful information in the dorsal horn.