Are We Looking in the Right Place? – Implications for Assessing Pain

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Summary

The use of behavioral indicators has advanced the assessment of pain in animals. However, effective assessment depends on establishing which behaviors indicate pain and which areas of the body need to be focused on to observe these indicators. Failure to focus on the correct place could mean that critical behaviors are missed. Recently, an eye-tracking study demonstrated that, irrespective of experience, observers focus first, more frequently, and longer on the face of rabbits when assessing post-ovariohysterectomy pain, and the general ability of observers to identify rabbits in pain was very poor. Alternatively, by identifying facial expressions related to pain in animals, we could increase the effectiveness of pain assessment. Facial expressions potentially associated with pain in rodents have been identified. A recent study has shown that facial expressions can be used to effectively assess post-surgical pain in mice, and changes in facial expression match those observed in validated pain behaviors.

Keywords: rabbit, rodent, facial expressions, behavior, pain

1 Background

Effective assessment of pain in laboratory animals is critical for both animal welfare and scientific validity. We have made considerable advances in the assessment of pain in a range of species by utilizing behavioral indicators. Roughan and Flecknell (2006) demonstrated that after a short period of training on which behaviors indicate pain in rats, observers (vets, technicians, and lay people) were more effectively able to identify rats in pain than when using more traditional techniques.

Although behavioral-based indicators have increased the effectiveness of pain assessment in many species, the improvement has not been as great as expected (i.e., 70%-80% successful identification of rats in pain; Roughan and Flecknell, 2006). Effective assessment of pain using behavior depends upon knowing which behaviors indicate pain and focusing on the correct areas of the animals' body to observe these indicators. Failure to observe all relevant body areas could mean that critical behaviors are missed. Although pain-related behaviors have been identified for a range of species, there has been little or no attempt to check whether observers focus on the correct location to see these behaviors (Leach et al., 2011). Ensuring that observers focus in the correct place has implications beyond pain assessment to any discipline that requires behavioral observation.

2 Eye-tracking study

Leach et al. (2011) recently carried out a study to investigate where on a rabbit's body observers focus their attention when attempting to assess pain following routine ovariohysterectomy (see Leach et al., 2011 for full details). Briefly, an automated eye-tracking system (Cambridge Research Systems, Rochester, UK) was used to assess the observation pattern of 151 participants (veterinarians, technicians, scientists, and lay people) when assessing pain in rabbits from video sequences. Each participant watched four one-minute video sequences of a rabbit either before or one hour after surgery. The video sequences were classified as showing no pain, mild, moderate, or severe pain by an independent experienced observer. In addition, each participant completed a short questionnaire asking them to classify each video sequence (no pain, mild, moderate, or severe pain) and provide their gender, occupation, and their rabbit-related experience. The automated eye-tracking system measured the frequency, duration, and latency to focus on five pre-determined areas of the rabbits' bodies (ears, face, back, abdomen, and hind-quarters).

Full details of the results are given in Leach et al. (2011). Briefly, the data showed that, irrespective of occupation, gender, and experience, participants focused significantly faster, more frequently, and for longer on the face than anywhere else. The

face was followed by the abdomen, which was observed significantly faster, more frequently, and for a longer period of time than the ears, back and hindquarters. Behavioral-based indicators of post-ovariohysterectomy pain in rabbits seem to be focused predominately in the back and hindquarters (Leach et al., 2009). The ability of the participants to accurately categorize each video sequence according to the degree of pain exhibited (according to an expert observer) was very poor (<25%), with gender, occupation, and rabbit- related experience having no effect. The ability to score pain was negatively correlated with the frequency of observation of the face but positively correlated with the latency of looking at the back and hind quarters, i.e., the participants who focused first on the back and hind quarters and less frequently on the face of the rabbit were more accurate in categorizing the video sequences.

The results demonstrate that in rabbits at least, observers seem to focus on the face of the rabbit when assessing pain, and that this occurs irrespective of experience. This fixation on the face appears to negatively affect the ability of the observers to assess pain in these animals, as the behavioral indicators of post-ovariohysterectomy pain predominately occur in the back and hindquarters (Leach et al., 2009), so participants are likely to miss them. Focusing on the face is not surprising, as humans focus on the faces of other people in order to assess their underlying emotional state, e.g., pain (Deyo et al., 2004).

3 Facial expressions and post-surgical pain

Alternatively, if we were able to identify facial expressions in animals that are associated with pain as in humans, then focusing on the face of an animal may actually increase the effectiveness of pain assessment that is based upon such expressions. Recent studies conducted in the Laboratory of Dr Jeffery Mogil have demonstrated convincingly that mice and rats exhibit facial expressions associated with routine nociceptive tests (Langford et al., 2010; Sotocinal et al., 2011). Recently, Leach et al. (manuscript submitted) carried out a study to determine whether the changes in facial expressions observed by Langford et al. (2010) are also observed following routine surgery in mice, and whether these can be used effectively to assess post-operative pain and to improve behavioral-based indicators (see Leach et al., manuscript submitted, for full details).

Briefly, eighteen male CD1 mice underwent routine scrotal approach vasectomy as part of Newcastle University's program for genetically modified mouse breeding. Therefore, no animals were directly used to collect the data. These animals were randomly allocated into one of three analgesic treatment groups (n=6 per group); (1) saline (2 ml/kg) administered subcutaneously 30 minutes prior to surgery, (2) meloxicam (20 mg/kg) administered subcutaneously 30 minutes prior to surgery, or (3) bupivicaine (5 mg/kg) administered by local infiltration of the scrotum intra-operatively. High definition video sequences (15 min) were taken before and one hour after surgery. All animals received an additional dose of effective analgesia (0.05 mg/kg buprenorphine) directly following the filming. The video sequences were initially analyzed in order to establish the frequency of validated mouse pain behaviors (Wright-Williams et al., 2007; Miller et al., 2011). Still images also were taken from the video sequences whenever the mice faced the camera. The resulting sixty images (30 pre- and 30 post- surgery, with 10 per treatment group in each) were scored by 21 treatment-blind participants (veterinarians, technicians, scientists, and lay people) using the Mouse Grimace Scale (MGS: Langford et al., 2010). In this study the Mouse Grimace Scale was composed of only 4 facial action units (orbital tightening, cheek bulge, nose bulge, and ear position), as whisker position was not clearly visible in the still images used.

Please see Leach et al. (manuscript submitted) for full details of the results. Briefly, there was a significant increase in the mean MGS score from pre to post vasectomy in all analgesic treatment groups, with the saline treated group demonstrating a 3-fold change from baseline, compared to a 2-fold change for the meloxicam and bupivicaine treated groups. There was no difference in the mean MGS scores between the treatment groups prior to surgery, but following surgery the mean MGS score was significantly higher in the saline compared to meloxicam and bupivicaine treated groups, with no difference between the latter two groups. An identical pattern was observed with the frequency of pain behaviors observed, with a significant increase from pre to post surgery and only a significant difference between saline compared to meloxicam and bupivicaine treated groups post-surgery. There was a high positive correlation between the change in pain-related behaviors and the change in MGS score from pre to post surgery, indicating that those animals exhibiting high frequencies of pain behaviors also exhibited the greatest change in facial expressions.

The results of this study demonstrate that facial expressions in mice change in response to surgery as well as to nociceptive tests, and the post-surgery change can be ameliorated by effective analgesic administration. Facial expressions are highly correlated with validated behavioral indicators of pain in mice undergoing vasectomy. Consequently, it seems likely that the changes in facial expression observed occur in response to post-surgical pain.

4 Conclusion

It appears that observers may have a tendency to focus on the face of animals when trying to assess pain and consequently could potentially miss some of the behaviors used to score pain. However, we could exploit this potential fixation on the face by scoring facial expressions associated with pain. The changes in facial expressions in response to pain were clearly visible and required less than 5 minutes training with the Mouse Grimace Scale manual for the still images to be accurately scored. The work of Langford et al. (2010) and Sotocinal et al. (2011) demonstrates that using facial expressions to assess pain appears to be reliable and accurate. Further research needs to be carried out

with other species and potentially painful procedures to further investigate both whether we focus on animal as well as human faces when assessing pain and whether facial expressions can effectively assess post-procedural pain at the cage or pen side.

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