Full title: The influence of rater training on inter- and intra-rater reliability when using the Rat Grimace Scale

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Running title: Rat Grimace Scale training

Abbreviations: Pain, Rats, Mice, Animal welfare, Analgesia
Abstract

Rodent grimace scales facilitate assessment of spontaneous pain and can identify a range of acute pain levels. Reported rater training in using these scales varies considerably and may contribute to observed variability in inter-rater reliability. This study evaluated the effect of training on inter-rater reliability with the Rat Grimace Scale (RGS). Two training sets, of 42 and 150 images, were prepared from several acute pain models. Four trainee raters progressed through 2 rounds of training, first scoring 42 images (S1) followed by 150 images (S2a). After each round, trainees reviewed the RGS and any problematic images with an experienced rater. The 150 images were then re-scored (S2b). Four years after training, all trainees re-scored the 150 images (S2c). Inter- and intra-rater reliability was evaluated using the intra-class correlation coefficient (ICC) and ICCs compared with a Feldt test. Inter-rater reliability increased from moderate (0.58 [95%CI: 0.43-0.72]) to very good (0.85 [0.81-0.88]) between S1 and S2b (p < 0.01) and also increased between S2a and S2b (p < 0.01). The action units with the highest and lowest ICCs at S2b were orbital tightening (0.84 [0.80-0.87]) and whiskers (0.63 [0.57-0.70]), respectively. In comparison to an experienced rater the ICCs for all trainees improved, ranging from 0.88 to 0.91 at S2b. Four years later, very good inter-rater reliability was retained (0.82 [0.76-0.84]) and intra-rater reliability was good or very good (0.78-0.87). Training improves inter-rater reliability between trainees, with an associated reduction in 95%CI. Additionally, training resulted in improved inter-rater reliability alongside an experienced rater. Performance was retained after several years. The beneficial effects of training potentially reduce data variability and improve experimental animal welfare.
Introduction

The effectiveness of a pain assessment scale lies in its validity (does a scale measure what is intended) and reliability (measurement error). Rodent grimace scales have renewed interest in measuring the affective component of pain and have been promoted as a means of overcoming the shortfalls of nociceptive threshold testing (Mogil & Crager, 2004; Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; De Rantere et al., 2016). There is increasing evidence that grimace scales discriminate painful and non-painful states in a range of acute pain models and interventions (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; De Rantere et al., 2016; Leach, 2012). However, there are conflicting reports regarding reliability when multiple raters score images (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; Faller et al., 2015; Mittal, 2016). Factors contributing to this variability may include a lack of structured training and variation in individual learning curves (Campbell et al. 2014; de Oliveira Filho, 2002; Roughan & Flecknell, 2006).

It is unclear what level of training is required to attain proficiency in using grimace scales. Most studies include minimal, non-specific descriptions of training (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; Leach et al., 2012; Faller et al., 2015; Mittal et al., 2016) and few report any measure of reliability (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; Mittal et al., 2016). Trainees progress at different rates during training to achieve proficiency in a task (Mittal et al., 2016; Campbell et al. 2014; Roughan & Flecknell, 2006); therefore, in addition to training, some assessment of score reliability is necessary. The impact of training on scoring reliability with the Rat Grimace Scale (RGS) has not been formally evaluated. The objective of this study was to assess the effect of training on inter-rater reliability when scoring was performed with single and multiple raters applying the RGS. We hypothesized that training would improve inter-rater reliability.
Materials and Methods

Two sets of training images were created from images collected during an unrelated project that had received institutional animal care and use committee approval from the University of Calgary Health Sciences Animal Care Committee (protocol IDs: AC13-0161 and AC13-0124) (De Rantere et al., 2016). This project used the following acute pain models: intraplantar carrageenan, Complete Freund’s adjuvant or plantar incision. RGS scores from these models are representative of the scale range (De Rantere et al., 2016). Animals were adult (> 10 weeks old) male Wistar (n = 34) rats, from a commercial source (Charles River Laboratories, Canada).

The methodology used to generate images was as previously described (Sotocinal et al., 2011). Briefly, still images were captured from high-definition video-recordings and cropped so that only the face was visible. Each image was presented on a single slide in presentation software (Microsoft PowerPoint, version 14.0, Microsoft Corporation, Redmond, WA, USA). Slide order was randomized and identifying information (animal ID, time point, model) removed.

Images were selected based on image quality alone, by an individual not involved with the study. Two unique sets of training images were created, of 42 (S1) and 150 (S2) images. Images were scored using the RGS (scale range 0-2 for each action unit) and the average score calculated from four action units: orbital tightening, nose/cheek flattening, ear changes, and whisker change. None of the 4 trainee raters recruited had previous experience with the RGS. All trainee raters were female undergraduate and graduate students (age range 20-25 years), studying veterinary medicine, biology (n = 2) and health sciences and were recruited when joining the research group as project students. No trainee raters had previous experience with rats, as experimental animal or pets, before beginning training. The experienced rater (DP) had used the RGS for several years with different models (De Rantere et al., 2016, Oliver et al., 2014).
All trainee raters followed the same scoring protocol: S1 images were scored independently by each individual, using the training manual provided by Sotocinal et al. (2011) alongside a training manual from our laboratory (Pang, 2018). Raters were encouraged to record comments for any images they found difficult to score. Following S1 scoring, raters reviewed their scores as a group with an experienced rater, discussing recorded comments and areas of inconsistency. Images with the most variation between raters were selected for review. The primary goal of the discussion was to improve standardization of scoring images assigned a score of 0 or 2. Disagreement in scores was tolerated provided differences between raters did not exceed 1 point on the scale. The standard of scoring was set by the experienced rater, following establishment of the technique within the laboratory with the support of the Mogil laboratory (McGill University).

Once review of S1 scoring was complete, S2 images were scored independently by each individual and comments recorded as before (S2a). The S2 image set was then scored independently a second time (S2b) after a facilitated group discussion with the experienced rater (as per the S1 image set discussion). Approximately 15-30 images were reviewed during group discussions, with 2-3 weeks between reviews. Intra-rater reliability was assessed by asking the trainee raters to independently re-score the S2 image set (S2c) with access to the training manual. Scoring S2c took place 4 years after initial training. The order of the images was randomized from S2b. At the time of S2c scoring, trainee rater 1 had not used the RGS in 10 months and trainee raters 3 and 4 had not used it in three years. Trainee rater 2 was still in the research group and actively using the RGS. All trainee raters were asked if they remembered any previous scores or images from the data set.
Intraclass correlation coefficients (ICCs, MedCalc version 12.6.1.0, MedCalc Software, Ostend, Belgium) were calculated to measure the reliability of RGS scoring between and within raters for the individual action unit scores and average RGS scores. An absolute model was used for the ICC calculation and single measure reported. This was done for each dataset (S1, S2a, S2b and S2c). ICCs were also calculated for the comparison between individual rater scores and those of the experienced rater (DP) to determine reliability of an individual rater. Planned comparisons were pre-established: calculated ICCs were compared with a Feldt test for S1 versus S2b, S1 versus S2a, S2a versus S2b and S2b versus S2c (critical F set at alpha = 0.01 and differences considered significant if the observed F value was greater than the critical F value) (Feldt et al., 1987; Kuzmic, 2015). ICCs were also calculated between the rater's own scores (S2b and S2c) to assess intra-rater reliability over time. Interpretation of the ICC followed the same divisions as used previously: "very good" (0.81–1.0), "good" (0.61–0.80), "moderate" (0.41–0.60), "fair" (0.21–0.40), "poor" (< 0.20) (Oliver et al., 2014). During the training process, raters were said to be proficient when calculated ICCs ± 95%CI overlapped with those published in a study reporting inter-rater reliability (Oliver et al., 2014) and obtained an ICC of at least 0.80 (Haidet et al., 2009). To assess the potential impact of scores memorized during group discussion between S2a and S2b introducing bias into the ICC calculation for S2b, images with the greatest scoring variability at S2a (those with a difference of 2 points between any 2 raters and therefore the most likely to have been discussed) were removed and the ICCs for S2b recalculated. Data are presented as ICC (± 95%CI) and a corrected p value for multiple comparisons of ≤ 0.017 was considered significant. Scoring accuracy was assessed by comparing the expert rater's scores for images collected at baseline and 6-9 hours after treatment (when a peak in RGS scores could be expected for the models studied (De Rantere et al., 2016); paired t test with alpha set at 0.05) from the S2
images. The datasets generated from this study and training manual are available in the Harvard Dataverse repository (Pang, 2018).

**Results**

Four raters completed the study. All training images were scored by every rater, and all scores included in the subsequent analysis.

**Inter-rater reliability**

Training was associated with a progressive improvement in inter-rater reliability and narrowing 95%CI (Fig. 1). The first training round (S1) resulted in a moderate ICC for the average RGS scores, with wide 95%CI (0.58 [0.43-0.72]). The increase in average RGS ICC between S1 and S2a (0.68 [0.58-0.76]) was not statistically significant (\(F_{0.01;149,41} = 1.88\), observed \(F = 1.31\), \(p > 0.05\)). A significant improvement was observed at S2b (0.85 [0.81-0.88]) compared with S1 (observed \(F = 2.8\)) and S2a (\(F_{0.01;149,149} = 1.47\), observed \(F = 2.13\), \(p < 0.01\) for both comparisons). The resultant S2b ICC was classified as very good and comparable with published values (Fig. 1)(Oliver et al., 2014).

A similar pattern of improvement was observed in the scores of individual action units (Table 1). Significant increases in ICCs were observed between S1 and S2b for orbital tightening (observed \(F = 1.94\)), ear changes (observed \(F = 2.14\)) and nose/cheek flattening (observed \(F = 2.21\), \(p < 0.01\) all comparisons), but not whisker changes (observed \(F = 1.65\), \(p > 0.05\)). And between S2a and S2b: orbital tightening (observed \(F = 1.81\)), ear changes (observed \(F = 1.96\) and nose/cheek flattening (observed \(F = 1.72\), \(p < 0.01\) all comparisons), but not whisker changes (observed \(F = 1.35\), \(p > 0.05\)). At all stages, orbital tightening had the highest ICC, improving from 0.69 to 0.84. Following training, ICCs for individual action units fell within the good or very good range (Table 1).
Comparing individual rater performance against the experienced rater showed considerable variation following the first training round with ICCs ranging from fair to good. All trainee raters showed improvement with training (Table 2).

There were 28 images (19%) with score differences between raters of 2 points at S2a. Removing these scores had a minimal effect on the recalculated ICCs for S2b (average RGS scores were 0.85 [0.81-0.88] and 0.86 [0.83-0.89] for 150 and 122 images, respectively).

There was a significant increase in RGS scores between baseline (n = 41, 0.45 ± 0.07) and 6-9 hours after treatment (n = 29, 0.92 ± 0.08, p < 0.001, 95%CI of mean difference 0.27 to 0.68), at which time the mean RGS score exceeded a published analgesic intervention threshold (Oliver et al., 2014).

When the images were re-scored four years after initial training (S2c), the ICC was very good for the averaged RGS scores (0.82 [0.76-0.84]) and proficiency was maintained from S2b (observed F = 1.20, p > 0.05). Between S2b and S2c there were no significant differences for nose/cheek flattening (observed F = 1.24, p > 0.05) and whisker changes (observed F = 1.30, p > 0.05, Table 1). However, inter-rater reliability from S2b was not maintained and decreased significantly for orbital tightening (observed F = 1.50, p < 0.01) and ear changes (observed F = 1.50, p < 0.01).

All raters maintained similar proficiency with the expert rater (observed F < 1.31, p > 0.05) except for rater 4 (observed F = 2.20, p < 0.01; Table 2).

Intra-rater reliability

The ability of a rater to score reliably over time was good or very good with ICCs ranging from 0.78 to 0.87 for the average RGS (Table 3). The intra-rater reliability of individual action units ranged from moderate to very good depending on the action unit and rater. Two trainee raters (2 and 4) reported that they did not recognize any images or remember previous scores while the remaining raters (1 and 3) reported recognizing a few images but did not remember scores.
Discussion

Our results suggest that reliability is limited when training is limited to reviewing the training manual, improving when feedback and discussion with an experienced rater are included. The high level of reliability and proficiency achieved from training can be maintained for several years.

Little is known regarding the need for, or role of, rater training in the use of rodent grimace scales. Where training has been described, it ranges from reviewing the grimace scale training manuals (Leach et al., 2012; Faller et al., 2015) to a single training session of variable length (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; De Rantere et al., 2016) or multiple training sessions (Mittal et al., 2016). Few studies describe an assessment of reliability (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; Mittal et al., 2016). The results of this study show that an assessment of reliability is necessary to confirm that training will lead to proficiency as well as standardized scoring.
The rate at which individuals achieve proficiency in a task is highly variable and, as such, it is erroneous to assume that participating in training guarantees proficiency. Neither a single training session nor repeated attempts at a task ensure proficiency (Campbell et al. 2014; de Oliveira Filho, 2002; Roughan & Flecknell, 2006). The length and intensity of training should depend on the difficulty of the mastering the tool and the proficiency of the trainee (Haidet et al., 2009). Additionally, proficiency should not be assumed just because a rater feels confident using a scale following training (Björn et al., 2017). Instead, it is important to test the actual proficiency of raters, and a simple approach is to assess inter-rater reliability (Streiner & Norman, 2008). This provides assurance that scoring has reached the desired standard, that variability is at an acceptable level and enables rogue raters to be identified (Mittal et al., 2016; Brondani et al., 2013). Identification of rogue raters during training allows for further testing and assessment or removal from participation in scoring (Mittal et al., 2016; Mullard et al., 2017). Ensuring reliability and standardizing scoring will reduce data variability and consequently, animal use. An alternative approach is to use a single rater; however, it is still useful to compare the performance of a single rater against that of an experienced rater, or a standard set of scores, to confirm reliability and consistency over time (Oliver et al., 2014). The presence of systematic bias may negatively affect data interpretation and pain management (Faller et al., 2015).

Orbital tightening had the highest associated ICC following the initial round of scoring, which was maintained throughout training. In contrast, the reliability of whisker scoring remained relatively low throughout training. These results support previous findings that assessing the whisker change action unit is more difficult for raters than orbital tightening (Oliver et al., 2014).
Four years after training, with variable use of the RGS during this time, the inter- and intra-rater reliability of the average RGS was maintained. This indicates that raters can retain scoring proficiency and score consistently with each other, with themselves and achieve the standard set by the expert rater. This agrees with a previous study showing that a single rater maintained scoring reliability after a break of six months (Oliver et al., 2014). Nevertheless, the observed reductions in ICC for two of the action units indicate that some degree of re-training may be beneficial.

A limitation of this study was re-scoring the 150 image set in the final training round, with the potential for memorized scores assigned during the group discussion following the second training round being applied rather than a rater scoring independently. We feel this is unlikely due to the large number of images scored, the similar appearance of rodent faces from similar strains, the time elapsed between review rounds, the small number of images reviewed during group discussion and the nature of the group discussion, where disagreement between raters was acceptable. The minimal difference in ICCs after removal of the 28 image scores supports this assertion as well as the maintained quality of scores after 4 years.

Images for training were selected on the basis of quality rather than to allow comparison between treatment groups. This limits any assessment of construct validity but the comparison of baseline and predicted peak pain periods indicates that accuracy was preserved.

Conclusion
These data show that reliance on access to the available manuals for rater training may be insufficient. Formal training improves inter-rater reliability and is likely to reduce data variability if rater proficiency is assessed before embarking on data collection. Collaborative training between research groups would ensure similar levels of rater proficiency and improve the reproducibility of research. Inclusion of clear descriptions of rater training and assessment would help in evaluating study results. Lastly, once raters achieve proficiency, this may be maintained over several years even without scoring during the intervening period.

Acknowledgements

The authors wish to thank Susana Sotocinal of the Mogil Laboratory, University of McGill, for invaluable assistance in establishing the Rat Grimace Scale in our laboratory and reviewing the selection of images in our training manual, and Kent Hecker and Grace Kwong (University of Calgary) for statistical advice.
References


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Legends

Figure 1. Average group ICCs for each of the four datasets (with 95%CI) with reference values (Oliver et al., 2014).

Table 1. Group Intra-class Correlation Coefficients (ICC) for each of the datasets.

<table>
<thead>
<tr>
<th>Action Unit</th>
<th>S1</th>
<th>S2a</th>
<th>S2b</th>
<th>S2c</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbital tightening</td>
<td>0.69 [0.56-0.80]a</td>
<td>0.71 [0.63-0.78]b</td>
<td>0.84 [0.80-0.87]h, b, c</td>
<td>0.76 [0.70-0.81]c</td>
<td>0.92 [0.89-0.95]</td>
</tr>
<tr>
<td>Ear changes</td>
<td>0.40 [0.25-0.56]a</td>
<td>0.45 [0.35-0.54]b</td>
<td>0.72 [0.66-0.77]h, b, c</td>
<td>0.58 [0.43-0.68]c</td>
<td>0.62 [0.51-0.72]</td>
</tr>
<tr>
<td>Nose/Cheek flattening</td>
<td>0.36 [0.21-0.52]a</td>
<td>0.50 [0.41-0.58]b</td>
<td>0.71 [0.65-0.76]h, b</td>
<td>0.64 [0.57-0.70]c</td>
<td>0.62 [0.51-0.72]</td>
</tr>
<tr>
<td>Whisker change</td>
<td>0.39 [0.26-0.55]</td>
<td>0.50 [0.42-0.58]</td>
<td>0.63</td>
<td>0.52 [0.41-0.57-0.70]</td>
<td>0.52 [0.39-0.63]</td>
</tr>
</tbody>
</table>
S1, S2a and S2b are the first, second and third training round, respectively. S2c was scored 4 years after initial training. Data are ICC single [95%CI]. Within a row, identical superscript letters indicate significant differences between the different training rounds, p < 0.01. Reference values are from Oliver et al. (2014).

**Table 2.** Agreement of individual raters when compared to an experienced rater (DP).

<table>
<thead>
<tr>
<th>Image set</th>
<th>Rater 1 vs DP</th>
<th>Rater 2 vs DP</th>
<th>Rater 3 vs DP</th>
<th>Rater 4 vs DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.41 [0.06-0.66]&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.70 [0.50-0.83]&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.62 [0.36-0.79]&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42 [0.13-0.64]&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2a</td>
<td>0.84 [0.79-0.88]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75 [0.68-0.82]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.68 [0.25-0.84]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65 [0.38-0.79]&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2b</td>
<td>0.89 [0.85-0.92]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.88 [0.84-0.91]&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.91 [0.88-0.94]&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.90 [0.87-0.93]&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2c</td>
<td>0.87 [0.82-0.90]</td>
<td>0.86 [0.82-0.90]</td>
<td>0.86 [0.80-0.90]</td>
<td>0.78 [0.71-0.83]&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are ICC single [95%CI]. Within a column, matching superscript letters indicate significant differences (p < 0.01).

**Table 3.** Intra-class Correlation Coefficients (ICC) for intra-rater reliability for each trainee rater four years after initial training.

<table>
<thead>
<tr>
<th>Action Unit</th>
<th>Rater 1</th>
<th>Rater 2</th>
<th>Rater 3</th>
<th>Rater 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.85 [0.78-0.90]</td>
<td>0.87 [0.82-0.90]</td>
<td>0.86 [0.79-0.90]</td>
<td>0.78 [0.71-0.84]</td>
</tr>
<tr>
<td>Orbital tightening</td>
<td>0.72 [0.53-0.82]</td>
<td>0.86 [0.82-0.90]</td>
<td>0.85 [0.78-0.89]</td>
<td>0.75 [0.63-0.83]</td>
</tr>
<tr>
<td>Ear changes</td>
<td>0.45 [0.30-0.58]</td>
<td>0.49 [0.11-0.70]</td>
<td>0.74 [0.66-0.81]</td>
<td>0.71 [0.61-0.79]</td>
</tr>
<tr>
<td>Nose/Cheek flattening</td>
<td>0.45 [0.32-0.57]</td>
<td>0.68 [0.56-0.77]</td>
<td>0.74 [0.60-0.82]</td>
<td>0.63 [0.53-0.72]</td>
</tr>
<tr>
<td>Whisker change</td>
<td>0.77 [0.70-0.83]</td>
<td>0.69 [0.55-0.78]</td>
<td>0.53 [0.27-0.69]</td>
<td>0.47 [0.34-0.59]</td>
</tr>
</tbody>
</table>

Data are ICC single [95% CI].