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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.

1. 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 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, euvolemia, 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 opiod 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

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provide a proof of principle to develop image-based facial expression and body dimension readouts to quantify sickle pain.

2. Material and methods

2.1. 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 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.

2.2. 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 × 1536 pixels) and iPhone 5S (Apple Inc., Cupertino, California; 1280 × 960 pixels). The Plexiglas surface contained a gridded template (unit measure: 1 cm × 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 min to each temperature and videotaped for 5 min 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.

2.3. Quantification of physical parameters

2.3.1. 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 Fig. 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].

2.3.2. 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 Fig. 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

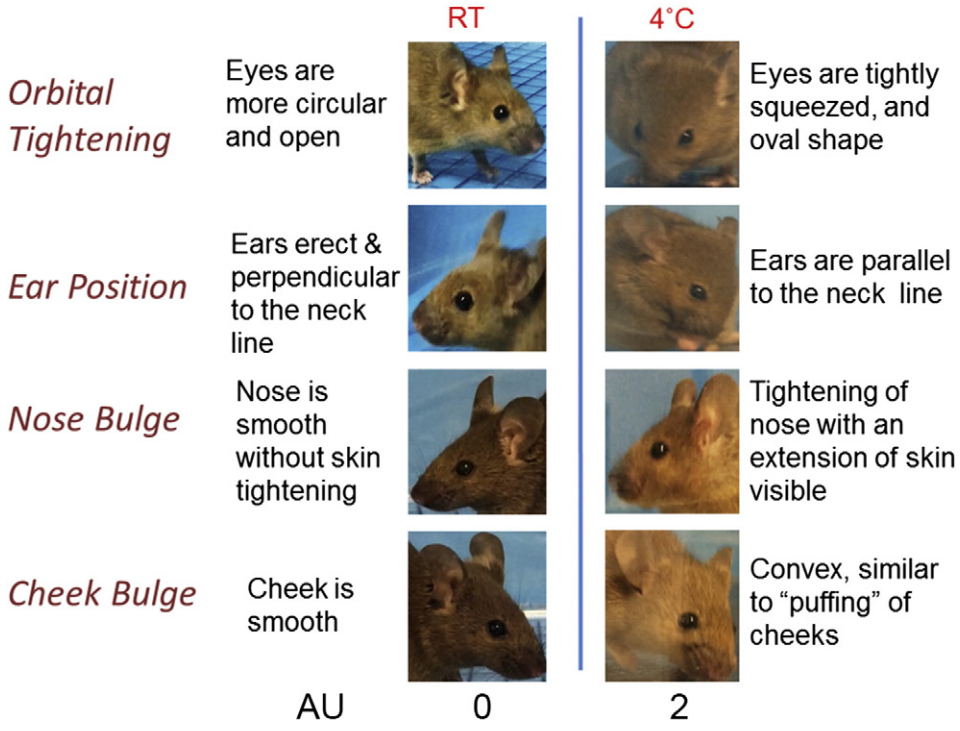

Fig. 1. Changes in mouse facial expression in response to cold. Description and representative images for each AU at RT and on cold plate (4 °C) are shown for the parameters measured for overall average MGS score. Abbreviations: AU, action units; MGS, mouse grimace scale; RT, room temperature.