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Original Research

Use of the Rat Grimace Scale to Evaluate Neuropathic Pain in a Model of Cervical Radiculopathy

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Although neck and low-back pain are common sources of neuropathic pain with high societal costs, the pathophysiology of neuropathic pain is not well-defined. Traditionally, most rodent pain studies rely on evoked reflex-based testing to measure pain. However, these testing methods do not reveal spontaneous pain, particularly early after injury. The rat grimace scale (RGS) for quantifying spontaneous pain has been validated after visceral, incisional, orthopedic, and inflammatory insults but not neuropathic pain. The current study used a rat model of radiculopathy to investigate the time course of RGS, the effect of the NSAID meloxicam on RGS, and the reliability and consistency of RGS across testers. RGS values at baseline and at 3, 6, 24, and 48 h after cervical nerve root compression (NRC) that induced robust evoked pain responses were compared with those obtained after sham surgery. The RGS was also evaluated at 6 h after NRC in another set of rats that had received meloxicam treatment prior to surgery. At 6 h, NRC induced higher RGS scores (1.27 ± 0.18) than did sham surgery (0.93 ± 0.20), and scores remained above baseline for as long as 48 h. Treatment with meloxicam before NRC reduced RGS at 6 h to sham levels, which were lower than those of injury without treatment. The RGS was associated with very good interobserver reliability (intraclass correlation coefficient, 0.91) and excellent internal consistency (Cronbach α, 0.87). These findings suggest that RGS is a useful approach to identifying and monitoring acute neuropathic pain in rats.

Abbreviations: ICC, intraclass correlation coefficient; NRC, nerve root compression; RGS, rat grimace scale

Neck pain is extremely common, with a 12-mo prevalence as high as 71.5% and with as many as 14% of workers reporting that their ability to work is limited by their neck pain.[18] Furthermore, as many as 75% of patients report incomplete resolution or recurrence of their neck pain at 5 y after their original complaint.[32] In addition, neck pain contributes substantially to the $61.2 billion in the annual cost of chronic pain conditions, due largely to lost work time.[57] Neck pain can be of soft tissue or neuropathic origin, and treatment options are often palliative or only modestly effective, largely due to an incomplete understanding of the complex pathophysiology of neuropathic pain.[5,17] Animal models have been invaluable in providing platforms to better understand the neuropathic pain mechanisms. However, most such pain models measure 'sensory hyperphenomena,' such as allodynia or hyperalgesia, by using reflex-based measures induced through stimulation by mechanical von Frey filaments and thermal testing causing paw withdrawal.[35,37,40,58,60] Although chronic-pain sufferers do exhibit evoked pain, only 64% report mechanical sensitivity and even fewer (38%) report thermal sensitivity, whereas nearly all describe ongoing, tonic, spontaneous pain.[36] Further complicating the use of evoked reflex responses as proxies for pain is the fact that reflex-based techniques have assessed nociception in decerebrate animals,[61] suggesting that these methods do not reflect the higher affective components of the painful experience that are usually the primary clinical complaint.[5,33,35,58] In fact, the over-reliance on reflex-based, rather than spontaneous, measures of pain is one of the main reasons cited for the failure of many novel analgesics that have good efficacy in preclinical animal trials but that do not translate well into humans.[5,19,35,37] For these reasons, there is growing emphasis on using nonevoked measures to evaluate pain in animal models.[15,35,37,40,48,56,58,60]

Several techniques have been developed to evaluate spontaneous pain in animals through the analysis of facial expression.[8,56] The Grimace Scale was originally developed as a measure of spontaneous pain in mice,[28] and that same approach has since been able to detect lower levels of pain than do standard cageside observation or automated behavioral analysis.[16,30] The Grimace Scale is based on 4 facial features—orbital tightening, nose or cheek flattening, ear change, and whisker change—with each feature rated as not present (score, 0), moderate (1), or severe (2).[28] Grimace scales are increasingly being used across many species, including rats,[10,14,24,25,29,31,44,46] horses,[13] cats[20] and rabbits,[26] because these measures are less time-consuming and often more specific for pain than are more complex ethogram-based techniques.[28,30,56] Although the Rat Grimace Scale (RGS) has been used to evaluate spontaneous pain after insults that produce inflammatory,

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surgical, and orthopedic pain, it has not been used in neuropathic pain models.[28]

Cervical radiculopathy is a common cause of pain in humans, with an annual incidence of 83.2 per 100,000 people of all age groups and sexes.[11] Most commonly due to nerve root impingement from spondylosis or disc extrusion, radiculopathy can produce neck and limb pain, weakness, numbness, and impaired mobility.[11,47] Like many neuropathic conditions, little guidance is available regarding treatment options for radicular pain, a situation that contributes to the large financial burden and societal impact associated with pain.[1,3,11,32] We have developed and characterized a model of cervical radiculopathy in the rat to study the pathophysiology of neuropathic pain and to evaluate potential treatments for that condition.[22,39,50,54,59] A transient compression of the right C7 dorsal nerve root is used to impose robust and sustained mechanical and thermal hyperalgesia and allodynia in the forepaw.[9,41,43,54] We hypothesized that the RGS can detect increases in ongoing neuropathic pain in rats after nerve root compression that typically induces evoked sensitivity responses and provide a relevant clinical metric for evaluating early neuropathic pain due to this injury. Several pain models exhibit increased grimace within hours of an initiating painful insult that coincides with the onset of evoked sensitivity,[12,14,44,56] suggesting that facial grimace scores may be a reliable early indicator of pain. However, whether and to what extent nonevoked pain is induced early after a neuropathic injury is unknown.

In the current study, we used the RGS to evaluate pain after nerve root compression in our model of neuropathic pain.[9,21,22,42,59] Moreover, to evaluate the potential for testing effects of analgesic interventions in mitigating pain, we also designed an analgesic trial study. Pain from radiculopathy is thought to occur at least partly to an inflammatory response,[11,49,50] and painful nerve root compression also induces spinal glial activation and inflammation.[21,22,54,59] NSAID are recommended nonsurgical treatment options for cervical radiculopathy, particularly in the early stages of injury.[11,52] Meloxicam is a cyclooxygenase-2–selective NSAID that is commonly used in veterinary practice and has well-established dose parameters in laboratory rats.[6,7] As such, we also tested whether meloxicam treatment affected the grimace score in rats after nerve root compression. Lastly, we analyzed the interrater reliability and internal consistency of the RGS for use with neuropathy.[56]

Materials and Methods

Three complementary studies were performed. The first experiment characterized the time course of the RGS in an established model of radicular injury. Building on that time course, which showed an increase in RGS values early after injury, we performed a separate study in which we administered meloxicam to determine its effects on spontaneous pain as measured by RGS at a single time point after nerve root compression. RGS data from the meloxicam experiment were evaluated by, and compared between, 3 raters to assess the reliability and consistency of this approach to assess ongoing pain in a rat model of neuropathic radiculopathy.

Animals. All animals were adult male HsdHot:Holtzman Sprague–Dawley rats (n = 23; weight at acquisition, 275 to 349 g; Harlan Laboratories, Indianapolis, IN). Rats were housed in pairs matched for treatment in standard 28 cm^3 polycarbonate caging (AnCare, Bellmore, NY), with 0.25-in. corncob bedding (Bed-o'Cobs, The Andersons, Maumee, OH) and unrestricted access to food (no. 5001; LabDiet, St Louis, MO) and water (acidified to pH 3). Rats were housed in an AAALAC -accredited vivarium under a 12:12-h light:dark cycle in a temperature-controlled environment in accordance with recommendations set forth in the *Guide for Care and Use of Laboratory Animals*, 8th edition.[23] Rats were maintained on a ventilated rack in an environment monitored through quarterly screening of dirty-bedding sentinels and were confirmed to be free of rat parvovirus, H1 virus, Kilham rat virus, parvovirus NS1, rat minute virus, rat theilovirus, rat respiratory virus, *Mycoplasma*, reovirus 3, PVM, Sendai virus, fur mites, and pinworms. Housing temperature was held between 68 to 79 °F (20.0 to 26.1 °C), and humidity was controlled at 30% to 70%; these ranges are in accordance with parameters set forth in the *Guide*.[23] Cages were changed at least twice each week and were autoclaved between uses. All rat use and procedures were approved by the IACUC of the University of Pennsylvania and done in accordance with the Committee for Research and Ethical Issues of the International Association for the Study of Pain.[62]

Surgical procedures. All surgeries were performed between 0900 and 1200. Nerve root compression was performed as previously described.[9,21,22,49,50,54,59] Rats were anesthetized with inhalation isoflurane (4% for induction, 2% to 3% for maintenance; Phoenix Pharmaceutical, St Joseph, MO) in oxygen and placed in a prone position. A midline incision was made from the base of the skull to the dorsal spinous process of the second thoracic vertebra. The dorsal cervical musculature was bluntly dissected to allow visualization of the cervical spine. A right dorsal hemilaminectomy at the level of C6C7 was performed to expose the C7 dorsal nerve root on the right side. A small opening was made in the dura and a microvascular clip (occlusion pressure, 10 g; World Precision Instruments, Sarasota, FL) was used to apply compression to the right C7 nerve root for 15 min (n = 7, nerve root compression [NRC] group). Sham procedures were performed in additional rats (n = 7, sham) to serve as surgical controls; these rats underwent hemilaminectomy and opening of the dura but not compression of the nerve root. After nerve root procedures, hemostasis was confirmed, and the deep muscle and fascial layers were closed with 3-0 polyglactin 910 suture in a simple continuous pattern; skin was closed with surgical staples. Rats were recovered in a clean cage with heat support and continuous monitoring.

To evaluate the effects of NSAID treatment on the measurements of the RGS, meloxicam was administered to another set of rats that underwent NRC; these rats (n = 7, MxNRC group) received a single injection of meloxicam (2 mg/kg SC; Bimeda, Oakbrook Terrace, IL), which was diluted with physiologic sterile saline to a volume of 1 mL, at the time of anesthetic induction, immediately prior to surgery and NRC. To control for the possible effects of anesthesia, an additional group of 2 rats underwent 30 min of isoflurane exposure. All other procedures of NRC surgery were identical to those described earlier.

Weight, activity, and incision appearance were monitored daily for 3 d after anesthesia and surgery, and any rats that lost more than 20% of their body weight or that showed severely decreased activity levels were to be euthanized for humane reasons. All rats were closely monitored daily after surgery, and the following clinical observations were considered grounds for removing an animal from the study: any signs of lethargy, ruffled hair coat, weight loss of more than 10% of the original weight, decreased

motor activity, anorexia for more than 24 h, swelling at the surgical site, and sensitivity to touch. No rats had to be removed from the study for these criteria.

Digital videorecording and image capture. For each time point of assessment, rats were placed individually in a $23 \times 10 \times 10$ cm^3 transparent acrylic chamber (E-Z Anesthesia, Palmer, PA) with a wire mesh cover to allow ventilation. Each rat was videotaped for 30 min (HDR-CX380/B High-Definition Handycam, Sony, Tokyo, Japan) positioned approximately 20 cm from the chamber. Personnel remained out of visual contact with the rats for the duration of the recording session. Rats were videorecorded at baseline (0 h) prior to surgery and at 3, 6, 24, and 48 h after surgery.

The procedures for acquiring images and scoring according to the RGS were adapted from a previous study.[56] Briefly, videos were acquired as Advanced Video Coding High-Definition video files (.m2ts), and still images of the face of the rat were captured from the video files. Video files were captured at 3-min intervals throughout each entire video session by using the Windows Snipping Tool (Microsoft, Redmond, WA), resulting in a total of 10 images for each rat at each time point. The observer who captured the images was blinded to the treatment group. No images taken while the rats were grooming, sleeping, or exhibiting active sniffing activity were used, and only images encompassing in-focus views of all relevant anatomy for the RGS were used. If an image could not be captured due to any of the reasons listed above, the video was advanced until the next available image, and the 3-min intervals were reset.

Image scoring. Images were saved as png files and copied into PowerPoint 2013 (Microsoft) with one image as a separate slide for evaluation. A neutral-colored shape was placed over the cranial cervical region to blind scorers to the surgery status of the rat (Figure 1). A PowerPoint macro (http://www.tushar-mehta.com/powerpoint/randomslideshow/ index.htm) was used to randomize the order of image presentation. Images were presented in random order to each rater and were scored for 4 separate action units: orbital tightening, nose or cheek flattening, ear change, and whisker change (Figure 1). According to established scoring methods,[28,29,56] each action unit in each image was rated on a score of 0 (for an absence of grimace for that action unit) to a value of 2 (present and severe).[28,56] Raters were instructed to score action units as 'not scored' when they unable to score the image presented. The average of all action units receiving a score was taken as the individual rat's RGS for that session.[14,31]

For the time-course study, a single experienced rater who was blinded to the groups scored all of the images obtained from the rats receiving NRC or sham procedures at all time points (0, 3, 6, 24, 48 h). In the study with meloxicam, all of the groups were scored by using the images from the 6-h time point only, given the findings from the time-course study. To determine the reliability and consistency of RGS in this radiculopathy model, images were scored by 2 additional observers who performed analyses in a blinded fashion. Those additional observers were trained by the experienced observer, who performed the time-course analyses during a single 1-h session which included a practice scoring session with 28 images to establish a scoring consensus between scorers. All 3 scorers have worked with laboratory rats in a professional capacity for more than 6 y either as researchers or as a veterinarian.

Evoked sensitivity testing. Ipsilateral forepaw mechanical hyperalgesia was assessed prior to surgery (baseline, day 0) and on postoperative days 1 and 7, as previously described.[9,53] Response thresholds were measured by using a series of von Frey filaments of increasing strengths ranging from 0.6 to 26 g (Stoelting, Wood Dale, IL). The lowest strength of filament to evoke a response was recorded as the response threshold, providing that the next filament also elicited a response. If a rat was nonresponsive to all filaments, the maximum filament strength (26 g) was recorded as the threshold. Each testing session consisted of 3 rounds with a rest period of at least 10 min between rounds. The threshold for each rat on each testing day was identified by averaging the results from individual rounds. After evoked testing on day 7, rats were euthanized and perfused, and nervous tissue was collected for future analysis.

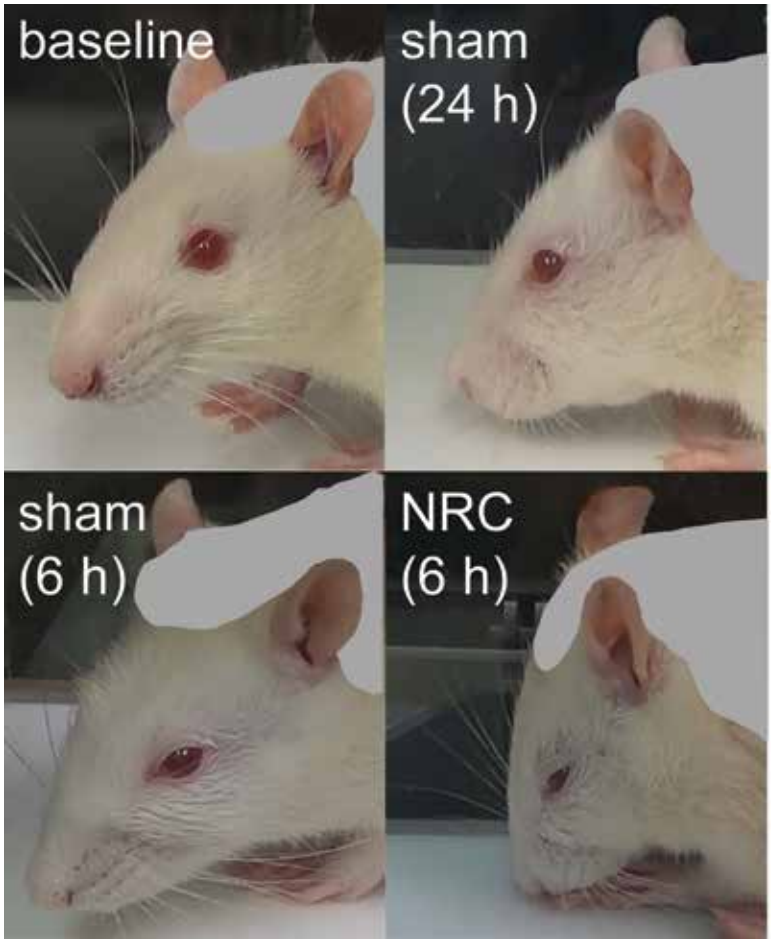
Statistical analyses. Because data were determined to be normally distributed according to the Shapiro–Wilk test, parametric statistical tests were used. For the time-course study, the RGS score was compared between NRC and sham groups over time by using repeated-measures ANOVA and post-hoc Tukey testing. In addition, the RGS score at 6 h was compared between all 4 groups (NRC, sham, MxNRC, and anesthesia only) by using a separate ANOVA and post-hoc Tukey test. All statistical analyses were performed by using JMP11 (SAS Institute, Cary, NC), with significance defined as a P value of less than 0.05. RGS values are reported as mean ± 1 SD.

To evaluate the interrater reliability between the scorers, intraclass correlation coefficients (ICC) for overall RGS and by individual action unit were compared across all treatment groups at 6 h. Interrater reliability was evaluated by using a 2-way mixed-effects model (StataMP 14, StataCorp, College Station, TX). Results for that analysis are reported as ICC (95% CI) and are interpreted with strength of agreement categorized as: moderate, less than 0.60; good, 0.61–0.80; and very good, 0.81 to 1.00.[2,27] To evaluate the internal consistency of the overall RGS score and the relative contributions of individual action units to scoring consistency, the Cronbach α coefficient was calculated as a conservative lower-bound estimate[45] and interpreted according to a previous reliability matrix,[45] with α scores greater than 0.8 considered 'excellent' for a sample size greater than 100 and a scale with fewer than 6 components.

Response thresholds for the evoked sensitivity tests were compared between NRC, MxNRC, and sham groups by using repeated-measures ANOVA with Bonferroni correction. Values are reported as mean ± 1 SD.

Results

For the time-course study, 700 images were collected from 14 rats at each of the 5 time points (0, 3, 6, 24, and 48 h) and were analyzed by an experienced blinded observer (BHP). All 4 action units were scored for all images. Overall, RGS scores were greater for the NRC group than the sham group at all time points; although this difference was nonsignificant ($P = 0.075$; Figure 2) when the groups were considered overall, significant differences are detected at individual time points. As early as 3 h after surgery, RGS values were significantly ($P < 0.0001$) higher than baseline for both the NRC (mean ± 1 SD, 1.24 ± 0.23) and sham (1.01 ± 0.28) groups and were 5 to 10 times greater than the corresponding scores at baseline (NRC, 0.14 ± 0.08; sham, 0.18 ± 0.11; Figure 2). RGS values at 6 and 24 h for both groups remained significantly ($P < 0.0041$) elevated over baseline (Figure 2). However, at 48 h after surgery, although the NRC RGS values were still



	baseline	sham (24 h)	sham (6 h)	NRC (6 h)
orbital tightening	0	0	1	2
nose/cheek flattening	0	1	1	1
ear change	0	1	1	2
whisker change	0	2	1	2
RGS value	**0**	**1**	**1**	**1.75**

Figure 1. Representative images and corresponding scores used to evaluate the rat grimace scale (RGS), with the corresponding scores for each action unit. The images were scored by using the 4 action units orbital tightening, nose or cheek flattening, ear change, and whisker change. A gray shape was placed over the mouse's dorsal cervical region (suture site) to blind scorers to its surgical status.

significantly greater than at baseline ($P = 0.014$), the RGS values in the sham group had returned to baseline levels (Figure 2). Of note, the RGS scores at 6 h after surgery were significantly ($P = 0.017$) greater in the NRC group (1.27 ± 0.18) than for the sham group (0.93 ± 0.20; Figure 2).

A total of 230 images from 23 rats were collected from the 4 treatment groups at 6 h after the surgical procedures (or anesthesia only) and were scored by 3 blinded observers. All images were scored for all 4 action units; no images had action units given a 'not scored' designation. The RGS scores for rats in both the MxNRC (mean ± 1 SD, 0.80 ± 0.12) and sham (0.90 ± 0.11) groups at 6 h were significantly ($P < 0.0001$) lower than the corresponding scores for the NRC group (1.22 ± 0.05; Figure 3). Furthermore, the RGS values for the anesthesia-only group (0.26 ± 0.2) were the lowest among all groups, a difference that was significant ($P < 0.0001$).

The reliability and consistency among the 3 blinded observers were very good. The overall ICC (95% CI) for scores between the 3 raters was 0.91 (0.88 to 0.93) for the RGS score for all rats and all time points (Table 1). The ICC for individual action unit scores exhibited a broad range depending on the action unit. Among the 4 action units, orbital tightening was scored most reliably, with an ICC of 0.92 (0.90 to 0.94), whereas nose or check flattening was the least reliably scored, with an ICC at 0.65 (0.56 to 0.72). The overall Cronbach α for RGS was 0.87 (Table 1). Exclusion of orbital tightening resulted in the smallest change in α (dropping α to 0.85), whereas excluding nose or cheek flattening and whisker change resulted in the greatest change (dropping α to 0.83).

Paw withdrawal thresholds for NRC were significantly lower than baseline values on both day 1 ($P < 0.0063$) and day 7 ($P < 0.0147$; Figure 4). Likewise, NRC thresholds were lower than those observed in the MxNRC group on both day 1 ($P < 0.0069$) and day 7 ($P < 0.0018$; Figure 4). Response thresholds at baseline did not differ between groups.

Discussion

This study is the first to use facial grimacing as a measure of nonevoked pain in a rat model of radiculopathy and demonstrates that the RGS is a useful tool for evaluating ongoing neuropathic pain early after the initial injury (Figures 1 and 2). Furthermore, treatment with the NSAID meloxicam before injury prevented RGS scores from changing from sham levels (Figure 3). RGS scores showed very good interrater reliability (Table 1) and excellent internal consistency (Table 1) among 3 blinded raters, thus validating the use of the RGS in our rat model. Evoked sensitivity

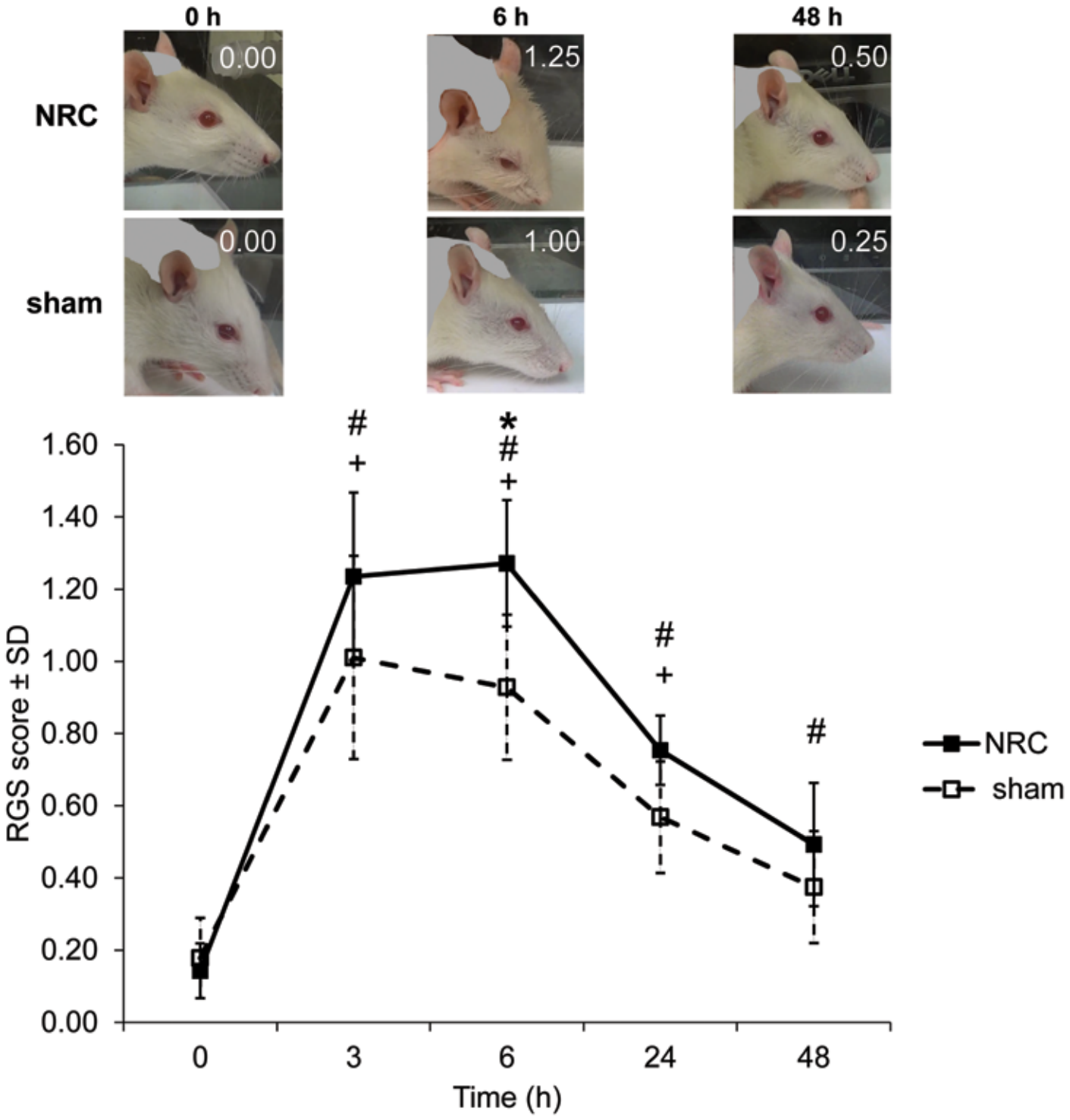


Figure 2. The RGS scores (mean ± 1 SD) were higher than baseline for both the NRC (#, $P < 0.0001$) and sham (+, $P \leq 0.0039$) groups at 3, 6, and 24 h after surgery. At 48 h, RGS values in the NRC remained significantly increased (#, $P = 0.014$) relative to baseline, whereas the RGS values in the sham group had returned to baseline levels. At 6 h, RGS values for the NRC group were significantly (*, $P = 0.017$) higher than those for sham rats. Representative images and corresponding quantification of RGS are shown at selected time points for the NRC and sham groups; images were scored by a single blinded rater.

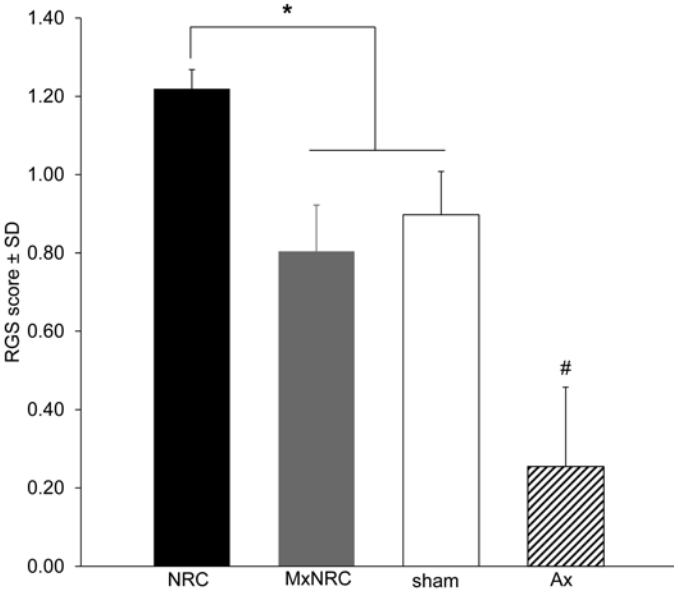


Figure 3. The RGS score (mean ± 1 SD) at 6 h was significantly (*, $P < 0.0001$) lower for rats that underwent nerve root compression and received meloxicam (MxNRC) and the sham surgery group than for the NRC group. RGS values for the anesthesia-only (Ax) group were lower (#, $P < 0.0001$) than those for all other groups.

Table 1. Interrater reliability (intraclass correlation coefficient) and internal consistency of scoring (Cronbach α) among 3 blinded raters using the RGS

	Intraclass correlation coefficient (95% CI)	Cronbach α
RGS overall	0.91 (0.88–0.93)	0.87
Orbital tightening	0.92 (0.90–0.94)	0.85
Nose or cheek flattening	0.65 (0.56–0.72)	0.83
Ear position	0.78 (0.72–0.82)	0.84
Whisker change	0.74 (0.67–0.79)	0.83

Regarding individual action units, reported reliability is for the indicated unit; that for consistency excludes the indicated unit.

testing showed that NRC induces mechanical hypersensitivity that begins on day 1 and persists until day 7, and meloxicam prevents the development of sensitivity (Figure 4). Both NRC and sham rats exhibited increased RGS scores as early as 3 h after surgery; these increases lasted as long as 24 h (Figure 2). However, by 48 h, only the group of rats that underwent NRC exhibited RGS values that exceeded baseline levels (Figure 2). Furthermore, additional differences between surgical groups were detected at 6 h (Figures 2 and 3). The fact that RGS scores increased even in the sham group suggests that the surgery alone can induce pain and thus highlights the need for surgical control groups. Because RGS scores for the NRC injury were greater than sham levels at 6 h, nerve root compression, which also induces robust increases in evoked sensitivity,[21,22,49,53,54,59] likely does in fact induce increased ongoing neuropathic pain; furthermore, the RGS is sensitive enough to detect this pain. Although behavioral differences between injury and sham were detected until day 1 after cervical nerve root injury by using evoked sensitivity tests[21,22,49,50,53,54,59] and even though the earliest evoked sensitivity time point was day 1

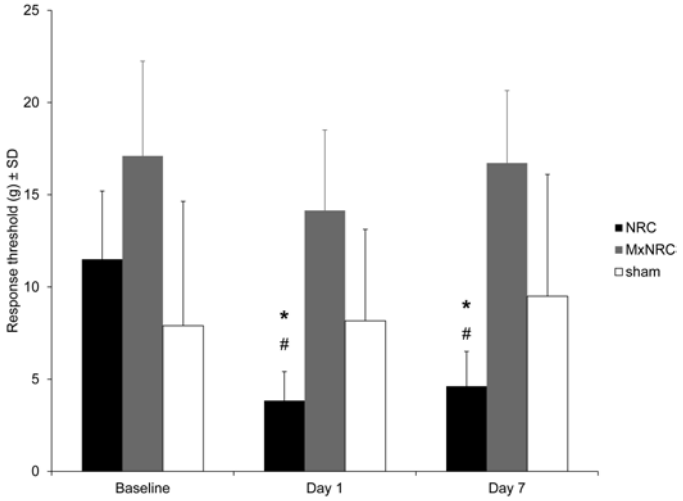


Figure 4. Evoked forepaw response thresholds (mean ± 1 SD) for the NRC group were significantly lower at day 1 (*, $P < 0.0063$) and day 7 (*, $P < 0.0147$) compared with baseline. In addition, withdrawal thresholds were significantly lower for the NRC group than the MxNRC group at day 1 (#, $P < 0.0069$) and day 7 (#, $P < 0.0018$). Baseline response thresholds did not differ between groups.

in the current study, our data suggest that such changes in pain may be present even earlier (Figure 2). Interestingly, nerve injury induces a breakdown of the blood–spinal cord barrier between 6 and 24 h after injury, and inflammatory cytokines are increased in the spinal cord as early as 1 h after NRC.[4,49,53] Rats undergoing NRC in the present study had heightened paw sensitivity on day 1 and remained sensitive at day 7 (Figure 4). Together with the RGS data, these findings suggest that neuropathic pain cascades are likely initiated within hours after nerve compression.

The RGS score peaked at 6 h after NRC (Figure 2), which is consistent with findings from other rodent models showing greatest postoperative facial grimacing at 4 to 8 h.[24,33,44,56] The RGS scores at 3 h were not significantly different between the NRC group and the sham group. Many rats slept through the majority of the video session at 3 h, likely because this time point was soon after surgery and reflects residual effects of anesthesia. Because of this effect, images were not collected at exactly 3-min intervals and were often taken shortly before or after sleep. This modification may have contributed to the increased variability that is evident in RGS scores at this time point (Figure 2). The RGS levels at 48 h after NRC remained increased over baseline but no longer differed from those from the sham rats (Figure 2). Perhaps ongoing neuropathic pain persists beyond the time when facial grimacing occurs and that grimace scores underestimate the actual duration of pain, as would be suggested by the persistence of evoked sensitivity in our rats at days 1 and 7 after injury. Factors such as facial muscle fatigue and prey animals' drive to conceal discomfort and illness may contribute to this phenomenon.[33,56] Taken together, these data suggest that RGS is a reliable measure of the onset of pain but that additional behavioral tests may be needed to measure the persistence of pain.

A single dose of meloxicam at the time of surgery prevented the reduction in paw-withdrawal thresholds that was observed in NRC alone on days 1 and 7 compared with baseline values (Figure 4), well past the time frame when RGS is most effective at detecting ongoing neuropathic pain. Although evoked responses

cannot be relied on as a robust measure of postoperative pain, these data demonstrate that early analgesic intervention can have beneficial effects in animals that last well beyond the period when the drug is likely to be present in its active form. Although meloxicam reduced the RGS of injured rats to sham levels, scores for all surgical groups remained elevated relative to those of the anesthesia-only controls (Figure 3). These data indicate that the RGS is more sensitive to surgery rather than to anesthesia. Facial grimace studies in mice and Wistar rats at comparable time points after a procedure show no effect of isoflurane anesthesia on grimace score.[10,16,24] Small increases in facial grimacing have been observed in mice exposed to isoflurane anesthesia alone, but that measurement was taken at just 1 h after exposure and was attributed to orbital tightening, likely due to decreased alertness from the lingering effects of anesthesia.[33] A similar effect may have occurred in our study. Because the effects of timing and magnitude of the meloxicam dose on the RGS score are not the focus of the current investigation, additional studies would be helpful to determine the effects of multimodal analgesia or alternative doses of meloxicam on pain in rats with radiculopathy to better translate these findings for clinical applications.

Overall, the RGS is a reliable and consistent method for quantifying pain. The interrater reliability for the overall RGS score between 3 blinded scorers is very good, with an ICC of 0.91 (Table 1).[2,27] Each action unit individually has a lower ICC than when all components are averaged together, indicating that the RGS scoring system is most reliable when all action units are combined. Nose or cheek flattening was the least reliable action unit in our model, with an ICC of 0.65 (Table 1), which is classified as substantial reliability.[27] This observation is consistent with the finding that nose or cheek flattening is the least reliable action unit in Wistar rats experiencing inflammatory pain.[56] Although not unacceptably low, the decreased reliability of nose or cheek flattening may reflect the greater influence of camera angle (head-on compared with profile; rat nose up compared with nose down) on this compared with other action units, such as orbital tightening, which are more easily quantified from any angle (Figure 1). Other studies in both mice and rats have reported whisker position as the least reliable action unit.[30,33,44] However, this discrepancy may be due to differences between rat strains. Some action units may be more difficult to quantify in one age, stock, or strain of rat compared with another.[24] Strain, sex, and time of day all influence grimace scores in mice.[34]

The overall internal consistency of the RGS with all action units combined was excellent, with a Cronbach's α coefficient of 0.87 (Table 1).[45] Exclusion of each action unit only slightly reduced the internal consistency, with a maximal difference of 0.04 for exclusion of nose or cheek flattening and whisker change (Table 1). This lack of effect suggests that each action unit contributes relatively equally to the consistency of the scoring system and that no single action unit drives the scoring in this model. Interestingly, whisker change was among the stronger contributors (Table 1), which is in contrast to a previous report, in which whiskers had a much lower effect on scoring consistency than did the other action units.[44] This discrepancy may be due to conformational differences in facial features between the rat strains used in the previous study (Sprague–Dawley)[44] and the current study (Holtzman). Although our findings demonstrate reliability and consistency of RGS scoring acutely in this radicular injury model, studies evaluating spontaneous pain by other quantifiable behavioral outcomes, particularly for later time points, are still needed. Nevertheless, behaviors such as licking, grooming, and rearing are reported to be correlated with grimace in vasectomized mice.[30] In addition assessing additional behaviors such as hunched posture, piloerection, body shakes, twitching, back arching, and sleep–wake disturbances[38,46,60] might provide valuable information relating to the duration and severity of spontaneous pain in treated and untreated animals in our model.

Several groups in this study did not receive perioperative analgesia. However, all rats in the study were monitored closely after surgery, with the intent to euthanize rats that showed evidence of undue distress. Previous work in this injury model showed that inflammatory cytokine levels increase in the spinal cord as early as 1 h after nerve root compression.[49,51] Given those results, administering preemptive or postoperative analgesics, even early after injury, would alter bimolecular responses and behavioral outcomes, particularly in the current study, in which differences in grimace were detected at 6 h after surgery (Figures 2 and 3). Furthermore, in the interest of reducing animal numbers and maximizing the information gained from each rat, tissues from the rats in the current study were collected for future analysis of pain-related markers at day 7. The prior administration of analgesics would affect those responses.[12]

Additional evaluations of spontaneous pain in rodents at later time points and with other chronic pain conditions are needed, given that facial grimacing likely underestimates the duration of spontaneous pain.[28,33,56] Although grimace scoring is a useful post hoc technique to detect pain, it may not be useful as a clinical decision-making tool. One of the greatest benefits of this technique is that the scorer can be out of the room during data acquisition, thereby reducing the likelihood of scorer–subject interactions and stress-induced analgesia.[55] This possibility is supported by the finding that mouse grimace scores assigned by observers in real time are lower than those assigned based on still images.[34] Despite the apparent lack of applicability of the grimace scale for animal care staff in evaluating pain levels on a day-to-day basis, the use of facial coding to detect pain has clinical utility in refining our understanding of acute pain and postoperative analgesic dosing.[16,24,26,30,46] The present study shows that the RGS is an effective tool to quantify ongoing neuropathic pain in a model of cervical radiculopathy and that preemptively delivered meloxicam attenuates this pain. Further studies will determine the downstream effects meloxicam exerts on central nervous tissue and how these relate more broadly to the pathophysiology of neuropathic pain.

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