Methods
Mice were given their diet pellet after removing their regular diet of mouse chow from their cages at a fixed time of day and monitored weekly for weight changes. A comprehensive behavioral analysis was performed weekly, including ventral plane videography for gait analysis, open field testing, clapping reflex scoring, and measurements of rotarod and wire hang performance. Upon consuming the entire pellet, mouse cages were replenished with regular mouse chow ad libitum until the next feeding time. Mice were exposed daily to BSSG for 18 weeks and at the end of the experiment animals were perfused and processed for histology. Anterior horn cells were visualized with fast cresyl violet staining for Nissl substance or FITC-choline acetyltransferase (ChAT).

Summary of Results
AlS2 null mice exhibit deficits in leg extension reflex and grip strength irrespective of BSSG exposure. Mice deficient in AlS2 gene show subtle differences in gait patterns and decreased muscle strength compared to their wild type counterparts. Exposure to BSSG results in a shorter stride and faster, more frequent steps during locomotion irrespective of genotype. AlS2 deficient mice are less active, exhibiting slower and wider turns during meandering. Conclusion: BSSG effects gait dynamics while AlS2 genotype exerts its effects on open field activity.

1. Motor Skills

A baseline was obtained for 12 weeks where mice were subjected to the battery of behavioral tests prior to administration of the BSSG diet. Baseline testing consistently showed a robust leg extension when suspended by the tail, and no significant differences between genotype on rotarod performance, although KO mice showed a slightly longer latency to fall off of the rotarod. After administering the BSSG diet, mice did not show significant differences in performance on the rotarod or leg extension until the twelfth week of feeding. Towards the endpoint of the study, WT mice performed better than KO when exposed to BSSG (p < 0.05). Abnormalities in leg extension began to manifest at the twelfth week after administration of BSSG feeding, where WT consistently scored higher than KO for the control fed group (p < 0.05).

During baseline testing, wild type mice consistently performed better on the hanging wire test of motor strength (p < 0.001). This significance is not due to animal weight differences, as mice from both genotypes had very similar body weights throughout each week of testing. After administering the BSSG diet, wild type mice continued to perform better than KO on the grip strength test after exposure to BSSG (p < 0.001) while animals maintained similar body weights across groups.

2. Gait Dynamics and Activity

**AlS2** mice exposed to BSSG exhibit shorter stride and faster, more frequent steps during locomotion irrespective of genotype. (A) Stride frequency, also cadence, is the number of times per second that a paw takes a complete stride. BSSG-fed animals have a higher stride frequency in motion. (B) Stance duration is the time the paw is in contact with the belt during motion, and is shorter in BSSG-fed animals. (C) Stride duration is the time for one complete stride of one paw, and is shorter for BSSG-fed animals. (D) Stride length is the distance between successive strides of the same paw, and is shorter for BSSG-fed animals. (p < 0.05)

**AlS2** mice are hypoactive. Two-way ANOVA revealed a significant effect of genotype in measures of distance travelled, waist, and movement duration.

3. Motor Neuron Pathology

Examination of spinal anterior horn cells reveals neuropathological features resembling clinical ALS. **Top row:** Immunohistochemistry of motor neurons in the lumbar cord stained with ChAT. Mice exposed to BSSG show a trend towards decreased motor neurons in the anterior horn. **Middle row:** Higher magnification at Resed lamina IX. **Bottom row:** BSSG exposure leads to neuropathological features such as abnormalities in the protein synthesizing system of motor neurons of the spinal cord. Quantitative examination of motor neurons in the lumbar anterior horn was performed using cresyl violet stained sections. Neurons were identified by the presence of a nucleolus and Nissl substance, and included if their size was greater than 20 μm. Spinal cords from mice with exposure to BSSG showed increased chromatolysis as visualized by light microscopy. (E) Apparently normal and healthy anterior horns cell with prominent nucleolus, darkly staining Nissl substance, and processes. (F) Motor neuron with central chromatolysis and displaced nucleus but with normal width. Satisfiafa also observed, characterized by the appearance of glial cells in proximity to the soma. (G) Stained motor neurons that appear irregularly distended with a decrease in overall width. (H) Anterior horn cells from a wild type mouse exposed to BSSG showing presence of both apparently normal and chromatolytic motor neurons in close proximity. Scale bar: 40 μm.

References

This work was supported by grants from the ALS Association, ALS Society, and National Institutes of Health. G.L. is the recipient of a Scottish Eye Foundation and University graduate student award. Personal thanks to A.J.K. for his unfailing support, helpful advice, and fond memories.