

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

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12 Running title: Rat Grimace Scale training

13

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

15 Abstract

16 Rodent grimace scales facilitate assessment of spontaneous pain and can identify a range of acute
17 pain levels. Reported rater training in using these scales varies considerably and may contribute
18 to observed variability in inter-rater reliability. This study evaluated the effect of training on in-
19 ter-rater reliability with the Rat Grimace Scale (RGS). Two training sets, of 42 and 150 images,
20 were prepared from several acute pain models. Four trainee raters progressed through 2 rounds of
21 training, first scoring 42 images (S1) followed by 150 images (S2a). After each round, trainees
22 reviewed the RGS and any problematic images with an experienced rater. The 150 images were
23 then re-scored (S2b). Four years after training, all trainees re-scored the 150 images (S2c). Inter-
24 and intra-rater reliability was evaluated using the intra-class correlation coefficient (ICC) and
25 ICCs compared with a Feldt test. Inter-rater reliability increased from moderate (0.58 [95%CI:
26 0.43-0.72]) to very good (0.85 [0.81-0.88]) between S1 and S2b ($p < 0.01$) and also increased be-
27 tween S2a and S2b ($p < 0.01$). The action units with the highest and lowest ICCs at S2b were or-
28 bital tightening (0.84 [0.80-0.87]) and whiskers (0.63 [0.57-0.70]), respectively. In comparison to
29 an experienced rater the ICCs for all trainees improved, ranging from 0.88 to 0.91 at S2b. Four
30 years later, very good inter-rater reliability was retained (0.82 [0.76-0.84]) and intra-rater reliabil-
31 ity was good or very good (0.78-0.87). Training improves inter-rater reliability between trainees,
32 with an associated reduction in 95%CI. Additionally, training resulted in improved inter-rater re-
33 liability alongside an experienced rater. Performance was retained after several years. The bene-
34 ficial effects of training potentially reduce data variability and improve experimental animal wel-
35 fare.

36

37 Introduction

38 The effectiveness of a pain assessment scale lies in its validity (does a scale measure what is in-
 39 tended) and reliability (measurement error). Rodent grimace scales have renewed interest in
 40 measuring the affective component of pain and have been promoted as a means of overcoming
 41 the shortfalls of nociceptive threshold testing (Mogil & Crager, 2004; Langford et al., 2010;
 42 Sotocinal et al., 2011; Oliver et al., 2014; De Rantere et al., 2016). There is increasing evidence
 43 that grimace scales discriminate painful and non-painful states in a range of acute pain models
 44 and interventions (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; De Rantere et
 45 al., 2016; Leach, 2012). However, there are conflicting reports regarding reliability when multi-
 46 ple raters score images (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; Faller et
 47 al., 2015; Mittal, 2016). Factors contributing to this variability may include a lack of structured
 48 training and variation in individual learning curves (Campbell et al. 2014; de Oliveira Filho,
 49 2002; Roughan & Flecknell, 2006).

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

61 **Materials and Methods**

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

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

74 Images were selected based on image quality alone, by an individual not involved with the study.
75 Two unique sets of training images were created, of 42 (S1) and 150 (S2) images. Images were
76 scored using the RGS (scale range 0-2 for each action unit) and the average score calculated from
77 four action units: orbital tightening, nose/cheek flattening, ear changes, and whisker change.

78 None of the 4 trainee raters recruited had previous experience with the RGS. All trainee raters
79 were female undergraduate and graduate students (age range 20-25 years), studying veterinary
80 medicine, biology (n = 2) and health sciences and were recruited when joining the research group
81 as project students. No trainee raters had previous experience with rats, as experimental animal or
82 pets, before beginning training. The experienced rater (DP) had used the RGS for several years
83 with different models (De Rantere et al., 2016, Oliver et al., 2014).

84 All trainee raters followed the same scoring protocol: S1 images were scored independently by
 85 each individual, using the training manual provided by Sotocinal et al. (2011) alongside a training
 86 manual from our laboratory (Pang, 2018). Raters were encouraged to record comments for any
 87 images they found difficult to score. Following S1 scoring, raters reviewed their scores as a
 88 group with an experienced rater, discussing recorded comments and areas of inconsistency. Im-
 89 ages with the most variation between raters were selected for review. The primary goal of the
 90 discussion was to improve standardization of scoring images assigned a score of 0 or 2. Disa-
 91 greement in scores was tolerated provided differences between raters did not exceed 1 point on
 92 the scale. The standard of scoring was set by the experienced rater, following establishment of
 93 the technique within the laboratory with the support of the Mogil laboratory (McGill University).
 94 Once review of S1 scoring was complete, S2 images were scored independently by each individ-
 95 ual and comments recorded as before (S2a). The S2 image set was then scored independently a
 96 second time (S2b) after a facilitated group discussion with the experienced rater (as per the S1
 97 image set discussion). Approximately 15-30 images were reviewed during group discussions,
 98 with 2-3 weeks between reviews. Intra-rater reliability was assessed by asking the trainee raters
 99 to independently re-score the S2 image set (S2c) with access to the training manual. Scoring S2c
 100 took place 4 years after initial training. The order of the images was randomized from S2b. At the
 101 time of S2c scoring, trainee rater 1 had not used the RGS in 10 months and trainee raters 3 and 4
 102 had not used it in three years. Trainee rater 2 was still in the research group and actively using the
 103 RGS. All trainee raters were asked if they remembered any previous scores or images from the
 104 data set.

105 Intraclass correlation coefficients (ICCs, MedCalc version 12.6.1.0, MedCalc Software, Ostend,
 106 Belgium) were calculated to measure the reliability of RGS scoring between and within raters for
 107 the individual action unit scores and average RGS scores. An absolute model was used for the
 108 ICC calculation and single measure reported. This was done for each dataset (S1, S2a, S2b and
 109 S2c). ICCs were also calculated for the comparison between individual rater scores and those of
 110 the experienced rater (DP) to determine reliability of an individual rater. Planned comparisons
 111 were pre-established: calculated ICCs were compared with a Feldt test for S1 *versus* S2b, S1 *ver-*
 112 *sus* S2a, S2a *versus* S2b and S2b *versus* S2c (critical F set at $\alpha = 0.01$ and differences consid-
 113 ered significant if the observed F value was greater than the critical F value) (Feldt et al., 1987;
 114 Kuzmic, 2015). ICCs were also calculated between the rater's own scores (S2b and S2c) to assess
 115 intra-rater reliability over time. Interpretation of the ICC followed the same divisions as used
 116 previously: “very good” (0.81–1.0), “good” (0.61–0.80), “moderate” (0.41–0.60), “fair”
 117 (0.21–0.40), “poor” (< 0.20) (Oliver et al., 2014). During the training process, raters were said
 118 to be proficient when calculated ICCs $\pm 95\%CI$ overlapped with those published in a study re-
 119 porting inter-rater reliability (Oliver et al., 2014) and obtained an ICC of at least 0.80 (Haidet et
 120 al., 2009). To assess the potential impact of scores memorized during group discussion between
 121 S2a and S2b introducing bias in to the ICC calculation for S2b, images with the greatest scoring
 122 variability at S2a (those with a difference of 2 points between any 2 raters and therefore the most
 123 likely to have been discussed) were removed and the ICCs for S2b recalculated. Data are present-
 124 ed as ICC ($\pm 95\%CI$) and a corrected p value for multiple comparisons of ≤ 0.017 was considered
 125 significant. Scoring accuracy was assessed by comparing the expert rater's scores for images col-
 126 lected at baseline and 6-9 hours after treatment (when a peak in RGS scores could be expected
 127 for the models studied (De Rantere et al., 2016); paired t test with α set at 0.05) from the S2

128 images. The datasets generated from this study and training manual are available in the Harvard
129 Dataverse repository (Pang, 2018).

130 **Results**

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

133 **Inter-rater reliability**

134 Training was associated with a progressive improvement in inter-rater reliability and narrowing
135 95%CI (Fig. 1). The first training round (S1) resulted in a moderate ICC for the average RGS
136 scores, with wide 95%CI (0.58 [0.43-0.72]). The increase in average RGS ICC between S1 and
137 S2a (0.68 [0.58-0.76]) was not statistically significant ($F_{0.01;149,41} = 1.88$, observed $F = 1.31$, $p >$
138 0.05). A significant improvement was observed at S2b (0.85 [0.81-0.88]) compared with S1 (ob-
139 served $F = 2.8$) and S2a ($F_{0.01;149,149} = 1.47$, observed $F = 2.13$, $p < 0.01$ for both comparisons).
140 The resultant S2b ICC was classified as very good and comparable with published values (Fig.
141 1)(Oliver et al., 2014).

142 A similar pattern of improvement was observed in the scores of individual action units (Table 1).
143 Significant increases in ICCs were observed between S1 and S2b for orbital tightening (observed
144 $F = 1.94$), ear changes (observed $F = 2.14$) and nose/cheek flattening (observed $F = 2.21$, $p < 0.01$
145 all comparisons), but not whisker changes (observed $F = 1.65$, $p > 0.05$). And between S2a and
146 S2b: orbital tightening (observed $F = 1.81$), ear changes (observed $F = 1.96$) and nose/cheek flat-
147 tening (observed $F = 1.72$, $p < 0.01$ all comparisons), but not whisker changes (observed $F =$
148 1.35 , $p > 0.05$). At all stages, orbital tightening had the highest ICC, improving from 0.69 to 0.84.
149 Following training, ICCs for individual action units fell within the good or very good range (Ta-
150 ble 1).

151 Comparing individual rater performance against the experienced rater showed considerable varia-
152 tion following the first training round with ICCs ranging from fair to good. All trainee raters
153 showed improvement with training (Table 2).

154 There were 28 images (19%) with score differences between raters of 2 points at S2a. Removing
155 these scores had a minimal effect on the recalculated ICCs for S2b (average RGS scores were
156 0.85 [0.81-0.88] and 0.86 [0.83-0.89] for 150 and 122 images, respectively).

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

161 When the images were re-scored four years after initial training (S2c), the ICC was very good for
162 the averaged RGS scores (0.82 [0.76-0.84]) and proficiency was maintained from S2b (observed
163 $F = 1.20$, $p > 0.05$). Between S2b and S2c there were no significant differences for nose/cheek
164 flattening (observed $F = 1.24$, $p > 0.05$) and whisker changes (observed $F = 1.30$, $p > 0.05$, Table
165 1). However, inter-rater reliability from S2b was not maintained and decreased significantly for
166 orbital tightening (observed $F = 1.50$, $p < 0.01$) and ear changes (observed $F = 1.50$, $p < 0.01$).
167 All raters maintained similar proficiency with the expert rater (observed $F < 1.31$, $p > 0.05$) ex-
168 cept for rater 4 (observed $F = 2.20$, $p < 0.01$; Table 2).

169 **Intra-rater reliability**

170 The ability of a rater to score reliably over time was good or very good with ICCs ranging from
171 0.78 to 0.87 for the average RGS (Table 3). The intra-rater reliability of individual action units
172 ranged from moderate to very good depending on the action unit and rater. Two trainee raters (2
173 and 4) reported that they did not recognize any images or remember previous scores while the
174 remaining raters (1 and 3) reported recognizing a few images but did not remember scores.

175 Discussion

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

180 Little is known regarding the need for, or role of, rater training in the use of rodent grimace
181 scales. Where training has been described, it ranges from reviewing the grimace scale training
182 manuals (Leach et al., 2012; Faller et al., 2015) to a single training session of variable length
183 (Langford et al., 2010; Sotocinal et al., 2011; Oliver et al., 2014; De Rantere et al, 2016) or mul-
184 tiple training sessions (Mittal et al., 2016). Few studies describe an assessment of reliability
185 (Langford et al., 2010; Sotocinal al., 2011; Oliver et al., 2014; Mittal et al., 2016). The results of
186 this study show that an assessment of reliability is necessary to confirm that training will lead to
187 proficiency as well as standardized scoring.

188 The rate at which individuals achieve proficiency in a task is highly variable and, as such, it is
 189 erroneous to assume that participating in training guarantees proficiency. Neither a single training
 190 session nor repeated attempts at a task ensure proficiency (Campbell et al. 2014; de Oliveira Fil-
 191 ho, 2002; Roughan & Flecknell, 2006). The length and intensity of training should depend on the
 192 difficulty of the mastering the tool and the proficiency of the trainee (Haidet et al., 2009). Addi-
 193 tionally, proficiency should not be assumed just because a rater feels confident using a scale fol-
 194 lowing training (Björn et al., 2017). Instead, it is important to test the actual proficiency of raters,
 195 and a simple approach is to assess inter-rater reliability (Streiner & Norman, 2008). This provides
 196 assurance that scoring has reached the desired standard, that variability is at an acceptable level
 197 and enables rogue raters to be identified (Mittal et al., 2016; Brondani et al., 2013). Identification
 198 of rogue raters during training allows for further testing and assessment or removal from partici-
 199 pation in scoring (Mittal et al., 2016; Mullard et al., 2017). Ensuring reliability and standardizing
 200 scoring will reduce data variability and consequently, animal use. An alternative approach is to
 201 use a single rater; however, it is still useful to compare the performance of a single rater against
 202 that of an experienced rater, or a standard set of scores, to confirm reliability and consistency
 203 over time (Oliver et al., 2014). The presence of systematic bias may negatively affect data inter-
 204 pretation and pain management (Faller et al., 2015).

205 Orbital tightening had the highest associated ICC following the initial round of scoring, which
 206 was maintained throughout training. In contrast, the reliability of whisker scoring remained rela-
 207 tively low throughout training. These results support previous findings that assessing the whisker
 208 change action unit is more difficult for raters than orbital tightening (Oliver et al., 2014).

209 Four years after training, with variable use of the RGS during this time, the inter- and intra-rater
 210 reliability of the average RGS was maintained. This indicates that raters can retain scoring profi-
 211 ciency and score consistently with each other, with themselves and achieve the standard set by
 212 the expert rater. This agrees with a previous study showing that a single rater maintained scoring
 213 reliability after a break of six months (Oliver et al., 2014). Nevertheless, the observed reductions
 214 in ICC for two of the action units indicate that some degree of re-training may be beneficial.
 215 A limitation of this study was re-scoring the 150 image set in the final training round, with the
 216 potential for memorized scores assigned during the group discussion following the second train-
 217 ing round being applied rather than a rater scoring independently. We feel this is unlikely due to
 218 the large number of images scored, the similar appearance of rodent faces from similar strains,
 219 the time elapsed between review rounds, the small number of images reviewed during group dis-
 220 cussion and the nature of the group discussion, where disagreement between raters was accepta-
 221 ble. The minimal difference in ICCs after removal of the 28 image scores supports this assertion
 222 as well as the maintained quality of scores after 4 years.
 223 Images for training were selected on the basis of quality rather than to allow comparison between
 224 treatment groups. This limits any assessment of construct validity but the comparison of baseline
 225 and predicted peak pain periods indicates that accuracy was preserved.

226 **Conclusion**

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

234 **Acknowledgements**

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

239

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296 Legends

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

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

Action Unit	S1	S2a	S2b	S2c	Reference val- ues
Orbital tightening	0.69 [0.56- 0.80] ^a	0.71 [0.63- 0.78] ^b	0.84 [0.80- 0.87] ^{a,b,c}	0.76 [0.70- 0.81] ^c	0.92 [0.89- 0.95]
Ear changes	0.40 [0.25- 0.56] ^a	0.45 [0.35- 0.54] ^b	0.72 [0.66- 0.77] ^{a,b,c}	0.58 [0.43- 0.68] ^c	0.62 [0.51- 0.72]
Nose/Cheek flat- tening	0.36 [0.21- 0.52] ^a	0.50 [0.41- 0.58] ^b	0.71 [0.65- 0.76] ^{a,b}	0.64 [0.57- 0.70]	0.62 [0.51- 0.72]
Whisker change	0.39 [0.26- 0.55]	0.50 [0.42- 0.58]	0.63 [0.57-0.70]	0.52 [0.41- 0.62]	0.52 [0.39- 0.63]

S1, S2a and S2b are the first, second and third training round, respectively. S2c was scored 4 years after initial training. Data are ICCsingle [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).

Image set	Rater 1 vs DP	Rater 2 vs DP	Rater 3 vs DP	Rater 4 vs DP
S1	0.41 [0.06-0.66] ^{a,b}	0.70 [0.50-0.83] ^a	0.62 [0.36-0.79] ^a	0.42 [0.13-0.64] ^a
S2a	0.84 [0.79-0.88] ^a	0.75 [0.68-0.82] ^b	0.68 [0.25-0.84] ^b	0.65 [0.38-0.79] ^b
S2b	0.89 [0.85-0.92] ^b	0.88 [0.84-0.91] ^{a,b}	0.91 [0.88-0.94] ^{a,b}	0.90 [0.87-0.93] ^{a,b,c}
S2c	0.87 [0.82-0.90]	0.86 [0.82-0.90]	0.86 [0.80-0.90]	0.78 [0.71-0.83] ^c

Data are ICCsingle [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.

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

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Data are ICC single [95% CI].