● REVIEW

Amyotrophic lateral sclerosis as a synaptopathy

Matthew J. Fogarty[1, 2, *]

1 Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA
2 School of Biomedical Sciences, The University of Queensland, St. Lucia, Australia

Abstract

The synapse is an incredibly specialized structure that allows for the coordinated communication of information from one neuron to another. When assembled into circuits, steady streams of excitatory and inhibitory synaptic activity shape neural outputs. At the organismal level, ensembles of neural networks underlie behavior, emotion and memory. Disorder or dysfunctions of synapses, a synaptopathy, may underlie a host of developmental and degenerative neurological conditions. There is a possibility that amyotrophic lateral sclerosis may be a result of a synaptopathy within the neuromotor system. To this end, particular attention has been trained on the excitatory glutamatergic synapses and their morphological proxy, the dendritic spine. The extensive detailing of these dysfunctions in vulnerable neuronal populations, including corticospinal neurons and motor neurons, has recently been the subject of original research in rodents and humans. If amyotrophic lateral sclerosis is indeed a synaptopathy, it is entirely consistent with other proposed pathogenic mechanisms – including glutamate excitotoxicity, accumulation of misfolded proteins and mitochondrial dysfunction at distal axon terminals (cortico-motor neuron and neuromuscular). Further, although the exact mechanism of disease spread from region to region is unknown, the synaptopathy hypothesis is consistent with emerging die-forward evidence and the prion-like propagation of misfolded protein aggregates to distant neuronal populations. Here in this mini-review, we focus on the timeline of synaptic observations in both cortical and spinal neurons from different rodent models, and provide a conceptual framework for assessing the synaptopathy hypothesis in amyotrophic lateral sclerosis.

Key Words: motor neuron; motor cortex; corticospinal; excitotoxicity; synaptic transmission; dendrites; dendritic spines; neuromuscular junction

Synapses and Amyotrophic Lateral Sclerosis

The synapse is an incredibly specialised structure that allows for the coordinated communication of information from one neuron to another. When assembled into circuits, the steady streams of synaptic excitatory, synaptic inhibitory and modulatory inputs shape neural outputs. At the organismal level, ensembles of various neural networks underlie behaviour, emotion and memory. Synapses consist of discrete pre- and postsynaptic domains that are under tight homeostatic regulation, yet remain responsive to changes in activity. Increasingly, evidence suggests that perturbations of synaptic structure and function, a synaptopathy, may underlie a host of neurological diseases, from developmental conditions such as autism and schizophrenia to neurodegenerative diseases including Alzheimer's and Huntington's diseases. There is a possibility that amyotrophic lateral sclerosis (ALS) may be a result of synaptopathy within the neuromotor system. To this end, a particular attention has been trained on the excitatory glutamatergic synapses and their morphological proxy, the dendritic spine. Within the last two years, extensive detailing of these dysfunctions in vulnerable neuronal populations has been a subject of original research in rodents (Fogarty et al., 2016a, b, 2017; Handley et al., 2017; Jiang et al., 2017), and humans (Genc et al., 2017) and the focus of a recent exhaustive review (Fogarty, 2018). Here in this review, we focus on the timeline of these observations in both cortical and spinal neurons from different rodent models, and provide a conceptual framework for assessing the synaptopathy hypothesis in ALS. Literature searches were performed on public databases (Pubmed) using the term 'synapses in ALS', with emphasis on presymptomatic studies.

In ALS, a clinically heterogeneous condition afflicting the neuromotor system; corticospinal motor neurons (CSMNs), motor neurons (MNs), neuromuscular junctions (NMJs), and the corticospinal tract degenerate inexorably (Vucic et al., 2014). The loss of CSMNs, MNs and NMJs leads to progressive muscle weakness followed by death, usually from respiratory muscle insufficiency (Vucic et al., 2014). In ALS, not all MNs are vulnerable, with slow and fast fatigue-resistant (type S and FR) MNs resilient and fast intermediate and fast fatigueable (FInt and FF) MNs more susceptible (Fogarty, 2018). Furthermore, MN populations that do not have extensive corticospinal inputs, such as the MNs within the trochlear and Onuf's nuclei are resilient to demise, underscoring the importance of the motor cortex to the disease. Contemporaneous pathology of CSMNs and MNs is pathognomic for ALS, with cases being divided into known genetic forms (~10%, based on family trees) and sporadic forms (~90%, apparently random incidence). Gross cortical post mortem pathology and subcellular dendritic and synaptic degenerations of CSMNs are highly conserved across sporadic and familial variants of ALS (Genc et al., 2017). Frustratingly, clinical presentation and progression is remarkably varied, with upper and lower limb weakness (~75%), bulbar signs (speech and swallowing difficulties ~25%) and a proportion of patients with frontotemporal dementia (20–50%) complicating diagnosis and prognosis (Vucic et al., 2014). This heterogeneity of disease has necessitated the development of

animal models based on the genetic forms of ALS in order to probe specific pathophysiological pathways. The suspected culprits in the aetiology of ALS include glutamate excitotoxicity, reactive oxygen species generation, protein misfolding and aggregation, mitochondrial dysfunction, endoplasmic reticulum stress, abnormal axon transport, neuroinflammation and metabolic stress (Vucic et al., 2014; Fogarty, 2018). The spread of degeneration in ALS is also unknown, with multiple myogenic and neurogenic factors flaming the controversy surrounding the 'die-forward' and 'die-backward' hypotheses (Vucic et al., 2014; Fogarty, 2018). Recently, Braak et al. (2013) classified ALS as a disease of neurons with large axons with discrete stages of trans-synaptic spread, starting with CSMNs, followed by MNs and progressing to extramotor areas (Braak et al., 2013; Brettschneider et al., 2013). This pathological insight is consistent with cortical hyperexcitability being an early clinical feature of ALS (Vucic et al., 2014). This concept also provides for a mechanism of transferring misfolded protein aggregates to distant populations of neurons, prion-like transmission (Braak et al., 2013), and is consistent with the idea of ALS being a synaptopathy spreading from the motor cortex. Indeed, in rats with superoxide dismutase (SOD)1 mutations corrected only in cortical neurons, ALS symptom onset was delayed and survival extended (Thomsen et al., 2014). These and the aforementioned early motor cortex synaptic abnormalities reinforce the concept of trans-synaptic spread and die-forward aetiology. If ALS is a synaptopathy, it has the advantages of being entirely compatible with glutamate excitotoxicity, the accumulation of misfolded proteins and mitochondrial dysfunction at distal axons (CSMN to MN and MN to NMJ). It does not however, explain the contribution of neuroinflammation to the disease process, explain the initial protein misfolding, account for non-cell-autonomous influences or some of the muscle-specific modifiers of disease progression.

Almost invariably, the earliest detected abnormalities of various rodent models involve synaptic dysfunctions (van Zundert et al., 2008; Fogarty et al., 2015, 2016a, b, 2017; Fogarty, 2018). These dysfunctions uniformly favour increased excitation compared to inhibition ratios in CSMNs and MNs, a phenomenon entirely consistent with observations of reduced inhibition and cortical hyperexcitability in clinical cohorts (Fogarty, 2018). The mechanisms underlying disordered synaptic structure and function of CSMNs and MNs centre on excitotoxicity and Ca^{2+} overloading paradigms, though some suggest that these phenomena merely prime vulnerable neurons towards susceptibility to future stressors (Fogarty, 2018).

Early Synaptic Dysfunction of CSMNs within the Motor Cortex

The morphology and function of synapses are abnormal in ALS, with the nature of changes dependent on where along the disease timeline observations are made. These changes include the altered density and morphology of dendritic spines, often sites of excitatory neurotransmission within the central nervous system, particularly in pyramidal cells such

as CSMNs (Genc et al., 2017; Fogarty, 2018). Post mortem analysis of human Betz cells (putative CSMNs) in ALS sufferers clearly shows dendritic spine degeneration (Genc et al., 2017; Fogarty, 2018), a phenotype mirrored in a variety of rodent models at disease endpoints (Jara et al., 2012; Fogarty et al., 2017). Of more utility in understanding pathogenesis is the assessment of synaptic activity and synapse morphology (dendritic spine density and shape) at earlier disease stages. The increased frequency of spontaneous excitatory synaptic inputs and/or a reduction in the frequency of inhibitory synaptic inputs onto putative CSMNs from the layer V motor cortex has been observed in wobbler, SOD1 and transactive response DNA-binding protein 43 (TDP-43) models of ALS (Nieto-Gonzalez et al., 2011; Fogarty et al., 2015, 2016). Importantly, these observations were made during or immediately after postnatal development, but before motor symptom onset and CSMN loss (Jara et al., 2012; Fogarty, 2018). In SOD1^{G93A} rodents, these electrophysiological alterations occur in concert with dendritic spine loss and precede the degenerative retraction of the apical dendritic arbor (Fogarty et al., 2015).

Decreased dendritic spine densities are observed in CSMNs (Jara et al., 2012; Fogarty et al., 2015, 2016a, b; Genc et al., 2017; Handley et al., 2017) and a variety of other cortical neurons (Fogarty et al., 2016a; Handley et al., 2017), and are a hallmark of synaptic dysfunction, and excitotoxicity. In the SOD1^{G93A} high-expressor line, with a remarkably more aggressive development of symptoms and neuronal (CSMN and MN) loss compared to other models, there appears to be no period of increased spine density (Fogarty et al., 2015, 2016a). By contrast, in the TDP-43^{Q331K} model, the course of disease is slower, and at the time points assessed, increased excitatory synaptic neurotransmission coincides with increased spine density (Fogarty et al., 2016b). In MNs of SOD1^{G93A} mice, increased functional synaptic excitation and increased dendritic spine density is followed by a period of spine loss (van Zundert et al., 2008; Fogarty et al., 2017; Fogarty, 2018). Notably, MNs death in this strain occurs before observable CSMN loss. The timeline of the structural and functional synaptic abnormalities of CSMNs and MNs in relation to neuronal death in SOD1^{G93A} mice is summarised in **Figure 1**.

Early Synaptic Dysfunction of MNs within the Brainstem and Spinal Cord

The earliest synaptic changes in MNs of SOD1^{G93A} mice have been observed in hypoglossal MNs, with increased glutamatergic synaptic transmission during the first week of age (van Zundert et al., 2008). In both hypoglossal and spinal MNs, dendritic spine density increases are evident between from ~8–15 days postnatally (Fogarty et al., 2017). In adult spinal cord, the functional synaptic abnormalities seem to persist (Jiang et al., 2017), yet the lumbar MN dendritic spine density is similar between control and SOD1 mice (Fogarty et al., 2017) at similar ages (**Figure 1**). In lumbar MNs, by the time substantial MN loss has occurred, there are less dendritic spines and those that remain display a degenerative pheno-
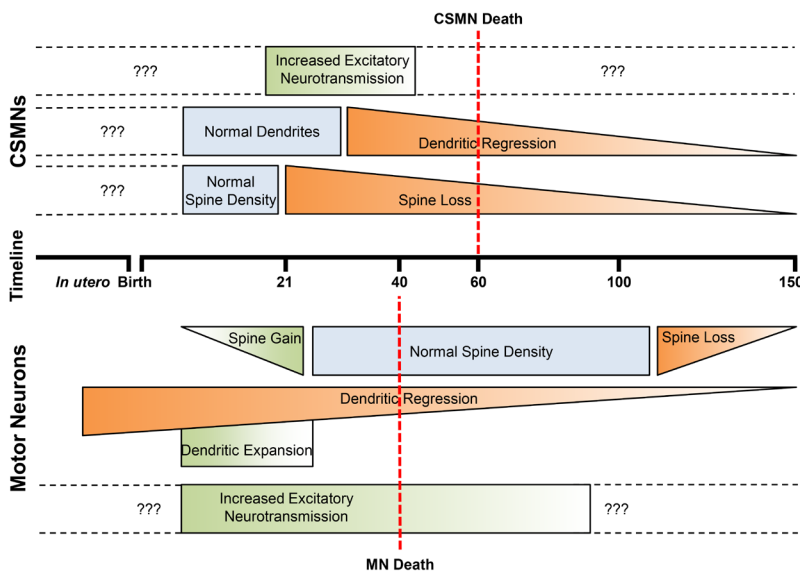
Figure 1 Timeline of synaptic dysfunction and neuronal death in the SOD1^{G93A} ALS model.
The top portion depicts the earliest CSMN changes, starting from ~21 days postnatally, with increased excitatory synaptic neurotransmission concomitant with spine loss. Following this, dendritic regression commences ~28 days postnatally. Importantly, these abnormalities precede CSMN loss at ~60 days postnatally. Currently, very little is known about embryologically abnormalities in the CSMNs of these mice. The bottom portion depicts the earliest changes in MNs starting from ~17 days *in utero*, with dendritic restriction. From ~4 days postnatally, synaptic dysfunctions, such as increased dendritic spine densities and increased excitatory neurotransmission increased excitatory synaptic neurotransmission are evident. These changes precede spinal MN loss at ~40 days postnatally. In some MN populations, such as hypoglossal MNs, spine density and dendritic arbors are increased throughout the duration of disease. Current knowledge of synaptic activity and dendritic spines on MNs during embryological development in SOD1G93A mice is minimal. SOD: Superoxide dismutase; ALS: amyotrophic lateral sclerosis; CSMN: corticospinal motor neuron; MN: motor neuron.

type (Fogarty et al., 2017). By contrast, the spine density of hypoglossal MNs remains elevated across the entire lifespan of the SOD1^{G93A} mice (Fogarty et al., 2017). Sadly, in these morphological assessments, no stratification of MNs into the vulnerable FInt an FF type and resilient S and FR types (Fogarty, 2018) was made.

Changes in NMJs at the presynaptic axon terminal and postsynaptic receptor domain are also perturbed in ALS, with NMJ dysfunction preceding gross motor deficits in SOD1 (Rocha et al., 2013; Arbour et al., 2015) and TDP-43 models (Chand et al., 2018). These synaptic alterations include neurotransmitter release (quantal content and frequency) abnormalities, decreased facilitation of neuromuscular transmission, impaired maintenance by Schwann cells and altered relationship of pre- and postsynaptic anchoring structures. The effects are not limited to synaptic transmission and include impaired axonal transport (Vilmont et al., 2016) and impaired trophic signalling (Williams et al., 2009; Taetzsch et al., 2017). The lack of axonal transport and muscle trophic interaction accelerates progression of ALS (Williams et al., 2009; Taetzsch et al., 2017). Additionally, these changes appear earlier and more strikingly in NMJs of the vulnerable type FInt and FF motor units (Frey et al., 2000). Although important, anatomical changes at the NMJ usually occur subsequent to MN loss (Steyn et al., 2013) and NMJ synaptopathy does not appear as early as CSMN or MN phenotypes.

Compensation for various maladaptations may be more likely to occur in the resilient populations. An important advancement on previous work would be to assess these synaptic changes with respect to motor unit types. The ratio of excitatory to inhibitory synapses is greater in type FInt and FF MNs compared to S and FR (Fogarty, 2018), and may provide the link between underlying excitotoxicity and differential vulnerability.

Future Perspectives

The main driver of both CSMN and MN pathology at pre-symptomatic stages is SOD1^{G93A} mice seems to be excessive glutamatergic synaptic transmission (van Zundert et al., 2008; Fogarty et al., 2015; Jiang et al., 2017; Fogarty, 2018), with other models showing similar results (Nieto-Gonzalez et al., 2011; Fogarty et al., 2016b; Fogarty, 2018). The functional abnormalities of CSMNs and MNs are mirrored by changes in the dendritic spine densities of these neurons in multiple ALS models (Jara et al., 2012; Fogarty et al., 2015, 2016a, b, 2017; Handley et al., 2017; Fogarty, 2018). Taken together, these functional and structural pathologies fit the criteria of a synaptopathy.

Of course, many of the observations presented here are phenomenological and are complicated by an extended timeline of degeneration – even in the aggressive ALS models. A major limitation of many of the electrophysiological studies presented here (van Zundert et al., 2008; Nieto-Gonzalez et al., 2011; Fogarty et al., 2016b; Jiang et al., 2017) and elsewhere (Fogarty, 2018), is the lack of multiple time-points being assessed. With one notable exception using two ages, albeit both pre-symptomatic (Fogarty et al., 2015), single window observations have been the norm, precluding determination of cause and effect. The idea that some of the early changes observed in ALS models are compensatory, as opposed to pathogenic has recently gained traction (Leroy and Zytnicki, 2015; Fogarty, 2018). Accordingly, a major limitation of the synaptopathy hypothesis is the fact that the neurons assessed, or for post mortem studies, those that remain, may exhibit helpful compensatory abnormalities. In this interpretation, synaptic changes are not pathological, with the neurons unable to compensate being vulnerable to loss (Leroy and Zytnicki, 2015). One way to test these competing scenarios would be to undertake a classical gain or loss of function experiment, at the time when the synaptic pertur-

bation is being established, *i.e.*, from the first week of birth until ~P30. This could be achieved pharmacologically by using riluzole, effectively reducing excitatory neurotransmission (Bellingham, 2011) or kainite, a known neuroexcitant (Choi et al., 1987). Dosing of riluzole or kainate at therapeutic (Bellingham, 2011) or toxic doses (Blizzard et al., 2016) respectively, for the first month postnatal would be one strategy to untangle causality and correlation. One would suspect that increased synaptic excitation (kainite treatment) during the critical period would worsen deleterious effects and lead to earlier onset, shorter disease durations and greater loss of vulnerable CSMNs and MNs. Accordingly, if the excitatory synaptic imbalance was ameliorated during the critical period by riluzole, longer delays until symptom onset, longer disease duration and preservation of vulnerable CSMNs and MNs would be expected.

Clearly, there is much still to discover about the pathogenesis and aetiology of ALS. With a focus on the neuronal types that are pathognomic for ALS, namely CSMNs and MNs, there is a rational and compelling case for the disease being a synaptopathy. A clear passage from observable synaptic phenomena to underlying mechanism remains tantalisingly within reach.

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