Title: Automated continuous behavioral monitoring and traditional behavioral testing reveal early phenotypes in a novel SOD1-G85R knock-in mouse model of ALS

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Dominant mutations in the gene encoding Superoxide dismutase 1 (SOD1) are a major cause of familial amyotrophic lateral sclerosis (fALS). Mouse models are a vital tool in revealing fALS pathogenic mechanisms and developing therapeutics for sporadic ALS. Until recently, models have been limited to transgenic mice overexpressing mutant human SOD1 alleles. While valuable, such transgenic lines rely on overexpression and do not reflect endogenous expression patterns and regulation of these alleles. In particular, it is not clear to what extent these models are useful for delineating early abnormalities. To address these gaps we created a knock-in mouse model harboring the $SOD1^{G85R}$ fALS mutation on the murine ortholog of the human SOD1 gene. We have characterized these animals using Automated Continuous Behavioral Monitoring (ACBM, Jhuang et al., 2010; White et al. 2018). During ACBM mice are video recorded at 30 frames/second for five days (total of ~1.3×10^7 frames/mouse/session). Behavioral assessment is then performed using a supervised, machine learning-based, computer algorithm to assign 1 of 8 designated behaviors to each frame. We assessed walking, hanging, rearing, drinking, eating from hopper, eating by hand, grooming and resting in male homozygous and heterozygous $SOD1^{G85R}$ mice in three sessions over 1-6 months of age. In parallel, we have characterized the mice using traditional behavioral assessments including weight monitoring, wire hang, open field, grip strength, and rotorod. We find that homozygous $SOD1^{G85R}$ mice exhibit robust weight and behavioral deficits as early as one month of age—much earlier than those reported for the transgenic $SOD1^{G85R}$ lines. Notably, $SOD1^{G85R}$ heterozygous mice also display these similar deficits, albeit to a lesser extent and/or at later times. We have also observed and characterized a core strength deficit that appears at one month of age and a tremor phenotype that appears at 6 months of age in homozygous animals. In summary, the combined use of ACBM and traditional behavioral measures has revealed behavioral abnormalities at early time in this novel $SOD1^{G85R}$ knock-in model. We propose that the knock-in $SOD1^{G85R}$ ALS disease model will be useful for characterizing early defects that could be important for predicting conversion in human fALS patients as well as revealing biomarkers useful for detecting presymptomatic changes and developing treatments for sporadic ALS.