The Assessment of Pain Using Facial Expressions in Laboratory Rodents

Matt Leach

Comparative Biology Centre, Medical School, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK.
Matthew.Leach@newcastle.ac.uk

Background

Pain in animals is of considerable public concern, particularly where animals are used in biomedical research. Pain can compromise not only animal welfare, but also the validity of scientific results. In order to alleviate pain, we need to be able to assess its severity and duration effectively [1]. If we cannot do this then we are unable to provide adequate pain management or to develop more effective and humane endpoints.

Behavioural indicators are increasingly being considered as effective cage-side indices of pain in many laboratory animal species, including rats [2], mice [3] and rabbits [4]. However, they have limitations:

- Behaviour may offer only a direct measure of an animal's response to the sensory afferent barrage (nociceptive input) rather than the emotional consequences of pain ('how pain makes animals feel') [5].
- Although behavioural indices are considered to offer an accurate and reliable assessment of pain in many species [6], there is still room for improvement.
- A recent study, investigating how we observe rabbits, showed that observers focus predominately on the face rather than on body areas where behavioural indices of pain are observed [7], reducing the effectiveness of behavioural indicators.
- The identification, validation and recording of behavioural indicators of pain is time consuming, which has led to pain behaviours only being identified for a very limited range of procedures in a small number of laboratory animal species.

The recent development of the Mouse Grimace Scale (MGS: [8]) and Rat Grimace Scale (RGS: [9]), which use facial expressions to assess pain, may overcome these difficulties. These studies demonstrate that mice and rats undergoing routine rodent nociceptive tests exhibit characteristic changes in facial expressions. Preliminary data from Langford et al. [8] raises the possibility that facial expression could indicate the affective component of pain in animals as it does in humans. Lesioning of the rostral anterior insula (implicated in the affective component of pain in humans) prevented changes in facial expression but not abdominal writhing (behavioural marker of nociception) in mice. The authors found both the MGS and RGS to be very accurate (72-97%) consistent and reliable (Interclass correlation: 0.9 respectively) both between and within observers. The assessment of pain using facial expressions should be less time consuming to apply than full behavioural scoring, allowing effective indicators of pain to be rapidly identified for a greater range of procedures. All of the indicators are located in one small area (i.e. the face), so exploiting the human tendency to focus on animal faces when assessing pain.

Assessment of facial expressions

In order to assess pain using facial expressions, images of mice and rats taken before and after potentially painful procedures are scored using the MGS and RGS respectively. The MGS is composed of 5 facial action units; orbital tightening, cheek bulge, nose bulge, ear position and whisker position (please see Langford et al. [8] for further details). The RGS is composed of 4 facial action units; orbital tightening, nose/cheek flattening, ear position and whisker position (please see Sotocinal et al. [9] for further details). Each facial action unit is scored on a 0 to 2 scale (0 = not present, 1 = moderately present, 2 = obviously present) by treatment-blind observers using mouse or rat grimace scale pictograms. A cumulative "grimace" score is then calculated for each image by simply adding the scores for each facial action unit that comprises the grimace scale.

Although the work of Langford et al. [8] and Sotocinal et al. [9] has demonstrated that mouse and rat facial expressions change in response to routinely used acute and chronic nociceptive tests, there has been little attempt to assess how facial expressions change in response to pain following routine surgical or other painful procedures. In addition, no attempt has been made to correlate changes in facial expression with the behavioural indices that we currently consider the most relevant to assessing post-operative and other post-procedure pain.

Assessment of post-surgical pain

We therefore have carried out a number of studies in rodents to determine whether routine surgical procedures are also associated with changes in facial expressions, and whether facial expressions are as effective for assessing post-surgical pain and analgesic efficacy as the established behavioural indices in mice and rats.

Study 1 aimed to determine if routine vasectomy in CD1 mice (n=18) induced changes in the MGS, and whether these could be used to effectively assess post-surgical pain and the efficacy of routinely used analgesia (20ml/kg saline [*sc*], 20mg/kg meloxicam [*sc*], 5mg/kg Bupivicaine [*li*]). In this study pain was assessed using MGS and validated behavioural indicators of pain [3]. The results showed that vasectomy did induce significant changes in the MGS, and that both the MGS and the scoring of pain behaviours identified clear differences between the pre- and post-surgery periods and between the animals receiving analgesia or saline post-operatively. Both these assessments exhibited a high positive correlation with each other. The MGS demonstrated high accuracy and reliability within and between observers.

Study 2 aimed to determine if routine laporatomy in Wistar (n=16) and Lister Hooded (n=16) rats induced changes in the RGS, and whether these could be used to effectively assess post-surgical pain and the efficacy of routinely used analgesia (0.4ml/kg saline [*sc*], 2mg/kg meloxicam [*sc*]). In this study pain was assessed using RGS and validated behavioural indicators of pain [2]. The results showed that laporatomy did induce significant changes in the RGS, and that both the RGS and the scoring of pain behaviours identified clear differences between the pre- and post-surgery periods and between the animals receiving analgesia or saline post-operatively. Both these assessments exhibited a high positive correlation with each other. The RGS demonstrated high accuracy and reliability within and between observers.

In order to reduce any unnecessary suffering in the saline treated groups (no analgesia), a rescue analgesia protocol was in place, where these animals received analgesia immediately after the data was collected (~ 1h post-surgery) to ensure that they went untreated for short a period as possible. The studies were conducted under licence from the UK Home Office and the research programme and protocols were reviewed and approved by the University of Newcastle's local ethical review process.

Conclusions

The results of these studies suggest that the assessment of facial expressions offers a means of assessing post-surgical pain in rodents that is as effective as behavioural pain assessment. The assessment of pain using facial expressions was easy and rapid to carry out, required minimal training (less than 5 minutes), and provided a reliable and accurate means of assessing pain following vasectomy and laporatomy in mice and rats respectively. However, further research needs to be carried out into the effectiveness of facial expressions for assessing pain following different procedures and in different species.

References

1. Hawkins, P. (2002). Recognising and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. *Laboratory Animals* **36,** 378–395.

2. Roughan, J.V., Flecknell, P.A. (2001). Behavioural effects of laparotomy and analgesics effects of ketoprofen and carprofen in rats. *Pain* **90,** 65-74.

3. Wright-Williams, S.L., Courade, J.P., Richardson, C.A., Roughan, J.V., Flecknell, P.A. (2007). Effects of vasectomy surgery and meloxicam treatment on faecal corticosterone levels and behaviour in two strains of laboratory mouse. *Pain* **130,** 108-118.

4. Leach, M.C., Allweiler, S., Richardson, C.A., Roughan, J.V., Narbe, R., et al. (2009). Behavioural effects of ovariohysterectomy and oral administration of meloxicam in laboratory housed rabbits. *Research in Veterinary Science* **87,** 336-347.

5. Flecknell, P.A. (2011). Affective state and quality of life in mice. *Pain* **152,** 963-964

6. Roughan, J.V., Flecknell, P.A. (2006). Training in behaviour-based post-operative pain scoring in rats - An evaluation based on improved recognition of analgesic requirements. *Applied Animal Behaviour Science* **96,** 327-342.

7. Leach, M.C., Coulter, C.A., Richardson, C.A., Flecknell, P.A. (2011). Are we looking in the wrong place? Implications for behavioural-based pain assessment in rabbits (*Oryctolagus cuniculi*) and beyond? *PloS ONE* **6,** 1-9.

8. Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond. T,E., et al. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* **7,** 447-449.

9. Sotocinal, S.G., Sorge, R.E., Zaloum, A., Tuttle, A.H., Marton, L.J., et al. (2011). The rat grimace scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain* **7,** 55.