

# IMMUNOTHERAPY TO TREAT BLADDER PAINFUL SYNDROME

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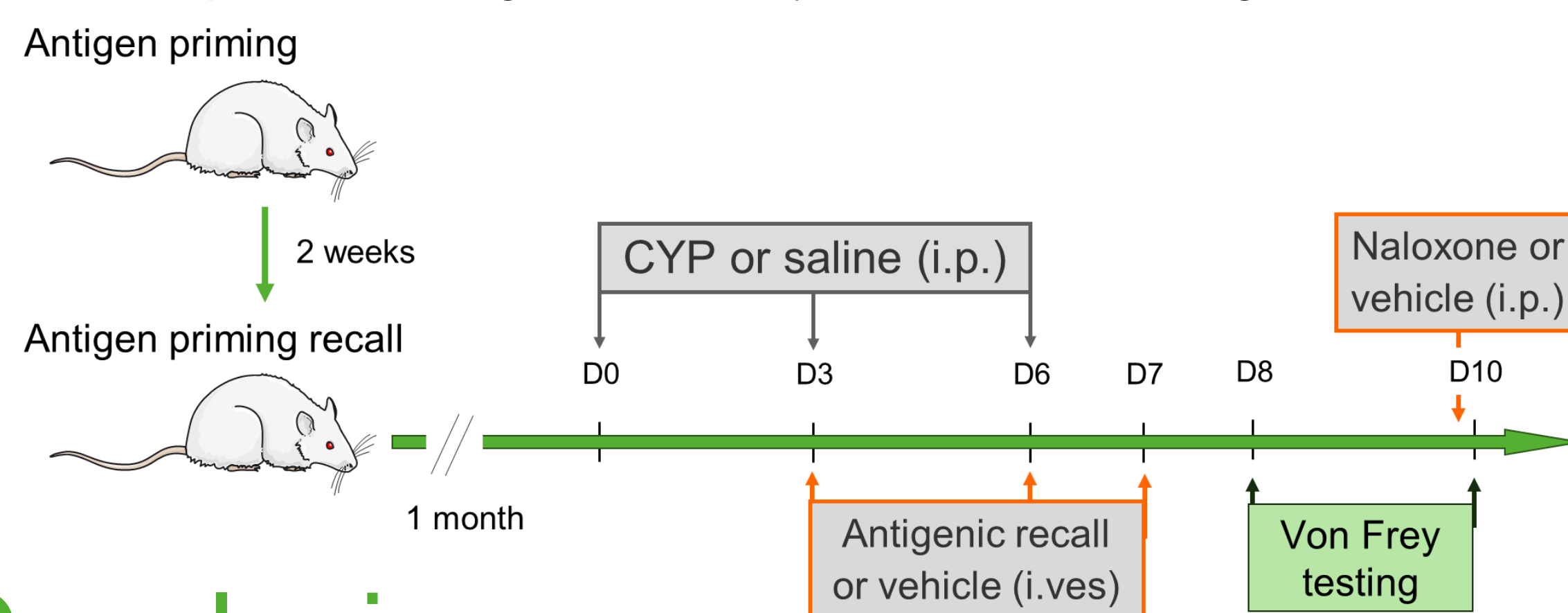
## Objectives

IC/BPS is a chronic disorder mainly characterized by bladder inflammation and pelvic pain. The efficacy of current treatments for IC/BPS is still largely insufficient, in particular for pain relief. Therefore, an anti-nociceptive approach with limited side-effects would offer a major therapeutic progress. We propose a new anti-nociceptive strategy exploiting the opioid-mediated analgesic properties of T lymphocytes <sup>(1)</sup>, to treat bladder pain.

**This strategy is based on several mucosal antigens recall of previously vaccinated rats in a chronic model of cyclophosphamide (CYP)-induced cystitis, a well established model for bladder inflammation and vesical pain<sup>(2)</sup>.**

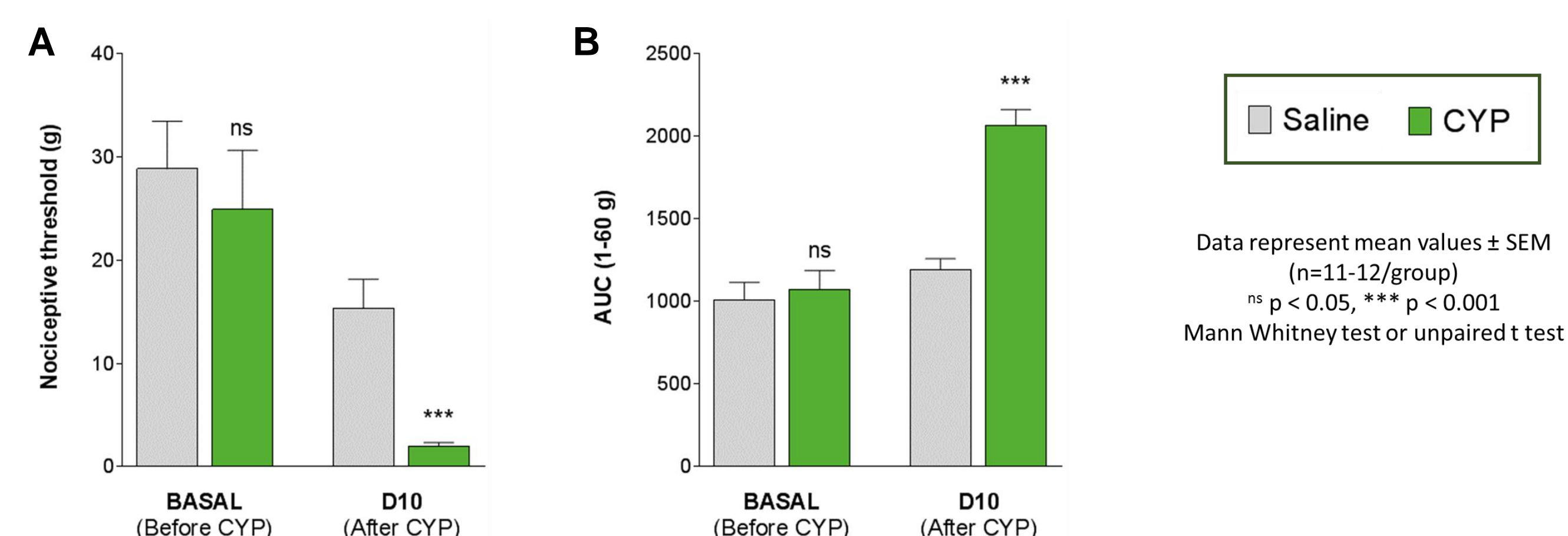
## Methods

- **Animals:** Female Sprague-Dawley rats (~ 7 weeks old).
- **Antigen priming** was performed intraplantar for ovalbumin (OVA) antigen or subcutaneous for BCG vaccine.
- **Chronic cystitis** was induced by 3 injections of CYP (40 mg/kg, i.p., D0, D3 and D6), control rats receiving saline (5 mL/kg) under the same conditions.
- **Antigenic recall** was performed intravesically, 3, 6 and 7 days after the first CYP injection with specific cognate antigen or irrelevant antigen. For OVA experiment, OVA was the cognate antigen and BSA was the irrelevant antigen (Irr. Ag). For BCG experiment, BCG medac was the cognate antigen, and OVA was the irrelevant antigen.
- **Nociceptive behavioral response to mechanical stimuli** was measured using calibrated von Frey filaments of binding forces ranging from 1 to 60 g. Vesical sensitivity of the lower abdomen to von Frey mechanical stimulation was determined 8 and 10 days after the first CYP-injection. On day 10, 30 min before pain evaluation, rat received naloxone-methiodine (10 mg/mL, i.p.)
- Nociceptive threshold was defined as the von Frey force in grams at which a first score of at least 1 was obtained. Area under the curve (AUC) has been calculated by plotting individual nociceptive scores against von Frey forces from 1 to 60 g.



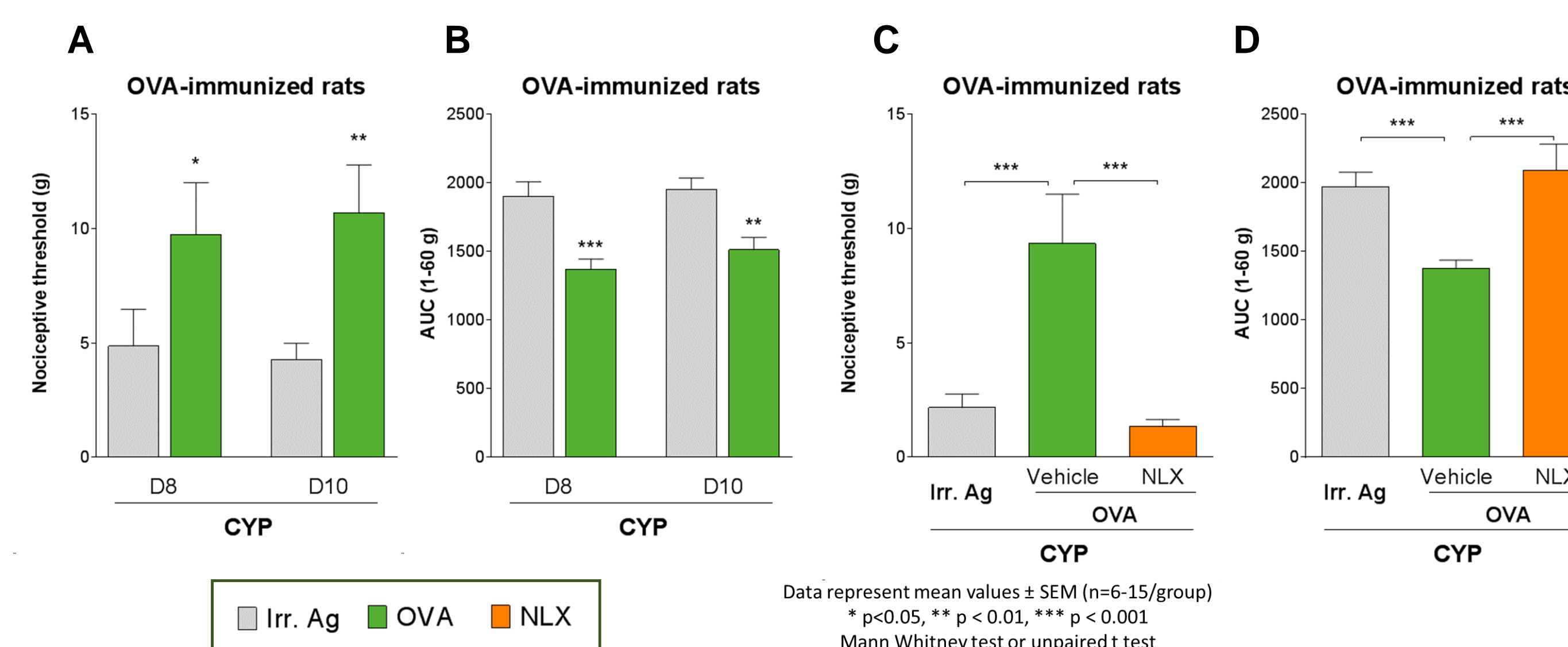
## Results

### 1. Chronic administration of CYP induces vesical pain up to 10 days<sup>(2)</sup>



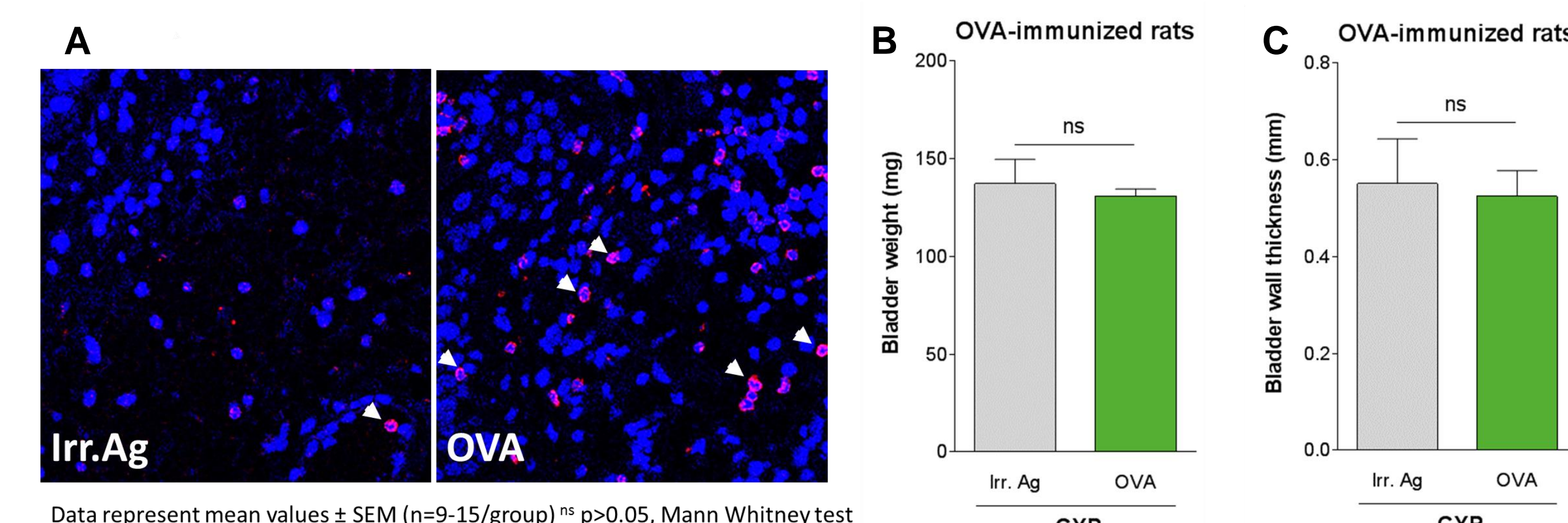
In naive rats, three administrations of CYP induced a decrease in nociceptive threshold (Fig. 1A) and an increase in AUC 1-60 g (Fig. 1B) compared to saline rats.

### 2. Intravesical recall with OVA alleviates CYP-induced chronic vesical pain via endogenous opioids pathway



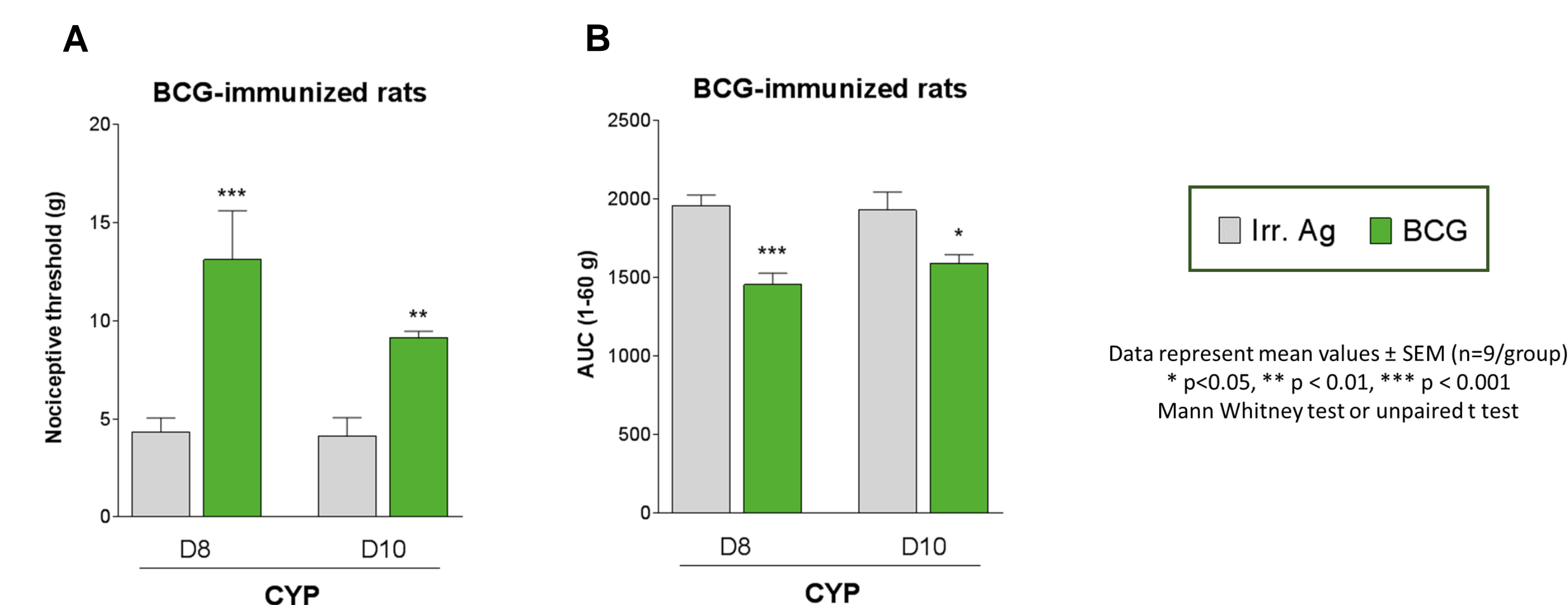
Compared to irrelevant antigen, OVA recall significantly increased nociceptive threshold (Fig. 2A) and decreased AUC (Fig. 2B) in CYP treated-rats. Naloxone (an opioid receptor antagonist) treatment reversed this analgesic effect (Fig. 2C and 2D).

### 3. Antigenic recall with OVA increases T lymphocytes, without any changes in inflammatory parameters



In bladder section of OVA immunized rat, OVA recall showed an increase in T lymphocytes density compared to irrelevant antigen (Fig. 3A). No inflammatory effect was observed as reflected by the lack of effect on bladder weight or bladder wall thickness (Fig. 3B and 3C, respectively).

### 4. Intravesical administration of BCG induces analgesic effects



Compared to irrelevant antigen, BCG recall significantly increased nociceptive threshold (Fig. 4A) and decreased AUC (Fig. 4B) in CYP treated-rats. This result demonstrated an analgesic effect of antigenic recall with a commonly used vaccine.

## Conclusions

Three CYP administrations induced chronic bladder pain and inflammation <sup>(2)</sup>. In this model, a secondary T cell response to vaccine antigens locally administered into inflamed mucosa, prevents from pain. This analgesic effect associated with the recruitment of T lymphocytes induced by intravesical OVA was reversed by naloxone-methiodide (a general opioid receptor antagonist unable to cross blood-brain barrier), indicating that vesical pain inhibition was dependent on the local release of endogenous opioids. These effects were also observed using BCG vaccination providing new insight into BCG therapy for relieving bladder pain in IC/BPS patients.

**Considering the universality of BCG vaccination programs worldwide, our results open new therapeutic avenue in the treatment of chronic bladder pain. This strategy might be rapidly developed since the therapeutic protocol consisting in intravesical administration of live mycobacterium into the bladder is already commonly used for bladder cancer treatment.**

## References

- (1) Boué et al. Gastroenterology, 146:166–175, 2014.
- (2) Chabot S, et al., 2016. 31<sup>st</sup> Annual EAU Congress, N° 420 (Munich, Germany)