



## EXPLORING THE ANALGESIC SYNERGY OF OPIOIDS AND NSAIDS IN POSTOPERATIVE PAIN MODULATION USING BEHAVIORAL ASSESSMENTS IN MICE

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### INTRODUCTION:

After surgery, over 50% of patients experience significant, persistent pain[1], with some individuals developing chronic discomfort after procedures like cesarean sections (15%)[2]. or hysterectomies (32%) [3]. Ibuprofen and morphine stand out as prominent choices for postoperative pain management. Ibuprofen, an NSAID, offers anti-inflammatory and analgesic benefits. Morphine provides strong relief for moderate to severe pain. Their pain relief efficacy varies with pain type and severity. Improving postoperative pain management is imperative to address immediate discomfort and to prevent long-term surgical complications.

### OBJECTIVE:

To investigate the distinct effects of opioid and NSAID analgesics, as well as their combination, on post-operative pain management by analyzing differences in sensory hyperalgesia, pain at rest and movement-evoked pain behaviors.

### MATERIALS AND METHODS:

**Animals:** Female CD-1 mice (24-30 g) were used.

**Laparotomy:** After undergoing gaseous anesthesia (isoflurane), shaved and properly disinfected in the abdominal area, the surgical procedure was performed. Laparotomy consisted of a 1.5 cm horizontal incision in the lower abdomen until it reached the abdominal cavity. Appropriate sutures were used to close the abdominal musculature, peritoneum, and skin. Drug evaluation was performed 3.5 h after surgery.

### Evaluation of sensory hypersensitivity:

Sensory hypersensitivity was evaluated using the von Frey test applied 2 mm from the surgical wound.

### Assessment of pain at rest:

Pain at rest was assessed by quantification of facial expressions classified as pain by an artificial intelligence algorithm in 15 min video recordings.

### Assessment of pain on movement:

Movement-evoked pain was assessed by measuring the exploratory activity with an infrared actimeter that quantifies time spent rearing (s) during 15 min.

### Drug administration:

Ibuprofen (8-32 mg/kg), Morphine (0.125-0.5 mg/kg), were administered subcutaneously 1 hour before the pain evaluation

### Statistics:

GraphPad Prism 8 program was used to perform a one-way analysis of variance(ANOVA) followed by Bonferroni. The differences were considered significant when  $p < 0.05$ .

### Bibliography:

- [1]Rose,J.et al.Lancet Glob Health 3 Suppl 2, S13–S20 (2015)
- [2] Jin, J., et al. BMC Anesthesiol 16, 99 (2016)
- [3] Brandsborg, B., et al. Anesthesiology (2007)

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### ASSESSMENT OF SENSORY HYPERALGESIA

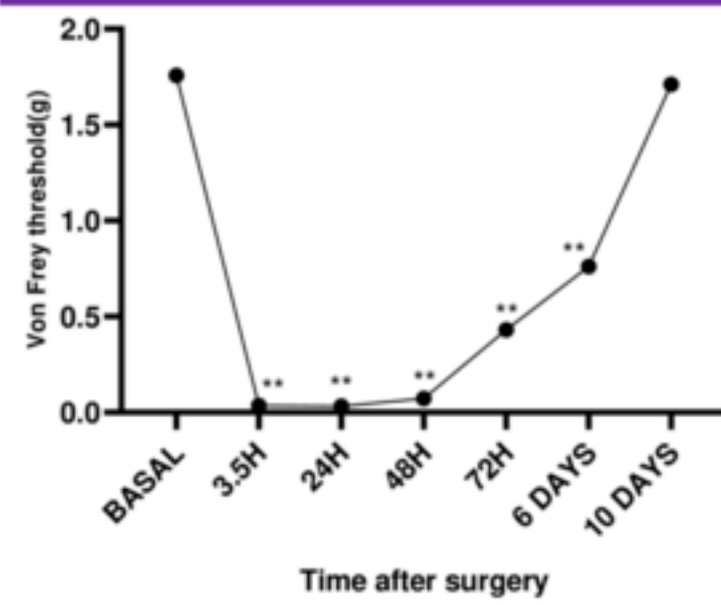


Fig. 1. Temporal course of sensory hypersensitivity using the von Frey test. The graph shows the timecourse values of control sham animals(basal) and laparotomized animals. Mechanical threshold drops to 0.06 g at 3.5 h after laparotomy and is maintained until day 3, after which it begins to recover. On day 10 it returns to normal sensory levels. Differences between control (sham) and animals with laparotomy: \*\* $p < 0.01$  (1-way ANOVA followed by Bonferroni).

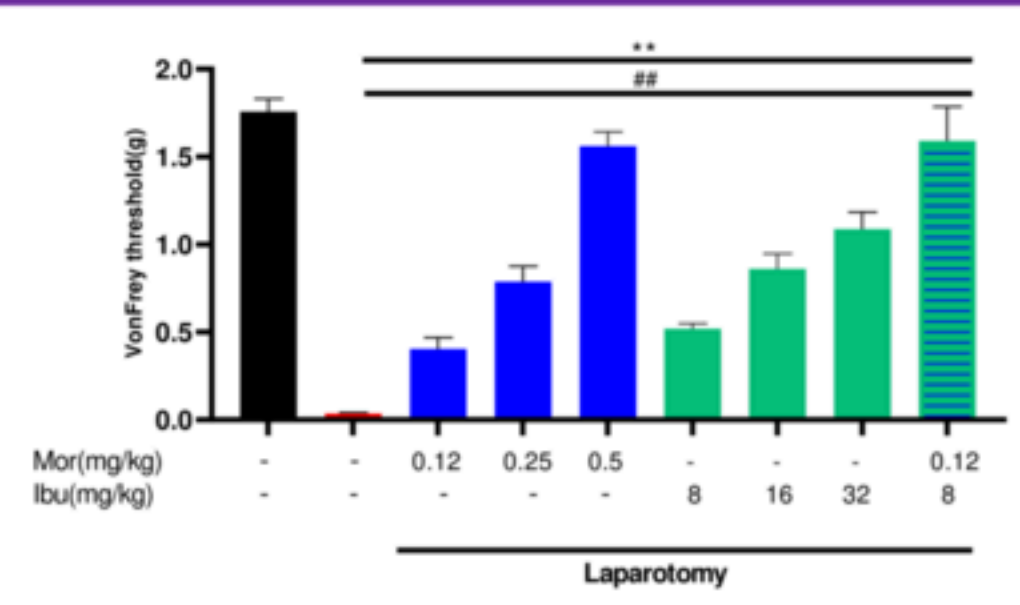


Fig. 2. Effect of Ibuprofen (Ibu) and Morphine (Mor) in laparotomy induced mechanical allodynia evaluated by the von Frey test (3.5 h after surgery). Differences between control animals and animals with laparotomy: \*\* $p < 0.01$ ; and differences between the values of laparotomized animals treated with Morphine, Ibuprofen or combination Morphine + Ibuprofen, 1 h before the evaluation: ## $p < 0.01$  (1-way ANOVA followed by Bonferroni).

**LAPAROTOMY SIGNIFICANTLY INDUCES MECHANICAL ALLODYNIA. ADMINISTERING MORPHINE AND IBUPROFEN MITIGATES THESE EFFECTS IN A DOSE-DEPENDENT MANNER. NOTABLY, COMBINING OPIOIDS WITH IBUPROFEN RESULTS IN A SYNERGISTIC INTERACTION THAT ENHANCES THE ANALGESIC EFFICACY OF BOTH DRUGS.**

### ANALYSIS OF PAIN AT REST USING ARTIFICIAL INTELLIGENCE

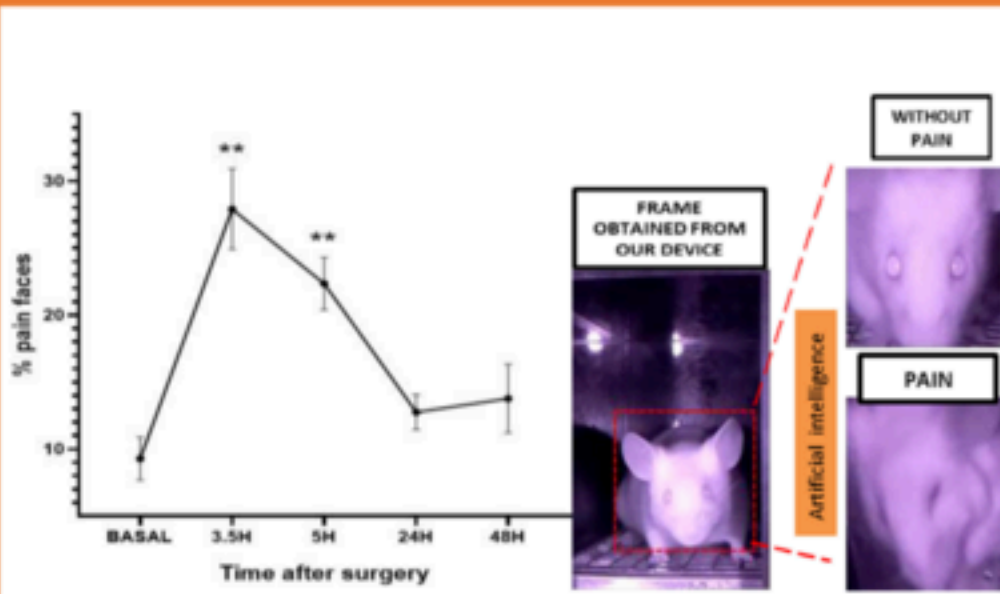


Fig. 3. Temporal course of pain at rest estimated by quantification of facial expressions classified as pain. The graph represents the timecourse of laparotomized animals. Each point represent the percentage of frames that our artificial intelligence algorithm classified as pain related expressions in 15 min of recording. Differences between control animals (basal) and animals with laparotomy: \*\* $p < 0.01$  (1-way ANOVA followed by Bonferroni).

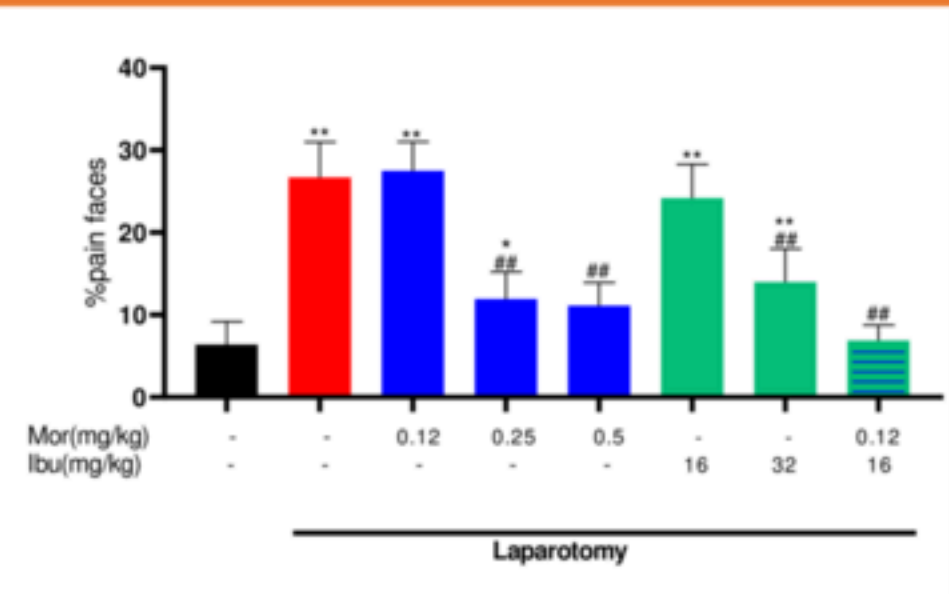


Fig. 4. Effect of Ibuprofen (Ibu) and Morphine (Mor) in pain at rest measured by facial expressions of pain. Bars represent the percentage of frames that our artificial intelligence algorithm classified as pain related expressions in 15 minutes of recording, evaluated 3.5 hours after surgery. Differences between control animals(sham) and animals with laparotomy: \*\* $p < 0.01$ ; and between the values of laparotomized animals treated with Morphine, Ibuprofen or combination Morphine + Ibuprofen, 1 h before the experiment: ## $p < 0.01$  (1-way ANOVA followed by Bonferroni).

**CAUSES PAIN AT REST. THE ADMINISTRATION OF MORPHINE AND IBUPROFEN REVERSED THESE EFFECTS IN A DOSE-DEPENDENT MANNER. THE COMBINATION OF OPIOIDS WITH IBUPROFEN PRODUCED A SYNERGISTIC EFFECT, ENHANCING THE ANALGESIC EFFICACY OF BOTH DRUGS.**

### ASSESSMENT OF MOVEMENT-EVOKED PAIN

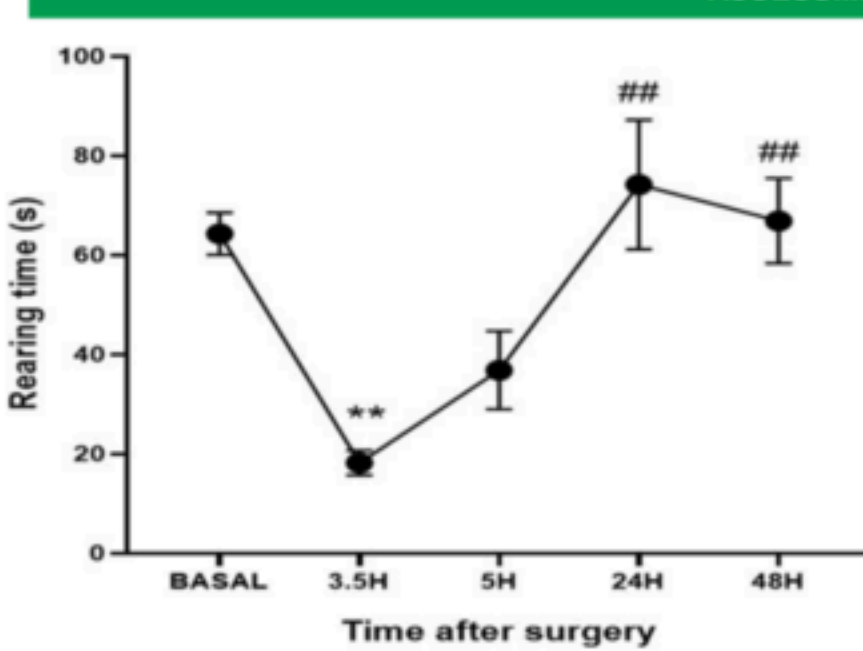


Fig. 5. Temporal course of locomotor activity, used as an index of movement-evoked pain. The graph represents the timecourse of laparotomized animals. Each point represents the value of time spent rearing during an evaluation of 15 min. Differences between control animals (basal) and animals with laparotomy: \*\* $p < 0.01$ ; differences between 3.5 h after laparotomy and other times after laparotomy: ## $p < 0.01$  (1-way ANOVA followed by Bonferroni).

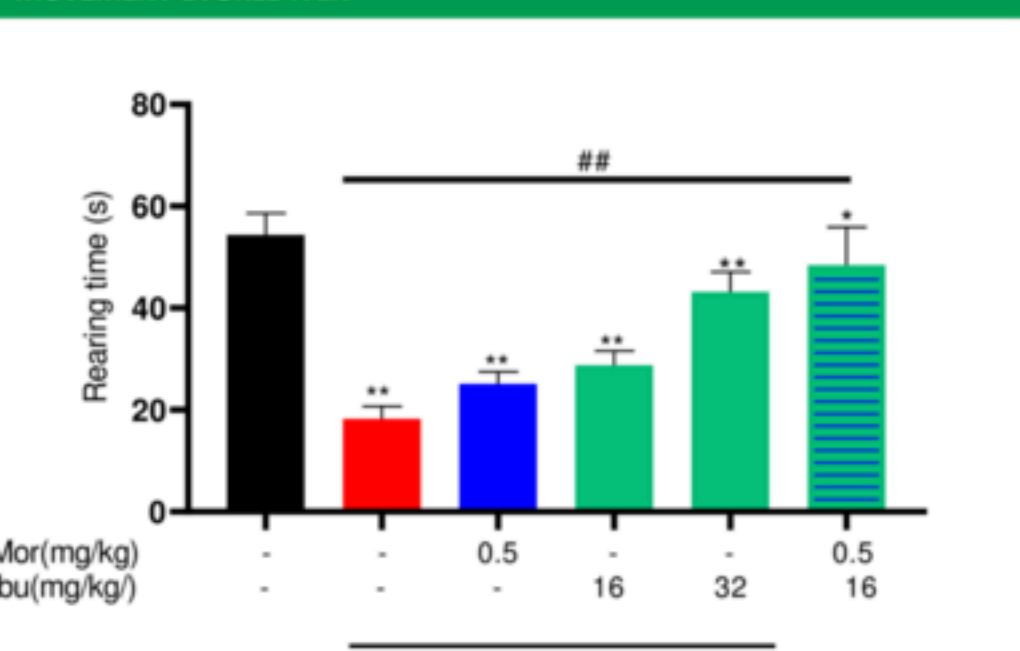


Fig. 6. Effect of Ibuprofen(Ibu) and Morphine(Mor) in movement-evoked pain. Bars represent the time spent rearing after placing the animals in an infrared actimeter for 30 min. The experiment is performed 3.5 h after surgery and 1 h after subcutaneous administration of the drug. Differences between control animals (sham) and animals with laparotomy: \*\* $p < 0.01$ ; differences between the values of laparotomized animals treated with Morphine, Ibuprofen or combination Morphine + Ibuprofen, 1 h before the test ## $p < 0.01$  (1-way ANOVA followed by Bonferroni).

**OUR STUDY SHOWS THAT OPIOIDS AND NSAIDS EFFECTIVELY REDUCE POST-OPERATIVE PAIN, WITH THEIR COMBINATION OFFERING ENHANCED RELIEF AND REDUCING MECHANICAL ALLODYNIA, PAIN AT REST AND MOVEMENT-EVOKED PAIN MORE THAN EITHER ALONE. THIS SUGGESTS THAT A COMBINED APPROACH COULD SIGNIFICANTLY IMPROVE PAIN MANAGEMENT AND PATIENT RECOVERY IN POST-OPERATIVE CARE.**

**LAPAROTOMY PRODUCES A DECREASE IN LOCOMOTOR ACTIVITY. THIS DECREASE IS REVERSED BY IBUPROFEN AND MORPHINE IN A DOSE-DEPENDENT MANNER. THE COMBINATION OF OPIOIDS WITH IBUPROFEN PRODUCED A SYNERGISTIC EFFECT, ENHANCING THE ANALGESIC EFFICACY OF BOTH DRUGS.**

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