Institut de la Vision

10 YEARS OF FIGHTING BLINDNESS

Mu-opioid receptor, a therapeutic target for corneal inflammatory pain relief?

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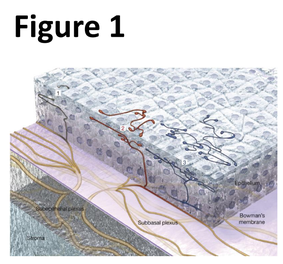
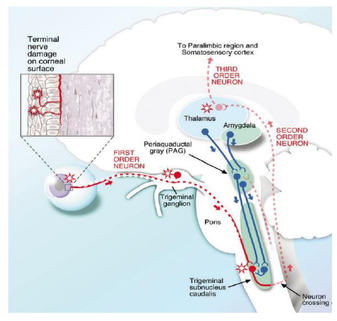
Background and aim

Ocular pain following corneal injury is frequently observed in clinic but the management of this debilitation condition remains today a therapeutic challenge in ophthalmology. The neural pathway implicated in corneal pain experience starts in the cornea, the most densely innervated epithelium by nociceptors in human body (Müller, *Exp Eye Res.*, 2003) . The corneal innervation is provided by sensitive neurons located in the ophthalmic branch (V1) of trigeminal ganglion (TG, Fig. 1) (Marfurt, *J Comp Neurol.,* 1987; Launay, *Exp Eye Res.*, 2015). Somatic pain can be modulated by exogenous and endogenous opioid peptides, which bind to mu-(MOR), delta-(DOR) and kappa-(KOR) opioid receptors widely expressed by the peripheral and central nervous system (Mansour, *J Comp Neurol.*, 1994). Morphine, opioid receptor agonist, remains as the gold standard for treating somatic pain, however, their systemic application give rise to unwanted secondary effects. To overcome these limitations arranging from its action in the central nervous system, topical morphine have been proposed for ocular pain (Peyman, *Br J Ophthalmol.,* 1994; Stiles, *Am J Vet Res.* 2003; Faktorovich, *J Refract Surg.*, 2010). These studies revealed that enkephalins, can be effective for reducing the hypernociceptive responses observed after corneal injuries, highlighting the idea that MOR and DOR are good target candidates to alleviate corneal pain. In this context, we recently demonstrated that increasing endogenous enkephalin levels reduced ocular pain in a opioid receptor-mediated manner (Reaux-Le Goazigo, 2019). However, there are no study evaluating the expression of MOR in cornea and ophthalmic branch of the trigeminal ganglion as well as its relation with TRPV1 at TG level or the potential analgesic effects of specific MOR selective agonists, such as DAMGO, on corneal pain relief. Therefore, the objective of the present work is the study of **MOR distribution in cornea and TG and its relation with TRPV1 both in naïve and under corneal inflammatory pain conditions.**

Figure 1



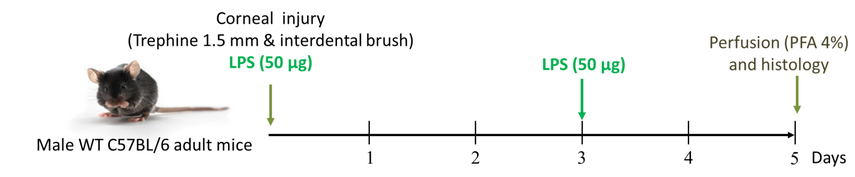
Rosenthal, *Ocul Surf.*, 2009.
Belmonte, *Ocul Surf.*, 2017.



Methodology:

Animals: Male WT C57BL/6 mouse and cynomolgus macaque (*Macaca fascicularis*)

Preclinical model of corneal inflammatory pain: Corneal scraping/LPS Model: Mice were submitted to a corneal scraping followed by of lipopolysaccharide (LPS) at day 1 and day 3.



All animal procedures were performed in strict accordance with institutional guidelines for the care and use of experimental animals.

Drugs: DAMGO (E7384, Sigma), Naloxone methiodide (N129, Sigma), capsaicin (M2028, Sigma)

Samples: TG and cornea were obtained from mice and macaque and used for histology.

Immunolabeling: Samples were incubated with antibodies against:

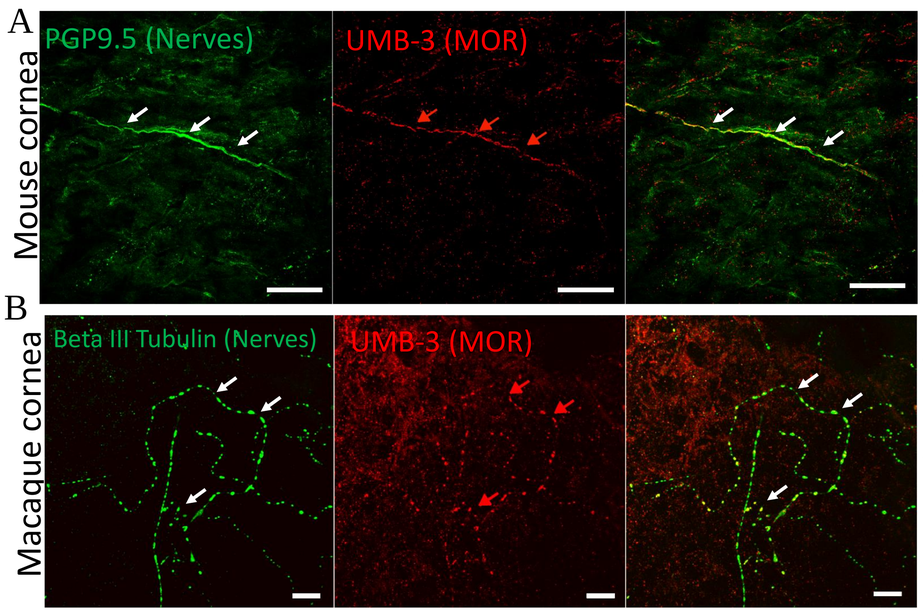
- Neuronal marker: PGP9.5 (ab8189, Abcam), beta III tubulin (ab78078, Abcam), Pan neuronal marker (Millipore, MAB2300)
- MOR (ab134054, Abcam) characterized by Lupp et al., 2011.

***In situ* hybridization:** RNAscope® method, MOR and TRPV1 mRNA probe, https://acdbio.com/.

Image acquisition: Confocal, epifluorescence and Nanozoomer microscopes.

Image analysis: ImageJ (Fiji) software, **statistical analysis**: Prism software

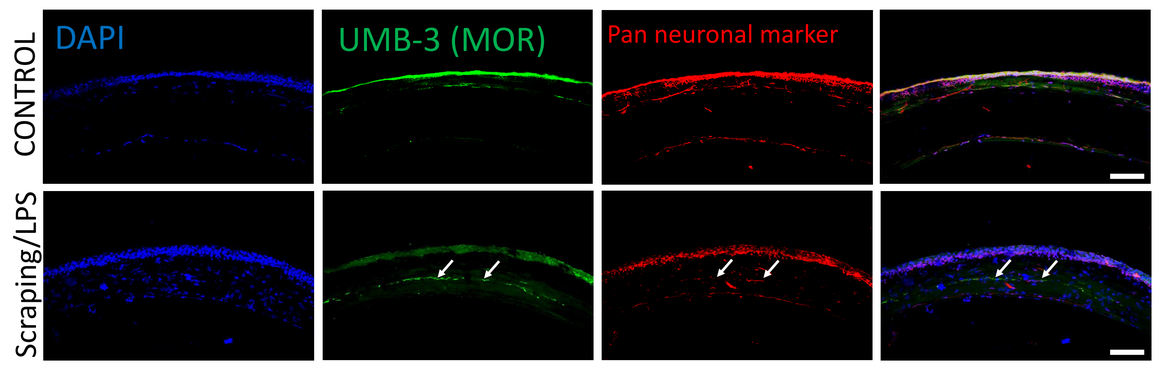
Result 1: MOR is detected in corneal nerves in WT mice and macaque



(A) Double-immunofluorescence against PGP9.5 (nerves, green) and MOR (red) . The technique was performed in whole-mount corneas from WT mice.

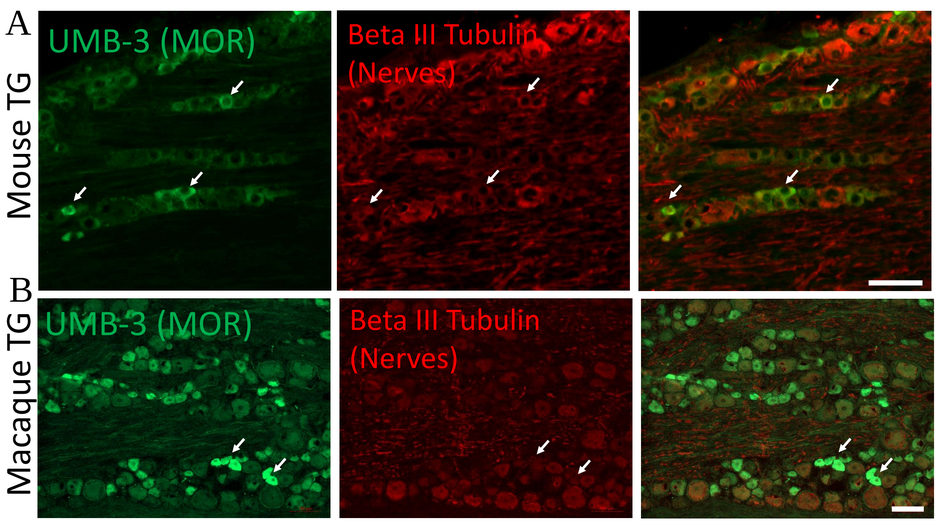
(B) MOR (red) and beta III tubulin (green) neuronal marker detection by immunolabeling on free floating sections from cynomolgus macaque cornea. Scale bars : 25 µm

Result 2: Corneal MOR expression is increased during inflammatory pain



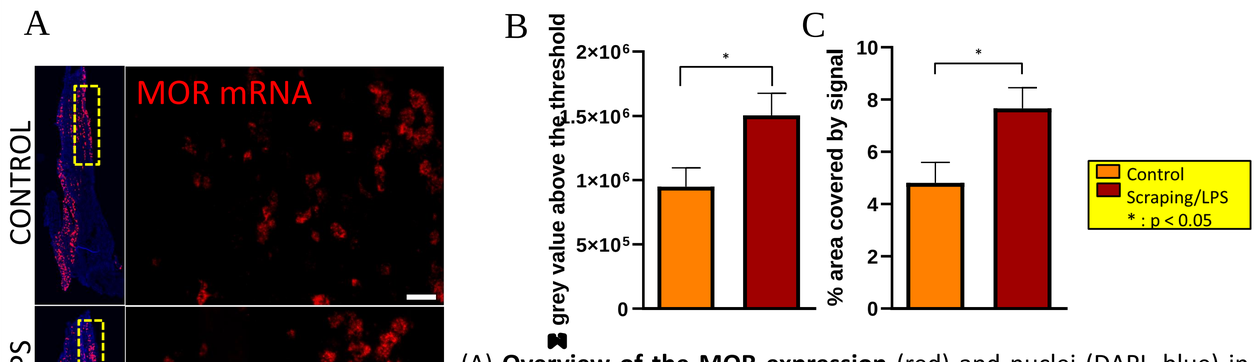
Immunostaining against MOR (red) and Pan neuronal marker (green) in frozen cornea sections of control mice and scraping/LPS pain model . DAPI staining (blue) shows the complete disorganization of the corneal layers in scraping/LPS cornea. MOR labeling is higher in corneal nerves of scraping/LPS animals (arrows). Scale bars: 50 µm

Result 3: MOR is expressed in primary afferent neurons of the ophthalmic branch (V1) of trigeminal ganglion in WT mice and macaque.



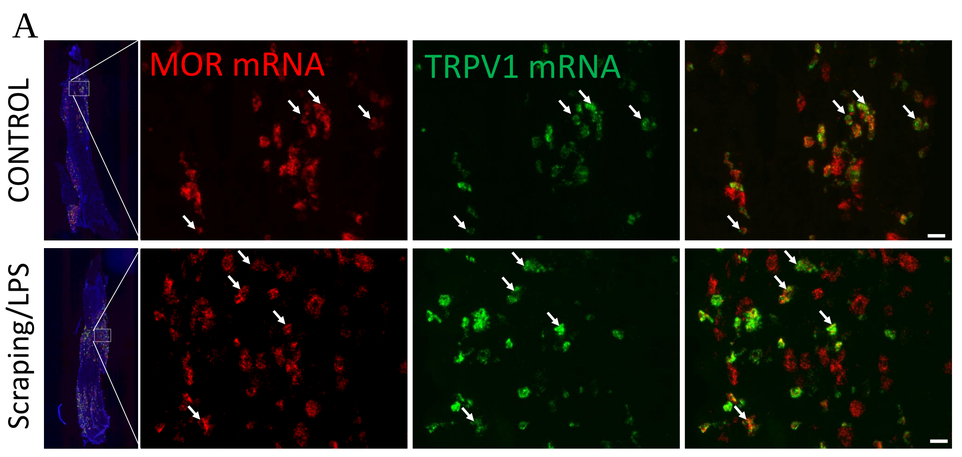
Immunostaining of MOR (red) and beta III tubulin (green) in frozen sections from mouse and macaque TG. Images show the presence of the MOR in primary afferent neurons at the level of ophthalmic branch (V1) of TG in both mice (A) and primates (B). Arrows show the of MOR-immunoreactivity in beta tubulin-positive neurons. Scale bars: 100 µm

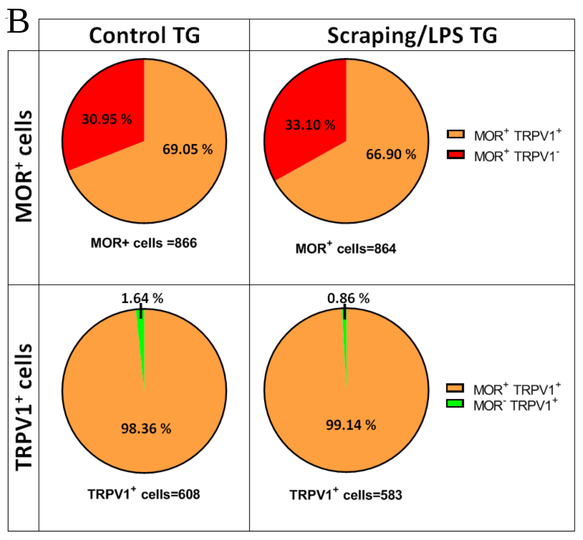
Result 4: MOR expression is upregulated under painful conditions in the ophthalmic branch of the trigeminal ganglion



(A) **Overview of the MOR expression** (red) and nuclei (DAPI, blue) in the TG from control vs scraping/LPS mouse revealed by RNAScope method. Dashed-line rectangle represents the ophthalmic branch of the trigeminal ganglion (V1). Scale bars: 50 µm. **(B, C) Quantitative analysis of MOR mRNA levels**. **(B) % area covered by signal.** Area with pixels whose grey level is above the threshold. **(C) Summation of all grey values** above the threshold. Both parameters showed an increase in MOR mRNA expression in Scraping/LPS animals. n=4 in each group. Unpaired t-test was applied for comparison between groups.

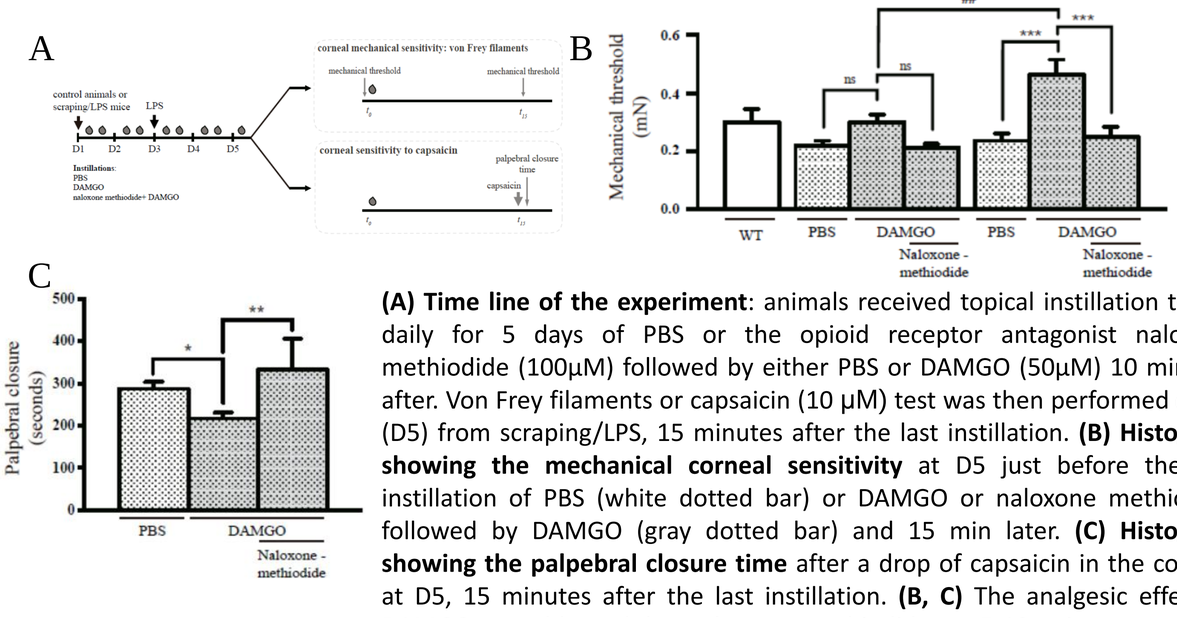
Result 5: MOR is expressed in TRPV1⁺ trigeminal neurons.





(A) Overview of the MOR (red) and TRPV1 (green) RNA expression in the TG revealed by RNAScope Method. Right: Extension of the zone delimited by a square in the left image, showing the expression of MOR and TRPV1 mRNA in the ophthalmic branch of trigeminal ganglion. Co-expression between MOR and TRPV1 (arrows). Scale bars: 50 µm. **(B) Quantification of the co-expression between MOR and TRPV1.** Almost all the TRPV1⁺ cells were also MOR positive. There were no significative differences between control and Scraping/LPS animals. Chi square was applied for comparison between groups.

Result 6: Repeated topical instillations of DAMGO decrease corneal pain



(A) Time line of the experiment: animals received topical instillation twice-daily for 5 days of PBS or the opioid receptor antagonist naloxone methiodide (100µM) followed by either PBS or DAMGO (50µM) 10 minutes after. Von Frey filaments or capsaicin (10 µM) test was then performed day 5 (D5) from scraping/LPS, 15 minutes after the last instillation. **(B) Histogram showing the mechanical corneal sensitivity** at D5 just before the last instillation of PBS (white dotted bar) or DAMGO or naloxone methiodide followed by DAMGO (gray dotted bar) and 15 min later. **(C) Histogram showing the palpebral closure time** after a drop of capsaicin in the cornea, at D5, 15 minutes after the last instillation. **(B, C)** The analgesic effect of DAMGO was blunted by naloxone methiodide. n=5-10 mice per group. Differences between groups were analyzed using 1-way ANOVA test. * : $p < 0.05$

Conclusions:

The present study shows for the first time the presence of MOR in corneal nerve fibers in mice and primate and reveals it as reliable target for ocular pain treatment with topical drugs. The experiments revealed an increase of MOR expression in the cornea and the TG during inflammatory pain, enhancing the availability of MOR at corneal nerve endings. Topical application of a MOR agonist, DAMGO, exerts analgesic effects in inflammatory corneal pain, postulating it as reliable topical treatment for corneal pain. Furthermore, activation of MOR in corneal neurons blunts the activation of capsaicin receptor TRPV1.

Altogether, this study provides novel information about the corneal and trigeminal distribution of MOR in control and pain conditions and highlights its key role in the therapeutic treatment of inflammatory ocular pain.

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