LABORAS™: An automated measure of formalin paw licking behaviour and concurrent locomotor activity in rats

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1. Introduction

The formalin paw test is a model of continuous (tonic) pain response to local injection of formalin into the paw. Formalin elicits a biphasic response. The initial response is derived from direct stimulation of nociceptors resulting in C-fibre firing (Puig and Sorkin, 1996). Subsequently, a delayed inflammatory response is observed in response to formalin-induced peripheral tissue damage. Concurrently, the intense barrage of C fibre firing causes central sensitisation or wind up of the dorsal horn pain signalling pathways (Dubuisson and Dennis 1977; Tjolsen et al., 1992).

The nociceptive response to formalin injection can be tracked by scoring the duration and frequency of licking of the affected limb. An initial response fades over 5-10 min before a second phase is observed lasting for around 30-40 min (Wheeler-Aceto et al., 1990). Indeed, hind paw licking is considered an optimal single parameter for tracking the pain response to intraplantar formalin injection (Abbott et al., 1995; Heughan and Sawynok 2002).

Although the formalin test does not model any particular pathological pain state, the involvement of multiple mechanisms allows detection of a wide range of drug classes with different modes of action in a relatively rapid screen. More recently it has been noted that the pharmacological sensitivity of the formalin paw test for anticonvulsants at least, correlates well with responses in the Bennett and Xie (chronic constriction injury model; CCI) model of neuropathic pain (Vissers et al., 2006). The formalin paw test has therefore been widely used as a pre-screen for activity in models of neuropathic pain (Blackburn-Munro and Erichsen 2005). The formalin paw test is typically carried out via labour intensive and subjective experimenter/observer scoring of behavioural response.

LABORAS™ (Laboratorium Animal Behaviour Observation, Registration and Analysis System, Metris B. V. Hoofddorp, The Netherlands) offers a method of monitoring rat formalin paw licking activity automatically. LABORAS™ is a fully automated device for the differentiation between several behavioural elements and locomotor activity of individually housed rats or mice. The system consists of sensor platforms and an analogue to digital converter linked to a computer. Each sensor platform consists of a triangular shaped sensing plate, which rests on two orthogonally placed force transducers and a third fixed point, attached to a heavy base plate. Each force transducer transforms the mechanical vibrations caused by animal movement into electrical signals which are amplified, filtered and digitized by the LABORAS™ system. Characteristic movements have their own unique patterns in terms of vibrational signals which the LABORAS™ software is able to interpret. The LABORAS™ cages consist of two halves to allow detection of small vibrations. Thus, the base is separate from the sides and lid and sits directly on the sensor platform, whilst the sides and the lid (including food hopper and water bottle if required) hang from supports on the base plate.

The primary measures recorded by LABORAS™ for the formalin paw model are hind limb licking duration and frequency. Locomotion parameters are also monitored and are broken down into maximum speed, average speed when moving, average speed over the whole time bin and distance travelled.

The aims of this study were to compare the LABORAS™ system with a human observer on paw licking behaviour, and to assess whether LABORAS™ dissociates an effect on hind paw licking from a potential effect on locomotor activity following administration of gabapentin or morphine in the rat formalin paw lick model.

The LABORAS™ system

2. Methods

Male Sprague-Dawley (SD) rats (approximately 200g at the time of dosing, Harlan UK) were group housed in cages of 6 prior to the study. Animals were maintained under a 12 h light/dark cycle, where temperature and humidity were controlled according to UK Home Office guidelines. Animals were allowed free access to food and water.

Animals received a 50 µL, subplantar injection of formalin (2.5% v/v in saline) and were placed immediately into the LABORAS™ cages without sawdust or bedding where behavioural monitoring was then initiated. After 40 minutes, the animals were removed and the cages were wiped clean after application of "Trigene" disinfectant spray (Medichem International, Sevenoaks, UK) ready for the next batch of six rats.

The behaviour of 26 animals was monitored after subplantar administration by LABORAS™ and also video recorded to allow subsequent assessment of hind paw licking duration by human observer. Correlation analysis between LABORAS™ and human observer was then carried out on 204 5-minute time bins of paw licking duration data.

A separate study was carried out in which gabapentin (12.5, 25 and 50 mg/kg ip) or vehicle (0.9% saline) was administered 2 hours pretest, whilst morphine (5 mg/kg ip) or vehicle was administered 20 min pretest. All animals were counter-dosed. Formalin paw licking and locomotor activity were monitored by LABORAS™. Acute phase responses were analysed 0-10 min post formalin, and second phase responses were analysed 15-30 min post formalin administration.

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3. Results and Discussion

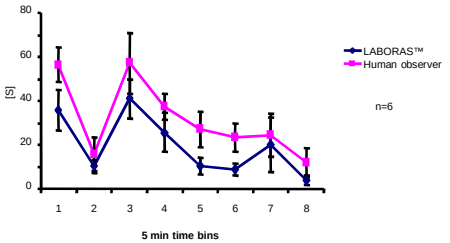
Correlation analysis: Human observer and LABORAS™ monitoring of time spent paw licking during 5 min time bins after rat subplantar formalin injection

Number of XY Pairs	204
Pearson r	0.57
95% confidence interval	0.47 to 0.65
P value (two tailed)	P<0.0001

A correlation plot of 5 min time bins shows a highly significant, large correlation (Cohen, 1988) between LABORAS™ and human observer .

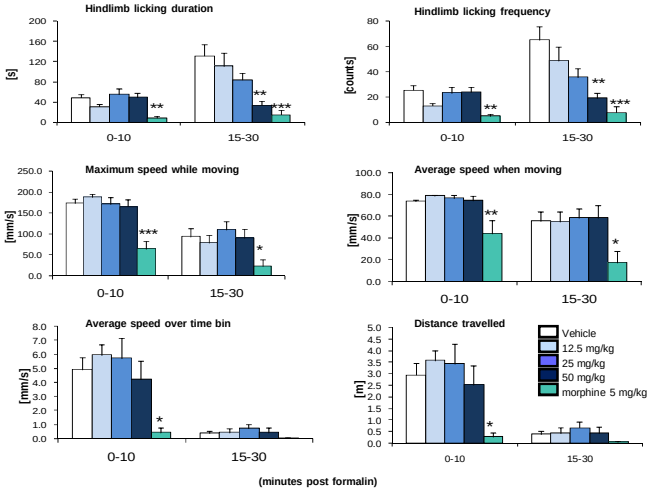
LABORAS™ therefore provides an automated method of continuously monitoring paw lick behaviour in a valid, faithful and objective manner in the rat formalin paw lick model.

Time course comparison of time spent paw licking monitored by human observer and LABORAS™ following rat subplantar formalin injection



Following subplantar formalin injection LABORAS™ detects the biphasic paw licking response recorded by human observer. All data cited as mean ± s.e.m.

Effect of gabapentin (12.5 – 50 mg/kg ip 2 h pre-test) and morphine (5 mg/kg ip, 20 min pretest) in the rat formalin paw lick model – monitored by LABORAS™



*, **, *** p<0.05, 0.01, 0.001 different from vehicle by Dunnett's test following 1-way ANOVA. All data cited as mean ± s.e.m.

LABORAS™ has detected a reduction in the second phase paw licking response to subplantar formalin administration following gabapentin administration, with a significant reduction produced by a dose of 50 mg/kg ip. This profile is in keeping with literature reports and predictive of activity in neuropathic pain (Yoon and Yaksh 1999; Heughan and Sawynok 2002). Over the dose range tested, gabapentin did not affect any speed measures or distance travelled, indicating a pharmacological dissociation between paw licking and locomotor activity in this model.

In contrast, morphine, at a dose of 5 mg/kg ip, significantly reduced both phases of the paw licking response, and also reduced all locomotor measures.

4. Conclusions

1. **LABORAS™ provides an automated method of continuously monitoring paw lick behaviour in a valid, faithful and objective manner in the rat formalin paw lick model.**
2. **LABORAS™ is very sensitive to the effects of gabapentin in the rat formalin paw lick model.**
3. **LABORAS™ generates robust quantitative data, the type of which can be subject to thorough statistical analysis such as isobolographic analysis for drug interaction studies.**

References

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