

# Clinical Trials in the G93A Mouse: Measuring Time to Onset of Disease and Survival

## Determination of End Stage (Survival)

- Natural death is not an end point. For humane reasons, progressive deterioration of the animal's health leading to death is not allowed.
- Survival is defined as the age at which the mouse demonstrates complete paralysis of one hind limb or is unable to right itself within 30 seconds of being placed on its side. At that time, the animal is sacrificed.

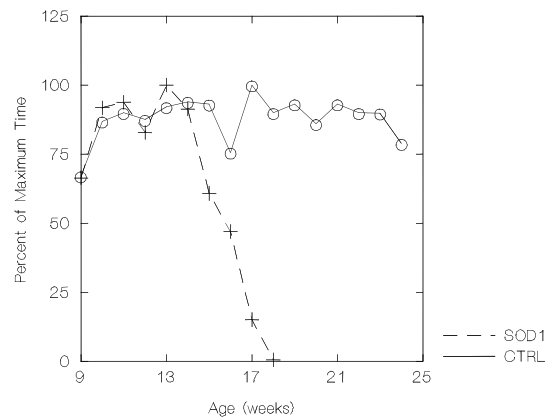


## Determination of Onset of Clinical Symptoms

Onset of clinical symptoms is detectable by 70-90 days of age. These mice show muscle wasting, abnormal splay of the hind limbs and tremor.

**A. Rotorod.** We have used the rotorod to identify the time of onset of clinical signs.

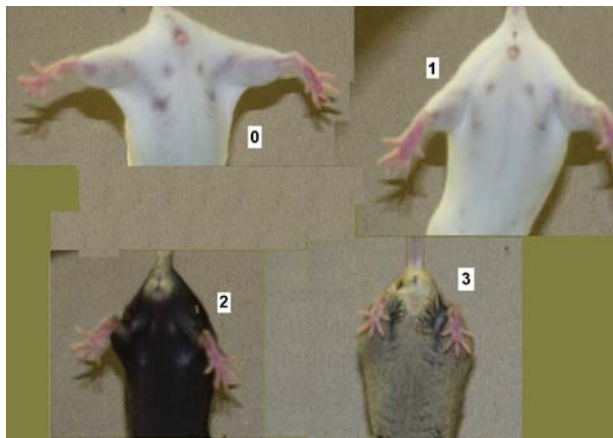
Rotorod data of controls (n=13) and SOD1 (n=15) animals starting at 60 days of age (Figure 1). For each animal 100% is taken as its maximum time in seconds that the animal remained on the rotorod. The rotorod was set at a constant rotation of 20 rpm, once the animal reached 5 minutes, it was removed from the apparatus and returned to its cage. Each animal was tested a minimum of two trials each week.



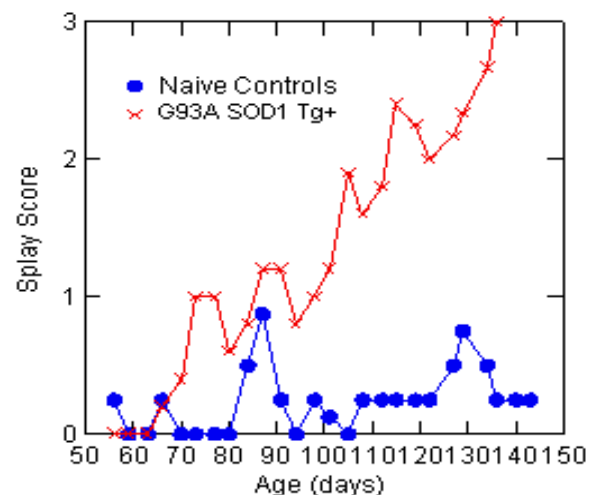
**B. Combined score from three assessments:**

### 1. Splay Test

(0=normal; 1=mild; 2=moderate; 3=severe)



**Splay vs Age in Female B6/SJL mice**



## 2. Beam Test

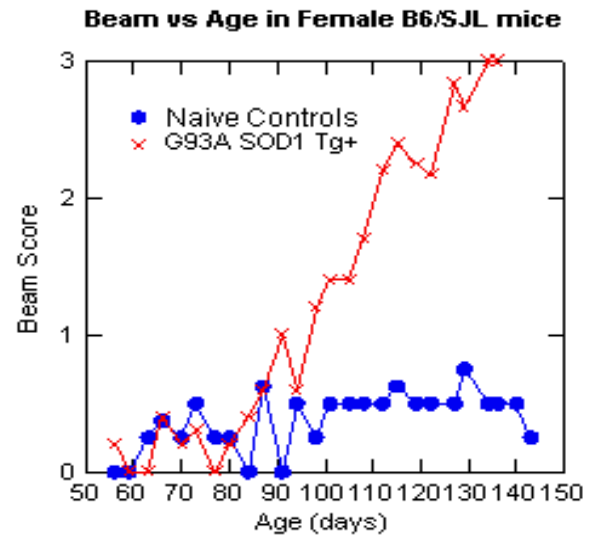
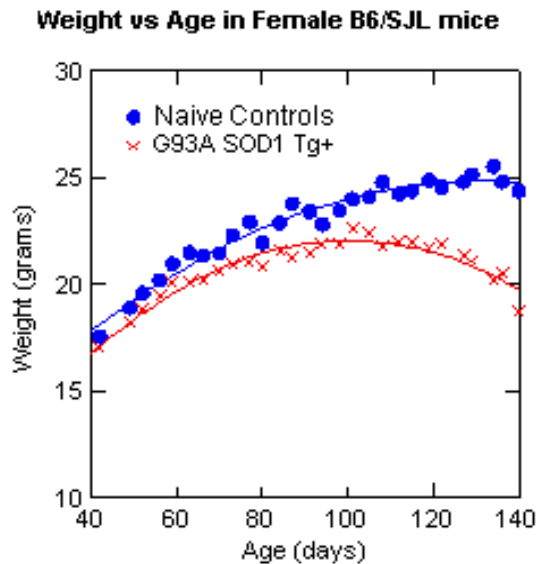
The mouse is placed on the edge of a plexiglas sheet (~0.3 inches wide) and observed while walking.

- 0 = Normal
- 1 = Mildly impaired (uses side of the cage)
- 2 = Moderately impaired (is barely able to walk)
- 3 = Severely impaired (is not able to walk and falls)



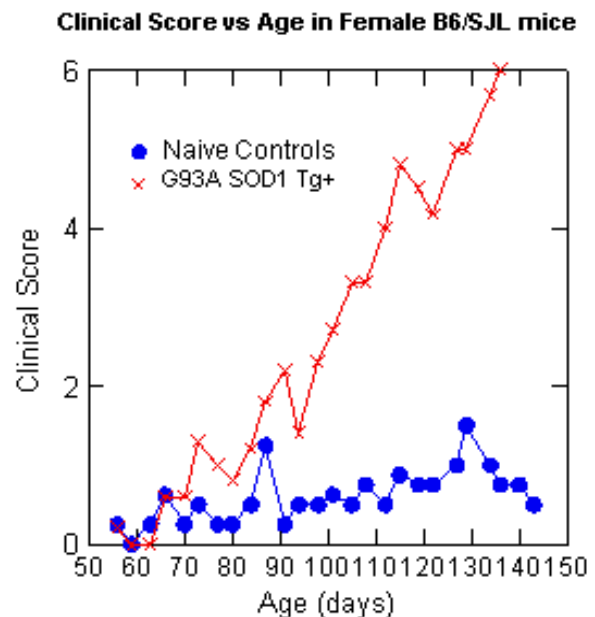
Example: Mildly impaired mouse (uses the side of the beam), giving a score = 1.

## 3. Weight



## The Determination of Disease Onset

- Weight Onset was defined as the maximum of the weight data (zero of the first derivative) when fitted to a second order polynomial (parabola).
- Clinical onset was defined as the day that the sum of splay and beam test reached a value equal to 2 without going below 2.



## References

Deitch, J.S., Alexander, G.M. Del Valle, L. and Heiman-Patterson, T.D. (2002) GLT-1 glutamate transporter levels are unchanged in mice expressing G93A human mutant SOD1. *J. Neurol. Sci.*, **193**:117-126.

Alexander, G.M., Erwin, K.L., Byers, N., Deitch, J.S., Augelli, B.J., Blankenhorn, E.P. and Heiman-Patterson, T.D. (2004) Effect of transgene copy number on survival in the G93A SOD1 transgenic mouse model of ALS. *Mol. Brain Res.*, 130:7-15.

Heiman-Patterson, T.D., Deitch, J.S., Blankenhorn, E.P., Erwin, K.L., Perreault, M.J., Alexander, B.K., Byers, N., Toman, I. and Alexander, G.M. (2005) Background and gender effects on survival in the TgN (SOD1-G93A)1Gur mouse model of ALS. *J. Neurol. Sci.*, **236**:1-7.

