The bigger they are the harder they fall: size-dependent vulnerability of motor neurons in amyotrophic lateral sclerosis

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Edited by: Ole Paulsen & Gregory Funk

Amyotrophic lateral sclerosis (ALS) is the most common disease of motor neurons (MNs) and involves the death of corticospinal neurons within the motor cortex and MNs within the brainstem and spinal cord. This loss causes an inexorable progression of muscular atrophy and weakness, resulting in death from reparatory complications within ∼2–3 years from diagnosis. The proposed mechanism(s) underlying ALS pathogenesis are myriad, though sadly very little consensus has emerged on the primacy of any one culprit. Despite a lack of clarity, it is well documented in patients and multiple ALS rodent models that cortical hyperexcitability and MN synaptic abnormalities precede MN death and muscle weakness (van Zundert *et al.* 2008). Secondly, there is a difference in the relative vulnerability to degeneration of MNs within specific brainstem and spinal cord pools, with evidence suggesting that smaller MNs are resilient regardless of the particular pool they inhabit (Leroy *et al.* 2014). Together these suggest a neuron-centric pathogenesis in ALS.

The motor unit is the final common pathway in neuromotor control and allows muscle force production to match the requirements for a desired motor behaviour. Motor units can be classified into four types: (i) slow, low force producing, fatigue resistant (type S); (ii) fast, low force producing, fatigue resistant (type FR); (iii) fast, higher force producing, fatigue intermediate (type FInt); and (iv) fast, highest force producing, fatigable (type FF) motor units. Each motor unit type comprises MNs of different sizes and intrinsic excitabilities, type S and FR motor units having the smallest, more excitable MNs, while type FInt and FF motor units have larger, less excitable (due to high capacitance) MNs. Thus, MN size determines the orderly recruitment of motor units and activity of their constituent muscle fibres. Though extensive developmental, ageing and injury studies show that MN size is highly plastic, there is a dearth of studies examining somal plasticity during diseases such as ALS.

In a recent study published in *The Journal of Physiology*, Dukkipati and colleagues (2018) investigated the plasticity of somal sizes in the $SOD1^{G93A}$ mouse model of ALS across four time points: postnatal day (P) 10 (subcellular functional and morphological abnormalities), P30 (earliest MN losses), P90 (frank MN loss and muscle weakness) and P120–140 (hindlimb paralysis and euthanasia). Their aim was to determine if MNs within the spinal cord exhibited consistent somal size alterations across the disease period, and if resilient type S MNs exhibit different patterns of plasticity compared to vulnerable MNs. Depending on the pattern of changes, somal plasticity could be interpreted as either contributing to pathogenesis, or a homeostatic mechanism to ameliorate extrinsic synaptic excitability alterations.

Using a combination of VAChT, Kv2.1 and Nissl immuno-labelling, the authors describe significant somal enlargements of lumbar MNs ($L_{4–6}$) at P10 (∼6% increase) and P30 (∼16%) in the $SOD1^{G93A}$ mutants. At P90, there was no difference in the somal sizes of lumbar MNs in $SOD1^{G93A}$ mutants and controls. By P120–140, there was a significant reduction (∼40%) in MN sizes in $SOD1^{G93A}$ mutants. A subset of MNs at P30 had somal 3-dimensional volumes assessed, with commensurate increases in lumbar MNs of $SOD1^{G93A}$ mutants. Though volumetric analysis is useful, MN capacitance and recruitment are based on somal surface areas (Fogarty *et al.* 2018), and 3-dimensional surface area estimations would be more informative.

Somal sizes were also investigated at the cervical ($C_{2–5}$) and sacral ($S_{1–3}$) regions. At P30, $SOD1^{G93A}$ mutants exhibited a ∼7% reduction in cervical MN size. By contrast, the MN somal sizes within the sacral pool were unchanged in $SOD1^{G93A}$ mutants compared to controls. Thus, there is evidence for regional changes, potentially related to the relative vulnerability of certain MN populations within the spinal cord.

Clinically, the incidence and prevalence of ALS is greater in males than in females, though in the elderly, the incidence is similar. At P10 there was no difference in MN somal sizes between male and female controls. However, male $SOD1^{G93A}$ mutants at this age had somal sizes increased ∼6%. By contrast, in female $SOD1^{G93A}$ mutants MN somal sizes were reduced by ∼9%, compared to controls.

The disambiguation of MN types by size alone remains problematic, and the authors endeavoured to distinguish type S MNs with SK3 immuno-labelling, with all type F MNs identified by a lack of SK3 reactivity. At P10, only the type F MNs were enlarged (∼7%) in $SOD1^{G93A}$ mutants. There were no differences in type S MN somal sizes, and no difference in the relative proportion of SK3 positive or SK3 negative MNs. At P120–140, type S and type F MNs in $SOD1^{G93A}$ mutants were reduced in somal size by ∼40% compared to controls. The relative proportion of SK3 positive to SK3 negative motor neurons was altered, with an almost fourfold increase in SK3 positive MNs in the $SOD1^{G93A}$ mutant, indicative of the selective death of type F MNs. Additional *in silico* computational modelling showed a reduction of MN firing frequencies of the vulnerable MN types when somal size was increased by 12, 16 or 20% compared to control.

In their paper, Dukkipati and colleagues quite rightly point out that vulnerability and resilience of MNs in ALS are related to MN type. However, in contrast to their hypothesis in lumbar MNs, they seem to reconsider their sophisticated approach and presume a somatotopic relationship to vulnerability in cervical and sacral MNs. For instance, the authors cite evidence that respiratory failures occur at the end-stage of disease, thus cervical MNs (within which the phrenic nucleus, innervating the diaphragm, is contained) are resilient compared to lumbar MNs in ALS. In reality, preservation of respiratory behaviours is highly indicative of the resilience of type S and FR motor units, recruited to perform the incessant, highly active ventilatory behaviours (∼30% duty cycle, compared with ∼8% for limb units), necessitating the recruitment of fatigue-resistant motor units (Fogarty *et al.* 2018). It is highly likely that maximal straining/expulsive behaviours (including cough, vomiting and sneezing) are impaired earlier in ALS

DOI: 10.1113/JP276312

progression, and that the type FInt and FF MNs recruited to perform these tasks are vulnerable early, similar to lumbar MNs. Studies of the underlying motor unit deficits in sarcopenia favour this interpretation (Fogarty *et al.* 2018), and are supportive of the overall argument for size-related selective vulnerability in ALS propounded by the authors. Granted, though ALS-resilient MN pools exist (trochlear, Onuf's), this is likely due to the relative proportions of constitutive motor unit types rather than some peculiarity of anatomy. Documenting these phenomena for a variety of motor pools (and sexes!) in the meticulous manner in which the current authors did for the lumbar pool would be extremely revealing.

A stated aim was to differentiate MN somal plasticity between resilient and vulnerable populations. Vulnerable type F MNs had earlier somal MN changes than type S, with SK3 negative MN somal sizes increased from P10. Type S MNs did not exhibit specific changes until P120–140, though type-specific MN somal plasticity was not examined at intermediate time points. In past studies where neonatal MN type was differentiated functionally, only type S MNs from $SOD1^{G93A}$ demonstrated differences to controls (Leroy *et al.* 2014), though somal sizes were unchanged in all MN types. As type FR motor unit functional properties are similar to type S (Fogarty *et al.* 2018), there is utility in separating these MNs from FInt and FF MNs in future studies.

Determining if somal plasticity of MNs during ALS progression is pathogenic or compensatory was a key aim of this study. The consensus from this work and clues from past studies (albeit without credence to MN type) is suggestive of a compensatory mechanism. The authors' simulations show that increased somal size at P10 and P30 is consistent with reduced intrinsic excitability, concordant with functional studies (Delestree *et al.* 2014; Leroy *et al.* 2014). At this time point, there is an altered excitatory/inhibitory balance of synaptic inputs onto $SOD1^{G93A}$ MNs (van Zundert *et al.* 2008). It has been known for decades that an increased excitatory/inhibitory ratio occurs on type FF MNs compared to type S (Delestree *et al.* 2014). This phenomenon is entirely consistent with observed functional and morphological alterations in past studies of MNs in the $SOD1^{G93A}$ model and with the new information provided by the current study (van Zundert *et al.* 2008; Delestree *et al.* 2014; Leroy *et al.* 2014; Dukkipati *et al.* 2018).

In conclusion, Dukkipati and colleagues, along with others in the field (Delestree *et al.* 2014; Leroy *et al.* 2014; Dukkipati *et al.* 2018) are to be commended for the renaissance of motor unit size-dependent investigations in ALS. The nuances of MN resilience and vulnerability in conditions involving MN death are certainly related to their type-dependent properties (Leroy *et al.* 2014; Dukkipati *et al.* 2018; Fogarty *et al.* 2018), particularly their relative plasticity in response to pathophysiological stressors or altered synaptic milieu. If progress is to be made in the understanding of ALS aetiology and in the advent of curative treatments, then considering MNs within the spinal cord as interchangeable relays must be abandoned. To this effect, future work on MN survival in various ALS models must consider size-dependent physiological characteristics.

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Additional information

Competing interests

No conflicts, either real or perceived, exist in regards to this work.

Funding

The National Health and Medical Research Council of Australia has funded the CJ Martin Early Career Fellowship to M.J.F.

Acknowledgements

My thanks to Dr Gary C. Sieck for enthusiastic discussions relating to this manuscript.