Diet and companionship modulate pain via a serotonergic mechanism

Huy Tran¹,6, Varun Sagi¹,6, Sarita Jarrett², Elise F. Palzer³, Rajendra D. Badgaiyan⁴ & Kalpna Gupta¹,5

Treatment of severe chronic and acute pain in sickle cell disease (SCD) remains challenging due to the interdependence of pain and psychosocial modulation. We examined whether modulation of the descending pain pathway through an enriched diet and companionship could alleviate pain in transgenic sickle mice. Mechanical and thermal hyperalgesia were reduced significantly with enriched diet and/or companionship. Upon withdrawal of both conditions, analgesic effects observed prior to withdrawal were diminished. Serotonin (5-hydroxytryptamine, 5-HT) was found to be increased in the spinal cords of mice provided both treatments. Additionally, 5-HT production improved at the rostral ventromedial medulla and 5-HT accumulated at the dorsal horn of the spinal cord of sickle mice, suggesting the involvement of the descending pain pathway in the analgesic response. Modulation of 5-HT and its effect on hyperalgesia was also investigated through pharmaceutical approaches. Duloxetine, a serotonin-norepinephrine reuptake inhibitor, showed a similar anti-nociceptive effect as the combination of diet and companionship. Depletion of 5-HT through p-chlorophenylalanine attenuated the anti-hyperalgesic effect of enriched diet and companionship. More significantly, improved diet and companionship enhanced the efficacy of a sub-optimal dose of morphine for analgesia in sickle mice. These findings offer the potential to reduce opioid use without pharmacological interventions to develop effective pain management strategies.

Chronic pain occurs in a sizeable population of patients with a variety of chronic conditions¹–⁵. Opioids remain the mainstay of chronic pain treatment⁶. Besides known side effects of opioids, including constipation and respiratory depression, social liabilities and premature deaths due to opioid abuse are a leading health concern⁷–¹¹. A therapeutic strategy to effectively treat chronic pain without opioids remains a major unmet need¹²,¹³.

An effective alternative to opioids has not yet been found, partly because pain perception has both physiological and affective components¹⁴. A focus on medications for pain management has limited appreciation of the affective and psychological components of pain perception and their downstream biological pathways¹⁵. Affective state significantly alters pain perception and response to analgesics¹⁶–¹⁸. Thus, psychiatric conditions like depression and anxiety; and, environmental, cultural, emotional, and psychological factors influence pain perception by altering the affective state. Moreover, pain itself causes depression and anxiety¹⁹–²¹, which in turn lowers the pain threshold²². This interdependence of pain and affect makes treatment of chronic pain challenging²³.

This challenge also provides an opportunity to control pain by modulating psychosocial environment. Psychological interventions are effective in controlling and even abolishing persistent pain²⁴. Techniques such as hypnotism and mindfulness have been successfully used to modulate pain in sickle cell disease (SCD) and other conditions²⁵–²⁷. Success of these interventions led us to hypothesize that psychosocial enhancement could be an alternate strategy to treat chronic pain. This strategy, if successful, could eliminate widespread use of opioids that has become a major health concern all over the world.

SCD is a common genetic disorder, known for its complex and distinctive pain characteristics⁹,²⁸. Acute pain in SCD is caused by aggregation of sickled red blood cells (RBC) blocking the vasculature, leading to vaso-occlusive crisis (VOC) and severe pain requiring hospitalization⁹. A significant proportion of people with SCD experience chronic pain, which may increase with age²⁹,³⁰. The global burden of SCD is primarily shouldered by communities with limited resources in Sub-Saharan Africa, Middle East, and India³⁰. In the United States, the

¹ Division of Hematology, Oncology and Transplantation, Department of Medicine, Vascular Biology Center, University of Minnesota, Minneapolis, MN, USA. ²Northwestern University, Evanston, IL, USA. ³Biostatistical Design and Analysis Center, Clinical and Translational Sciences Institute, University of Minnesota, Minneapolis, MN, USA. ⁴Department of Psychiatry, Long School of Medicine, University of Texas Health Science Center, San Antonio, Texas, USA. ⁵Hematology/Oncology, Department of Medicine, University of California, Irvine and Southern California Institute for Research and Education, VA Medical Center, 5901 East 7th St, Long Beach, CA 90822, USA. ⁶These authors contributed equally: Huy Tran and Varun Sagi. ⁷email: kalpnag@hs.uci.edu
Companionship and improved nutrition reduce hyperalgesia. Effect of diet and/or companionship on hyperalgesia in male sickle mice. We observed significantly reduced mechanical, heat, and cold hyperalgesia after three weeks of treatment in male sickle mice in the RD/C+, SD/C−, and SD/C+ treatment groups compared to the RD/C− group (Fig. 1a–c). However, we did not observe a significant reduction in musculoskeletal hyperalgesia after three weeks of treatment for mice in the RD/C+, SD/C−, and SD/C+ treatment groups compared to the RD/C− group (Fig. 1d). Mice in the RD/C+ and SD/C− groups showed levels of hyperalgesia comparable to those in the SD/C+ group after just three weeks of treatment. This is significant as the SD/C+ treatment group was maintained on the sickle diet and companionship since weaning, while those in the RD/C+ and SD/C− groups were only provided enriched diet or a companion for three weeks after being on the regular diet without companionship before treatment initiation. Control mice expressing normal human hemoglobin A, which do not have constitutive hyperalgesia, did not show a significant change in hyperalgesia with different treatments (Fig. 1e–h). These data show that improving the nutritional requirements and companionship can independently reduce pain/hyperalgesia in sickle mice.
Figure 1. Effect of enriched diet and companionship on hyperalgesia in male sickle and control mice. Mice were fed specific diets from birth until approximately 8 months of age as indicated on the left side of each figure. Diet and/or companion introduction or withdrawal was done for three weeks following baseline testing; specific treatment groups are listed on the right side of the figure. Hyperalgesia measures for all mice are shown for mechanical hyperalgesia as PWF in response to 1.0 g von Frey filaments; heat hyperalgesia as PWL in response to a heat stimulus; cold hyperalgesia as PWF per 2 min on a cold plate at 4 °C and deep tissue/musculoskeletal hyperalgesia as grip force exerted by forelimbs. (a–d) Male sickle mice (HbSS) given sickle or regular diet in the presence or absence of a female companion. (e–h) Male control mice (HbAA) given sickle or regular diet in the presence or absence of a female companion. (i–l) Male sickle mice (HbSS W) fed sickle diet in the presence of a female companion followed by withdrawal of sickle diet replaced with regular diet and/or withdrawal of the female companion. Regular diet without companionship, RD/C−, nSS = 12, nAA = 8; regular diet with companionship, RD/C+, nSS = 20, nAA = 11; sickle diet without companionship, SD/C−, nSS = 13, nAA = 10; sickle diet with companionship, SD/C+, nSS = 20, nAA = 11; withdrawn from sickle diet, W RD/C+, nSS = 5; withdrawn from companionship, W SD/C−, nSS = 6; withdrawn from sickle diet and companionship, W RD/C−, nSS = 20, nAA = 8; mixed linear models with Tukey’s post-hoc test, *p < 0.05, **p < 0.01, ***p < 0.001 compared to HbSS RD/C−; †p < 0.05, ††p < 0.01, †††p < 0.001 compared to HbSS SD/C+. PWF, paw withdrawal frequency; PWL, paw withdrawal latency.
Nutrition and companionship activated the descending pain pathway. Effect of diet and/or companionship on 5-HT and DA in whole spinal cord lysates of male sickle mice. High Performance Liquid Chromatography (HPLC) of neurotransmitters in spinal cord lysate confirmed an increase in 5-HT in whole spinal cords of sickle mice in the SD/C− and the SD/C+ treatment groups compared to those in the RD/C− group, though these increases were not significant (Fig. 2a). We observed a reduction of 5-HT in sickle mice in the RD/C+ group compared to those in the RD/C− treatment groups, but this decrease was also not significant (Fig. 2a). However, we found significantly higher levels of 5-HT in whole spinal cords of mice in the SD/C− and SD/C+ groups compared to the RD/C+ treatment group, suggestive of a contribution of sickle diet to elevated 5-HT independent of companionship. This contribution of sickle diet is further validated by a significantly lower 5-HT level in the W RD/C− group when compared to the SD/C+ group (Fig. 2a). DA levels in whole spinal cords of sickle mice did not differ significantly between treatment groups (Fig. 2b). Thus, sickle diet contributes to 5-HT in the spinal cord of male sickle mice. It is likely that a greater effect occurs in the dorsal horn where neurotransmitters are released and therefore it may not be truly reflected in whole spinal cord lysates.

Effect of diet and/or companionship on hyperalgesia in female sickle mice. Several challenges including irregular menstrual cycles (which influence pain), and pregnancy risk upon addition of a male companion interfered with examining hyperalgesia in female sickle mice. To prevent the risk of alterations due to pregnancy, older female mice at a mean age of approximately 9 months were tested. However, some mice still became pregnant and significant intra-group variability in hyperalgesia precluded from drawing conclusions (Fig. S1).

Effect of diet and/or companionship on 5-HT and DA in the brain. Confocal images showing distribution of 5-HT and DA in the dorsal horn. (e) Quantification of 5-HT level in the dorsal horn. (f) Quantification of DA level in the dorsal horn. (g, h) Confocal images showing distribution of 5-HT and DA in the brain. (i) Quantification of 5-HT level in the RVM area of the brain. (j) Quantification of DA in the PAG area of the brain. Male HbSS-BERK and HbAA-BERK mice between 8 and 10 months of age were used. Regular diet without companionship, RD/C−, a: nSS = 9, nAA = 8, c–f: nSS = 7, g–j: nSS = 6; regular diet with companionship, RD/C+, a: nSS = 6, nAA = 10, c–f: nSS = 8, g–j: nSS = 6; sickle diet without companionship, SD/C−, a: nSS = 9, nAA = 9, c–f: nSS = 9; g–j: nSS = 6; sickle diet with companionship, SD/C+, a: nSS = 7, nAA = 9, c–f: nSS = 9, g–j: nSS = 8; withdrawn from sickle diet and companionship, W RD/C−, a: nSS = 10, nAA = 7, c–f: nSS = 7, g–j: nSS = 6; one-way ANOVA with Bonferroni’s post-hoc test, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar (c and g) 500 µm, (d and h) 20 µm. RVM, rostral ventromedial; PAG, periaqueductal grey; i.r, immunoreactivity.

Effect of circulating corticosterone levels in response to diet and companionship. Stress is an aggravating factor in several chronic conditions and has been associated with fluctuations in pain level in patients dealing with chronic pain. We therefore examined whether improved diet and companionship could lead to changes in levels of corticosterone, a stress indicator, in sickle mice. Corticosterone levels were significantly reduced in sickle mice in the RD/C+ group compared to those in the RD/C− group (Fig. 3). Reduced levels of corticosterone were also observed in the SD/C− and SD/C+ groups compared to RD/C−, but these were not statistically significant (Fig. 3). Lower corticosterone levels were found...
Figure 1: Distribution of 5-HT and DA in different brain regions.

(a) Graph showing the concentration of 5-HT (ng/g tissue) across different groups. 
(b) Graph showing the concentration of DA (ng/g tissue) across different groups.

c) HbSS Spinal Cord

d) HbSS 5-HT

(e) Dorsal Horn 5-HT

(f) Dorsal Horn DA

(g) HbSS Brain

(h) HbSS RVM 5-HT

(i) RVM 5-HT

(j) PAG DA
in sickle mice in the W RD/C− group compared to the SD/C+ group, although this decrease was not statistically significant. The sickle mice in the W RD/C− group, however, did show a significantly lower level of corticosterone compared to the SD/C− or RD/C− treatment groups (Fig. 3). Such a significant drop in corticosterone level suggests impaired compensatory stress response as a result of withdrawal of SD and companionship.

Serotonin-norepinephrine reuptake inhibitor (SNRI, 5-HT-NE), duloxetine, ameliorates thermal hyperalgesia in sickle mice. Since increased 5-HT levels, resulting from improved nutrition and companionship, were associated with analgesia in sickle mice, we examined whether a pharmacological strategy to increase 5-HT levels in the nervous system could also ameliorate hyperalgesia in sickle mice. We used duloxetine, an SNRI, which has been shown to be effective in reducing pain and improving health-related quality of life (HRQoL) in patients with knee pain due to arthritis in a Phase III clinical trial.

Male and female sickle mice fed RD and housed without a companion (RD/C−) uniformly showed a significant decrease in heat and cold hyperalgesia 30 min after treatment with 3 and 10 mg/kg duloxetine, returning to baseline levels 6–8 h post injection (Fig. 4b–c, f–g). No significant change in mechanical or musculoskeletal hyperalgesia was observed in either male or female sickle with one-time duloxetine treatment. After 9-day treatment with duloxetine at 3 mg/kg, the anti-hyperalgesic response for heat and cold hyperalgesia remained without causing tolerance in both male and female sickle mice (Fig. 4j–k, n–o). Additionally, long term treatment with 3 mg/kg duloxetine led to significantly lower musculoskeletal hyperalgesia in female sickle mice, which was not observed with one-time treatment (Fig. 4p). These data suggest intervention with 5-HT increasing pharmacologics can ameliorate hyperalgesia in both male and female sickle mice with a greater effect on female mice without causing tolerance.

p-chlorophenylalanine (PCPA), inhibitor of tryptophan hydroxylase, a rate limiting enzyme in the biosynthesis of 5-HT, induces hyperalgesia in sickle mice. A decreased level of 5-HT and an associated increase in hyperalgesia were observed in sickle mice following withdrawal of both the sickle diet and companionship (W RD/C−). We tested whether pharmacologically targeted endogenous depletion of 5-HT could also increase hyperalgesia.

At baseline, mechanical, heat, cold and musculoskeletal hyperalgesia in sickle mice in the SD/C+ group were significantly lower compared to sickle mice in the RD/C− group (Fig. 5). After three days of PCPA treatment, mechanical, heat, cold, and musculoskeletal hyperalgesia in SD/C+ sickle mice were elevated to the level of RD/C− sickle mice. One week after discontinuing PCPA treatment, mechanical, heat, and cold hyperalgesia in SD/C+ sickle mice returned to their baseline levels, but musculoskeletal hyperalgesia remained increased when compared to SD/C+ baseline before PCPA treatment (Fig. 5). These data validate the role of 5-HT in ameliorating hyperalgesia and the effectiveness of SD and companionship in elevating 5-HT.

Nutritional diet and companionship improves opioid analgesia. High doses of opioids are often required to treat sickle pain compared to analogous pain in other conditions. Therefore, we examined whether modulation of the descending pain pathway via improved diet and companionship could reduce opioids needed to treat pain in sickle mice.

A sub-optimal dose of 10 mg/kg of morphine in RD/C− sickle mice was less effective in reducing mechanical, heat, cold, and deep tissue hyperalgesia compared to the higher dose of 20 mg/kg (Fig. 6). However, hyperalgesia was significantly ameliorated by a sub-optimal dose (10 mg/kg) of morphine in SD/C+ sickle mice (Fig. 6). RD/C− sickle mice required 20 mg/kg of morphine to achieve a comparable effect, twice the dose required by SD/C+. A 37.5 mg/kg of morphine in RD/C− sickle mice showed synergistic effects with SD/C+ mice in reducing mechanical and cold hyperalgesia (Fig. 6; inset b–c).
Figure 4. Effect of acute and chronic duloxetine treatment on hyperalgesia in male and female sickle mice. All mice were fed regular diet and housed singly without a companion. Hyperalgesia measures for all mice are shown for, mechanical hyperalgesia as PWF in response to 1.0 g von Frey filaments; heat hyperalgesia as PWL in response to a heat stimulus; cold hyperalgesia as PWF per 2 min on a cold plate at 4 °C and deep tissue/musculoskeletal hyperalgesia as grip force exerted by forelimbs. Effect of an acute single dose of duloxetine at 3 and 10 mg/Kg or vehicle (a–h) and of chronic administration of 3 mg/Kg/day duloxetine or vehicle for 9 days (i–p), in male and female sickle mice on hyperalgesia. Acute duloxetine treatment in male sickle mice (a–d), nSS vehicle = 8, nSS 3 mg/kg duloxetine = 8, nSS 10 mg/kg duloxetine = 8; Acute duloxetine treatment in female sickle mice (e–h), nSS vehicle = 8, nSS 3 mg/kg duloxetine = 8, nSS 10 mg/kg duloxetine = 8; Chronic duloxetine treatment in male sickle mice (i–l), nSS vehicle = 6, nSS 3 mg/kg duloxetine = 6, nSS 10 mg/kg duloxetine = 6; Chronic duloxetine treatment in female sickle mice (m–p), nSS vehicle = 8, nSS 3 mg/kg duloxetine = 8, nSS 10 mg/kg duloxetine = 13; mixed linear models with Tukey’s post-hoc test, *p < 0.05, ***p < 0.001 compared to vehicle. D1, D3, D5, D7, and D9, day 1, 3, 5, 7, and 9 respectively; PWF, paw withdrawal frequency; PWL, paw withdrawal latency.
C+ sickle mice (Fig. 6). Therefore, improved nutrition and companionship which reduce stress improve the response to opioid therapy.

**Discussion**

Psychological factors are known to alter pain perception in a variety of conditions. We observed that both the SD and companionship individually and in combination reduced hyperalgesia in sickle mice. This suggests that persistent chronic pain can be attenuated by enriching environmental conditions. We also found that removing companionship alone or both companionship and the SD led to restored hyperalgesia. This observation is consistent with reports of elevated pain in response to negative emotion and poor diet and reduction in opioid overdose with behavioral interventions. It is believed that poor nutrition alters the leptin/ghrelin hormone balance, resulting in development of psychiatric symptoms such as anxiety and depression. Therefore, HRQoL including malnutrition, loneliness, stigmatization, etc. and adverse life events can sustain or worsen hyperalgesia.

Multiple neurotransmitters including 5-HT and DA are involved in processing pain sensation and perception. These neurotransmitters modulate pain perception. In this context, it is significant that we observed increased 5-HT concentration in the spinal cord of the group that received enriched diet and companionship, particularly because the higher concentration was observed in the dorsal horns, where signals transmitted from the descending pain pathway are modulated. Since higher 5-HT concentration is associated with elevated mood, it appears that the positive mood induced by the SD and companionship increased 5-HT production which in turn induced analgesia.
The decreased level of 5-HT in sickle mice lacking SD in the RD/C− and RD/C+ treatment groups as compared to the RD/C+ and SD/C+ groups respectively demonstrates the important role of diet in production of 5-HT. Moreover, when paired with companionship (SD/C +), enriched diet elevated 5-HT to the highest level among treatment groups. Therefore, nutrition can modulate 5-HT in response to pain and/or distress. The observation of increased 5-HT in the SD/C+ group is an important finding. It suggests that pain modulation induced by the descending pathways is mediated by the 5-HT releasing system. To our knowledge, this is the first study to demonstrate 5-HT activating supraspinal control of nociception in the context of SCD.

Depletion of 5-HT in the brain by PCPA was previously shown to decrease pain threshold and unresponsiveness to morphine in healthy rodents\textsuperscript{42,43}. Similar hyperalgesia-induced effects were observed in sickle mice who were previously in the SD/C+ treatment group after short-term treatment with PCPA, implying the relationship between reduced 5-HT and chronic pain in sickle mice. These data support the role of 5-HT in modulation of sickle pain.

We confirmed the role of 5-HT in mediating analgesia by enhancing 5-HT availability in the synapses. Using a SNRI, duloxetine, produced an analogous effect to that of improved diet and companionship in sickle mice, which suggests that these benefits could be induced with SNRI drug treatment. Since pain in females is more challenging to manage than in males\textsuperscript{79–82}, therapies targeting the descending pain pathway may improve analgesic...
outcome in females. In SCD, females and older subjects were found to require neuropathic pain treatment and had longer hospital stays compared to males and younger patients\(^8\). Therefore, targeting of the descending serotonergic pathway may be beneficial in treating pain in these vulnerable populations of SCD.

We also observed reduced corticosterone levels in sickle mice with sickle diet and/or companionship. Since corticosterone is involved in stress response, this observation is consistent with reported stress-relieving effects of satisfying food\(^9,10\) and social bonding\(^11,12\). Stress has been known for decades to enhance pain perception, especially in patients having chronic pain\(^13-16\). Our observation of consistently high hyperalgesia in sickle mice lacking enriched diet and companionship further supports this concept. It is likely that the stress-relieving effect of enriched diet and companionship contributes to analgesic responses in sickle mice. A study of 121 married/partnered patients with colorectal cancer demonstrated that intimacy moderated the association between processing and depressive symptoms\(^17\). It was found that relatively high intimacy relationships were associated with lower depression and that the quality of relationship and emotional approach may enhance coping efforts. Therefore, strategies to reduce stress and improve emotion in patients may be helpful in managing pain and restoring HRQoL. However, rapid withdrawal of these sources of happiness significantly diminished the level of corticosterone and led to an increase in hyperalgesia in sickle mice. This dramatic depletion of corticosterone following the withdrawal period suggests a displeasure-induced impairment in psychological and/or metabolic states\(^18\). Therefore, withdrawal of a happy state may lead to worsening pain and inability to cope with distress.

We also performed analysis for depression and anxiety-like behaviors and did not observe any significant difference between AA and SS BERK mice from 3- to 8-months of age on 6 different tests, namely, (i) Elevated Plus Maze, (ii) Forced Swim Test, (iii) Tail Suspension Test, (iv) Novelty-induced Hypophagia, (v) Sucrose Preference Test, and (vi) Stress-induced Hyperthermia upon repeating 3 times with 4 different groups of male mice. All testing was performed in a double-blind manner with highly experienced experts at the Behavioral Phenotyping Core of the National Institute of Neurological Disorders and Stroke (NINDS) Center at the University of Minnesota. We are further continuing the longitudinal analysis on > 10 month-old male SS and AA BERK mice but high morbidity in male SS mice has delayed obtaining the large numbers of mice. Therefore, these data are not included herein.

Beside observing the influence of enriched diet and companionship in transmission and perception of pain in CNS, we also wanted to examine their effect on the pathology of peripheral tissues. We did not observe a significant difference in the pathology of different organs under different treatments (Fig. S2). High protein/ calorie diets have been shown to reduce organ injury and improve survival in sickle mice\(^19\). The difference in our observations could be due to irreversible organ damage in the older sickle mice we used. Increased circulating cytokines have been demonstrated in major depressive disorders and animal models of depression\(^20-22\). Increased proliferation and mobilization of immune cells, including bone-marrow derived monocytes in the bloodstream, is stimulated by chronic stress\(^23,24\). Social stress has been shown to compromise blood–brain barrier (BBB) integrity\(^25\). Activation of mast cells as well as 5-HT in the brain have been associated with increased BBB permeability under stress\(^26,27\). As a result, circulating cytokines and peripheral immune cells can diffuse into the brain under stress-induced conditions, promoting anxiety and depression\(^28,29\). Therefore, improved diet and companionship may attenuate chronic hyperalgesia via increased descending modulation from the RVM, and may also show protective effects on peripheral tissues by reducing stress-induced inflammatory response. This is particularly important for SCD, which is associated with depression, anxiety, cognitive impairment, “cytokine storm”, and increased bone-marrow derived hematopoietic and myeloid cells in the circulation\(^30-32\). Notably, circulating IL6 is highly elevated in sickle patients and sickle mice\(^33,34\). It has been shown that social stress stimulates IL6 diffusion into the brain, which acts on the nucleus accumbens resulting in depressive behaviors\(^35\). In a condition like SCD with a highly inflammatory microenvironment in the wake of a VOC, social stress may promote a vicious cycle of peripheral and central inflammation, depression, anxiety and cognitive impairment, all of which can potentiate pain perception via inhibition of 5-HT releasing mechanisms.

Our data suggest that an enhanced psychosocial environment through improved nutrition and companionship attenuates chronic hyperalgesia via increased descending modulation from the RVM involving 5-HT and DA. The observation of increased 5-HT in the SD/C+ group is an important finding of this study. It suggests that pain modulation induced by the descending pathways is mediated by the 5-HT activating system. Therefore, creating a happy environment with alternative and complementary strategies could improve response to analgesic therapies\(^36-38\). It is likely that the often life-long nature of pain, frequent hospitalization, socioeconomic stress and social stigma in SCD negatively modulates affective mechanisms and interferes with pain management, necessitating high doses of opioids\(^39\). Arginine supplementation showed a trend towards a decrease in VOC pain and a significant decrease in opioid use in children with SCD\(^40,41\). L-glutamine and ω-3 fatty acids also show promising outcomes in reducing VOC and hospital admissions in patients with SCD\(^42,43\). Specifically, ω-3 are known for their neuroprotective properties, potentially contributing to protection from sickle cell pathobiology\(^44,45\). Ready-to-use supplementary food (RUSF) has been shown to promote growth and improve hematological parameters with no influence on sickle cells\(^46\). The sickle diet contained increased arginine, ω-3 fatty acids and glutamate, compared to the regular diet. The observations on the effect of sickle diet on reducing pain are complemented by our recent observations on the improvement in survival of pups up to 5 months of age of sickle mice fed sickle diet compared to regular diet\(^47\). In this study, feeding sickle diet to the parents significantly improved the survival of male offsprings, suggesting the vulnerability of male survival in utero and post-natally and sensitivity to the environmental changes. Therefore, some of the effects of diet may be due to an improved metabolic state in SCD.

In conclusion, we show that analgesia is mediated by the 5-HT releasing system acting in the descending pain pathway. Since opioids also work partly on this system, these results suggest that a serotonergic enhancement strategy could be a substitute for opioid use for treating chronic pain. Chronic treatment with opioids leads to addiction, dose escalation, and reduced efficacy due to development of tolerance\(^48,49\). Enhancement of 5-HT release in these vulnerable populations of SCD may provide a valuable strategy for pain management in SCD.
activating system either by psychosocial enrichment or by pharmacological manipulation could induce similar levels of analgesia without causing addiction and tolerance associated with opioid treatment. Additionally, dietary improvement and companionship led to increased analgesic effectiveness of a sub-optimal dose of morphine.

Materials and methods

Study design. This study aimed to modulate the psychosocial environment of male sickle mice and examined its effect on hyperalgesia. The psychosocial environment was modulated by providing companionship with a female, enriched diet, and 5-HT regulators either independently or in combination as described below in brief and/or in the accompanying supplementary information. After behavioral evaluation, molecular analyses of 5-HT, DA and corticosterone were performed in tissues collected from male mice exposed to different diets and/or absence/presence of a female companion as described in the “Study groups” below and in the “Results” section.

Animal handling and procedures. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota and were conducted in accordance with the statutes of the Animal Welfare Act and the guidelines of the Public Health Service as issued in the Guide for the Care and Use of Laboratory Animals. We used male and female transgenic BERK sickle mice expressing human sickle hemoglobin (HbSS-BERK) and control mice expressing normal human hemoglobin A (HbAA-BERK). Homozygous HbSS-BERK sickle mice with knockout of murine α and β globins carry transgenes for human α and β globins, and express >99% human hemoglobin Sβ. These mice feature a severe disease pathology that resembles human sickle cell anemia (SCA), involving hemolysis, reticulocytosis, anemia, extensive organ damage, shortened life span and pain. Control HbAA-BERK mice express human α and β globins exclusively without murine α and β globins. Mice were genotyped for the knockout of mouse globins and presence of human globins (Transnetx, Cordova, TN) and phenotyped by isoelectric focusing for the presence of homozygous HbS or HbA. The age of both sickle and control mice was between 6 and 10 months.

Study groups. Both sickle and control mice were divided randomly into the following four study groups based on whether they received the standard rodent, RD, or the sickle diet, SD, and whether they were housed with (C+) or without (C−) a companion of opposite sex. The groups were as follows: RD/C− mice received the standard diet without a companion after birth and throughout the duration of the study; RD/C+ received the standard diet with a companion for the study period after originally being on RD/C− after birth; SD/C− received sickle diet without a companion for the study period after originally being on SD/C− after birth; SD/C+ received both the sickle diet and a companion after birth and for the duration of the study. Three additional study groups examined the impact of withdrawal of the sickle diet, companionship or both. All the withdrawal groups used mice that were treated with both the sickle diet and a companion after birth up to the beginning of the study period, so the baseline reflects the SD/C+ treatment. The withdrawal groups were as follows: SS W SD/C− mice were withdrawn from companionship for the study period after originally being on SD/C+ from birth; SS W RD/C− mice were withdrawn from the sickle diet for the study period after originally being on SD/C+ from birth; SS W RD/C− mice were withdrawn from both the sickle diet and companionship for the study period after originally being on SD/C+ from birth.

Diet composition. The RD was a fixed formula regular rodent diet (Teklad Global 18% Protein Rodent Diet) obtained from Teklad Diets (Madison, WI, USA). The SD was a modified version of the Standard Mouse Diet 9F obtained from LabDiet (St.Louis, MO, USA) to better meet the nutritional requirements of sickle mice. The SD is enriched in protein (26.4% vs 18.6%) and fat content (11.1% vs 6.2%). SD also has greater amounts of minerals (potassium, magnesium, iron, zinc copper, and iodine), vitamins (vitamin A, D-3, niacin, folic acid, B-12 and choline chloride), amino acids (including arginine, and glutamic acid) and omega3 fatty acid content compared to RD. Mice were fed ad libitum with their respective diets.

Pharmacological agents. Duloxetine (Dr. Reddy's Laboratory Ltd, India), a 5-HT-NE reuptake inhibitor, was dissolved in distilled water and administered intraperitoneally to male and female HbAA-BERK and HbSS-BERK mice in the RD/C− treatment group. A single dose at 3, 10, and 15 mg/kg was injected prior to behavioral testing to assess acute analgesic effect. A dose of 3 mg/kg/day of duloxetine was administered to HbSS-BERK mice in the RD/C− treatment group for nine consecutive days to assess the analgesic effect and tolerance behavior over a long-term treatment period. PCPA (Sigma Aldrich, St. Louis, MO, USA), a 5-HT depletion agent, was prepared and administered as previously described. HbSS-BERK mice in the RD/C− and SD/C+ treatment groups received 100 mg/kg of PCPA each day for 3 days immediately following behavioral testing. A final behavioral assessment was conducted one week after discontinuation of the last PCPA administration.

Morphine sulfate (West-Ward, Eatontown, NJ, USA) dissolved in normal mouse saline at 10 mg/ml was injected subcutaneously to HbSS-BERK mice in the RD/C− and SD/C+ treatment groups. Mice in the RD/C− treatment group were administered either a suboptimal dose of 10 mg/kg or a regular dose of 20 mg/kg. Mice in the SD/C+ treatment group were administered the suboptimal dose of 10 mg/kg. Following injection with morphine, all mice were subjected to behavioral testing to investigate the effect of diet and companionship on opioid analgesia.
Behavioral testing. Mice were acclimated to handling and testing protocols in a quiet room at controlled temperature of 26–27 °C before being tested for mechanical, thermal (heat and cold), and musculoskeletal hyperalgesia (grip force). A minimum of 5 min was given between each test to prevent carry-over hyperalgesia.

Mechanical Hyperalgesia: The paw withdrawal frequency (PWF), evoked by 10 applications of a 1.0 g von Frey monofilament (Stoelting Co., Wood Dale, IL, USA) to the plantar surface of each hind paw for one to two seconds with a five second inter-stimulus interval, was measured to determine mechanical sensitivity.

Thermal Hyperalgesia: For heat sensitivity, a stimulus generated by a radiant bulb was applied to the plantar surface of the hind paw, and paw withdrawal latency (PWL), to the nearest 0.1 s, was recorded once the mouse withdrew its paw in response to the stimulus. For cold sensitivity, the PWF on a 4 °C cold plate (Stoelting Co., Wood Dale, IL, USA) over a period of 2 min was determined.

Grip Force: Musculoskeletal hyperalgesia was assessed by peak forepaw grip to a computerized grip force meter (SA Maier Co., Milwaukee, WI, USA). Mice were made to pull on a wire-mesh gauge with their forepaws. The peak force exerted in grams (g) was recorded.

5-HT and DA analysis by HPLC. Spinal cords were harvested following euthanasia, swiftly frozen, and stored at a temperature of −80 °C prior to sample preparation. Frozen spinal cords were homogenized with 0.5 ml of 0.2 M perchloric acid and incubated at 4 °C for 30 min. The homogenates were centrifuged at 20,000g for 15 min at 4 °C, and the supernatant was collected and adjusted to pH 3.5 by using 1 M sodium acetate, and then filtered using a 3000 NMWL centrifugal system (Merck Millipore, Billerica, MA, USA). The filtrate was analyzed on an Eicom Pak SC-3ODS column (ID 3.0 × 100 mm) with AC-ODS Precolumn packing material. The mobile phase was composed of 80% 0.1 M citrate-acetate buffer (pH 3.5) containing 220 mg/l sodium octane sulfonate, 5 mg/l EDTA and 20% (v/v) methanol. A graphite electrode (WE-3G, Gasket GS-25) served as the working electrode and was set at +750 mv (Eicom) versus an Ag/AgCl reference electrode. The flow rate was set at 340–400 µl/min.

5-HT and DA analysis by immunostaining. A brain block containing midbrain and brain stem was sectioned coronally at 6 µm by cryostat to collect the PAG and RVM. Landmarks of the PAG and RVM were identified based on the Allen mouse brain atlas. Cervical spinal cord, PAG, and RVM at 6 µm thickness were labelled with rabbit anti-DA (Abcam, Cambridge, MA, USA) and rat anti-5-HT (Santa Cruz, Dallas, Texas). Cy2-conjugated donkey-anti-rabbit and Cy3-conjugated donkey-anti-rat secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) were used to detect immuno-reactivity, and samples were mounted with Vectashield H-1000 (Vector Labs, Burlingame, CA, USA). Images were captured using Olympus IX70 inverted microscope (Olympus Corporation, Center Valley, PA) under 60X objective. The total area of fluorescence corresponding to the labeled regions was measured using Image J (NIH). Data was collected and expressed as total area of fluorescent pixels as described previously.

Corticosterone analysis by ELISA. Whole blood was collected by cardiac puncture into Eppendorf tubes (Eppendorf North America, Hauppauge, NY). Blood was clotted for 30 min at room temperature before centrifuging for 10 min at 2000 × g. Serum samples were collected and analyzed using the Corticosterone ELISA kit (R&D systems, Minneapolis, MN). Assay results were collected and calculated using the Synergy HT plate reader and Gen5™ 1.0 data analysis software (BioTek).

Statistical analysis. Analyses reported in Figs. 2 and 3 used one-way repeated-measures analysis of variance (ANOVA) with the Bonferroni post-hoc test and were implemented using Prism (v 7.0c, GraphPad Prism Inc., San Diego, CA). For analyses reported in Figs. 1, 4, 5, and 6, a three-way ANOVA was performed, but no significant three-way interactions were present. Thus, mixed linear models were used correcting for multiple comparisons using Tukey’s post-hoc test. These analyses were done using the lme4 package (v. 1.1-21) in the R system (v. 3.4.0).

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 30 May 2018; Accepted: 8 January 2021
Published online: 01 February 2021

References
1. Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B. & Touboul, C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain 136, 380–387 (2008).
2. Breivik, H., Collett, B., Ventafridda, V., Cohen, R. & Gallacher, D. Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. Eur. J. Pain 10, 287–287 (2006).
3. Koulouris, A. I., Banim, P. & Hart, A. R. Pain in patients with pancreatic cancer: Prevalence, mechanisms, management and future developments. Dig. Dis. Sci. 62, 861–870 (2017).
4. Dureja, G. P. et al. Prevalence of chronic pain, impact on daily life, and treatment practices in India. Pain Pract. Off. J. World Inst. Pain 14, E51–E62 (2014).
5. Edwards, R. R., Dworkin, R. H., Sullivan, M. D., Turk, D. & Wasan, A. D. The role of psychosocial processes in the development and maintenance of chronic pain disorders. J. Pain Off. J. Am. Pain Soc. 17, 770–792 (2016).
6. Rosenblum, A., Marsch, L. A., Joseph, H. & Portenoy, R. K. Opioids and the treatment of chronic pain: Controversies, current status, and future directions. Exp. Clin. Psychopharmacol. 16, 405–416 (2008).
7. Okie, S. A flood of opioids, a rising tide of deaths. N. Engl. J. Med. 363, 1981–1985 (2010).
11. Mokdad, A. H. 
10. Daoust, R. 
9. Tran, H., Gupta, M. & Gupta, K. Targeting novel mechanisms of pain in sickle cell disease. 
8. Gupta, M., Msambichaka, L., Ballas, S. K. & Gupta, K. Morphine for the treatment of pain in sickle cell disease. 
12. Premkumar, L. S. Targeting TRPV1 as an alternative approach to narcotic analgesics to treat chronic pain conditions. 
15. Bonakdar, R. A. Integrative pain management. 
14. Porreca, F . & Navratilova, E. Reward, motivation, and emotion of pain and its relief. 
19. Burke, A. L. J., Mathias, J. L. & Denson, L. A. Psychological functioning of people living with chronic pain: A meta-analytic review. 
20. Brown, J. L., Sheffield, D., Leary, M. R. & Robinson, M. E. Social support and experimental pain. 
21. Harris, A., Parker, N. & Barker, C. Adults with sickle cell disease: Psychological impact and experience of hospital services. 
24. Turk, D. C., Audette, J., Levy, R. M., Mackey, S. C. & Stanos, S. Assessment and treatment of psychosocial comorbidities in pain patients. 
25. McCracken, L. M., Gauntlett-Gilbert, J. & Vowles, K. E. The role of mindfulness in a contextual cognitive-behavioral analysis perspective. 
29. Smith, W . R. 
31. Hassell, K. L. Population estimates of sickle cell disease in the U.S. 
32. Bediako, S. M. Psychosocial aspects of sickle cell disease: A primer for African American psychologists. 
33. Martinowich, K. & Lu, B. Interaction between BDNF and Serotonin: Role in mood disorders. 
35. Bravo, J. A., Dinan, T. G. & Cryan, J. F . Early-life stress induces persistent alterations in 5-HT1A receptor and serotonin transporters' mRNA expression in the adult rat brain. 
37. Bressan, R. A. & Crippa, J. A. The role of dopamine in reward and pleasure behaviour—Review of data from preclinical research. 
40. Blackburn-Munro, R. Hypothalamo–pituitary–adrenal axis dysregulation as a contributory factor to chronic pain and disability. 
43. Bernhard, R. A. Brain mapping pain. 
45. Wurtman, R. J., Hefti, F . & Melamed, E. Precursor control of neurotransmitter synthesis. 
47. Fernstrom, J. D. & Fernstrom, M. H. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. 
48. Benedetti, M. 
49. Blackburn-Munro, G. Pain in the brain: Are hormones to blame? Trends Endocrinol. Metab. 
50. Blackburn-Munro, G. & Blackburn-Munro, R. Pain in the brain: Are hormones to blame? Trends Endocrinol. Metab. 
51. Paszty, C. et al. Transgenic knockout mice with exclusively human sickle hemoglobin and sickle cell disease. 
52. Wang, S. et al. Expression of central glucocorticoid receptors after peripheral nerve injury contributes to neuropathic pain behaviors in rats. 
53. Paszty, C. et al. The role of serotonin in human mood and social interaction: Insight from altered tryptophan levels.
54. Van Houdenhove, B. Psychosocial stress and chronic pain. *Eur. J. Pain* 4, 225–228 (2000).
55. Elzinga, B. M.  
57. Uchio, Y.  
58. Neville, A., Soltani, S., Pavlova, M. & Noel, M. Unravelling the Relationship between Parent and Child PTSD and Pediatric Chronic Pain: the Mediating Role of Pain Catastrophizing. *J. Pain Off. J. Am. Pain Soc.* https://doi.org/10.1016/j.jpainsymptom.2017.10.004 (2017).
56. Saltzman, W., Hogan, B. K. & Abbott, D. H. Diminished cortisol levels in subordinate female marmosets are associated with altered central drive to the hypothalamic–pituitary–adrenal axis. *Biol. Psychiatry* 60, 843–849 (2006).
57. Uchio, Y. et al. A randomized, double-blind, placebo-controlled Phase III trial of duloxetine in Japanese patients with knee pain due to osteoarthritis. *J. Pain Res.* 11, 809–821 (2018).
58. Neville, A., Soltani, S., Pavlova, M. & Noel, M. Unravelling the Relationship between Parent and Child PTSD and Pediatric Chronic Pain: the Mediating Role of Pain Catastrophizing. *J. Pain Off. J. Am. Pain Soc.* https://doi.org/10.1016/j.jpainsymptom.2017.10.004 (2017).
59. Merlin, J. S. et al. ‘Two Pains Together’: Patient perspectives on psychological aspects of chronic pain while living with HIV. *PLoS ONE* 9, e11765 (2014).
60. Block, A. R., Ohnmeiss, D. D., Guyer, R. D., Rashbaum, R. F. & Hochschuler, S. H. The use of presurgical psychological screening to predict the outcome of spine surgery. *Spine J. Off. J. N. Am. Spine Soc.* 1, 274–282 (2001).
61. Rudy, T. E., Lieber, S. J., Boston, J. R., Gourley, L. M. & Baysal, E. Psychosocial predictors of physical performance in disabled individuals with chronic pain. *Clin. J. Pain* 19, 18–30 (2003).
62. Hinrichs-Rockett, A. et al. Psychosocial predictors and correlates for chronic post-surgical pain (CPSP)—A systematic review. *Eur. J. Pain* 13, 719–730 (2009).
63. Mathur, V. A. et al. Disease-related, nondisease-related, and situational catastrophizing in sickle cell disease and its relationship with pain. *J. Pain Off. J. Am. Pain Soc.* 17, 1227–1236 (2016).
64. Rhudy, J. L. & Meagher, M. W. Fear and anxiety: Divergent effects on human pain thresholds. *Pain* 84, 65–75 (2000).
65. Kenntner-Mabiala, R. & Pauli, P. Affective modulation of brain potentials to painful and nonpainful stimuli. *Psychophysiology* 42, 559–567 (2005).
66. Weich, K. & Tracey, I. The influence of negative emotions on pain: Behavioral effects and neural mechanisms. *Brain Body Med.* 47, 987–994 (2009).
67. Coffin, P. O. et al. Behavioral intervention to reduce opioid overdose among high-risk persons with opioid use disorder: A pilot randomized controlled trial. *PLoS ONE* 12, e0183354 (2017).
68. Klok, M. D., Jakobsdottir, S. & Drent, M. L. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review. *Obes. Rev.* 8, 21–34 (2007).
69. Haleem, D. J., Haque, Z., Inam, Q.-A., Ikram, H. & Haleem, M. A. Behavioral, hormonal and central serotonin modulating effects of injected leptin. *Peptides* 74, 1–8 (2015).
70. Spencer, S. J. et al. Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety after acute stress. *Biol. Psychiatry* 72, 457–465 (2012).
71. Bair, M. J., Robinson, R. L., Katon, W. & Kroenke, K. Depression and pain comorbidity: A literature review. *Arch. Intern. Med.* 163, 2433–2445 (2003).
72. Bardin, L. The complex role of serotonin and 5-HT receptors in chronic pain. *Behav. Pharmacol.* 22, 390–404 (2011).
73. Harmen, C. J. Serotonin and emotional processing: Does it help explain antidepressant drug action?. *Neuropharmacology* 55, 1023–1028 (2008).
74. Sommer, C. Serotonin in pain and analgesia. *Mol. Neurobiol.* 30, 117–125 (2004).
75. Wood, P. B. Role of central dopamine in pain and analgesia. *Expert Rev. Neurother.* 8, 781–797 (2008).
76. Song, Z., Utenius, C., Meyerson, B. A. & Linderoth, B. Pain relief by spinal cord stimulation involves serotonergic mechanisms: An experimental study in a rat model of mononeuropathy. *Pain* 147, 241–248 (2009).
77. Carruba, M. O. et al. Catecholamine and serotonin depletion from rat spinal cord: Effects on morphine and footshock induced analgesia. *Pharmacol. Res.* 25, 187–194 (1992).
78. Bardin, L., Lavarenne, J. & Eschaller, A. Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats. *Pain* 86, 11–18 (2000).
79. Weiss, D. K. et al. Gender differences in pain and healthcare utilization for adult sickle cell patients: The PiSCES Project. *J. Womens Health* 2002(15), 146–154 (2006).
80. Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B. & Riley, J. L. Sex, gender, and pain: A review of recent clinical and experimental findings. *J. Pain Off. J. Am. Pain Soc.* 10, 447–485 (2009).
81. Mogil, J. S. & Bailey, A. L. Sex and gender differences in pain and analgesia. *Prog. Brain Res.* 186, 141–157 (2010).
82. Mogil, J. S. Sex differences in pain and pain inhibition: Multiple explanations of a controversial phenomenon. *Nat. Rev. Neurosci.* 13, 859–866 (2012).
83. Brandow, A. M., Farley, R. A., Dasgupta, M., Hoffmann, R. G. & Panepinto, J. A. The use of neuropathic pain drugs in children with sickle cell disease is associated with older age, female sex, and longer length of hospital stay. *J. Pediatr. Hematol. Oncol.* 37, 10–15 (2015).
84. Orlotani, D., Garcia, M. C., Melo-Thomas, L. & Spadari-Bratfisch, R. C. Stress-induced endothane response and anxiety: The effects of comfort food in rats. *Stress Med.* 17, 211–218 (2014).
85. de Oliveira, C. et al. Hypercaloric diet modulates effects of chronic stress: A behavioral and biometric study on rats. *Stress Med.* 18, 514–523 (2015).
86. Ruis, M. A. et al. Housing familiar male wildtype rats together reduces the long-term adverse behaviour and physiological effects of social defeat. *Psychoneuroendocrinology* 24, 285–300 (1999).
87. Dronjak, S., Gavršlović, I., Filipović, D. & Radićević, M. B. Mobilization and cold stress affect sympa-thoadrenomedullary system and pituitary-adrenocortical axis of rats exposed to long-term isolation and crowding. *Physiol. Behav.* 81, 409–415 (2004).
88. Sternbach, R. A. Pain and ‘hasses’ in the united states: Findings of the nuprin pain report. *Pain* 27, 69–80 (1986).
89. Davis, M. C., Thummala, K. & Zautra, A. J. Stress-related clinical pain and mood in women with chronic pain: Moderating effects of depression and positive mood induction. *Ann. Behav. Med.* 48, 61–70 (2014).
90. Corcoran, L., Roche, M. & Finn, D. P. The role of the brain's endocannabinoid system in pain and its modulation by stress. *Int. Rev. Neurobiol.* 125, 203–255 (2015).
91. Hannibal, K. E. & Bishop, M. D. Chronic stress, cortisol dysfunction, and pain: A psychoneuroendocrine rationale for stress management in pain rehabilitation. *Phys. Ther.* 94, 1816–1823 (2014).
92. Reese, J. B., Lepore, S. J., Handorf, E. A. & Haythornthwaite, J. A. Emotional approach coping and depressive symptoms in colorectal cancer patients: The role of the intimate relationship. *J. Psychosoc. Oncol.* 35, 578–596 (2017).
93. Yehuda, R. & Seckl, J. Minireview: Stress-related psychiatric disorders with low cortisol levels: A metabolic hypothesis. *Endocrinology* 152, 4496–4503 (2011).
94. Manzi, E. A. et al. High protein diet attenuates histopathologic organ damage and vascular leakage in transgenic murine model of sickle cell anemia. *Exp. Biol. Med.* 239, 966–974 (2014).
95. Miller, A. H. & Raison, C. L. The role of inflammation in depression: From evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.* 16, 22–34 (2016).
96. Ménard, C., Pfau, M. L., Hodes, G. E. & Russo, S. J. Immune and neuroendocrine mechanisms of stress vulnerability and resilience. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 42, 62–80 (2017).
97. Hodes, G. E., Kana, V., Menard, C., Merad, M. & Russo, S. J. Neuroimmune mechanisms of depression. Nat. Neurosci. 18, 1386–1393 (2015).

98. Hodes, G. E. et al. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc. Natl. Acad. Sci. U. S. A. 111, 16136–16141 (2014).

99. Powell, N. D. et al. Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via β-adrenergic induction of myelopoesis. Proc. Natl. Acad. Sci. USA 110, 16574–16579 (2013).

100. Menard, C. et al. Social stress induces neurovascular pathology promoting depression. Nat. Neurosci. 20, 1752 (2017).

101. Esposito, P. et al. Acute stress increases permeability of the blood–brain barrier through activation of brain mast cells. Brain Res. 888, 117–127 (2001).

102. Sharma, H. S. & Dey, P. K. Impairment of blood–brain barrier (BBB) in rat by immobilization stress: Role of serotonin (5-HT). Indian J. Physiol. Pharmacol. 25, 111–122 (1981).

103. Wöhlé, E. S., Powell, N. D., Godbout, J. P. & Sheridan, J. F. Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. J. Neurosci. Off. J. Soc. Neurosci. 33, 13820–13833 (2013).

104. Campbell, C. M. et al. An evaluation of central sensitization in patients with sickle cell disease. J. Pain Off. J. Am. Pain Soc. 17, 617–627 (2016).

105. Carroll, C. P. et al. Chronic opioid therapy and central sensitization in sickle cell disease. Am. J. Prev. Med. 51, S69–77 (2016).

106. Lamming, C. E. D. et al. Spontaneous circulation of myeloid-lymphoid–initiating cells and SCID-repopulating cells in sickle cell crisis. J. Clin. Investig. 111, 811–819 (2003).

107. Taylor, S. C., Shacks, S. J., Mitchell, R. A. & Banks, A. Serum interleukin-6 levels in the steady state of sickle cell disease. J. Interferon Cytokine Res Off. J. Int. Soc. Interferon Cytokine Res. 15, 1061–1064 (1995).

108. Barak, Y. The immune system and happiness. Autoimmun. Rev. 5, 523–527 (2006).

109. Wilson, E. R. H. et al. Do illness perceptions and mood predict healing time for burn wounds? A prospective, preliminary study. J. Psychosom. Res. 71, 364–366 (2011).

110. Johnson, R. A., Meadows, R. L., Haubner, J. S. & Sevedge, K. Animal-assisted activity among patients with cancer: effects on mood, fatigue, self-perceived health, and sense of coherence. Oncol. Nurs. Forum 35, 225–232 (2008).

111. Schweitzer, M., Gilpin, L. & Frampton, S. Healing spaces: Elements of environmental design that make an impact on health. J. Altern. Complement. Med. 10, S7 (2004).

112. Coskaley, A. B. & Mahoney, E. K. Creating a therapeutic and healing environment with a pet therapy program. Complement. Ther. Clin. Pract. 15, 141–146 (2009).

113. Morris, C. R. et al. A randomized, placebo-controlled trial of arginine therapy for the treatment of children with sickle cell disease hospitalized with vaso-occlusive pain episodes. Haematologica 98, 1375–1382 (2013).

114. Sins, J. W. R., Mager, D. J., Davis, S. C. A. T., Biemond, B. J. & Fijnvantdka, K. Pharmacotherapeutic strategies in the prevention of acute, vaso-occlusive pain in sickle cell disease: A systematic review. Blood Adv. 1, 1598–1616 (2017).

115. Hyacinth, H. I. The injured brain might need more fat! EBioMedicine 33, 12–13 (2018).

116. Hyacinth, H. I. Sickle-cell anaemia needs more food?. Lancet Haematol. 5, e130–e131 (2018).

117. Jahagirdar, O. B. et al. Diet and gender influence survival of transgenic Berkley sickle cell mice. Haematologica 104, e331 (2019).

118. Kohli, D. R. et al. Pain-related behaviors and neurochemical alterations in mice expressing sickle hemoglobin: Modulation by cannabinoids. Blood 116, 456–465 (2010).

119. Hyacinth, H. I., Gee, B. E. & Hibbert, J. M. The role of nutrition in sickle cell disease. Nutr. Metab. Insights 3, 57–67 (2010).

120. Iyengar, S., Webster, A. A., Hemrick-Luecke, S. K., Xu, J. Y. & Simmons, R. M. A. Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats. J. Pharmacol. Exp. Ther. 311, 576–584 (2004).

121. Dong, H. W. The Allen Reference Atlas: A Digital Color Brain Atlas of the C57Bl/6j Male Mouse (Wiley, New York, 2008).

122. Vincent, L. et al. Mast cell activation contributes to sickle cell pathobiology and pain in mice. Blood 122, 1853–1862 (2013).
Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021