Physiology & Behavior 125 (2014) 17–20

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Relationships between tongue motility, grip force, and survival in SOD1-G93A rats

Susan E. Smittkamp [a], Heather N. Spalding [a], Jordan W. Brown [b], Hung-Wen Yeh [c], John A. Stanford [a,b,*]

[a] Department of Molecular & Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, United States
[b] The Kansas Intellectual and Developmental Disabilities Research Center, University of Kansas Medical Center, Kansas City, KS 66160, United States
[c] The Department of Biostatistics, University of Kansas Medical Center, Kansas City, KS 66160, United States

HIGHLIGHTS

- SOD1-G93A rats exhibit heterogeneity with regard to bulbar motor deficits.
- Forelimb deficits were greater in SOD1-G93A rats with tongue motility deficits.
- Hindlimb deficits were similar across SOD1-G93A rats.
- Disease progression was worse for rats with tongue motility deficits.

ARTICLE INFO

Article history:
Received 20 June 2013
Accepted 19 November 2013

Keywords:
Amyotrophic
Spinal
Bulbar
Preclinical models
Survival
Cluster analysis

ABSTRACT

Most preclinical studies of amyotrophic lateral sclerosis (ALS) have focused on spinal symptoms, despite the importance of bulbar deficits in progression of the disease. We sought to determine how bulbar deficits are related to spinal deficits and survival in the SOD1-G93A rat model of ALS. We examined forelimb and hindlimb grip force and tongue motility in SOD1-G93A rats using statistical cluster analysis. Decrements in forelimb grip force, hindlimb grip force, and tongue motility were used to cluster affected rats into groups. The analysis clustered one group that exhibited primarily forelimb deficits (forelimb group) and a second group that exhibited forelimb and tongue motility deficits (forelimb + bulbar group). The analysis did not identify a distinct hindlimb phenotype group because all SOD1-G93A rats exhibited deficits in hindlimb grip force. Rats in the forelimb + bulbar group exhibited earlier and greater forelimb deficits, and earlier mortality than rats without bulbar deficits. Hindlimb deficits were similar in both groups. There was a significant correlation between forelimb grip force and tongue motility deficits, but not between forelimb and hindlimb deficits. These data extend clinical findings of a more rapid disease progression in individuals with bulbar symptoms to the SOD1-G93A rat model of ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of upper and lower motor neurons that progresses rapidly from muscle weakness and atrophy, to paralysis and eventual death. Disease onset can occur in the arms or legs (spinal onset) or in the muscles of the face (bulbar onset). A bulbar onset, due primarily to its effects on oropharyngeal muscles, is associated with a poorer prognosis [1–3]. In humans, the site of onset is approximately equally-distributed between arms, legs and bulbar muscles [4]. Determining the mechanisms that account for relationships between site of onset and disease progression would be important advance in ALS research [5]. Linking disease heterogeneity in a preclinical model to the full clinical range of motor deficits seen in ALS would be an important translational advance toward achieving this goal.

Approximately 90% of ALS cases occur sporadically; in the remaining cases, the disease is inherited in an autosomal dominant manner (i.e., familial ALS; [6,7]). Transgenic rodent models harboring a variety of human SOD1 mutations have been developed to study the pathogenesis and progression of ALS [8,9]. Like ALS, these models vary in disease onset, progression, and survival. While the SOD1-G93A mouse is the most widely studied model, SOD1-G93A rats are believed to exhibit greater heterogeneity with regards to endstage limb paralysis [10]. Until recently, preclinical studies with SOD1-G93A rodents have focused almost exclusively on spinal deficits. We and others have reported orolingual motor deficits in SOD1-G93A mice [11–13] and rats [14] that are analogous to bulbar deficits in ALS. A previous study reported heterogeneity with regard to spinal deficits that relates to survival in SOD1-G93A rats [10]. It is unknown, however, how bulbar

* Corresponding author at: Department of Molecular & Integrative Physiology, University of Kansas Medical Center, Mail Stop 3051, 3901 Rainbow Blvd., Kansas City, KS 66160, United States. Tel.: +1 913 588 7416; fax: +1 913 588 5677.
E-mail address: jstanford@kumc.edu (J.A. Stanford).

0031-9384/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.physbeh.2013.11.010

deficits fit into this heterogeneity. The purpose of this study was to answer this question by applying statistical cluster and correlation analyses to behavioral data from our recent study in which we examined forelimb and hindlimb grip force, as well as tongue force and motility over the lifespan of SOD1-G93A rats [14]. These analyses were beyond the scope of our previous study.

2. Materials and methods

2.1. Subjects

Animals were bred from Sprague–Dawley (SD) female and hemizygous TgN(SOD1-G93A)L26H (SOD1-G93A) male rats (SD background) obtained from Taconic. Twelve SOD1-G93A (n = 9 males, n = 3 females) and 8 wild-type littermates (n = 3 males, n = 5 females) were used. All work was approved by the KUMC Institutional Animal Care and Use Committee with methods carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. See Ref. [14] for further details.

2.2. Procedure

Tongue motility and force were evaluated 3 times/week [14]. Water restricted rats licked water from an 18 mm diameter aluminum disk rigidly attached to the shaft of a Model 31 load cell (0–250 g range, Sensotec, Columbus, OH). A LabMaster interface (Scientific Solutions; Solon, OH) received the analog signals from the load cells, converted the signals to digital form and routed the signals to a computer. Computer software recorded the force output of the transducer/operandum at 100 samples/s and with a force resolution of 0.2 g equivalent weights. The computer controlled a peristaltic pump that delivered water to the center of the lick disk through a 0.5-mm-diameter hole. The force sensitivity and sampling rate made it possible to resolve the force–time waveforms of each individual lick. Tongue force and motility (licks/s) were evaluated for each rat for each session until animals were euthanized (~250 days of age). Although they exhibited no tongue force deficits, SOD1-G93A rats exhibited early differences in tongue motility (~95 days of age) that progressed to frank deficits at ~185 days of age [14].

Fore- and hindlimb grip force was also evaluated 3 times/week [14]. Using an animal grip strength system (San Diego Instruments), rats were passed over a metal mesh grid connected to a force transducer. Rats gripped the grid with either their fore- or hindlimbs and then were tugged gently away until the grip was released. The peak force in grams was recorded by the transducer. Three fore- and 3 hindlimb trials were performed during each test session. The highest of the 3 values for each was recorded for analysis. SOD1-G93A rats exhibited forelimb and hindlimb grip force deficits between 175 and 185 days of age [14].

2.3. Data analysis

Behavioral data were collected as described above and previously [14] for each rat until overt limb paralysis was observed (typically a single forelimb or hindlimb). Once this occurred, the date was noted and the animal was euthanized. Because SOD1-G93A rats did not exhibit tongue force deficits, tongue motility (licks/s) was used as the measure of bulbar function. Magnitude of tongue motility, forelimb grip force and hindlimb grip force deficits were determined by dividing values during the symptomatic stage (day 230) by presymptomatic values. These proportion ratios were then entered into a K-means cluster analysis using SYSTAT software (Chicago, IL). Two cluster groups were used based on the rule-of-thumb $k \approx \sqrt{n}/2$ [15]. This choice was verified by the fact that two cluster groups accounted for a greater amount of variance (combined F = 48.592) than three (F = 41.135) or four (F = 42.786) groups. After the analysis identified two groups, data for tongue motility, forelimb and hindlimb grip force, and body weight were analyzed using mixed between-groups (cluster group) and within-subjects (time) ANOVA. Only the two SOD1-G93A cluster groups were analyzed statistically. Although data were collected 3 days/week, ANOVAs were conducting using data from ~14 day intervals. A survival analysis was also performed as a function of cluster group using SYSTAT, which provided Kaplan–Meier (KM) estimates for the median survival time as well as the 95% CI for each cluster. We then performed correlational analysis (Pearson's) to determine relationships between changes in dependent variables at endstage (using the day 230 ratios that were used in the cluster analyses).

3. Results

Graphs showing the clustering of SOD1-G93A rats into two phenotypic groups are presented in Fig. 1. Cluster analysis resulted in a group that exhibited primarily forelimb grip force deficits and no bulbar deficits (i.e., the "Forelimb" group; n = 5) and a group that exhibited forelimb grip force deficits and bulbar deficits (i.e., the "Forelimb + Bulbar" group; n = 7). Rats in both groups exhibited hindlimb grip

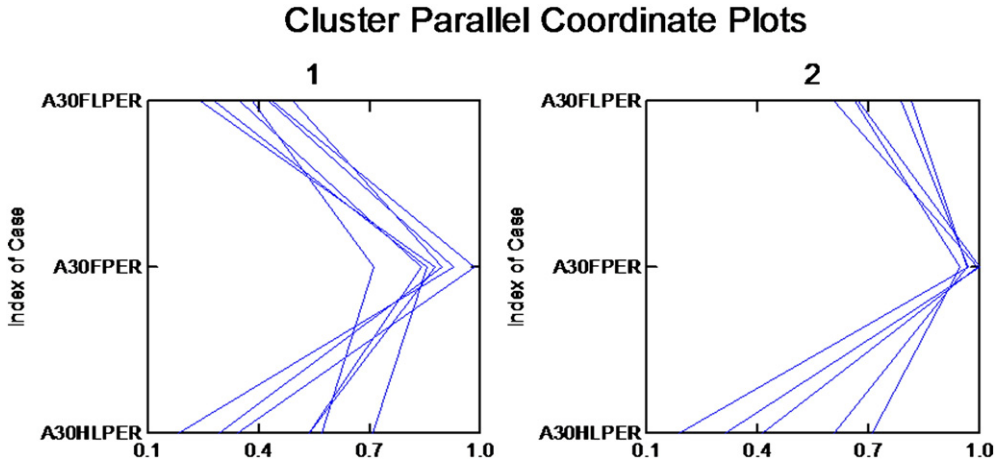

Fig. 1. Parallel coordinate plots of the two groups identified by K-means cluster analysis. Deficit modality (A30FLPER = forelimb grip force, A30FPER = tongue motility, A30HLPER = hindlimb grip force) is on the y-axis and proportion of baseline motor function (deviation to left = greater deficit) is on the x-axis. Cluster group 1 (forelimb + bulbar group) exhibited greater forelimb grip force and tongue motility deficits than Cluster group 2 (forelimb only group). Hindlimb grip force deficits were similar between the two clustered groups.