Formalin Test (rats)

LABORAS™ Let the computer score!

The automated Formalin Test developed by Metris is a special module for LABORAS for automated recording and analysis of the hind limb licking response after local injection with formalin into one of the hind limbs. This module replaces and standardizes traditional time-consuming human observations. Researchers from several companies conclude that the LABORAS hind limb licking algorithm provides a fast, consistent and reliable measurement for the assessment of analgesic effects of compounds.

INTRODUCTION

The automated Formalin Test was developed and validated together with Gedeon Richter Ltd. Neuropharmacology Department and was later validated by several other pharma companies and CRO's such as Merck, AstraZeneca, Amgen and Allergan. The Formalin Test module for LABORAS™ makes it possible to measure the Formalin related pain behavior **in freely moving** rats. By using LABORAS™ is also possible to automatically analyze several normal behaviors of the rat too (such as locomotion, immobility, rearing, grooming, eating and drinking). LABORAS™ is the only automated system that measures the Formalin Pain response in freely moving rats and determines also the other behaviors of the rats.

To verify the performance of the formalin response detection algorithm a special experiment was prepared. During data acquisition with LABORAS™, a video camera recorded the rat from the long side of the cage. After data acquisition, the pain response of the rats was automatically analyzed with LABORAS™ by classifying hind limb licking episodes and duration using the special Formalin Test algorithm. The video recording of the measurement was independently scored by three observers. The results of the LABORAS™ analysis and the observers' scores were compared.

The LABORAS™ hind limb licking module was pharmacologically validated by using different doses of four reference analgesic compounds (morphine, MK-801, lacosamide, pregabalin).

RESULTS

Functional validation

No significant difference could be detected between the LABORAS™ hind limb licking module and the mean of the three human observers (Figures 1, 2, 3 4 and 5).

Pharmacological validation

The LABORAS™ hind limb licking module revealed ED_{50} values that were in accordance with previous findings.

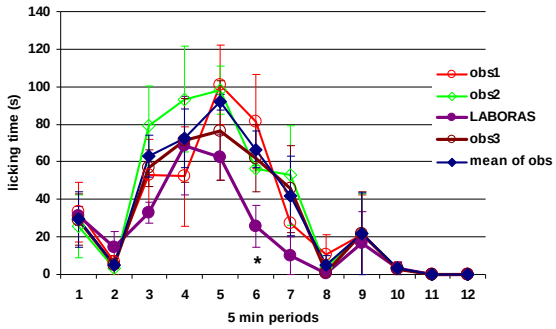

Fig. 1 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with **saline** (n=3-3).

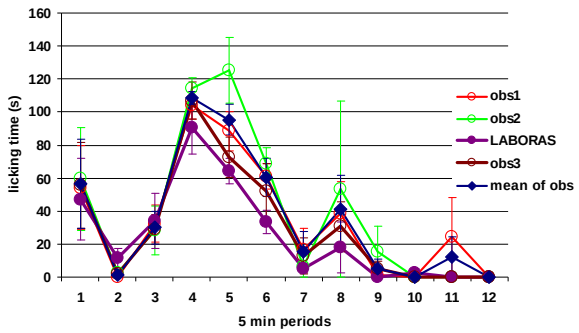

Fig. 2 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with **vehicle** (n=3-3)

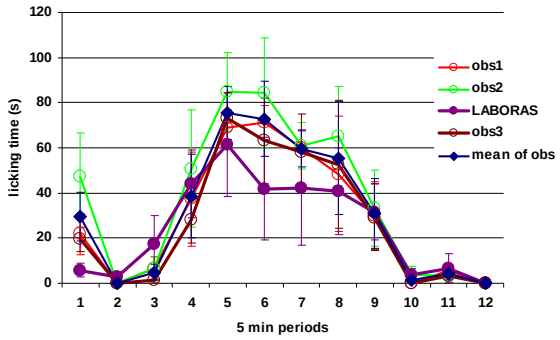

Fig. 3 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with **treatment A** (n=3-3)

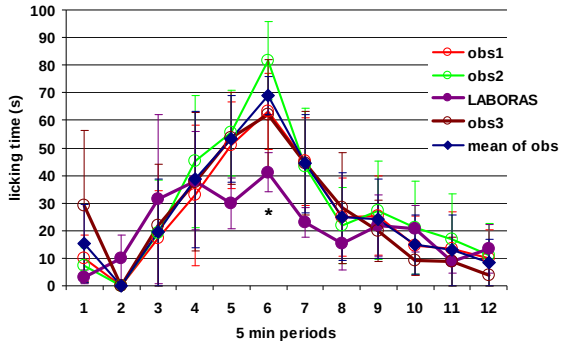

Fig. 4 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with **treatment B** (n=3-3)

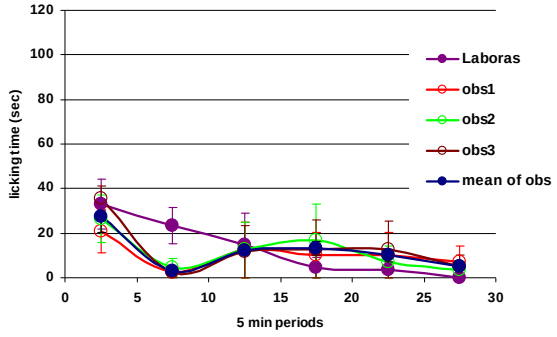

Fig. 5 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with **treatment C** (n=3-3)

DISCUSSION AND CONCLUSIONS

It is well established in the literature that the formalin hind limb licking response curve is biphasic. After formalin injection an increased time of licking can be measured until 5 or 10 minutes which is called phase 1. Between 10 and 15 min licking behavior almost completely disappears, and after 15 min following formalin injection phase 2 can be observed wherein hind limb licking is increased again up to 30-35 min. The results are in correspondence with these previous findings. Either LABORAS formalin measurement or observers' scores resulted in the biphasic formalin response curve mentioned above.

When evaluating the LABORAS™ formalin result and observers' scored data it was clear that the difference between observers and LABORAS is not higher than can be measured between observers. The highest difference can be found at the descending part of 2nd phase. This is the part of pain related licking behavior where animals are licking their hind limb frequently for a short time. There is a possibility of a small systematic error made by LABORAS™ or by observers, as well. Probably, the longer reaction time of humans can make such a difference. The difference is small and does not disturb reliable assessment of inhibition by a drug which was the main goal of the test.

Dr. Kristina Kostás *et al.*[1], Gedeon Richter Ltd., Dept. Neuropharmacology, Budapest, Hungary:

"Our conclusion is that the LABORAS™ hind limb licking software provides a fast and reliable measurement to assess the effects of analgesic compounds. The ED_{50} values measured with the LABORAS™ hind limb licking module are in agreement with previous findings".

More information

For more information or other behaviors automatically detected by LABORAS, please visit the LABORAS page on our website.