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Central sensitization associated with fetal hemoglobin levels in adults with sickle cell anemia

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Ethical Issues

Written informed consent was required of all enrolled subjects after protocol approval by the Institutional Review Board at the National Institutes of Health. The study was registered with [Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01441141) (identifier NCT01441141).

Conflict of Interest

The authors report no conflicts of interest.

Author Contributions

Conceived and designed study: DSD, CS, GRW, IB, JGT. QST and research coordination: KV, CS, KC, AC. Statistical analysis: KV, MQ, MAW, JGT. Data interpretation: DSD, KV, CS, KC, MQ, AC, LD, ANS, JAH, MAW, GRW, IB, JGT. Logistical support and QST training: AC, LD, GRW, ANS, JAH, IB. Drafted manuscript: JGT with contributions from DSD and KV. All authors contributed to manuscript writing and editing.

Abstract

Background and aims—Pain is the hallmark of sickle cell anemia (SCA), presenting as recurrent acute events or chronic pain. Central sensitization, or enhanced excitability of the central nervous system, alters pain processing and contributes to the maintenance of chronic pain. Individuals with SCA demonstrate enhanced sensitivity to painful stimuli however central mechanisms of pain have not been fully explored. We hypothesized that adults with SCA would show evidence of central sensitization as observed in other diseases of chronic pain.

Methods—We conducted a prospective study of static and dynamic quantitative sensory tests in 30 adults with SCA and 30 matched controls.

Results—Static thermal testing using cold stimuli showed lower pain thresholds ($p=0.04$) and tolerance ($p=0.04$) in sickle cell subjects, but not for heat. However, SCA subjects reported higher pain ratings with random heat pulses ($p<0.0001$) and change in scores with temporal summation at the heat pain threshold ($p=0.002$). Similarly, with the use of pressure pain stimuli, sickle cell subjects reported higher pain ratings ($p=0.04$), but not higher pressure pain tolerance/thresholds or allodynia to light tactile stimuli. Temporal summation pain score changes using 2 pinprick probes (256 and 512 mN) were significantly greater ($p=0.004$ and $p=0.008$) with sickle cell, and delayed recovery was associated with lower fetal hemoglobin ($p=0.002$ and 0.003).

Conclusions—Exaggerated temporal summation responses provide evidence of central sensitization in SCA.

Implications—The association with fetal hemoglobin suggests this known SCA modifier may have a therapeutic role in modulating central sensitization.

Keywords

Sickle cell anemia; pain; central sensitization; temporal summation; fetal hemoglobin

1. Introduction

Pain is the most common manifestation of sickle cell disease (SCD). The onset of acute painful vaso-occlusive crises (VOCs) coincide with a post-natal fetal hemoglobin decline, and frequency is impacted by age, sex, fetal hemoglobin and homozygous SCD [1–7]. Diary studies demonstrate children with SCD experience pain, however, frequency and opioid use increase in adolescence [2, 6]. In an adult pain diary study (PiSCES), 55% of subjects reported pain on more than half of days and 29% had daily pain, where more frequent pain correlated with hospital utilization [3, 8]. In older patients, acute SCD pain is often superimposed on chronic pain [9].

Occlusion of sickled erythrocytes during hypoxia within the microcirculation is believed to be the proximate cause of acute VOCs. However, it is not known if factors other than hypoxia influence chronic SCD pain [4]. Recurrent VOCs, opioid-induced hyperalgesia, genetic polymorphisms and central sensitization likely contribute to variation in SCD pain [10–15]. Murine SCD models and individuals with SCD show increased sensitivity to experimental pain similar to that described in other groups with chronic pain [12, 16–18]. Recent murine and human studies have observed central sensitization in SCD [10, 14, 18–

22]. Central sensitization is a process where repeated nociceptive signals from the periphery affect and alter pain processing in the central nervous system, leading to amplification of pain. Manifestations include hyperalgesia and allodynia, an increased area of hyperalgesia extending beyond the initial area of injury, and aftersensations following resolution of initial injury [23]. Many of these features are often observed by clinicians who treat SCD.

Quantitative sensory testing (QST) measures responses to experimental stimuli and the function of neural pain processing pathways [24]. Static end points include pain thresholds, pain tolerance, and pain ratings (e.g., 0 to 10) in response to a noxious stimulus. Temporal summation assesses excitatory elements of central nervous system pain processing [25].

QST has been used to characterize many chronically painful conditions, but its use in SCD has only recently developed. QST in SCD shows increased sensitivity to painful stimuli [12, 18–21, 26–30] which is heightened by age [18, 30, 31]. [27]. While SCD studies have demonstrated increased sensitivity by static QST methods, the magnitude of these differences were significantly smaller than in fibromyalgia, [14, 18, 27, 28, 32]. Recent QST studies exploring central sensitization in SCD are promising [14], however its relationship with known disease modifiers has not been evaluated [4, 13, 14, 19, 20]. Indeed, recent resting state functional MRI showed a significant correlation between anti-nociceptive brain connectivity and fetal hemoglobin in low pain burden SCD patients [15].

In this study, we compared static and dynamic QST responses between adults with SCA and matched normal volunteers. We hypothesized that adults with homozygous SCD would show greater evidence of central sensitization owing to the high prevalence of chronic pain in older patients. We observed that central sensitization a feature of sickle cell pain, and surprisingly that temporal summation was lessened in the setting of higher fetal hemoglobin, a determinant of disease severity [4].

2. Materials and Methods

2.1. Study Population

Sickle cell anemia (homozygous SS or S β^0) subjects who consented to the Bethesda Sickle Cell Cohort Study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT00011648) Identifier: NCT00011648) during 2011 were approached sequentially for participation in a new protocol focused on pain. Other SS subjects expressing interest in the study were also enrolled, yielding a random cross sectional sample. Inclusion criteria were 1) sickle cell anemia (SCA), 2) African ancestry, and 3) residence within 50 miles of the National Institutes of Health so that participants could return for the pain testing as needed. Subjects were age and sex matched to healthy normal volunteers (NVs) identified by the NIH Clinical Center Recruitment Office (www.cc.nih.gov/recruit/volunteers.html). Inclusion criteria for NVs were 1) African ancestry and 2) absence of any disease associated with pain (e.g. fibromyalgia, chronic back pain, diabetes, etc.). NVs were excluded for any hemoglobinopathy including sickle trait or acute or chronic pain at initial evaluation. Subjects provided written informed consent to an IRB approved protocol ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT01441141) identifier NCT01441141) and underwent further screening which included a medical history, physical exam (including a neurologic exam)

and lab studies prior to being scheduled for QST. Recruitment and initial testing took place between November, 2012 and August, 2014 (Supplemental Figure 1).

2.2. Quantitative Sensory Testing

QST was performed in steady state without acute painful events requiring physician treatment in the two weeks prior to testing. QST as described below was performed by 2 team members (CS and KV) to minimize investigator dependent variability. All verbal instructions to subjects were scripted. Testing for thermal thresholds, thermal temporal summation, algometry, and mechanical pinprick temporal summation were based upon a previously published QST protocol [33]. Thirteen out of 30 sickle cell subjects were on maintenance daily opioids which were not stopped for the study as it was determined to be ethically and medically inappropriate to withhold analgesics. Daily opioids were prescribed by their primary hematologists for management of typical daily SCD pain without an acute painful event (i.e. pain not requiring medical attention). Pain scores obtained during QST used the 20 point Gracely Pain Scale (GPS); with one score for pain and another for unpleasantness [34]. The Gracely Pain Scale was designed to measure the intensity and unpleasantness of the individual's pain sensation with the aid of verbal pain descriptors. The multiple verbal descriptors cover the entire pain range, minimizing floor and ceiling effects [35]. Scoring required that each subject reviewed the 13 verbal descriptors along a visual analogue scale (VAS) from 0 to 20. The location along with the VAS for a descriptor was used as the numerical score for that descriptor. The subject's score was determined by the descriptor selected as best representing their perceived pain or unpleasantness. The VAS creates a ratio scale for the descriptors, where the minimum VAS score is 0. The psychometric properties of the scales, assessed using ratio-scaling techniques, indicate that scores assigned to the descriptors demonstrate good internal consistency, reliability and objectivity [36]. Records of all hospitalizations during the 12 months prior to study were reviewed to confirm patient reported outcomes.

Thermal QST defined static temperature responses and dynamic thermal testing included random order heat pulses and temporal summation [25]. Thermal stimulus experiments used a Medoc Sensory Analyzer (model TSA 2001-II; Medoc Ltd., Israel) with a 2.56-cm² contact thermode. Static tests were administered at four sites on each forearm (nonhairy volar surface between the elbow and wrist of the distal forearm; approximately 4 cm between sites). At each forearm testing site, subjects indicated the thermal detection threshold (thermal stimuli first felt), the pain threshold (pain first felt from thermal stimuli), and pain tolerance (point at which unable to continue testing due to pain caused by heat and cold stimuli).

The dynamic thermal test was performed on the dominant volar forearm, slightly lateral to where static thermal tests were administered to avoid overlapping stimulation sites. The test first consisted of random order heat pulses and then individualized temperature heat pulses for temporal summation. The random thermal series included 14 pulses administered in random order between 32 and 44 °C (in 2 °C increments; each temperature tested twice). Individualized pulses included a series of 10 heat pulses with 5 second intervals between each pulse (the shortest interval allowed by the TSA 2001-II [33]). Four 10 pulse sequences

were tested: one at the subject's pain threshold, one 2°C below the threshold, one 2 °C above the threshold, and then one at 45 °C. Pain scores were recorded between each pulse for each series of 10 pulses. Additional pain ratings were obtained 15 seconds after cessation of the stimulus to characterize aftersensations. Thermal temporal summation was defined by a pain score = highest pain rating minus the first score [37].

Conditioned Pain Modulation (CPM) used the Medoc (thermode applied to nonhairy volar forearm) and either a room temperature water bath or a hot water bath (immersion of nondominant hand) [25]. The hot water bath was a modification from use of ice water which is not a feasible inhibitory painful stimulus in sickle cell anemia. The thermode temperature was where subjects reported a pain magnitude rating score as close to 11 (verbal descriptor "moderate") as possible. The thermode at this pain magnitude rating was the primary painful stimulus. The neutral water bath served as a habituation test. The hot water bath temperature was where subjects reported pain ratings of 12–14 (pain magnitude rating) after hand immersion (20 seconds). During CPM testing, the volar forearm thermode increased to the temperature for the pre-defined pain magnitude rating of 11 and returned to baseline. Subjects reported pain ratings every eight seconds during heating and twice during recovery.

Pressure pain testing used 2 instruments (1) a hand-held algometer (FDN200, Wagner Instruments, Greenwich, CT) and (2) a custom hydraulic pressure device (University of Michigan, Ann Arbor, MI) on the dominant thumbnail [38]. Algometer pressure increased at 0.5 kg/s administered with a 0.785 cm² algometer probe until the subject reached tolerance. Algometer pressure (kg) was recorded at pain threshold and tolerance. Handheld algometry pain threshold and tolerance were also performed at additional sites including at the dominant hand proximal forearm muscle extensors (probe applied between the olecranon process and the medial epicondyle) and the trapezius muscle (5 cm lateral to each side of the spinous process of C8). Tests at each site were performed twice. The hydraulic pressure device measured pain tolerance using a blunt pressure stimulus delivered to the nail bed by a 1 cm² hard-rubber probe, where the thumb of the dominant hand was inserted into a plastic housing connected to a hydraulic piston. Pressure was delivered by weights (kg) placed above the piston. The sequence and intensity of pressure delivery followed guidelines developed at University of Michigan.

Mechanical sensitivity was measured using electronic von Frey filaments (Model 2390 Series, IITC Life Science, Inc., Woodland Hills, CA) and weighted Oppera pinprick probes (Stoelting Inc., Wood Dale, IL)[33, 37]. Von Frey filaments measured static tactile sensation detection thresholds (light touch). Filaments with uniform 0.8mm diameter probes were applied to the nonhairy portion of the upper volar forearm with sequentially increased filament size until the touch sensation was detected. This was then repeated in reverse, moving from larger to smaller filaments until touch was no longer detected. Filaments ranged from 0.1 to 70 grams.

Mechanical pinprick pain scores were measured with 256 milliNewton (mN) and 512 mN probes on the dorsum of the dominant hand index and middle digits between the first and second interphalangeal joints, respectively. During testing, the probe was pressed onto the finger once followed by a pain score and a 1 minute break. Next, ten pinpricks were applied

at the same site with 1 second between each stimulus. Pain ratings were taken immediately after the first stimulus, after the 10 stimuli sequence and after completion of testing (at 15 and 30 seconds). This sequence was repeated three times with a 1 minute break between testing.

2.3. Statistical Analysis

At the time of study design no effect size data were available for power calculations for sickle cell anemia so chronic pain model of osteoarthritis was selected. The study was prospectively designed to recruit 30 subjects and 30 NVs (assuming 10% dropout) to yield 93% statistical power to detect the effect sizes observed for pressure pain threshold [39]. Analysis software included Instat (Graph Pad Software, San Diego, CA), Prism (Graph Pad Software), Microsoft Excel (Microsoft Corporation, Redmond, WA), and SVS (Golden Helix, Bozeman, MT). Continuous data for QST with multiple replicates were averaged for each subject, and means (with standard deviations) were then used for group comparisons. Comparisons of sickle cell and NV groups were made by Chi-square (categorical variables) or t-tests (Alternate Welch's t test where these groups had unequal variances or Mann-Whitney test for non-normally distributed data). ANCOVA (analysis of covariance) was used for analysis of the random order thermal temporal summation QST (Figure 1C). Mechanical temporal summation was assessed using the change in pain score (pain score) from the first to last stimulus, while thermal pain score subtracted the first score from the highest score from the train of 10 pulses [37]. Spearman correlation was used to determine if 2 variables were associated as a univariate analysis. Multivariable stepwise linear regressions using both backward and forward selection methods were used to determine if any of six variables that distinguished sickle cell anemia subjects from normal volunteers (Table 1; including sickle cell anemia status, hemoglobin, fetal hemoglobin, number of hospitalizations for pain treatment during the 12 months prior to enrollment, hydroxyurea dose (mg/kg/day) and opioid dose during the 24 hours prior to testing (morphine equivalents, mg)) were associated with specific pain tests in a final regression model. Regression models limited to subjects with sickle cell only included significant variables from models that analyzed all subjects (n=60). Final regressions were adjusted for gender and age, as these variables are known to modify pain and fetal hemoglobin [1, 4]. P values are presented without correction for significance at an $\alpha = 0.05$.

3. Results

3.1. Subject Demographics

Demographics for 30 SS subjects and 30 age and sex matched NVs are presented in Table 1. Mean age was 34.3 versus 33.7 years in sickle cell anemia and NVs, respectively. Sickle cell anemia subjects had a mean of 2.14 (range 0 to 5) hospitalizations for pain during the 12 months prior to enrollment and 60% reported hydroxyurea treatment. Thirteen subjects (43%) were on daily oral opioids at a mean dose of 46.9 mg morphine equivalents for treatment of routine daily sickle cell pain without an acute, severe painful event. Four out of the 13 subjects were on maintenance sustained release opioids. On the day of evaluation, sickle cell subjects had higher overall baseline self-reported pain scores (1.88 ± 2.58)

compared to normal volunteers (0.07 ± 0.37) using a 0–10 clinical scale despite the absence of acute crisis pain suggesting a high daily pain burden in adults with sickle cell anemia.

3.2. Thermal Quantitative Sensory Testing including Temporal Summation

Pain threshold (12.2°C versus 8.0°C in NVs, $p=0.04$) and pain tolerance (6.3°C versus 2.8°C in NVs, $p=0.04$) temperatures using cold stimuli were significantly different in sickle cell anemia although there were no difference in cold detection (when the cold stimulus was first felt or sensation detection) (Fig. 1A). No differences were noted for threshold or tolerance to heat (Figure 1B). Thresholds were not associated with age as observed in a prior pediatric study likely because all subjects in this study were adults (Spearman correlation, data not shown) [18].

Fourteen random thermal pulses between 32 and 44°C elicited higher pain scores in sickle cell subjects ($p<0.0001$) (Figure 1C). In preparation for temporal summation testing at the heat pain threshold, we again determined heat pain thresholds. As in Fig. 1B, there was again no difference in heat pain thresholds between SS participants and NVs (44.1°C versus 44.0°C , respectively; $p=0.94$). However, temporal summation pain scores after a pulse of ten stimuli at or near the pain threshold were significantly higher in the sickle cell group ($p=0.002$) (Fig. 1D) as well as significantly higher aftersensation pain scores 15 seconds after stimulation had concluded (Fig. 1E).

CPM testing with thermal stimuli screened for differences in the prevalence of chronic pain (Supplemental Fig. 2). Pre-determined pain magnitude ratings defined by a pain score of at least 11 was achieved at a significantly lower temperature in the sickle cell group (mean $46.2 \pm 2.3^{\circ}\text{C}$) than in NVs ($47.5 \pm 2.1^{\circ}\text{C}$, $p=0.02$) with the thermode on the volar forearm. The second noxious stimulus was hand immersion into a hot water bath. Again, sickle cell subjects reached this pre-determined pain magnitude rating at a lower temperature ($46.1 \pm 1.6^{\circ}\text{C}$ in $n=29$ versus $46.8 \pm 1.3^{\circ}\text{C}$ in $n=28$ NVs; $p=0.05$). However, there was no difference in pain scores between groups when using the Medoc pain magnitude rating temperature alone ($p=0.83$) or in combination with either a neutral control (hand immersion in room temperature water bath; $p=0.88$) or an inhibitory control (hand in hot water bath; $p=0.77$) (Supplemental Fig. 1).

3.3. Static Mechanical Pressure Pain

The algometer pressure pain threshold at the dominant thumb was significantly lower in sickle cell anemia (Fig. 2A; $p=0.04$), while pain tolerance was not (Fig. 2A). Tolerance with the operator independent hydraulic pressure device was identical to algometer tolerance, indicating the reproducibility of this mode of testing (Figure 2B). Pressure threshold testing on the forearm ($p=0.06$) and shoulder ($p=0.06$) approached statistical significance but tolerance was not different (Fig. 2C and D).

3.4. Mechanical Light Touch, Pin Prick Temporal Summation, and Fetal Hemoglobin

Light touch testing (von Frey) did not show differences between groups (Supplemental Table 1). Mechanical pinprick testing using different weighted probes (256 mN and 512 mN) showed both sickle cell and NV subjects experienced temporal summation comparing pain

scores for a single stimulus to the change in scores for a train of 10 pinpricks (pain score) (Table 2). Single stimuli with the pinprick probes showed no significant difference in pain between sickle cell and NVs ($p=0.38$ and $p=0.92$, respectively). Furthermore, sickle cell subjects experienced significantly higher pain scores after summation and aftersensation pain scores (Table 2). Using stepwise linear regression, pain scores (temporal summation) were independently associated with sickle cell anemia as expected and inversely associated with HbF (Table 3). These regression models had adjusted $r^2=0.25$ and 0.21 , respectively. To determine if HbF played any role in pain sensitivity, we assessed this association limited only to the sickle cell cohort which showed fetal hemoglobin was negatively associated with pain ($\beta = -0.338$, $p=0.006$ for 512 mN; $\beta = -0.368$, $p=0.005$ for 256 mN). Because another report suggested that nociceptive processing in SCD is affected by opioid therapy [40], regressions restricted to sickle cell subjects not treated with opioids ($n=17$) showed comparable results for association with HbF (256 pain score $\beta = -0.322$, standard error= 0.171 with $p=0.08$ and 512 pain score for $\beta = -0.365$, standard error= 0.178 with $p=0.06$), and suggest this association is independent of opioids.

4. Discussion

In this study comparing adults with homozygous SCD (Hb SS or sickle cell anemia) to age and sex matched controls we show increased heat and cold pain sensitivity and pressure hyperalgesia compared to normal volunteers as previously reported [18, 20, 27, 28]. We extended these observations by utilizing dynamic assessments of pain sensitivity, where we observed higher changes in pain scores and delayed recovery in response to thermal and mechanical temporal summation in sickle cell anemia. Slow recovery with prolonged aftersensations suggests excitatory changes within the central nervous system consistent with central sensitization [41]. More importantly, mechanical temporal summation was inversely associated with fetal hemoglobin, a known modifier of SCA pain and disease severity, but not with hospitalizations for acute pain or other hematologic variables (i.e. reticulocytes or total bilirubin) from these sickle cell subjects. This association suggests some elements of sickle cell pathophysiology may also impact the development of central sensitization in SCA.

The key finding in this study that adults with SCA have enhanced mechanical temporal summation responses, which are associated with fetal hemoglobin levels independent of the effects of opioids in sickle cell. Tests of temporal summation are being considered as potential clinical assessment tools for characterizing central sensitization in other chronic pain conditions [41, 42], although central sensitization is not uniformly observed in chronic pain [14, 43]. Temporal summation is specific for increased responses by spinal dorsal horn or other central neurons to painful stimuli [41, 44, 45]. Ezenwa et al. showed that sixty percent of SCD adults had centralized pain using an algorithm assessing allodynia and hyperalgesia [20], while another SCD study showed a prevalence of 25% [14]. These results that suggest central sensitization may be a common element of pain in SCD. Direct findings documenting central sensitization are seen in nociceptive dorsal horn neurons in SCD mice [19]. Indirect findings in mice, in particular, partial reversal of mechanical pain with a local anesthetic (i.e. a peripheral channel antagonist) also suggest contribution from non-peripheral factors [12, 21].

The association between mechanical temporal summation (pain scores) and fetal hemoglobin (HbF) (Table 3) was not unexpected because HbF modulates both SCA pain and crisis frequency [4]. This finding is also consistent with the recent report of an association between antinociceptive brain connectivity, higher fetal hemoglobin and low pain crisis hospitalization burden in children with SCD [15]. If causative, fetal hemoglobin induction with hydroxyurea could also help reduce the prevalence of chronic pain attributable to central sensitization. Alternatively, it is possible that this association could be due to a high rate of hydroxyurea treatment in this cohort and not a direct effect of fetal hemoglobin. Otherwise, peripheral nociceptor inhibition or central neuroactive agents are experimental treatments for pain states characterized by central sensitization [41, 46]. Interestingly, in our experiment the HbF effect was not observed with heat temporal summation. Pinprick is detected by myelinated A δ peripheral nociceptors, while heat utilizes unmyelinated C fibers [24]. Faster A δ fiber transmission to the spinal cord leads to more rapid excitatory input. In addition, the fixed 5 second heat pulse spacing interval may have contributed to the absence of disease severity associations, as summation pain is enhanced by shorter intervals [33, 41]. Together, these factors could explain the inability to detect co-variable effects. Nonetheless, the link between fetal hemoglobin and SCA central hyperalgesia needs to be confirmed in larger studies.

We did not observe differences for static heat pain thresholds or tactile allodynia in SCA. These results are in contrast with the heat pain hyperalgesia and light touch allodynia reported in SCD mice and heat pain hyperalgesia in children with SCD [12, 18, 21, 30, 31]. However, our results are similar to another QST study of adults with SCD, which likewise did not show increased heat pain thresholds [13]. We speculate that these inconsistent findings might be explained by different physiologic effects in children versus adults [13, 18]. Our sites for heat pain testing were also different than those used in the pediatric study (forearm versus hand) and may reflect site specific sensitivity [18]. Furthermore, CPM failed to demonstrate heat pain inhibition in NVs, indicating that hot water hand immersion in place of ice water failed to produce a valid test [47]. The change was made because cold has the potential to induce an SCD painful crisis [1]. African Americans also experience greater pain sensitivity and reduced CPM than Europeans, suggesting that our small pilot study might have been underpowered [48]. Our CPM protocol will require further modification to produce a valid test [20]. Perhaps testing different sensory afferents together (e.g. heat/cold or heat/mechanical) will produce the expected inhibition as was observed in 2 other studies of adults with SCD [13, 14]. Determining CPM responses is critical for further understanding descending modulation of pain transmission within the central nervous system in SCD [13, 14, 25, 41].

Finally, our subjects were limited to homozygotes (SS or SCA), as other genotypes have differences in clinical manifestations, in particular, the frequency of painful events [4]. Previous SCD studies have not controlled for genotype effects on experimental or clinical pain [13, 14, 18, 20, 27, 28]. Furthermore, we were likely only able to detect the HbF association because its expression is significantly higher in SCA compared to hemoglobin SC^{34,36}, another common genotype present in prior QST studies [13, 14, 18, 20, 27, 28, 49, 50]. The association was similarly not detected in studies of the S-Berk mouse, which does not express HbF [19]. In addition, QST responses and the pain hospitalization rate were not

correlated, consistent with prior observations [18]. Pain hospitalization rate is important because it is associated with mortality, although it underestimates daily pain [3, 4]. Several recent studies of adults with SCD assessed pain using diaries, but did not quantify this specific measure of healthcare utilization due to pain [13, 14]. Despite the lack of association with this measure of SCD severity, QST may still be a useful for clinical trials [4, 51]. Pain diaries will also be necessary to further assess the relationship between pain and QST responses as has been documented elsewhere [14].

This study has several limitations. Age, sex, opioids and genotype modulate SCD pain [1, 4]. We controlled for age and sex with matching, and controlled for genotype by only studying subjects homozygous for the sickle hemoglobin mutation. In addition, less than 50% of subjects reported taking opioids prior to QST. Opioid use in adults with SCD is complex because treatment could produce analgesia, tolerance or hyperalgesia [52]. Although neither the regression with all subjects, nor regression excluding those taking opioids led to different conclusions, it is not possible to preclude opioid induced responses, especially in patients experiencing high pain burden that necessitates daily therapy. Indeed, a study of 38 SCD patients with high or low central sensitization showed high group responses were associated with opioid therapy [40]. Therefore, larger studies designed to determine the effect of opioids in sickle cell are needed [53]. In addition, our power estimates were based on pressure pain thresholds in osteoarthritis due to the lack of published data in SCD during study design [54]. However, there is a need to better characterize effect sizes, variability due to different forms of SCD and statistical significance of pain measures in SCD in larger studies [53]. Finally, QST alone is unable to determine whether sickle cell hyperalgesia is of neuropathic or inflammatory origin [51].

5. Conclusions

We present temporal summation evidence for central sensitization as one mechanism underlying enhanced sickle cell sensitivity to pain [13, 14, 19, 20], and identify an association between increased temporal summation pain and fetal hemoglobin levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SCD sickle cell disease

SCA	sickle cell anemia
VOC	vaso-occlusive crisis

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Implications

The fetal hemoglobin association has not been previously been reported and it supports broad use of hydroxyurea. Therapeutic agents that modify pain transmission within the central nervous system should also be investigated as alternative to opioid therapy.

- Adults with sickle cell anemia show evidence of central sensitization.
- Central sensitization correlated with fetal hemoglobin levels.
- Central sensitization could be modulated by fetal hemoglobin induction.

Fig. 1. Static thermal pain threshold/tolerance testing and dynamic thermal temporal summation testing

(A) Cold testing was performed at sites of the volar forearm. Means with standard deviations are illustrated. (B) Heat testing was also performed on the forearm. Thermal detection threshold (sensation in the figure), pain threshold and pain tolerance means with standard deviation are illustrated. (C) Pain scores (0–20 scale) after each of 14 random order thermal pulses between 32 and 44°C. Sickle cell status ($p<0.0001$) and temperature ($p<0.0001$) were associated with pain scores (ANCOVA). (D) Temporal summation thermal testing at individualized heat pain thresholds shows enhanced pain scores in sickle cell anemia. pain scores are reported as means with standard deviations. (E) Aftersensations suggested by pain scores 15 seconds after thermal sequence pulses in panel D.

Fig. 2. Static pressure pain testing

Pressure pain testing was conducted with the use of a handheld algometer (A) or a hydraulic pressure device (B) on the dominant hand thumbnail. The pain threshold was where subjects first reported pain and the tolerance was the subject's maximum tolerated pain. Results for pain tolerance were nearly identical for both the algometer and the hydraulic pressure testing system. Pressure testing for threshold and tolerance was also performed on the arm (C) and shoulder (D). SS patients reported higher pain scores than normal volunteers. All illustrations are for means with standard deviations.

Table 1

Population demographics of adults with sickle cell anemia and matched normal volunteers.

Parameter	SS (n=30) Mean (SD)	Normal Volunteer (n=30) Mean (SD)	p value
Age (years)	34.3 (10.0)	33.7 (10.0)	0.81
Male (n)	15 (50%) ¹	15 (50%)	1.00 ²
WBC (K/uL)	8.43 (2.72)	5.34 (1.41)	< 0.0001
Hemoglobin (g/dL)	8.1 (1.2)	12.9 (1.4)	< 0.0001
Hb F (%)	10.9 (7.9)	0.0 (0.0)	–
Hb S (%)	70.8 (21.0)	0.0 (0.0)	–
Hb A (%)	14.5 (24.9)	97.1 (0.4)	< 0.0001
Platelets (K/uL)	328.2 (116.6)	236.4 (50.9)	0.0003
Reticulocytes (%)	12.1 (5.3)	1.3 (0.5)	< 0.0001
LDH (U/L)	495.1 (205.6) ³	174.2 (37.8) ³	< 0.0001
Creatinine (mg/dL)	0.58 (1.76)	0.93 (0.17)	< 0.0001
Hospitalizations for pain ⁴	2.14 (1.77)	0.0 (0.0)	–
Hydroxyurea (n)	18 (60%) ¹	0 (0%)	–
Hydroxyurea (mg/kg/day)	18.6 (9.4) ⁵	0.0 (0.0)	–
Opioid dose prior to testing (morphine equivalents, mg)	46.9 (88.8) ⁶	0.0 (0.0)	–
Pain score at evaluation ⁷	1.88 (2.58)	0.07 (0.37)	0.0006

¹ Number and percent of study population² 2 × 2 table³ N=29 sickle cell and N=28 normal volunteers (due to hemolyzed samples)⁴ Over the 12 months prior to enrollment⁵ Hydroxyurea dose average for n=18 subjects⁶ Morphine equivalent dose for n=13 subjects taking opioids during the 24 hours preceding QST⁷ Using a 0–10 clinical pain rating scale

Table 2

Pinprick mechanical pain is enhanced in sickle cell anemia.

Pinprick Probe	SS (n=30) Mean Pain Score ¹ (SD)	Normal Volunteer (n=30) Mean Pain Score (SD)	p value
256 mN probe			
Single stimulus	1.65 (1.98)	2.06 (2.21)	0.38
Ten stimuli	8.56 (5.40)	5.83 (4.31)	0.05
15 seconds post testing	4.62 (5.03)	1.20 (2.17)	0.0002
30 seconds post testing	3.68 (4.50)	0.61 (1.42)	<0.0001
Temporal summation (pain score)	6.91 (5.02) ²	3.77 (3.26) ³	0.004
512 mN probe			
Single stimulus	3.52 (2.55) ³	3.93 (3.72)	0.92
Ten stimuli	11.73 (5.57) ³	9.36 (5.38)	0.10
15 seconds post testing	8.00 (6.41)	3.67 (4.96)	0.005
30 seconds post testing	5.83 (6.40)	2.70 (4.36)	0.03
Temporal summation (pain score)	8.53 (5.03) ^{3,4}	5.43 (3.41) ⁵	0.008

¹ Gracely pain scale (0–20)

² Paired Wilcoxon non-parametric test comparing SS single to SS TS (pain score) P<0.0001.

³ Paired Wilcoxon test comparing NV single to NV TS (pain score) P=0.01.

³ N=29 subjects

⁴ Paired t-test comparing SS single to SS TS (pain score) P<0.0001.

⁵ Paired Wilcoxon non-parametric test comparing NV single to NV TS (pain score) P=0.03.

Table 3

Low fetal hemoglobin is associated with mechanical temporal summation in adults with sickle cell anemia.

Independent Variables ¹	β (Standard Error)	p value
256 nM probe TS (pain score)		
Sickle cell status	6.392 (1.427)	<0.0001
HbF (%) ²	-0.300 (0.904)	0.002
512 nM probe TS (pain score)		
Sickle cell status	6.673 (1.536)	<0.0001
HbF (%) ³	-0.318 (0.101)	0.003

¹Linear regression by stepwise backward elimination in all study subjects (both sickle cell anemia and normal volunteer, N=60) with variables including sickle cell anemia status, hemoglobin, HbF, number of hospitalizations for pain treatment during the 12 months prior to enrollment, hydroxyurea dose (mg/kg/day) and opioid dose during the 24 hours prior to QST (morphine equivalents, mg). Regressions were adjusted for age and gender.

²Regression limited to SCA subjects (N=30) also showed $\beta=-0.338$, $p=0.006$ when adjusting for age and gender.

³Regression limited to SCA subjects (N=29) also showed $\beta=-0.368$, $p=0.005$ when adjusting for age and gender.