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Quantification of pain in sickle mice using facial expressions and body measurements

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Abstract

Pain is a hallmark feature of sickle cell disease (SCD). Subjects typically quantify pain by themselves, which can be biased by other factors leading to overtreatment or under-treatment. Reliable and accurate quantification of pain, in real time, might enable to provide appropriate levels of analgesic treatment. The mouse grimace scale (MGS), a standardized behavioral coding system with high accuracy and reliability has been used to quantify varied types of pain. We hypothesized that addition of the objective parameters of body length and back curvature will strengthen the reproducibility of MGS. We examined MGS scores and body length and back curvature of transgenic BERK sickle and control mice following cold treatment or following treatment with analgesic cannabinoid CP55,940. We observed that sickle mice demonstrated decreased length and increased back curvature in response to cold. These observations correlate with changes in facial expression for the MGS score. CP55,940 treatment of sickle mice showed an increase in body length and a decrease in back curvature concordant with MGS scores indicative of an analgesic effect. Thus, body parameters combined with facial expressions may provide a quantifiable unbiased method for objective measure of pain in SCD.

Keywords

Pain; sickle cell disease; cannabinoid; mouse grimace scale; hyperalgesia

Introduction

Sickle cell disease (SCD) is the most common genetic disease caused by a single point mutation in the beta-globin chain of hemoglobin resulting in hypoxia induced

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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polymerization of hemoglobin S and sickling of red blood cells (RBCs) under low oxygen [1]. SCD is associated with chronic pain and unpredictable episodes of acute pain during “crises” when the sickle RBCs cause vasoocclusion, intense ischemic pain and end organ damage [2]. Pain can start in infancy and may continue throughout life resulting in increased morbidity and mortality. Pain in SCD is difficult to treat and the mainstream treatment is maintenance of normoxia, euvoemia, and opioids, which commonly require hospitalization [2]. The treatment of sickle cell pain crisis remains challenging due to unavailability of timely access to healthcare and fear of opioid addiction. This challenge is especially amplified in infants, children and mentally disabled, who cannot verbalize or quantify pain effectively, further delaying initiation of effective treatment, leading to prolonged discomfort and irreversible end organ damage in this vulnerable population. Currently there is no objective measure of pain, and subjects typically quantify pain by themselves, which may lead to overtreatment or under-treatment of pain. Patient’s quantification of their own pain may also be influenced by external factors (mood, stress, sleep, etc.). Early diagnosis and prompt treatment of sickle pain crisis can prevent prolonged suffering and irreversible ischemic end organ damage [2], therefore image based quantification of pain performed by the patient or caregivers is likely to increase early diagnosis. Furthermore, timely outpatient treatment of pain crisis is likely to shorten hospital stay and reduce cost of healthcare.

Transgenic sickle mice mimic the clinical disease replete with reticulocytosis, organ pathology, shortened survival and pain [3–7]. The HbSS-BERK sickle mice recapitulates the characteristic features of chronic sickle pain with increased sensitivity to touch, deep tissue pain and cold hyperalgesia, which progress with age [5, 8, 9]. Therefore, these sickle mice offer a unique advantage for performing translational studies leading to clinically relevant outcomes.

Elegant studies by Langford et al., demonstrated that image-based quantification of pain can be performed on mice [10]. Facial expressions are known to change in response to emotional alterations and pain in both humans and animals [10–12]. Additionally, we hypothesized that body dimensions also change with pain affecting the gait and length, because of the tendency to curl-up to find comfort when in pain. Since mice have a curved back, further curvature changes as well as length could be quantified mathematically on the images. We developed a simple mathematical model to quantify pain based on body curvature and length to support the facial expression readouts to finally provide a simple and reproducible method to quantify sickle pain. Our data provide a proof of principle to develop image-based facial expression and body dimension readouts to quantify sickle pain.

Material and Methods

Mice

Transgenic HbSS-BERK mice, called “sickle” mice henceforth, express >99% human sickle hemoglobin (HbS) were used. Sickle mice are homozygous knockouts for both murine α and β globins, and carry human α and β^S globins. These mice express severe phenotypic features of SCD including hemolysis, organ damage, and hyperalgesia. [5, 13] Control HbAA-BERK mice, Called “control” mice henceforth, express normal human hemoglobin A and are a progeny of the same breeders as sickle mice. Mice were phenotyped

for the presence of human sickle hemoglobin (HbS) and normal human hemoglobin A, by isoelectric focusing as described and genotyped using Transnetyx services [14]. All animal procedures were performed in compliance with the University of Minnesota Institutional Animal Care and Use Committee.

Image Capture

Mice were placed on a Plexiglas sheet on a tabletop in a glass chamber at room temperature (RT) and digitally videotaped using a Nikon Coolpix model L105 (Nikon Inc, Melville, NY; 2048 x 1536 pixels) and iPhone 5S (Apple Inc, Cupertino, California; 1280 x 960 pixels). The Plexiglas surface contained a gridded template (unit measure: 1 cm x 1 cm) to determine length. One camera was placed on the top of the chamber for a top-down view and the other camera was placed immediately outside of the glass chamber in front of the mouse. A light blue background was used to provide a contrast between mouse and background. Mice were acclimated for 5 minutes to each temperature and videotaped for 5 minutes before the treatment to obtain a baseline and after the treatment to examine the treatment effect. Color images were extracted. Still frames showing clear headshots were obtained from the videos using QuickTime Player 7 (Apple Inc.), and transported into Photoshop CS 6.0 (Adobe, San Jose, CA) and sized equally for coding.

Quantification of Physical Parameters

Mouse Grimace Scale (MGS) parameters—Images were coded in a blinded manner to quantify facial action units (AUs). All coders went through three training sessions using published images by Langford et al. [10], as a guide and unlabeled randomized images of ‘no pain’ and ‘pain’ faces of mice that had been exposed to room temperature or cold treatment, respectively. Individual, randomized and unlabeled facial images were presented on a large computer monitor. As described in Figure 1 four AUs (orbital tightening, nose bulge, cheek bulge, and ear position) were assessed for each mouse and condition using four randomized images of each mouse by three blinded ‘coders’. For MGS, the intensity of each AU was scored as follows: ‘0’ = absence; ‘1’ = moderate and ‘2’ = severe according to Langford et al. [10]. The average scores from three coders were calculated for each AU. In cases where the AU could not be observed, no score was given. Mice with more than two obscured AUs, did not receive an overall score. Cumulative pain calculations were made by averaging all AUs as described[10]. Images were randomized and all coders were blinded to the treatment and/or mouse genotype/gender. Intra and inter-coder variability was assessed by Cronbach’s alpha and Intraclass Correlation Coefficient (ICC) [10, 15].

Body parameters—Top-down view images were extracted from the videos using QuickTime Player 7 and length was measured using the pre-standardized grid template image placed on the surface below the mouse, two images were analyzed per mouse. To analyze spinal curvature, best-fit ellipses were constructed on the acquired images using EllipseFit (Vollmer F.W., <http://www.frederickvollmer.com/ellipsefit/>) as shown in Figure 2. Points were plotted on the arch of the spine of the mouse between the neck where the arch begins and at the end of the arch, just proximal to the base of the tail. Ellipsefit software calculates the best fit ellipse using an average sum of log probability distribution function of each individual point. From the lengths of major and minor axes (a and b respectively), for

the resulting best fit ellipse the eccentricity values (e) were calculated according to the formula: $e = \sqrt{(a^2 - b^2)/a}$. Eccentricity values are the measure of curvature for ellipses. Lower eccentricity values corresponds to a more curved body shape (more hunched posture, indicative of more pain), while larger differences corresponded to a more elliptical shape (less curved indicative of less pain).

Cold Treatment

Age-matched male and female mice were placed in a glass chamber on a Plexiglas surface with grid template at RT or on a cooled aluminum plate at 4.5 C of a Hot/Cold Plate (UGO Basile Model 35100, Stoelting Co., Wood Dale, IL). Under the cold condition, the grid template was placed above the mouse, in order to avoid insulating the mouse from the cold plate. Due to the difference in focus for the top view camera, a conversion ratio was used to obtain the actual length from observed length in the images. The conversion ratio was obtained by dividing the diameter of the cold plate in centimeters to the observed diameter of the cold plate when seen in an image. Conversion ratios were calculated for each mouse. The mice were videotaped for 5 minutes under each condition.

Drug Treatment

The cannabinoid receptor agonist, (-)-cis-3-[2-hydroxy-4(1,1-dimethylheptyl) phenyl]-trans-4-(3-hydroxypropyl) cyclohexanol (CP 55,940; Tocris Bioscience, BioTechnie, Minneapolis MN) called CP55,940 henceforth, was prepared in 2% Dimethyl sulfoxide (DMSO) and 98% normal saline as described [5]. Male sickle mice were separated into vehicle and treatment groups ($n=8/\text{group}$) and treated with 0.3 mg/kg CP 55,940 intraperitoneally or vehicle (2% DMSO and 98% saline). Mice were videotaped at RT for 5 minutes before injection to obtain the baseline and 1 hour after treatment to examine the effect of drug or vehicle.

Statistical Analysis

All data were analyzed using Prism software (v 5.0a, GraphPad Prism Inc., San Diego, CA) with the exception of the Cronbach's Alpha and ICC statistics, which were calculated using MATLAB R2015a (v 8.5, The MathWorks, Inc., Natick, MA). A two-way repeated measures analysis of variance (ANOVA) with Bonferroni's correction was used to compare the response between treatments in the cold plate experiment. An unpaired Student's t test was used to compare the response between treatments in the CP 55,940 experiment. A p -value of < 0.05 was considered significant. All data are presented as mean $\pm$ SEM. The Pearson correlation analysis was used to compare the relationship between body measurements and MGS score.

Results

Initially, six coders determined pain in mice using MGS (Fig. 2). However, the ICC ($\text{ICC}_{\text{average}} < 0.7$) and Cronbach's alpha values ($\alpha < 0.8$) were low due to the large intra-coder variability among three of the coders. The results from the three coders, who could reproducibly code images with low variability, were used. Due to intra-coder variability, we included length and curvature measurements which could be directly pulled from images,

and prevent coder-bias. Then we used the MGS scores as a validation of the length and curvature measurements.

Body Parameters can be used to quantify cold hyperalgesia

Cold hypersensitivity is common in mice and patients with sickle cell disease [5, 8, 16, 17]. Therefore, we elected to evaluate response to cold in sickle and control mice to determine whether sickle pain can be quantified objectively using body parameters. At RT both control and sickle mice showed similar lengths and back curvature but when placed on the cold plate both presented a significant decrease in their length compared to RT (Fig. 3A; $P < 0.0001$). However, when comparing the percent decrease of length between the mice, sickle mice exhibited a significantly higher percent difference from the RT value (Fig. 3B; $P < 0.0001$). Control mice exhibited $85.9\% \pm 1.5\%$ retention of their original length. Sickle mice exhibited $74.7\% \pm 1.5\%$ retention of their original length. The F statistics indicated a genotype x treatment interaction for length ($F_{1, 18} = 23.61$; $P = 0.0001$). Although control and sickle mice both exhibited a significant decrease in length, control mice may have responded only to the cold, while sickle mice length likely decreased due to cold and pain associated with increased sickling which was more evident when expressed as percent change.

Back curvature was quantified by measuring the major and minor axes of the fitted ellipse to determine the value for eccentricity (Fig. 2). A smaller difference indicates more/increased curvature and larger difference indicates less/decreased curvature. At RT, control and sickle showed similar values; which were significantly decreased on the cold plate (Fig 3C; $P < 0.0001$) and sickle mice showed a greater decrease of eccentricity compared to control mice. Curvature is inversely proportional to the eccentricity, indicating less hunched posture. When comparing differences from the RT value the percent difference between control and sickle mice was significant (Fig 3D; $P < 0.01$) although the genotype x treatment interaction for curvature was not significant ($F_{1, 36} = 2.265$; $P = 0.141$). Control mice exhibited a $83.38\% \pm 1.95\%$ difference, while sickle mice exhibited a $74.25\% \pm 3.28\%$ difference indicating a greater increase in curvature, which parallels what we found in the length measurement.

Facial expression of pain in sickle mice

The MGS has been proven to effectively quantify pain based on the readout of facial expressions, termed 'Action Units' exhibited by mice [10]. Mice were grouped by genotype and gender, to see if there is any bias between male and female mice when assessing the presence of a 'pain face'. The AUs, orbital tightening, nose bulge, cheek bulge, and ear position were analyzed in each mouse under cold and RT conditions and a final MGS score was computed from the average of all AUs. Orbital tightening exhibited significance between RT and cold for male sickle ($P < 0.01$) and female sickle ($P < 0.001$) but not for the male control and female control groups (Fig. 4A). Significant difference in orbital tightening was observed only on the cold plate when comparing sickle to control mice in both the male and female groups (Fig. 4A; $P < 0.01$, and < 0.001 , respectively). Nose bulge increased significantly between female sickle mice at RT and cold (Fig. 4B; $P < 0.01$) and between male control and male sickle mice on cold exposure (Fig. 4B; $P < 0.01$). Cheek bulge

demonstrated significant increase between sickle males, sickle females, and control males at RT compared to cold (Fig. 4C; $P < 0.001$, $P < 0.001$, $P < 0.05$ respectively). Ear position demonstrated significant difference between female sickle mice at RT and cold (Fig 4D; $P < 0.001$) and female control and sickle mice on cold plate (Fig 4D; $P < 0.001$). Only between sickle and control females was there a significant increase in cheek bulge and ear position on exposure to cold supporting the idea that there might be a gender bias in the intensity of facial features in response to nociceptive stimuli. Orbital tightening appeared to be the most sensitive AU along with cheek bulge with greater significant differences in groups following cold exposure (Fig. 4A and C), while nose bulge seemed to be the least sensitive AU. The F statistics indicated a gender x treatment interaction in sickle mice for ear position ($F_{1, 23} = 6.750$, $P = 0.0161$) and genotype x treatment interaction for female sickle and control mice for cheek bulge ($F_{1, 16} = 5.772$, $P = 0.0288$) and ear position ($F_{1, 16} = 15.05$, $P = 0.0013$).

When all of the AUs were combined for a final MGS score significance was observed between male control, male sickle, and female sickle at RT compared to cold (Fig. 4E; $P < 0.05$, $P < 0.001$, $P < 0.0001$). Cross genotype (sickle vs control) comparisons revealed significant differences upon exposure to cold for males and females (Fig 4E; $P < 0.05$ and $P < 0.0001$, respectively), while the percent differences in MGS score significantly increased only for female sickle mice in comparison to control mice (Fig 4F; $P < 0.05$, $F_{3, 37} = 4.227.07$, $P = 0.0109$). Also, only the final MGS score demonstrated significance between sickle males and females on the cold plate (Fig 4E; $P < 0.05$, $F_{1, 25} = 10.07$, $P = 0.0040$). This is in line with observations on sickle patients, since females with SCD report pain episodes of longer duration compared to males [18] and female sickle patients report higher pain intensity compared to males [19]. Earlier, we observed increased deep tissue hyperalgesia in female sickle mice as compared age-matched males [5].

Treatment with CP 55,940 decreases pain in sickle mice

Earlier we observed that the non-selective cannabinoid receptor agonist CP 55,940 attenuates deep tissue, mechanical and thermal hyperalgesia in HbSS-BERK sickle mice [5, 20]. We therefore examined if the length and curvature measurements would complement the behavioral measures in response to CP55,940 treatment. We observed a significant difference in length between CP 55,940 and vehicle-treated sickle mice (Fig 5A; $P < 0.001$). The length decrease from the untreated value in the vehicle group was $91.05\% \pm 1.0\%$ and the percent change for the treatment group was $107.7\% \pm 2.3\%$ (Fig. 5B; $P < 0.0001$). The difference between the Max-Min axes increased in the CP55,940 treated mice (Fig 5C; $P < 0.05$), indicative of decreased curvature and less pain. Vehicle treated mice had no percent difference in eccentricity ($97.31\% \pm 4.95\%$) while CP55,940 treatment increased the observed eccentricity from the untreated value ($125.3\% \pm 6.81\%$), which could be interpreted as reducing curvature by half indicative of significant amelioration of pain (Fig 5D; $P < 0.05$).

Validation of Treatment using MGS

MGS score was analyzed to validate the length and curvature measures following CP55,940 treatment (Fig 6). Orbital tightening and cheek bulge presented the most significant difference (Fig 6A and C; $P < 0.01$) between CP55,940 and vehicle treated sickle mice,

consistent with the observed differences in these AUs upon cold exposure above (Fig 4). Ear position was also significantly different between the treatments (Fig 6D; $P < 0.05$). Nose bulge did not show statistically significant difference after treatment (Fig 6B). Upon combining all the AUs we observed a significant difference in MGS score between vehicle and CP55,940 treatment groups (Fig 6E; $P < 0.01$). Mouse size is not a factor in the observed responses since there was no significant difference in body weight between vehicle and treated groups (30.1 ± 1.5 g and 34.0 ± 1.3 g, respectively). The analysis of correlation between body measurements and MGS for the pain measurements showed moderate inverse relationship in sickle mice (Fig.7).

Discussion

Image analysis of facial expression of mice reveals that pain and effectiveness of analgesia in various mouse models can be reliably quantified using the MGS. It has been used to evaluate pain in experimental myocardial infarction and post-vasectomy and in studies on analgesia in mice [21–24]. Here we report that an increase in cold sensitivity in sickle mice can be recorded using body parameters (length and back curvature) along with the MGS scale. In sickle mice, the difference in pain response to cold was demonstrated objectively through the percent change in length and curvature. The individual AUs along with the overall MGS score correlate to the observed change in body parameters, which support previous findings[10]. While we did not observe a difference between male and female control mice, the MGS score demonstrates a difference between male and female sickle mice upon cold exposure, which suggests that sensitivity to cold is gender biased. These results corroborate previous findings that mice and individuals with SCD show increased cold sensitivity [2, 5, 8, 9, 14, 16] Therefore, image analysis of body parameters along with facial expressions can be quantified for evaluating pain in mice.

Notably, female sickle mice showed a significantly higher response in MGS. This is important in the context of SCD, because female subjects as well as mice show increased pain as compared to males [5, 19, 25]. It could also be due to differences in the mechanisms of pain between the two genders as recognized recently [26–28]. Additionally, females are overall more expressive, which could contribute to the bigger changes in facial expressions upon cold stimulation. These possibilities favor using MGS in females for subtle changes and needs to be examined further.

CP 55,940, a cannabinoid receptor agonist, has been shown to decrease chronic hyperalgesia in sickle mice [5, 8]. We observed that sickle mice treated with CP 55,940 demonstrate an increase in length with a corresponding decrease in curvature as compared to vehicle treated mice correlating with the MGS scores. Although the changes in pain were small, because of no nociceptive stimulation, body parameters and the MGS were able to capture the effect of the treatment, which suggests that this method of pain quantification is sensitive and accurate and can be applied to observe responses to therapy.

Procedures such as the body measurements as described by us and facial expression readouts of freely moving mice also prevent the stress induced by restraint and noxious stimuli as reported by Langford et al [10]. However, facial readouts may be influenced by observer-

biased variability. In our experience, 50% of the observers showed significant intra-observer variability in coding the expressions, affecting the reproducibility of their readouts. These observers were excluded from coding, suggesting that a large pool of trained observers has to be recruited for facial expression readouts, and intra- and inter-coder reproducibility has to be established to successfully code for facial expressions. The objective measurements using body length and curvature provide accurate measures without the influence of coder variability and may be useful in validating facial expression readouts. We saw a modest correlation between the readouts of facial expression and body parameters. This could be due to a narrow scale of 1 and 2 for MGS, which reduces the sensitivity of the measure and the challenges in acquiring expressions on moving mice. In larger animals and in humans, we may observe improved correlation.

In conclusion, facial expression coding system developed by Langford and changes in body dimension described here can be successfully applied to quantifying pain in sickle mice. These parameters offer the advantage to quantify pain using images from a remote access. Several facial expression readout technologies are under development [29–32]. Quantification of pain using images utilizing remote access, appears to be a promising approach, but may require intense efforts on precluding false positive outcomes. It is known that response to therapy in SCD is more achievable if pain is treated as soon as it starts in the ‘primordial’ stage [2]. Since SCD affects individuals in lower socioeconomic classes, this pain quantification method if developed, may improve accessibility to health care, reduce hospitalization, improve outcomes and reduce costs. A previous study has demonstrated that facial expressions prove to be a valid mean of quantifying pain in humans [33]. Therefore, modeling of pain quantification in sickle mice herein provides a proof of analyzing pain using facial expressions.

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Figure 1. Changes in mouse facial expression in response to cold. Description and representative images for each AU at RT and on cold plate (4°C) are shown for the parameters measured for overall average MGS score. Abbreviations: AU, action units; RT, room temperature.

Figure 2.

Facial features and body parameters proposed to quantitate pain. Diagram showing the facial action units (AUs) measured for the Mouse Grimace Scale (MGS) score and positioning of the ellipse used to determine curvature of the back.

Figure 3.

Cold hyperalgesia leads to shortening of length and increased curvature of the back. (A) Cold exposure (red bars) reduced the length measurements in both sickle (SS) and control (AA) mice as compared to their measurements at RT (black bars). (B) Sickle mice showed significantly more reduced percent difference in length than control mice. (C) Cold exposure increased the curvature in both sickle and control mice compared to values at RT, curvature is inversely proportional to the eccentricity. (D) Sickle mice showed significantly greater percent difference in back curvature than control mice. Percent difference in lengths and curvature of sickle and control mice at 4°C are compared to their respective values at RT as 100%. Data given as mean $\pm$ SEM (n=10 per group). Mean age of each group of mice in months $\pm$ SEM for sickle mice: 5.4 ± 0.3 and for control mice: 5.5 ± 0.2 . Two-way ANOVA followed by Bonferroni's correction for multiple comparisons revealed a significant genotype $\times$ treatment interaction for the measured length but not eccentricity (A: $F_{1,18} = 23.61$; $P = 0.0001$; C: $F_{1,36} = 2.265$; $p = 0.141$). Unpaired t-test was used to determine significance between percent change values (B, D). * $P < 0.01$, ** $P < 0.0001$.

Figure 4.

Cold exposure increases AUs and MGS score differently in male and female sickle mice. (A) Orbital tightening significantly increases in both male and female sickle mice at 4°C compared to RT. Both sickle groups also showed significant increases from their respective controls at 4°C. (B) Nose bulge increased significantly in response to cold only in female sickle mice and between male sickle and control mice. (C) Cheek bulge significantly increased in both male and female sickle mice and only in male control mice at 4°C compared to RT. There was a difference between the female sickle and female control mice at 4°C. (D) Ear position AUs only significantly changed in female sickle mice at 4°C compared to RT, and only between sickle and control females at 4°C. (E) Average MGS score significantly increased in both male and female sickle mice and only in male control mice at 4°C compared to RT. Both sickle groups also showed significant increases from their respective controls at 4°C. There was an overall significant increase of the MGS score for female sickle mice when compare to male sickle mice at 4°C. (F) The percent differences in MGS score significantly increased only in female sickle mice in comparison to control mice. These differences at 4°C are compared to their respective values at RT as 100%. Data are means $\pm$ SEM (n=10 per group). Mean age of each group of mice in months

± SEM for sickle male mice: 6.2 ± 0.2 ; sickle female mice: 4.6 ± 0.0 ; control male mice: 6.1 ± 0.1 and control female mice: 4.9 ± 0.2 . Two-way ANOVA (A–E) or one-way ANOVA (F) followed by Bonferoni's correction for multiple comparisons was used to determine significance. F test analyses of sickle males x sickle females, sickle males x control males, sickle females x control females, and control males x control females, showed significant treatment interaction between male and female sickle mice for ear position (D: $F_{1, 23} = 6.750$, $P = 0.0161$) and overall MGS score (E: $F_{1, 25} = 10.07$, $P = 0.0040$) and sickle and control females for cheek bulge (C: $F_{1, 16} = 5.772$, $P = 0.0288$), ear position (D: $F_{1, 16} = 15.05$, $P = 0.0013$), overall MGS score (E: $F_{1, 15} = 20.05$, $P = 0.0004$) and % difference in MGS score (F: $F_{3, 37} = 4.277$, $P = 0.0109$) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Abbreviations: AU, action units; MGS, Mouse Grimace Scale

Figure 5.

CP 55,940 treatment of sickle mice increases length and decreases curvature of the back. (A) Length measurements increased in treated sickle mice compared to vehicle group. (B) Percent difference in length of CP 55,940 treated sickle mice is significantly greater than that of vehicle. (C) Back curvature decreased significantly in sickle mice following treatment compared to vehicle group, after CP55,940 treatment. (D) Percent change in back curvature (eccentricity) following CP 55,940 treatment was higher in sickle mice than in vehicle treated mice. Percent difference in length and eccentricity of treated and vehicle groups are relative to their respective values before injection as 100%. Data are means $\pm$ SEM ($n=8$ per group). Mean age of each group of mice in months $\pm$ SEM for vehicle: 5.6 ± 0.2 and CP55,940: 5.4 ± 0.2 . Unpaired t-test was used to determine significance. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

Figure 6.

CP 55,940 treatment decreases AU and MGS score in sickle mice. Orbital tightening (A), cheek bulge (C), ear position (D), and overall MGS score (E) significantly decreased in CP55,940 treated sickle mice compared to vehicle treated sickle mice. Nose bulge (B) was not significantly different between the CP55,940 and vehicle treated groups. Data are means $\pm$ SEM (n=8 per group). Mean age of each group of mice in months $\pm$ SEM for vehicle: 5.6 ± 0.2 and CP55,940: 5.4 ± 0.2 . Unpaired t-test was used to determine significance. * $P < 0.05$, ** $P < 0.01$. Abbreviations: AU, action units; MGS, mouse grimace scale.

Figure 7.

Correlation between body parameters and MGS in sickle mice. Pain determination using body parameters correlates to the MGS in sickle mice. (A) Length demonstrates an inverse correlation ($R = -0.7178$) with a value of $R^2 = 0.5153$, $P < 0.0001$. (B). Eccentricity demonstrates a moderate inverse correlation ($R = -0.5780$) and the resulting coefficient of value of $R^2 = 0.3341$, $P < 0.0001$. This inverse correlation demonstrates a tendency for high MGS scores to go with low body parameter values (and vice versa). Values are from all the mice used in this study including pre- and post-cold exposure and pre- and post-CP55,940 treatment. Abbreviations: MGS, mouse grimace scale.