Inferiority of LABORAS over incapacitance testing to measure spontaneous murine osteoarthritis pain

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Intro: There currently exist more than 10 methods for assessing pain in preclinical models of osteoarthritis (OA) (Piel 2014, Blaker 2016). Pain assessments include evoked responses and spontaneous behaviour, both of which have been measured after surgical induction of OA. Two commonly used spontaneous pain measures are Linton incapacitance testing and Laboratory animal behaviour observation registration and analysis system (LABORAS). Linton incapacitance testing determines the weight distribution through the hind limbs by calculating the percentage of weight borne through the operated vs non-operated limb. Previously, Linton incapacitance testing has shown two phases of pain: a post-operative one lasting 3 days and a late OA-pain phase starting around 10 weeks following surgery (Inglis 2008). LABORAS is an individual cage-based platform that detects 7 different types of spontaneous behaviours based on specific movements from the animals, which are detected by a vibration-sensitive platform and translated to a respective behaviour by the LABORAS software. LABORAS classifies behaviours into "climbing", "locomotion", "inactivity", "rearing", "grooming", "drinking", and "eating". Painful murine behaviour after OA surgery has been reported by several groups to date (Inglis 2008, Miller 2012, Sambamurthy 2017). However, reporting of LABORAS results in the literature to date is not standardized and consensus for outcome measures using this platform needs to be created. In this study we assessed the robustness and utility of LABORAS compared with spontaneous pain measured by Linton incapacitance testing in a surgical model of OA.

Methods: 10-week old male C57BL6 mice underwent either partial meniscectomy, destabilization of the medial meniscus to induce OA or sham surgery as previously described (Glasson 2007, Knights 2012). Inflammatory arthritis was induced in 10 male 10-week old DBA-1 mice by collagen-induced arthritis (CIA), as previously described (Williams 2004). Naïve DBA-1 mice were used as controls. Spontaneous painful behaviour

was assessed bi-weekly by Linton incapacitance testing as well as LABORAS over 12 weeks post-operatively. The experimenter was blinded to operation status. Statistical analysis was performed using multiple t-tests with a Holm-Sidak post hoc to adjust for multiple comparisons. At 12 weeks post surgery, a functional regression analysis using a Fourier series study was performed in the LABORAS cohort.

Results: Linton incapacitance testing after partial meniscectomy detected two phases of pain – a post-operative one and a late OA phase, evident from 8 weeks post-surgery, with statistical significance reached by week 10 post-surgery. Using LABORAS, a 24-hour recording of naïve animals from 11:00-11:00 showed an initial 3-hour high activity phase (11:00-14:00), with generally low activity levels during the daylight hours (14:00-19:00, 7:00-11:00) and higher activity levels during the hours of darkness (19:00-7:00). CIA mice showed a marked reduction of activity compared with naïve mice over the 12-hours of darkness (19:00-7:00), but not during the initial 3-hour exploratory phase (15:00-18:00). Focusing on the period of most activity (19:00-7:00) the following was recorded, for animals that underwent OA- or sham surgery:

1) A significant increase in 'immobility' within the first day of surgery in sham and PMX operated animals compared with naïve mice
2) When anaesthetic and analgesia was not controlled for post-operatively, operated mice exhibited decreased activity in 'immobility', 'grooming', and 'eating'.
3) No significant differences were measured for any activity at any further time point when correcting for multiple comparisons
4) No significant differences in any activity were measured in animals that had not previously been acclimatized to LABORAS at the time of established pain (i.e. first test period at 12 weeks post-operatively).

Conclusion: Linton incapacitance testing was able to detect two phases of pain in a statistically significant manner even when rigorously blinded to treatment (surgery) to avoid observer bias. Conversely, LABORAS, despite potentially offering greater objectivity, was unable to measure any pain behaviour other than the immediate post-operative phase. We were unable to replicate our own previous study, which was likely incorrect due to the failure to correct for multiple comparisons. We do not think LABORAS is a suitable methodology for measuring OA pain.

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