

# PHARMACOLOGICAL CHARACTERIZATION OF THE BEHAVIOURAL REGISTRATION SYSTEM LABORAS IN THE DETECTION OF FORMALIN-INDUCED NOCICEPTION

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## Introduction

Laboratory Animal Behaviour Observation, Registration and Analysis System (LABORAS™) is a newly developed apparatus for automated objective behavioral analysis in rodents. The formalin test is commonly used for evaluation of potential analgesic efficacy. However, manual analysis of pain behavior in the formalin model is time consuming and left to subjective influences of the experimenter.

## Aims of study

We evaluated the reliability of the LABORAS system in the formalin test by comparing data obtained automatically with the data obtained by manual scoring (licking time). In addition, we also validated the LABORAS system for efficacy against reference analgesic drugs.

## Materials & Methods

**Animals and drug administration:** Male Sprague-Dawley rats (n=10/group) received in separate experiments: Vehicle or Morphine (3.13, 6.25, 12.5 µmol/kg, SC); Pregabalin (30, 100, 300 µmol/kg, PO); MTEP (37.5, 75, 150 µmol/kg, PO); Naproxen (10, 30, 100 µmol/kg, PO); Retigabine (10, 30, 100 µmol/kg, PO); Flupirtine (50, 100, 200 µmol/kg, PO) prior to formalin injection.

**Formalin test:** Formalin (2.0%, 100 µl, sc) was injected into the dorsal side of the left hind paw.

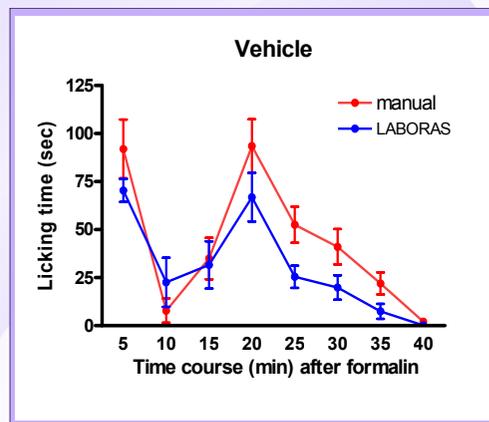
**Nociceptive behavior:** The nociceptive behavior was analyzed either by manual recording of the time spent licking of the formalin injected paw or automatically using the LABORAS system in connection with a software designed to detect formalin-induced behavior (paw licking). The formalin test consists of two phases: phase 1 (0-5 min) and phase 2 (15-35 min).

## Experimental set-up

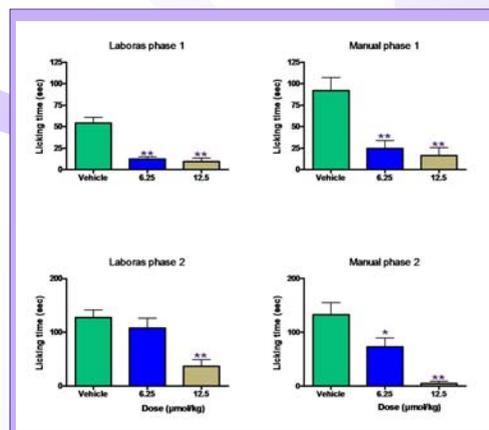


## Results

**Fig 1. Biphasic response pattern after formalin injections detected by LABORAS and after manual scoring of data in control animals**



**Fig 2. Morphine reduced formalin-induced responses in the LABORAS as well as after manual scoring**



The results are presented as mean ± SEM, n = 10/group. \*p<0.05, \*\*p<0.01, One-Way ANOVA, followed by Newman-Keuls test.

**Table 1. Pharmacological validation of LABORAS system in the formalin test**

Compound Dose - µmol/kg	Formalin - Manual scoring Efficacy max (%) MED (minimum effective dose - µmol/kg)	Formalin - LABORAS Efficacy max (%) MED (minimum effective dose - µmol/kg)
Morphine 3.1, 6.25, 12.5 (SC)	50-80 % (6.25µmol) ~100% (12.5 µmol) 6.25 µmol/kg (ph1-2)	60-80%(6.25µmol) ~100% (12.5 µmol) 3.1 µmol/kg (ph1); 6.25µmol/kg (ph2)
Pregabalin 30, 100, 300 (PO)	67% 300 (ph2) Not active in phase 1	90% 30 (ph2) Not active in phase 1
MTEP 37.5, 75, 150 (PO) mGluR5 antagonist	50% (ph2) 75 (phase 2) Not active in phase 1	40% (ph2) 75 (phase 2) Not active in phase 1
Naproxen 10, 30, 100 (PO)	Not active (phase 1-2)	Not active (phase 1-2)
Flupirtine 50, 100, 200 KCNQ2/3 blocker	No data	50% (phase 1-2) 200 (phase 1-2)
Retigabine 10, 30, 100 (PO) KCNQ2/3 blocker	40% (phase 1-2) 100 (phase 1-2)	40% (phase 1-2) 100 (ph1) 30 (ph2)

## Summary

- We recorded a typical biphasic nociceptive behavior after formalin injection using either the LABORAS™ system or observers scored data.
- No statistical difference was observed between data generated by LABORAS™ data or the mean licking time recorded by the experimenter in either phase 1 or 2 of the formalin test.
- After pretreatment with morphine, retigabine or flupirtine LABORAS™ detected a significant dose-related reduction in nociception in both phases of the formalin test. Pregabalin and MTEP significantly reduced nociceptive behavior in phase 2 only, whereas naproxen was inactive.

## Conclusion

- LABORAS™ system provides a fast and reliable assessment of analgesic effects of compounds in the formalin test.