Locomotor mal-performance and gait adaptability deficits in sickle cell mice are associated with vascular and white matter abnormalities and increased oxidative stress in cerebellum

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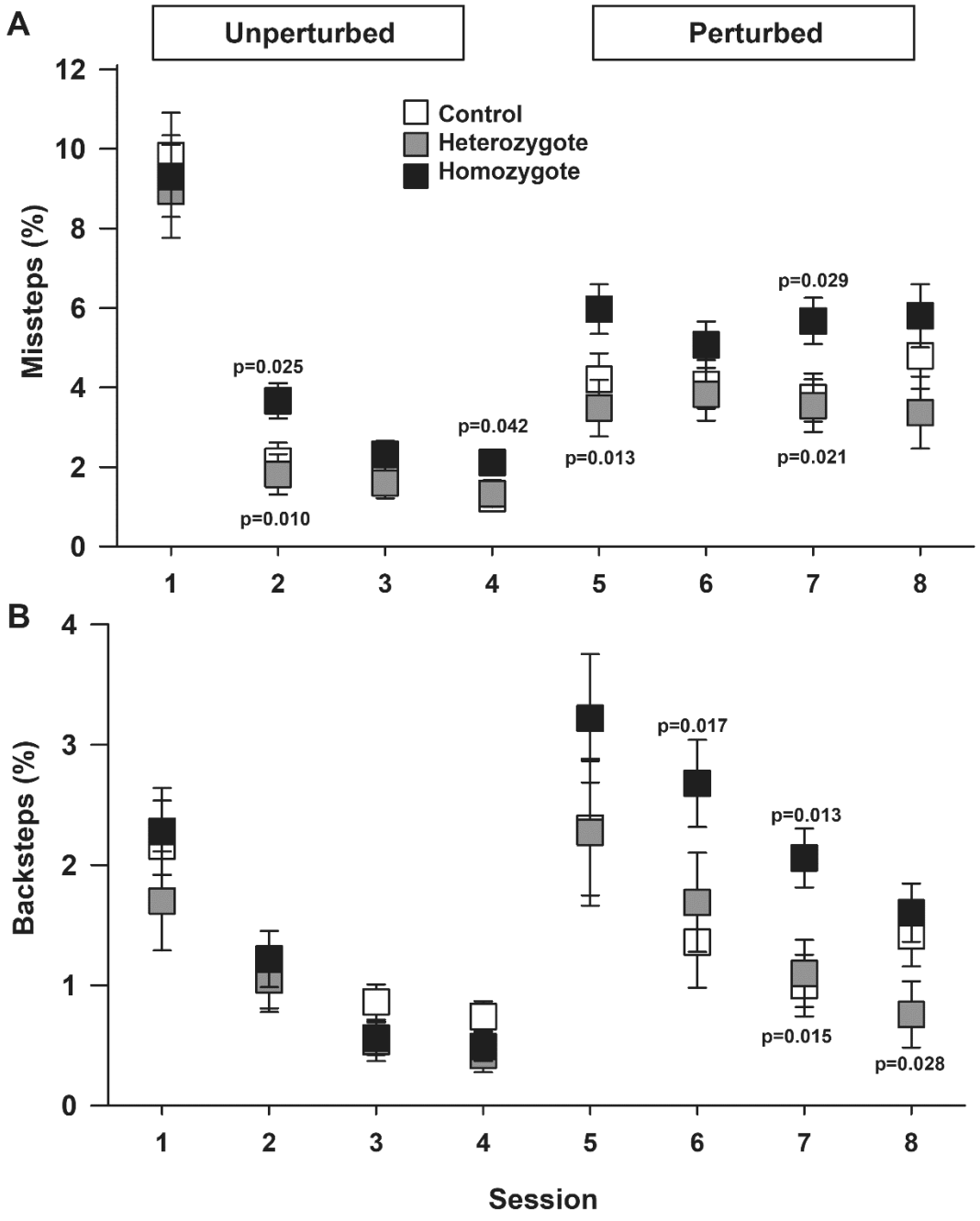
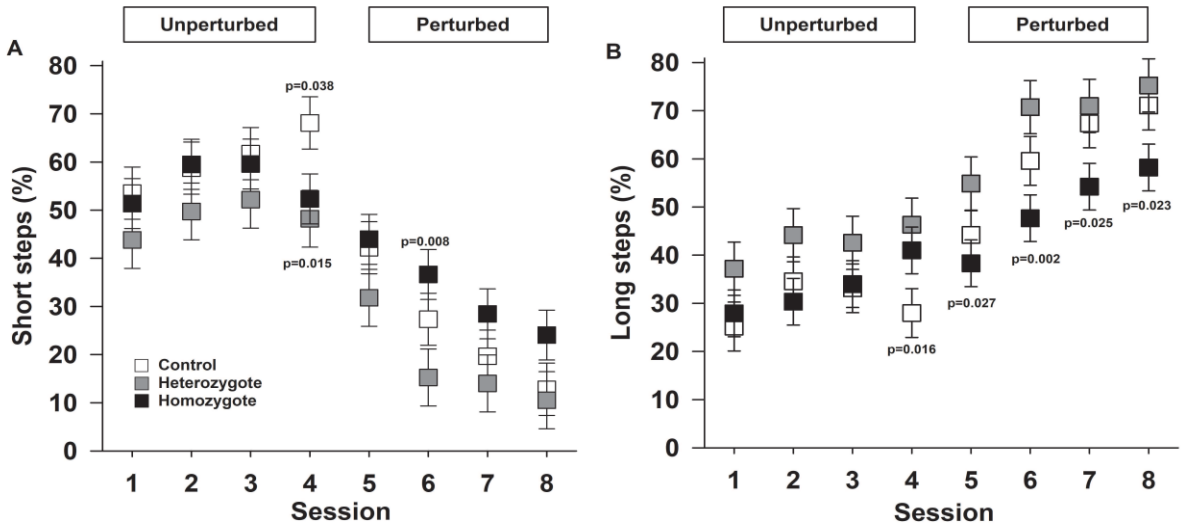


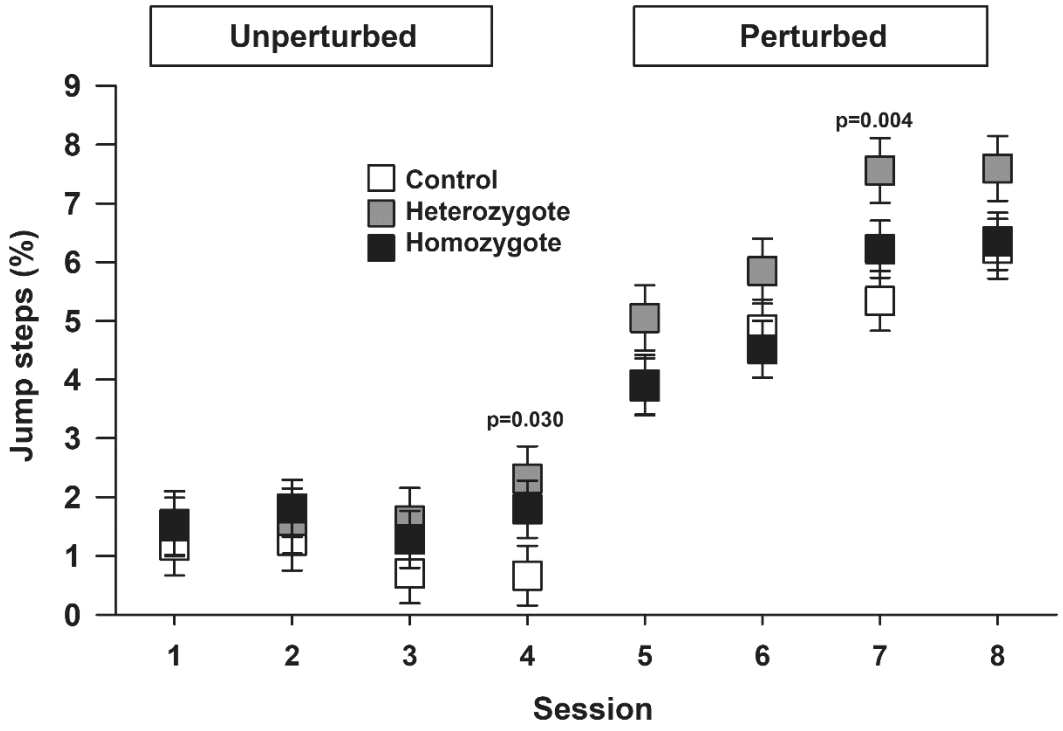
Supplemental Figure 1. Sickle cell mice have significant locomotor miscoordination and decreased motivation. Data are shown as least-square means±standard error of the least-square means for male and female combined for each genotype. P values reflect ad hoc pairwise genotype comparisons for respective sessions. A. Controlling for session and sex, homozygous

Townes committed a higher percentage of missteps compared with heterozygotes and control animals (overall effect of genotype, p=0.031). Compared to controls, homozygous Townes mice had higher percentages of missteps on sessions two (p=0.025), four (p=0.042), and seven (p=0.021). Compared to heterozygotes, homozygous Townes mice had higher percentages of missteps on sessions two (p=0.010), five (p=0.013), and seven (p=0.021). B. During unperturbed sessions overall, control, heterozygous, and homozygous mice displayed similar percentage of backsteps. However, during perturbed sessions, compared to controls, homozygous Townes displayed higher percentage of backsteps in sessions six (p=0.017) and seven (p=0.0054) and compared to heterozygotes in sessions seven (p=0.015) and eight (p=0.028). N=10-11 per genotype including balanced numbers of age-matched male and females.



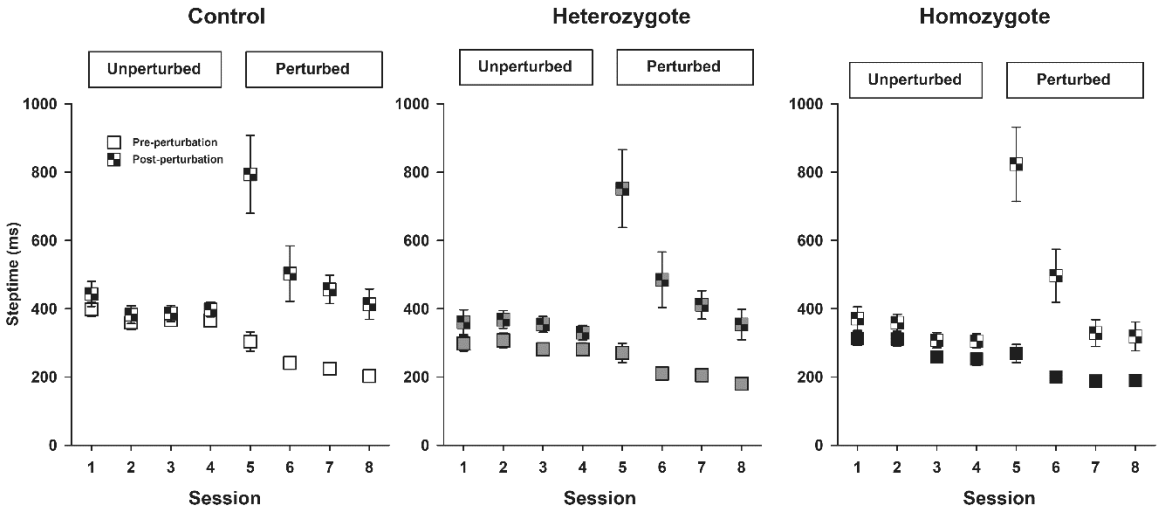
Supplemental Figure 2. Sickle cell mice have significant deficits in locomotor/gait adaptability Data are shown as least-square means±standard error of the least-square means for male and female combined for each genotype. P values reflect ad hoc pairwise genotype comparisons for respective sessions. The percentage of short and long steps reflect gait/stepping pattern, a reflection of multi-joint limb control and coordination capabilities when facing obstacles during perturbed sessions. Homozygous and heterozygous Townes have altered stepping patterns compared to controls. A. Overall controlling for sex and genotype, during perturbed sessions (five-eight) animals displayed decreases in percentage of short steps compared to the last unperturbed session (all, $p \leq 0.0001$). Controlling for sex, on the last unperturbed session, homozygous ($p=0.038$) and heterozygous ($p=0.0149$) Townes had a lower percentage of short steps, compared to controls. During perturbed session six, homozygous had a higher percentage of short steps compared to heterozygous ($p=0.008$). B. Regarding long steps, overall, controlling for session and sex, homozygous Townes had a lower percentage of long steps compared to heterozygotes ($p=0.015$, for overall effect of genotype). Controlling for

sex, on the last unperturbed session, heterozygous Townes had a higher percentage of long steps compared to controls ($p=0.016$). Controlling for sex, homozygous Townes had a lower percentage of long steps compared to heterozygotes on perturbed sessions five ($p=0.027$), six ($p=0.002$), seven ($p=0.025$), and eight ($p=0.023$). Thirty-one mice participated in the Erasmus Ladder test, N=10-11 per genotype including balanced numbers of age-matched male and females.



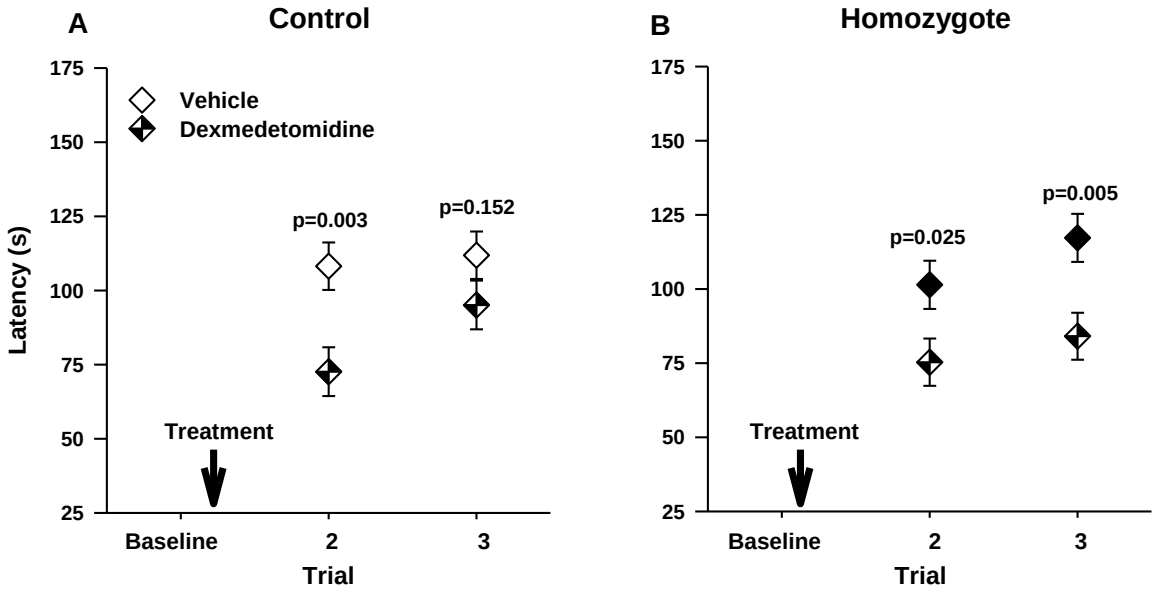
Supplemental Figure 3. Sickle cell mice have significant deficits in locomotor/gait adaptability Data are shown as least-square means±standard error of the least-square means for male and female combined for each genotype. P values reflect ad hoc pairwise genotype comparisons for respective sessions. Overall controlling for sex and genotype, during perturbed sessions (five-eight) animals displayed increases in percentage of jump steps compared to the last unperturbed session (all, $p \leq 0.0001$). There was also an overall effect of genotype ($p=0.046$) and controlling for session and sex, heterozygous Townes displayed a higher percentage of jumps compared to controls ($p=0.014$) but similar percentage compared to homozygotes ($p=0.103$). Controlling for sex, heterozygous Townes had a higher percentage of jump steps on the last unperturbed session ($p=0.030$) and on perturbed session seven ($p=0.004$) compared to controls. Thirty-one mice participated in the Erasmus Ladder test, N=10-11 per genotype

including balanced numbers of age-matched male and females.



Supplemental Figure 4. Sickle cell mice have intact motor learning. Using the Erasmus ladder, we examined adaptive cerebellar learning by comparing rung activation steptimes among genotypes. Data are shown as least-square means±standard error of the least-square means for pre and post perturbation steptimes of male and female combined in the control (A), heterozygote (B), and homozygote (C) groups. Over unperturbed sessions, homozygous and heterozygous had similar steptimes (all $p>0.191$). However, during the third and fourth unperturbed sessions, homozygous and heterozygous had overall shorter step times compared to controls (all $p<0.035$). Controlling for sex and genotype, over perturbed sessions, when obstacles were presented, overall, there were significant increases in post perturbation steptimes in sessions five ($p<0.0001$) and six ($p=0.002$) compared to session four. However, by sessions seven and eight, post perturbations steptimes decreased and were similar to those in session 4 (all $p>0.065$) in all genotypes indicating that controls, heterozygous, and homozygous Townes mice had similar adaptive cerebellar motor learning abilities ($p=0.0995$ for overall effect of genotype). Thirty-one mice participated in the Erasmus Ladder test, N=10-11 per genotype including

balanced numbers of age-matched male and females.



Supplemental Figure 5. Dexmedetomidine decreases latency to fall but does not affect motor learning. Data are shown as least-square means±standard error of the least-square means of latency to fall from the rod for male and female controls (A) and homozygotes (B) treated with dexmedetomidine or vehicle. P values reflect ad hoc pairwise treatment comparisons for respective trials. Controlling for baseline, sex, and genotype, dexmedetomidine-treated controls and homozygous Townes had significantly lower latency to fall compared to respective vehicle-treated mice (p=0.0006, for overall treatment effect). Interestingly, controlling for baseline, genotype, sex, and treatment, overall from trial two to trial three mice showed increases in latency to fall (p=0.0005 for overall effect of trial, supplemental Figure 5) indicating that dexmedetomidine did not impact motor learning. For each genotype, the p values on the figures reflect comparisons between vehicle- and dexmedetomidine treated mice. Forty mice participated in the rotarod test N=20 per genotype including balanced numbers of age-matched

male and females.