Characterization of a SOD1 Rat Model of ALS: Behavior and Electrophysiology

Alexis Zajicek[1], Brian Haner[1], Melville Osborne[1], Moriah Jacobson[2], Michael Carr[2], Taleen Hanania[1].

[1]PsychoGenics, Paramus, New Jersey, US. [2] Taconic Biosciences, Rensselaer, New York, US.

PsychoGenics Transforming CNS Discovery

TACONIC BIOSCIENCES

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disorder characterized by progressive motor neuron degeneration resulting in muscle weakness, paralysis, and eventually death. Mutations of the superoxide dismutase 1 (SOD1) gene are linked to familial and sporadic cases of ALS. In collaboration with Taconic Biosciences, PsychoGenics characterized a SOD1 G93A overexpressing (NTac:SD-Tg(SOD1G93A)L26H) rat model of ALS.

Male and female wild type (WT) and transgenic (Tg) rats were used in the study. Fifteen rats were enrolled in each group which allowed us to assess phenotypic differences in male and female rats. Body weight (BW), motor function, and nerve conduction changes were measured longitudinally starting at 16 weeks of age.

SOD1 rats show progressive decrease in BW and hindlimb grip strength starting at 22 weeks of age, whereas decreased locomotor and rearing activities occurred at 29 weeks of age. Deficits in rotarod performance were also seen in the SOD1 rats starting at 22 weeks of age. In general, the behavioral deficits were more robust in male SOD1 rats compared to female rats. Survival analysis showed that female rats survived longer than male rats.

Assessment of compound muscle action potential (CMAP) found that starting at 24 weeks of age, onset latency, which corresponds to the time from initiation of nerve stimulus to response, was increased in SOD1 rats. Peak amplitude and neuromuscular conduction velocity were reduced in SOD1 rats compared to WT rats.

Analysis of cerebrospinal fluid and plasma from SOD1 HMZ rats shows an increase of Neurofilament light (NFL) and inflammatory marker IL-6 in the plasma and CSF of SOD1 rats.

Together, these results suggest that this model can be used for screening novel therapeutics for ALS treatment.

METHODS

To characterize behavioral and molecular phenotypes, a total of 30 WT and 30 SOD1 mixed-sex rats were assessed (15M/15F). Body weight was taken once per week, and three times per week at the onset of symptoms. Locomotor efficacy was assessed by open field test, grip strength, rotarod, and PsychoGenics' proprietary gait analysis system, NeuroCube ®. Plasma and cerebrospinal fluid (CSF) were collected at humane endpoint or at 38 weeks of age. NFL was measured using a Quanterix Simoa NF-light V2 Advantage Kit. Inflammatory markers were measured in CSF and Plasma using a Meso Scale Discovery V-Plex Plus Proinflammatory Panel 2 Rat Kit.

RESULTS

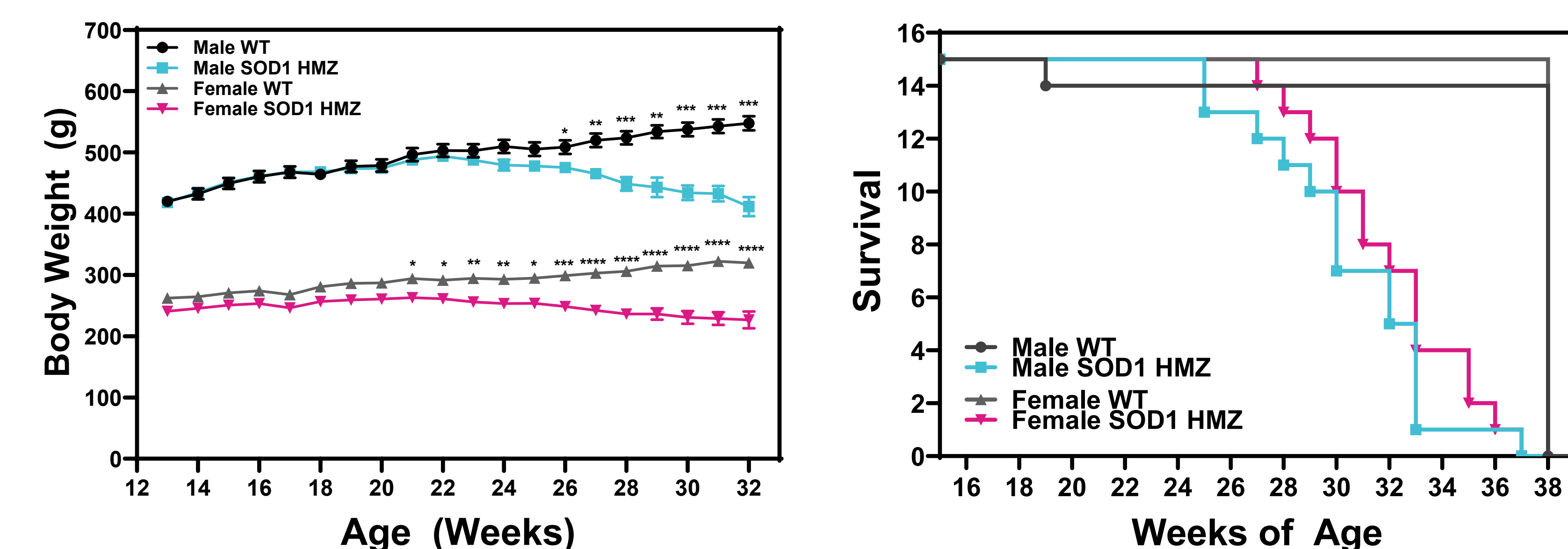### Body Weight and Survival are Decreased in SOD1 HMZ Rats

Figure 1: Average weekly body weight (left) and survival (right) of WT and SOD1 HMZ rats over time, separated by sex. * p<0.05, **p<0.01, *** p<0.001, **** p<0.0001, mixed-effects analysis with Tukey's post-hoc test.

Locomotion and Rearing are Decreased in Male SOD1 HMZ Rats
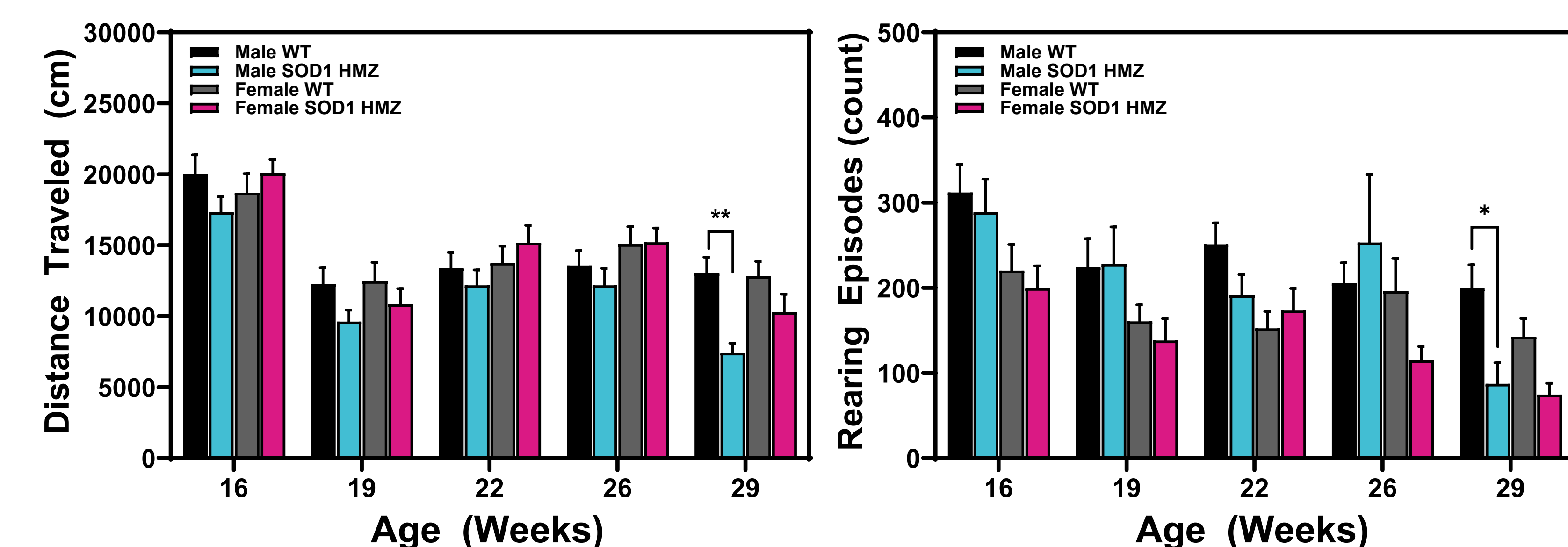

Figure 2: Locomotion and rearing of WT and SOD1 HMZ rats captured by infrared beam crossings over a 60-minute test period. * p<0.05, ** p<0.01, Mixed-effects analysis with Tukey's post-hoc test.

RESULTS

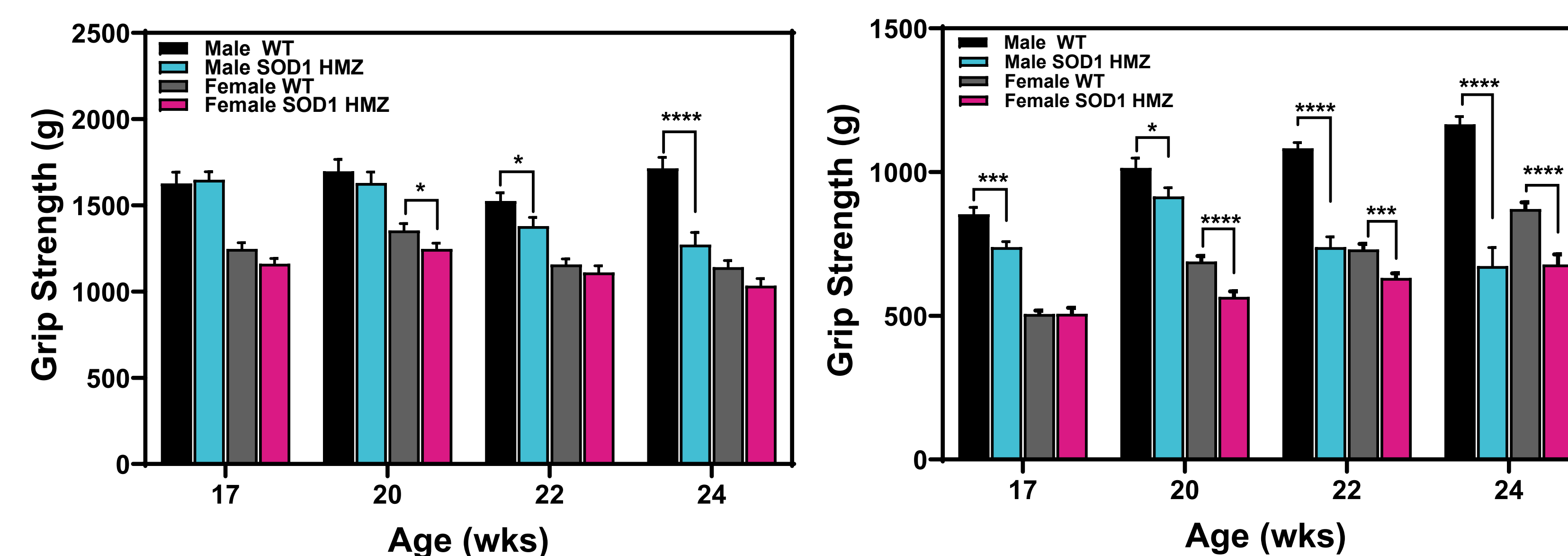### Forelimb and Hindlimb Grip Strength are Decreased in SOD1 HMZ Rats

Figure 3: Front limb (left) and hind limb (right) grip strength of SOD1 WT and HMZ rats measured over time. * p<0.05, *** p<0.001, **** p<0.0001, mixed-effects analysis with Tukey's post-hoc test.

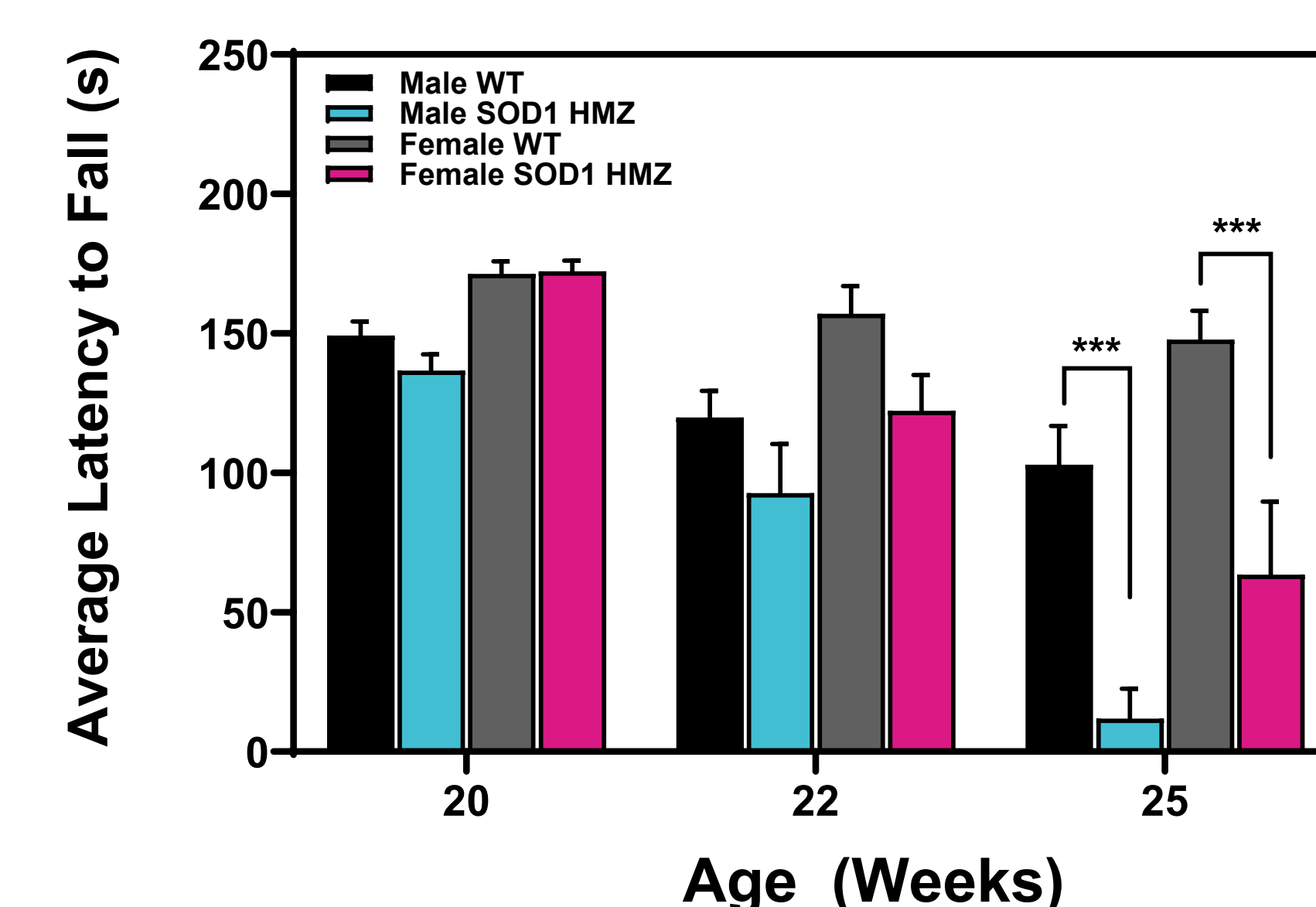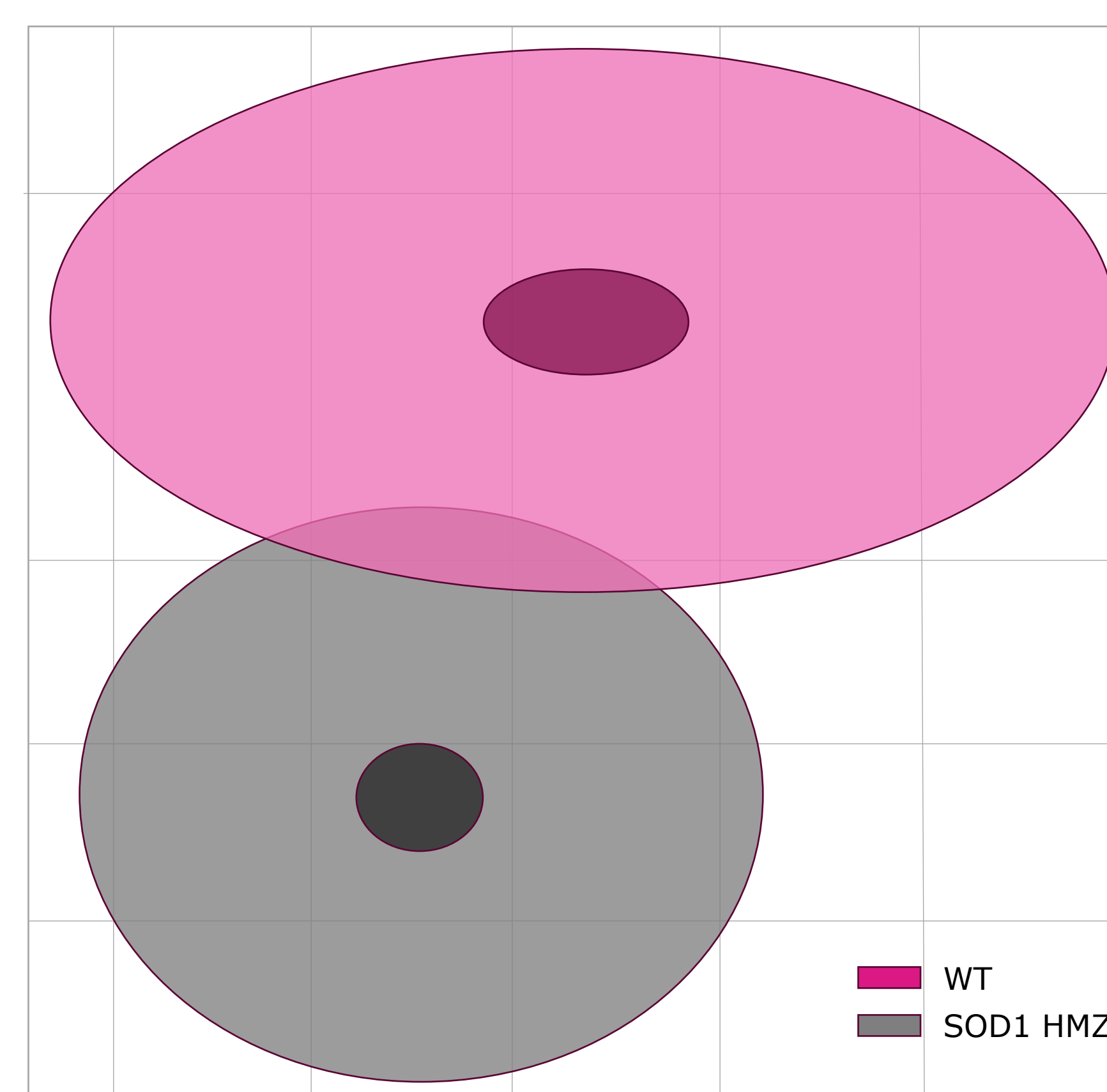### Rotarod Performance is Impaired in SOD1 HMZ Rats

Figure 4: Latency of WT and SOD1 HMZ rats to fall from an accelerating rotating rod, *** p<0.001, mixed-effects analysis with Tukey's post-hoc test.

NeuroCube® Gait Analysis Shows Feature Discrimination of SOD1 HMZ Rats

Age (Weeks)	Total	Gait	Rhythmicity	Paw Position	Body Motion
14	64.3%	60.8%	52.6%	51.1%	52.5%
22	60.8%	62.7%	55.6%	53.3%	53.2%
24	57.5%	58.6%	53.9%	52.1%	56.0%
26	74.8%	75.7%	55.9%	51.8%	57.7%

WT to SOD1 HMZ Discrimination (%)

Figure 5: De-correlated ranked feature analysis cloud plot of gait and speed features at 26 weeks of age (top) and feature discrimination indices prior to symptom development (14 weeks) and over time (22-24 weeks) (bottom) by PsychoGenics' NeuroCube® proprietary gait analysis system.

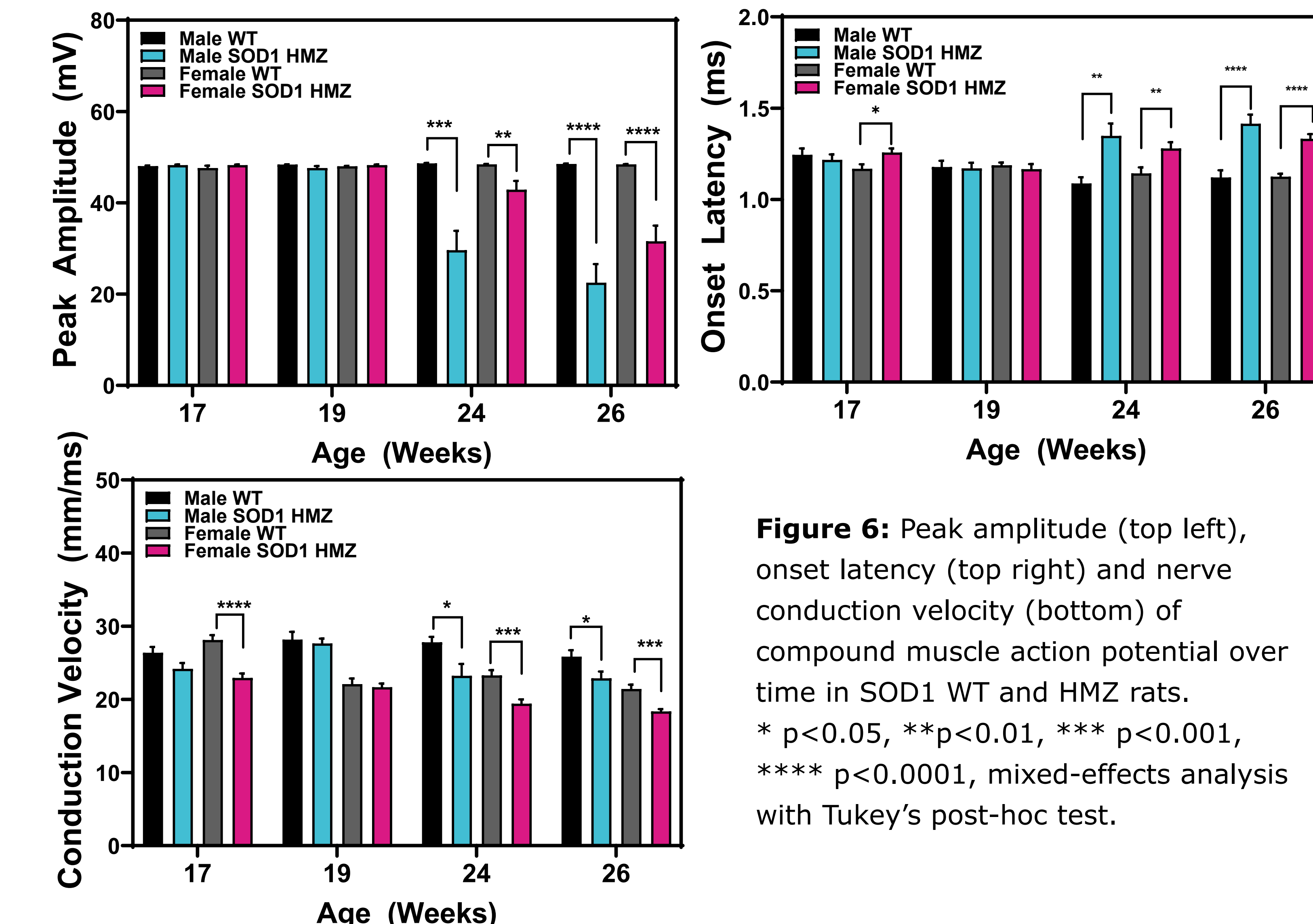### Compound Muscle Action Potential Deficits in SOD1 HMZ Rats

Figure 6: Peak amplitude (top left), onset latency (top right) and nerve conduction velocity (bottom) of compound muscle action potential over time in SOD1 WT and HMZ rats. * p<0.05, **p<0.01, *** p<0.001, **** p<0.0001, mixed-effects analysis with Tukey's post-hoc test.

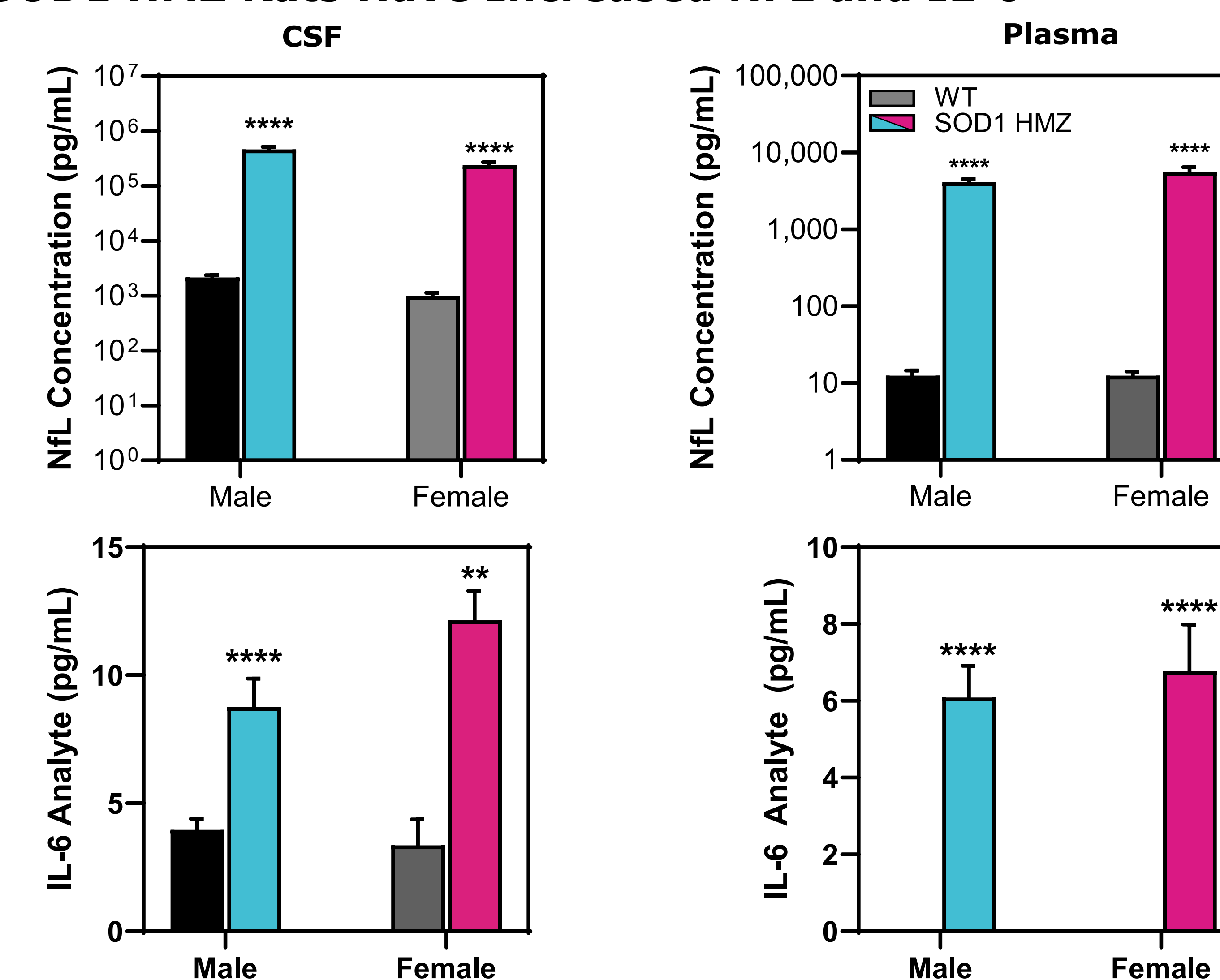### SOD1 HMZ Rats Have Increased NFL and IL-6

Figure 7: NFL and inflammatory marker IL-6 in CSF (left) and plasma (right) of male and female SOD1 hemizygous rats. ** p<0.01, **** p<0.0001, two-tailed unpaired t-test.

SUMMARY

In collaboration with Taconic Biosciences, PsychoGenics characterized a new rodent model of SOD1 G93A-overexpressing rats.

Hemizygous rats showed a progressive loss of body weight and increased mortality starting at 24 weeks of age. Deficits in locomotion during the open field test were seen in males at 29 weeks of age, and reduced grip strength was seen as early as 20 weeks, decreasing with age. Gait analysis by NeuroCube ® also showed reduced gait speed in males as early as 14 weeks, and changes in gait dynamics becoming apparent by 26 weeks.

Analysis of compound muscle action potential found that onset latency increased whereas conduction velocity and peak amplitude decreased in HMZ rats starting at 24 weeks of age in both males and females.

Cerebrospinal fluid of SOD1 HMZ rats showed an increase of NFL and inflammatory markers IL-6 and IL-1b (data not shown), and plasma showed an increase of NFL and IL-6. These results indicate that this model may be effective for the screening of novel therapeutic agents for ALS treatment.