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Cognitive and behavior deficits in sickle cell mice are associated with profound neuropathologic changes in hippocampus and cerebellum

Li Wang [a], Luis E.F. Almeida [a], Celia M. de Souza Batista [b], Alfia Khaibullina [a], Nuo Xu [a], Sarah Albani [a], Kira A. Guth [a], Ji Sung Seo [a], Martha Quezado [c], Zenaide M.N. Quezado [a,d,e,*]

[a] The Sheikh Zayed Institute for Pediatric Surgical Innovation, Children's Research Institute, United States
[b] Department of Nutritional Sciences, Howard University, Washington DC 20059, United States
[c] Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, United States
[d] Divisions of Anesthesiology and Pain Medicine, Children's National Health System, United States
[e] Center for Neuroscience Research, Children's Research Institute, Children's National Health System, School of Medicine and Health Sciences, George Washington University, Washington, DC 20010, United States

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ABSTRACT

Strokes are perhaps the most serious complications of sickle cell disease (SCD) and by the fifth decade occur in approximately 25% of patients. While most patients do not develop strokes, mounting evidence indicates that even without brain abnormalities on imaging studies, SCD patients can present profound neurocognitive dysfunction. We sought to evaluate the neurocognitive behavior profile of humanized SCD mice (Townes, BERK) and to identify hematologic and neuropathologic abnormalities associated with the behavioral alterations observed in these mice. Heterozygous and homozygous Townes mice displayed severe cognitive deficits shown by significant delays in spatial learning compared to controls. Homozygous Townes also had increased depression- and anxiety-like behaviors as well as reduced performance on voluntary wheel running compared to controls. Behavior deficits observed in Townes were also seen in BERKs. Interestingly, most deficits in homozygotes were observed in older mice and were associated with worsening anemia. Further, neuropathologic abnormalities including the presence of large bands of dark/pyknotic (shrunken) neurons in CA1 and CA3 fields of hippocampus and evidence of neuronal dropout in cerebellum were present in homozygotes but not control Townes. These observations suggest that cognitive and behavioral deficits in SCD mice mirror those described in SCD patients and that aging, anemia, and profound neuropathologic changes in hippocampus and cerebellum are possible biologic correlates of those deficits. These findings support using SCD mice for studies of cognitive deficits in SCD and point to vulnerable brain areas with susceptibility to neuronal injury in SCD and to mechanisms that potentially underlie those deficits.

1. Introduction

Cerebrovascular accidents are serious complications in sickle cell disease (SCD) patients and occur in 5 to 10% of children and approximately 25% of adults by the fifth decade of life (Helton et al., 2014; Ohene-Frempong et al., 1998; Vichinsky et al., 2010). Moreover, approximately 22% of SCD children also develop silent strokes, which are evident on magnetic resonance imaging but are not associated with overt clinical neurologic deficits (DeBaun et al., 1998). As a consequence of overt or silent strokes, SCD patients can have global neurocognitive impairment as well as sensory and motor deficits (DeBaun et al., 1998). However, there is mounting evidence to suggest that even in the absence of overt cerebrovascular disease and/or parenchymal cerebral abnormalities on imaging studies, children and adults with SCD who are neurologically intact can still have profound neurocognitive dysfunction (Vichinsky et al., 2010; Wang et al., 2001). Researchers showed that nearly 20% of infants and toddlers with SCD score more than two standard deviations below the normal control mean on cognitive and motor neurodevelopmental evaluations (Glass et al., 2013). Further, some SCD patients without cerebrovascular accidents also show reduced performance in standard global cognitive function, working memory, processing speed and executive function tests (Steen et al., 2003; Vichinsky et al., 2010; Wang et al., 2001). Therefore, a great deal of evidence indicates that central nervous system dysfunction is highly prevalent among SCD patients and can present as significant neurocognitive deficits.

* Corresponding author at: The Sheikh Zayed Institute for Pediatric Surgical Innovation, Center for Neuroscience Research, Children's Research Institute, Divisions of Anesthesiology and Pain Medicine, Children's National Health System, School of Medicine and Health Sciences, George Washington University, 111 Michigan Avenue, Washington, DC 20010, United States.
E-mail address: zquezado@childrensnational.org (Z.M.N. Quezado).
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In addition to cognitive deficits, researchers have shown that SCD patients can also present alterations in other behavior domains. While large epidemiologic studies are lacking, several investigations show that attention-deficit/hyperactivity disorder, clinical depression, and anxiety are common among children and adult SCD patients (Benton et al., 2011; Levenson et al., 2008; Lukoo et al., 2015; Wallen et al., 2014). Among children and adolescents with SCD admitted with vaso-occlusive episodes (VOEs), nearly 8% have some mental health diagnosis including mood and anxiety disorders and patients with those diagnoses have higher number of hospitalizations and prolonged length-of-stay (Myrvik et al., 2013). Therefore behavior alterations are prevalent in SCD, especially among patients who have frequent VOEs.

The mechanisms underlying cognitive deficits and behavior alterations in SCD are incompletely understood. In SCD patients who have had strokes, neurocognitive dysfunction can be partially explained by those cerebrovascular events. However, the mechanisms leading to cognitive dysfunction in SCD patients without strokes remain poorly understood. Few studies have examined the mechanism of brain dysfunction in SCD patients and researchers have shown that recurrent pain episodes, chronic hypoxia, worsening anemia, and aging correlate with the degree of cognitive deficits (Glass et al., 2013; Hogan et al., 2006; Steen et al., 2003; Vichinsky et al., 2010). However, these deficits and associations have not been consistently reported (Armstrong et al., 2013; Schatz and McClellan, 2006; Schatz and Roberts, 2007; Thompson et al., 2002; Wang et al., 1993). Therefore, continued investigations to elucidate mechanisms of brain dysfunction and behavioral deficits in SCD are needed to identify modifiable variables that contribute to those abnormalities.

Humanized SCD mice display hematologic abnormalities (leukocytosis, hemolytic anemia), pathologic features (kidney and liver dysfunction), and somatosensory alterations (sensitization of sensory nerve fibers) observed in humans with SCD (Cataldo et al., 2015; Garrison et al., 2012; Hanna et al., 2007; Kenyon et al., 2015; Kohli et al., 2010; Manci et al., 2006; Paszty et al., 1997; Wu et al., 2006). We hypothesized that SCD mice would have cognitive deficits and alteration in mood and emotionality. We tested these hypotheses by examining several behavior domains (cognitive function, anxiety, motivation, and depression) and seeking possible hematologic and neuropathologic correlates of the behavior changes observed in humanized SCD mouse strains.

2. Material and methods

We conducted this study after approval from the Animal Care and Use Committee from Children's National Health System, Washington, DC and in accordance with recommendations from the Guide for the Care and Use of Laboratory Animals.

2.1. Animals

We examined several behavior domain in two strains of humanized SCD mice, the B6;129-*Hba*$^{tm1(HBA)Tow}$ *Hbb*$^{tm2(HBG1,HBB*)Tow}$/*Hbb*$^{tm3(HBG1,HBB)Tow}$/J strain, here referred to as the Townes strain (Hanna et al., 2007; Wu et al., 2006), and the *Hba*^{tm1Paz} *Hbb*^{tm1Tow} Tg(HBA-HBBs)41Paz/J, the BERK strain (Paszty et al., 1997). Townes mice (homozygous, heterozygous, and controls) do not express mouse hemoglobin. Homozygous Townes mice express more than 96% and heterozygous approximately 30% of human sickle and 70% of human hemoglobin A (Kenyon et al., 2015; Wu et al., 2006). Details about breeding and genotyping (Supplementary Fig. 1 and Supplementary Table 1) of Townes and BERK SCD mouse strains are provided in the Supplementary files. Throughout experiments, animals had unrestricted access to mouse chow and water, and were housed in ventilated cages in a temperature- and humidity-controlled (21 °C) facility under a 12-h light–dark cycle. Female mice were housed together in order to synchronize estrous cycles and thus control for variability. Behavior experiments were not conducted on days when cages were changed in the animal facility as to avoid the effect of stress on behavior tasks.

2.2. Study design and experimental protocol

We evaluated behavior in SCD and respective control mice in a cross-sectional fashion using several cohorts of naïve BERK and Townes animals that had not been previously enrolled in other studies. All Townes cohorts included balanced numbers of age-matched male and female mice of each genotype. As BERK mice have high spontaneous mortality (Jackson Laboratory and personal observation in our animal facility), are of limited availability, and most homozygous males are used for breeding and maintenance of the colony, only female mice were available in sufficient numbers for a few of the behavior tests performed.

Behavioral testing was performed between 9:00 AM and 3:00 PM in a quiet and dedicated behavior laboratory room. During experiments, mice of all genotypes (heterozygotes, homozygotes, and controls) were included in each cohort in order to control for the effect of time and possible investigator variability. In order to avoid confounding effects of repeated handling and multiple behavior assays, each animal underwent only one behavior test, with one exception, i.e., animals undergoing voluntary wheel running test also underwent grip strength measurements.

2.3. Behavioral studies

2.3.1. Water T-maze

In order to examine the effect of SCD on cognitive learning and memory, we conducted the water T-maze test as previously described (Tanimura et al., 2008; Wang et al., 2011). A T-maze (San Diego Instruments, San Diego, CA) apparatus made of beige plastic (7.5 cm wide, 32 cm long, and 17 cm tall) was placed in a predetermined location in a dedicated behavior laboratory and spatial cues were provided by the furniture, additional equipment, and overhead lighting. The test included a pre-testing session aimed at identifying whether a mouse had turning biases [defined as five entries or more into the same arm (right or left) out of eight trials]. If a mouse showed turning bias during the pre-testing session, the escape platform was placed on the non-preferred arm during the testing sessions. Each trial started with placement of the mouse in the starting arm of the T-maze and was completed when the mouse either, reached and stayed on the platform for 2 s, or when 60 s had elapsed. When the mouse did not reach the platform within the allotted time, it was gently guided to it after 60 s. Once each trial was complete, the mouse was left on the platform for 15 s and subsequently returned to its cages. During each trial, a mouse showed a correct response if it navigated directly from the start arm to the platform without entering the arm without the platform. Spatial learning criterion was reached when a mouse showed correct responses during at least seven out of eight trials for three consecutive days. We also examined cognitive flexibility by testing reversal learning. On the day after each mouse reached spatial learning criterion, the reversal learning test was initiated. Testing procedures for reversal learning were similar except that the position of the platform was reversed. Criterion for reversal learning was reached when a mouse displayed at least seven correct responses in the eight trials for three consecutive days. During the T-maze test, we also measured latency to reach the platform in each trial during the first three days of spatial and reversal learning tests.

2.3.2. Elevated plus maze

In order to evaluate the effect of SCD on emotionality, we conducted the elevated plus maze test as previously described (Lister, 1987). Briefly, the elevated plus maze (San Diego Instruments, San Diego, CA) has a "+" shape and is composed of two open and two identical enclosed arms elevated 40 cm above the floor. During a single video-recorded