Riluzole exerts central and peripheral modulating effects in amyotrophic lateral sclerosis

Steve Vucic,1,2 Cindy Shin-Yi Lin,2,3 Benjamin C. Cheah,2 Jenna Murray,2 Parvathi Menon,1 Arun V. Krishnan2,3 and Matthew C. Kiernan2,4

1 Sydney Medical School Westmead, University of Sydney, Australia
2 Neuroscience Research Australia
3 School of Medical Sciences, Translational Neuroscience Facility, Faculty of Medicine, University of New South Wales, Australia
4 Prince of Wales Clinical School, University of New South Wales Sydney, Australia

Correspondence to: Professor Matthew C Kiernan,
Neuroscience Research Australia,
Barker Street,
Randwick,
Sydney,
NSW 2031,
Australia
E-mail: M.kiernan@unsw.edu.au

Riluzole, a benzothiazole derivative, has been shown to be effective in prolonging survival in amyotrophic lateral sclerosis. The mechanisms by which riluzole exerts neuroprotective effects in amyotrophic lateral sclerosis remains to be fully elucidated, although inhibition of glutamatergic transmission and modulation of Na+ channel function have been proposed. In an attempt to determine the mechanisms by which riluzole exerts neuroprotective effects, in particular to dissect the relative contributions of inhibition of glutamatergic transmission and Na+ channel modulation, the present study utilized a combination of cortical and peripheral axonal excitability approaches to monitor changes in excitability and function in patients with amyotrophic lateral sclerosis. Cortical assessment was undertaken by utilising the threshold tracking transcranial magnetic stimulation (TMS) technique and combined with peripheral axonal excitability studies in 25 patients with amyotrophic lateral sclerosis. Studies were performed at baseline and repeated when patients were receiving riluzole 100 mg/day. At the time of second testing all patients were tolerating the medication well. Motor evoked potential and compound muscle action potential responses were recorded over the abductor pollicis brevis muscle. At baseline, features of cortical hyperexcitability were evident in patients with amyotrophic lateral sclerosis, indicated by marked reduction in short interval intracortical inhibition (P < 0.001) and cortical silent period duration (P < 0.001), as well as an increase in the motor evoked potential amplitude (P = 0.01). Riluzole therapy partially normalized cortical excitability by significantly increasing short interval intracortical inhibition (short interval intracortical inhibitionbaseline 0.5 ± 1.8%; short interval intracortical inhibitionON riluzole 7.9 ± 1.7%, P < 0.01). In contrast, riluzole did not exert any modulating effect on cortical silent period duration (P = 0.45) or motor evoked potential amplitude (P = 0.31). In terms of peripheral nerve function, axonal excitability studies established that, relative to control subjects, patients with amyotrophic lateral sclerosis had significant increases in depolarizing threshold electrotonus [amyotrophic lateral sclerosisbaseline TEd (90–100 ms) 49.1 ± 1.8%; controlsTEd (90–100 ms) 45.2 ± 0.6%, P < 0.01] and superexcitability (amyotrophic lateral sclerosisbaseline 30.1 ± 2.3%; control subjects 23.4 ± 1.0%, P < 0.01) at baseline. Following institution of riluzole therapy there was a significant reduction in superexcitability (amyotrophic lateral sclerosisbaseline 30.1 ± 2.3%; amyotrophic lateral sclerosisON riluzole 27.3 ± 2.3%, P < 0.05) and refractoriness at 2 ms (amyotrophic lateral sclerosisbaseline 98.7 ± 10.7%; amyotrophic lateral sclerosisON riluzole 67.8 ± 9.3%, P < 0.001). In conclusion, the present study has established that riluzole exerts effects on both central and peripheral nerve function, interpreted as partial normalization of cortical hyperexcitability and reduction of
transient Na⁺ conductances. Taken together, these findings suggest that the neuroprotective effects of riluzole in amyotrophic lateral sclerosis are complex, with evidence of independent effects across both compartments of the nervous system.

Keywords: motor neuron disease; axon; excitotoxicity

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; SICI = short interval intracortical inhibition

Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and universally fatal neurodegenerative disorder of motor neurons in the spinal cord, brainstem and motor cortex (Kiernan et al., 2011; Turner et al., 2013). Although there is no cure for ALS, riluzole, a benzothiazole derivative, exerts neuroprotective effects and prolongs survival (Lacomblez et al., 1996; Bensimon et al., 2002). The mechanisms by which riluzole exerts these neuroprotective effects in ALS remains to be fully elucidated, although inhibition of glutamatergic transmission, either at a presynaptic level or postsynaptically through modulation of the ionotropic glutamate receptors, appears to be a key mechanism (Cheah et al., 2010).

Glutamate-mediated excitotoxicity has been postulated to be central to ALS pathogenesis, with motor neuron degeneration mediated by corticomotoneuronal hyperexcitability through transynaptic anterograde processes (Eisen et al., 1992). In support, transcranial magnetic stimulation studies have identified cortical hyperexcitability as an early feature in sporadic ALS, and that cortical hyperexcitability precedes the clinical onset of ALS (Caramia et al., 1991; Eisen et al., 1993; Prout and Eisen., 1994; Desiato et al., 2002; Mills, 2003; Vucic and Kiernan, 2006a, 2009, 2010; Vucic et al., 2008). Given that riluzole inhibits glutamatergic transmission, it may therefore be expected that riluzole could exert modulating effects on the transcranial magnetic stimulation parameters of cortical excitability. Such a finding would then provide an objective measure of drug efficacy in ALS.

The modulating effects of riluzole on cortical function in ALS, however, have been varied. Specifically, while some studies have reported that riluzole partially normalizes cortical excitability (Desiato et al., 1999; Stefan et al., 2001), others have failed to establish any cortical modulating effects (Sommer et al., 1999; Caramia et al., 2000). For instance, one may anticipate that any neuroprotective effects of riluzole would be minimal towards the later stages of the disease course. These discordant findings may be related to the study design, in particular the variability in the duration of riluzole therapy, and the timing of studies relative to disease course. In terms of discordant findings, previous transcranial magnetic stimulation studies utilized the constant stimulus transcranial magnetic stimulation technique for assessing cortical function, which may be limited by marked variability of the motor evoked potential amplitudes (Kiers et al., 1993). As such, a threshold tracking transcranial magnetic stimulation technique was recently developed to overcome this potential limitation and has been validated in healthy controls and applied across a wide array of neurological disorders including ALS (Vucic and Kiernan, 2006b; 2008, 2010, 2011).

In addition to direct anti-glutamatergic activity, riluzole appears to exert modulating effects on Na⁺ channel function (Cheah et al., 2010). Specifically, riluzole antagonizes persistent Na⁺ currents in the SOD1 G93A mouse model and stabilizes transient Na⁺ channels in their inactivated state (Benoit and Escande, 1991; Urbani and Belluzzi, 2000; Wang et al., 2008). Given that increased Na⁺ channel conductances may contribute to neurodegeneration through Ca²⁺-mediated processes (Stys, 2005, 2007), the Na⁺ channel blockade by riluzole may provide an additional neuroprotective mechanism in ALS. Of further relevance, riluzole may in part exert its anti-glutamatergic effects by inhibiting Na⁺ channel conductances and reducing glutamate release from presynaptic nerve terminals (Jimonet et al., 1994; Cheah et al., 2010).

The effects of riluzole on Na⁺ channel function in patients with ALS may be assessed by axonal excitability techniques, which provide unique information on membrane potential as well as nodal and internodal axonal ion channel conductances (Bostock et al., 1998; Kiernan et al., 2000; Burke et al., 2001; Krishnan et al., 2009). Specifically, axonal refractoriness reflects the function of nodal transient Na⁺ channels, while the strength–duration time constant (τSD) is a biomarker of nodal persistent Na⁺ conductances (Kiernan et al., 2000). As such, in an attempt to determine the mechanisms by which riluzole exerts neuroprotective effects in ALS and to dissect the relative contribution of Na⁺ channel modulation and direct glutamate inhibition, the present study combined central and peripheral nerve excitability techniques in a cohort of patients with sporadic ALS.

Materials and methods

Studies were undertaken on 25 patients with clinically possible or probable ALS (15 male, 10 female; mean age: 57.4 years, range 37–70) as defined by the revised El Escorial criteria (Brooks et al., 2000). The diagnosis of ALS was subsequently confirmed on longitudinal follow-up of patients. Patients underwent cortical (central) and axonal (peripheral) excitability studies, with the first study undertaken before commencement of riluzole therapy and a second study while receiving riluzole (100 mg/day). The studies were performed at a similar time of the day and in an identical clinical environment. None of the patients with ALS were receiving medications, such as baclofen, benzodiazepines or Na⁺ channel blocking agents, which could potentially interfere with the neuropathological results. Patients with ALS were clinically staged using the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) (Cedarbaum et al., 1999), and hand strength using the Medical Research Council (MRC) rating scale (O’Brien, 2004). Carpal tunnel was excluded in the ALS cohort. Informed consent to the procedures was provided by all patients,
Cortical excitability

Cortical excitability was undertaken by applying a 90 mm circular coil to the motor cortex with currents generated by two high-power magnetic stimulators connected through a BiStim (Magstim Co.). Paired-pulse threshold tracking transcranial magnetic stimulation was undertaken according to a previously reported technique (Vucic et al., 2006). Briefly, the motor evoked potential amplitude was fixed and changes in the test stimulus intensity required to generate a target response of 0.2 mV (±20%), when preceded by subthreshold conditioning stimuli, were measured (Vucic et al., 2006). The motor evoked potential response was recorded over the right abductor pollicis brevis muscle. Resting motor threshold was defined as the stimulus intensity required to maintain this target motor evoked potential response.

Maximum motor evoked potential amplitude (mV), motor evoked potential onset latency (ms) and cortical silent period duration were initially recorded with the stimulus intensity set to 140% of resting motor threshold. Three stimuli were delivered at this level of stimulus intensity. Central motor conduction time (in ms) was calculated according to the F-wave method (Mills and Murray., 1986). The cortical silent period duration was measured from the beginning of motor evoked potential to return of EMG activity (Cantello et al., 1992).

Short interval intracortical inhibition (SICI) and intracortical facilitation were measured according to a previously devised paired-pulse threshold tracking protocol (Vucic et al., 2006). SICI was determined by using subthreshold conditioning stimuli (70% resting motor threshold) at increasing interstimulus intervals as follows: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 7 ms. Intracortical facilitation was measured over the following interstimulus intervals: 10, 15, 20, 25 and 30 ms. Stimuli were delivered sequentially as a series of three channels: channel 1 tracked the stimulus intensity required to produce the unconditioned test response (i.e. resting motor threshold); channel 2 monitored the response to the subthreshold conditioning stimulus; and channel 3 tracked the stimulus required to produce the target motor evoked potential when conditioned by a stimulus equal in intensity to that on channel 2. Stimuli were delivered every 5–10 s and the computer advanced to the next interstimulus interval only when tracking was stable.

SICI was measured as the increase in the test stimulus intensity required to evoke the target motor evoked potential. Inhibition was calculated off-line as follows (Vucic et al., 2006):

\[
\text{Inhibition} = \frac{\text{Conditioned test stimulus intensity} - \text{resting motor threshold}}{\text{resting motor threshold}} \times 100
\]

Facilitation was measured as the decrease in the conditioned test stimulus intensity required to evoke a target motor evoked potential.

Peripheral studies

In the same sitting, axonal excitability studies were undertaken on the median motor nerve according to a previously described protocol utilizing multiple measures of axonal excitability (Kiernan et al., 2000). The median nerve was stimulated at the wrist using 5 mm non-polarizable Ag–AgCl electrodes (ConMed) with the cathode positioned over the skin crease and anode ~10 cm proximally over the lateral forearm. Stimulation was computer controlled and converted to current using an isolated linear bipolar constant current simulator (maximal output ±50 mA; DSS5, Digitimer). The compound muscle action potential responses were recorded from the abductor pollicis brevis with the active (G1) electrode positioned over the motor point and the reference (G2) electrode placed at the proximal phalanx 4 cm away. Test current pulses were applied at 0.5 s intervals and combined with either subthreshold polarizing currents or suprathreshold conditioning stimuli according to previously described protocol (Kiernan et al., 2000). The compound muscle action potential amplitude was measured from peak-to-peak, with the target set to 40% of maximum for all tracking studies. Proportional tracking was utilized to determine the changes in threshold current required to produced and maintain a target response (Bostock et al., 1998).

The following axonal excitability parameters were measured: (i) strength–duration time constant (r SD) and rheobase, determined according to Weiss’ formula (Weiss, 1901; Bostock et al., 1998); (ii) threshold electrotonus (TE) recorded with sub-threshold depolarizing currents at 10–20 ms [TEd (10–20 ms)], 40–60 ms [TEd (40–60 ms)], and 90–100 ms [TEd (90–100 ms)], and with hyperpolarizing currents at 10–20 ms [THE (10–20 ms)] and at 90–100 ms [THE (90–100 ms)]; (iii) hyperpolarizing current-threshold relationship (I/IV) calculated from polarizing current between −50 and −100%; and (iv) recovery cycle parameters including the relative refractory period (in ms), super-excitability (%) and late subexcitability (%). In addition to the parameters of axonal excitability, the compound muscle action potential onset latency and 20 F-wave responses were also recorded from which the neurophysiological index was derived according to a previously reported formula (de Carvalho and Swash, 2000).

Recordings of motor evoked and compound muscle action potential responses were amplified and filtered (3 Hz to 3 kHz) using a Nicolet-Biomedical EA-2 amplifier (Cardinal Health Viking Select version 11.1.0, Viays Healthcare Neurocare Group) and sampled at 10 kHz using a 16-bit data acquisition card (National Instruments PCI-MIO-16E-4). Responses were further filtered for electronic noise by using a Hum Bug (Hum Bug 50/60 Hz Noise Eliminator, Quest Scientific Instruments). Data acquisition and stimulation delivery were controlled by QTRACS software (TROND-F, version 16/02/2009, © Professor Hugh Bostock, Institute of Neurology, Queen Square, London, UK). Temperature was monitored with a purpose built thermometer at the stimulation site.

Statistical analysis

Cortical excitability in patients with ALS was compared to control data obtained from 30 age-matched control subjects (14 males; 16 females, mean 58 ± 1.7 years). Axonal excitability studies were compared with 35 age-matched controls (18 males; 17 females, mean age 57.5 ± 2.2 years). Baseline cortical and axonal excitability studies in the patients with ALS were compared with studies after commencing riluzole. Paired and unpaired Student’s t-test were used to determine differences between the means. Repeated measure ANOVA was used for assessing differences in SICI between groups, as this transcranial magnetic stimulation parameter was assessed over multiple time points. A P-value of < 0.05 was considered statistically significant. Results are expressed as mean ± standard error of the mean (SEM).

Results

Clinical features

At the time of assessment the mean disease duration in patients with ALS from symptom onset was 12.8 ± 1.9 months, confirming that the studies were undertaken towards the earlier stages of the disease.
disease course when the neuroprotective effects of riluzole was probably greatest (Table 1). The mean ALSFRS-R score was 41.2 ± 1.0 and the median Medical Research Council score from the target abductor pollicis brevis muscle was 5.0, confirming a good level of function for the tested muscle. Bulbar-onset disease was evident in 44% of patients, while limb-onset disease accounted for 56% of patients with ALS. The mean time interval between the two studies (before and while receiving treatment) was 46.6 ± 6.1 days (median 30 days). There was no significant change in the ALSFRS score between the two studies (ALSFRS-R baseline 41.1; ALSFRS-R ON riluzole 40.3 ± 1.1, P = 0.28). Riluzole was well tolerated by all patients with ALS from the present cohort, and none of the patients ceased the medication during the study period.

Cortical excitability

Before undertaking cortical excitability studies, peripheral disease burden was assessed at baseline and after commencement of riluzole. At baseline, compared to controls, there was a significant reduction of the compound muscle action potential amplitude (ALS 5.2 ± 0.5 mV; control subjects 9.6 ± 0.7 mV, P < 0.0001) and neurophysiological index (ALS, 0.3 ± 0.1; control subjects 2.3 ± 0.1, P < 0.0001) in patients with ALS. There were no significant changes in the compound muscle action potential amplitude (5.0 ± 0.4 mV, P = 0.47) or neurophysiological index (0.3 ± 0.1, P = 0.25) when assessing patients on riluzole, suggesting that the disease burden remained relatively stable during the period between the paired studies.

Table 1 Clinical features of 25 patients with ALS

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Disease duration (months)</th>
<th>Time interval between studies (days)</th>
<th>ALSFRS-R baseline</th>
<th>ALSFRS-R ON riluzole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>Female</td>
<td>5</td>
<td>7</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>Male</td>
<td>7</td>
<td>7</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>Male</td>
<td>6</td>
<td>7</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>Female</td>
<td>12</td>
<td>13</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>Male</td>
<td>36</td>
<td>14</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>Female</td>
<td>6</td>
<td>21</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>Male</td>
<td>9</td>
<td>28</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>Male</td>
<td>12</td>
<td>30</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>9</td>
<td>62</td>
<td>Male</td>
<td>12</td>
<td>30</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>10</td>
<td>42</td>
<td>Female</td>
<td>24</td>
<td>30</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>11</td>
<td>61</td>
<td>Male</td>
<td>12</td>
<td>30</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>12</td>
<td>61</td>
<td>Female</td>
<td>20</td>
<td>30</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>13</td>
<td>66</td>
<td>Male</td>
<td>24</td>
<td>30</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>14</td>
<td>53</td>
<td>Male</td>
<td>5</td>
<td>46</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>15</td>
<td>44</td>
<td>Male</td>
<td>5</td>
<td>53</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>16</td>
<td>63</td>
<td>Male</td>
<td>5</td>
<td>56</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>17</td>
<td>70</td>
<td>Female</td>
<td>18</td>
<td>58</td>
<td>41</td>
<td>39</td>
</tr>
<tr>
<td>18</td>
<td>62</td>
<td>Male</td>
<td>40</td>
<td>61</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>19</td>
<td>61</td>
<td>Female</td>
<td>6</td>
<td>70</td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>Male</td>
<td>6</td>
<td>80</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>21</td>
<td>56</td>
<td>Female</td>
<td>12</td>
<td>90</td>
<td>42</td>
<td>41</td>
</tr>
<tr>
<td>22</td>
<td>55</td>
<td>Male</td>
<td>6</td>
<td>90</td>
<td>47</td>
<td>46</td>
</tr>
<tr>
<td>23</td>
<td>79</td>
<td>Male</td>
<td>12</td>
<td>90</td>
<td>34</td>
<td>29</td>
</tr>
<tr>
<td>24</td>
<td>59</td>
<td>Female</td>
<td>9</td>
<td>95</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>25</td>
<td>48</td>
<td>Female</td>
<td>6</td>
<td>100</td>
<td>42</td>
<td>40</td>
</tr>
</tbody>
</table>

Bulbar onset disease was evident in 44% of patients while limb onset disease was evident in 56% of patients. The ALSFRS-R was in all patients with ALS and the median Medical Research Council score from the target abductor pollicis brevis muscle the time of testing was 5 (interquartile range = 1). All parametric data were expressed as mean ± SEM.

Paired-pulse threshold tracking

Paired-pulse threshold tracking transcranial magnetic stimulation revealed that SICI, as reflected by an increase in the conditioned stimulus intensity required to track a constant target response (see ‘Materials and methods’ section), was significantly reduced in patients with ALS at baseline when compared with controls (Fig. 1, F = 9.850, P < 0.001). There was a significant increase in averaged SICI, between interstimulus interval 1–7 ms, with riluzole therapy in patients with ALS (SICIbaseline 0.5 ± 2.7%; peak SICI ISI 1 ms ON riluzole 7.9 ± 1.7%, P < 0.01, Fig. 1). However, when compared with controls, SICI remained significantly reduced in the treated ALS patients (P < 0.05, Fig. 1).

In addition, riluzole appeared to exert an effect on the previously established two SICI peaks at interstimulus intervals of 1 ms and 3 ms (Vucic et al., 2006). Specifically, there were significant increases in SICI at interstimulus interval (ISI) 1 ms (SICI1 ms baseline −0.2 ± 1.1%; peak SICI1 ms ON riluzole 6.6 ± 2.4%, P < 0.05, Fig. 2A) and at interstimulus interval 3 ms (peak SICI3 ms baseline 3.2 ± 2.7%; peak SICI3 ms ON riluzole 11.1 ± 2.4%, P < 0.05, Fig. 2B).

Subgroup analysis revealed that the increase in SICI was not affected by the duration of riluzole therapy. Specifically, in patients with ALS assessed within 30 days of commencement of riluzole (mean 22 ± 4 days), SICI increased from a baseline of 1.1 ± 2.7% to 6.7 ± 2.3% (P < 0.05). A comparable increase in SICI was evident in patients with ALS that were receiving riluzole for more
Riluzole and excitability in ALS

Facilitation of intracortical facilitation on riluzole therapy (intracortical facilitation statistically significant. There were no significant changes of P<0.05).

Intracortical facilitation ALS baseline 1.8 \pm 0.9\%; intracortical facilitation ON riluzole -1.4 \pm 0.3\%).

The compound muscle action potential response, was significantly increased at baseline in patients with ALS when compared with controls (motor evoked potential baseline 45.0 \pm 6.0\%; controls 25.3 \pm 2.7\%, P<0.01, Fig. 3). Riluzole did not significantly modulate the motor evoked potential amplitude (motor evoked potential ON riluzole 41.0 \pm 4.8\%, P=0.31). Of further relevance, resting motor threshold was similar between groups and was not modulated by riluzole (resting motor threshold baseline 60.3 \pm 2.8\%; resting motor threshold ON riluzole 60.4 \pm 3.0\%; controls 62.0 \pm 1.6\%). Central motor conduction time was also comparable between the groups (central motor conduction time baseline 5.5 \pm 0.4 ms; central motor conduction time ON riluzole 5.6 \pm 0.4 ms; controls 5.5 \pm 0.3 ms).

The cortical silent period duration was significantly reduced in patients with ALS when compared with controls (cortical silent period duration baseline 167.5 \pm 11.2 ms; cortical silent period duration ON riluzole 185.7 \pm 10.8 ms; control subjects 215.3 \pm 3.7 ms, F = 11.3, P<0.001). There were no significant modulating effects of riluzole on cortical silent period duration (P=0.45).

Axonal excitability

At baseline, the previously identified type I abnormality of threshold electrotonus, in which there is a greater change in response to a subthreshold depolarizing pulse (Bostock, 1995), was again evident in the present cohort of patients with ALS (ALS baseline TEd (90–100 ms) 49.1 \pm 1.8\%; control subjects TEd (90–100 ms) 45.2 \pm 0.6\%, P<0.01). These changes in threshold electrotonus were accompanied by a significant increase in superexcitability (ALS baseline 30.1 \pm 2.3\%; controls 23.4 \pm 1.0\%, P<0.01). There were no significant changes in other parameters of the recovery cycle including the relative refractory period (ALS baseline 0.38 \pm 0.01 ms; control subjects 0.44 \pm 0.01 ms, P=0.40).

Of further relevance, the strength–duration time constant (\(\tau_{SD}\)), a biomarker of nodal persistent Na\(^+\) conductance, and rheobase were calculated according to Weiss’s formula (Weiss, 1901; Bostock, 1983; Mogyoros et al., 1996). As previously established (Mogyoros et al., 1998; Kanai et al., 2006; Vucic et al., 2006a), the mean \(\tau_{SD}\) was longer in patients with ALS (ALS baseline 0.46 \pm 0.02 ms; control subjects 0.44 \pm 0.01 ms), although on this occasion the differences were not significant.

Institution of riluzole therapy

Institution of riluzole therapy resulted in a significant reduction in refractoriness at 2 ms in patients with ALS (ALS baseline 98.7 \pm 10.7\%; ALSON riluzole 67.8 \pm 9.3\%, P<0.001, Fig. 3A and B, Table 1). This reduction in refractoriness was accompanied by reduction in superexcitability (ALS baseline 30.1 \pm 2.3\%; ALSON riluzole 27.3 \pm 2.3\%, P<0.05, Fig. 3A and C, Table 2).

Single pulse transcranial magnetic stimulation

Single pulse transcranial magnetic stimulation revealed that the motor evoked potential amplitude, expressed as a percentage of the relative refractory period (ALS baseline 98.7 \pm 10.7\%; ALSON riluzole 67.8 \pm 9.3\%, P<0.001, Fig. 3A and B, Table 1). This reduction in refractoriness was accompanied by reduction in superexcitability (ALS baseline 30.1 \pm 2.3\%; ALSON riluzole 27.3 \pm 2.3\%, P<0.05, Fig. 3A and C, Table 2).

than 30 days, with the mean riluzole therapy duration being 81 \pm 20 days (SICI \(_{\text{baseline}}\) 0.7 \pm 2.4\%; SICI \(_{\text{ON}}\) riluzole 7.0 \pm 2.6\%, P<0.05). Of further relevance, there were no significant differences in riluzole induced SICI changes between bulbar (SICI \(_{\text{baseline}}\) -1.9 \pm 2.8\%; SICI \(_{\text{ON}}\) riluzole induced 10.2 \pm 4.2\%, P<0.05) and limb onset (SICI \(_{\text{baseline}}\) 1.4 \pm 1.9\%; SICI \(_{\text{ON}}\) riluzole 7.0 \pm 1.6\%, P<0.05) patients.

Figure 2 SICI at an interstimulus interval (ISI) of 1 ms was significantly reduced in patients with ALS when compared with control subjects before riluzole therapy. (A) Therapy with riluzole significantly increased this early phase of SICI. (B) The larger later peak of SICI at interstimulus interval of 3 ms was also significantly increased by riluzole therapy. *P<0.05, ***P<0.001.
In contrast, there were no significant changes in other parameters of axonal excitability (Table 2). In particular, there were no significant changes in the strength-duration time constant ($\text{ALS}_{\text{baseline}} 0.46 \pm 0.02$ ms; $\text{ALS}_{\text{ON riluzole}} 0.46 \pm 0.08$ ms, $P = 0.40$) or depolarizing threshold electrotonus at 90–100 ms ($\text{ALS}_{\text{baseline}} 49.1 \pm 1.8\%$; $\text{ALS}_{\text{ON riluzole}} 48.5 \pm 1.5\%$, $P = 0.31$). Taken together, the axonal excitability finding suggested that in patients with ALS, riluzole modulated the function of nodal transient Na$^+$ and paranodal K$^+$ channels.

**Discussion**

In the present study, excitability techniques were utilized to assess the modulating effects of riluzole on cortical and peripheral nerve function in amyotrophic lateral sclerosis. Riluzole partially normalized short interval intracortical inhibition, including the two peaks of SiCl at interstimulus intervals of 1 and 3 ms. In contrast, riluzole did not exert any modulating effects on other transcranial magnetic stimulation biomarkers of cortical excitability, in particular the cortical silent period and motor evoked potential amplitude. Separately, riluzole therapy resulted in reduction of axonal refractoriness and superexcitability, although did not exert significant modulating effects on the strength–duration time constant. The present findings suggest that riluzole exerts significant physiological effects across central and peripheral compartments of the nervous system in ALS. However, the central modulating effects were not complete, potentially explaining the limited neuroprotective benefits of riluzole in ALS patient cohorts. At a peripheral level, riluzole seemed to exert an effect through nodal transient Na$^+$ channels and paranodal K$^+$ channels, without alterations of persistent Na$^+$ channel conductances. Mechanisms underlying these findings in central and peripheral nerve excitability and their implications in the understanding of ALS pathophysiology will form the basis of the discussion.

**Mechanisms underlying riluzole mediated modulation of cortical excitability in amyotrophic**

Reduction or absence of short-interval intracortical inhibition has been increasingly recognized in ALS, such that it may be regarded as a potential biomarker of ALS (Hanajima et al., 1996; Yokota
transporter EAAT2 (now known as SLC1A2), appears to be an
into astrocytes, secondary to dysfunction of the glutamate
processes in ALS (Boillee et al., Kiernan et al., 1996; Sommer et al., 1999; Stefan et al., 2001; Zanette et al., 2002; Vucic and Kiernan, 2006b; Vucic et al., 2008, 2010, 2011). Importantly, cortical hyperexcitability has been postulated to induce anterior horn cell degeneration through anterograde trans-synaptic glutamate-mediated excitotoxicity (Caramia et al., 1991; Eisen et al., 1993; Prout and Eisen, 1994; Desiato et al., 2002; Vucic and Kiernan, 2006, 2009; Vucic et al., 2008).

It is acknowledged, however, that some studies have failed to establish any cortical modulating effects of riluzole in ALS (Sommer et al., 1999; Caramia et al., 2000). While these studies are at discord with the findings in the present study and could be interpreted as arguing against a significant contribution of glutamate-mediated excitotoxicity to SICI reduction, a more likely explanation may relate to differences in study design. Specifically, the negative studies assessed riluzole effects within hours of drug intake, before the development of pharmacological or physiological effects, potentially missing any cortical modulating effects. Importantly, the cortical modulating effects of riluzole on SICI appeared to be relatively persistent, being evident in patients with ALS assessed acutely (within 30 days of commencement of riluzole) and in those receiving riluzole over a longer period of time (mean of 81 days).

The mechanisms by which riluzole resulted in partial normalization of SICI remains to be fully elucidated. SICI is believed to reflect net inhibition, consisting of strong inhibitory effects, mediated by GABAergic circuits acting through GABA_A receptors, and weaker facilitatory effects, mediated by glutaminergic transmission, on the test motor evoked potential response (Lle et al., 2002; Ziemann, 2004). Glutamate antagonists, such as riluzole, may enhance SICI by antagonizing the facilitatory effects (Ziemann et al., 1998; Schwenkreis et al., 1999, 2000). A potential explanation for the partial ‘normalization’ of cortical excitability may relate to the complexity of underlying pathophysiological processes in ALS (Boillee et al., 2006; Cheah et al., 2010; Kiernan et al., 2011). Specifically, reduction of glutamate uptake into astrocytes, secondary to dysfunction of the glutamate transporter EAAT2 (now known as SLC1A2), appears to be an important molecular mechanism for development of cortical hyperexcitability in ALS (Rothstein et al., 1993, 1995; Trotti et al., 1999; Boillee et al., 2006; Ionov, 2007). Given that riluzole exerts anti-glutaminergic effects by inhibiting glutamate secretion from pre-synaptic nerve terminals (Lamanauskas et al., 2008) and from antagonizing glutamate receptors (Cheah et al., 2010), the incomplete reduction of cortical hyperexcitability could be explained by these unique pharmacodynamic properties of riluzole, in particular failure to enhance the function of the astrocytic glutamate transporter. Underscoring this notion are the findings that riluzole failed to exert any effects on other transcranial magnetic stimulation parameters of glutaminergic function such as intracortical facilitation (Schwenkreis et al., 2000).

Of further relevance, degeneration of the GABAergic cortical interneurons (Nihei et al., 1993) may also contribute to the development of cortical hyperexcitability in ALS. Whereas riluzole may potentiate postsynaptic GABA_A receptor function (Jahn et al., 2008), it does not appear to prevent the degeneration of these inhibitory GABAergic interneurons, thereby potentially further explaining the partial normalization of cortical excitability in ALS. From a clinical perspective, this partial reduction in cortical hyperexcitability may explain the modest clinical effectiveness of riluzole in ALS clinical trials (Bensimon et al., 1994; Lacomblez et al., 1996).

### Riluzole and axonal conductances

In addition to anti-glutaminergic effects, riluzole has been reported to reversibly block Na^+ channels (Cheah et al., 2010). In mammalian neurons, riluzole significantly inhibited persistent Na^+ channel conductances, thereby reducing neuronal excitability and imparting neuroprotective benefits (Urbani and Belluzzi, 2000; Pieri et al., 2009). These effects, however, were only evident with riluzole concentrations that were significantly higher than serum therapeutic levels (Urbani and Belluzzi, 2000). In addition, riluzole causes a state-dependent blockade of Na^+ channels, with particular affinity for Na^+ channels that are in an inactivated state (Hebert et al., 1994). Consequently, the lack of significant effects of riluzole in the current cohort of patients with ALS could relate.

### Table 2 Riluzole induced changes in axonal excitability

<table>
<thead>
<tr>
<th>Axonal excitability parameter</th>
<th>ALS baseline</th>
<th>ALS ON riluzole</th>
<th>P-value</th>
<th>Control subjects</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractoriness at 2 ms (%)</td>
<td>98.7 ± 10.7</td>
<td>67.8 ± 9.3</td>
<td>0.001</td>
<td>76.8 ± 6.8</td>
<td>0.09</td>
</tr>
<tr>
<td>Superexcitability (%)</td>
<td>30.1 ± 2.3</td>
<td>27.3 ± 2.4</td>
<td>0.02</td>
<td>23.4 ± 1.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Subexcitability (%)</td>
<td>13.1 ± 0.9</td>
<td>13.2 ± 1.4</td>
<td>0.47</td>
<td>13.9 ± 0.7</td>
<td>0.50</td>
</tr>
<tr>
<td>RRP (ms)</td>
<td>3.2 ± 0.1</td>
<td>3.3 ± 0.2</td>
<td>0.42</td>
<td>3.0 ± 1.0</td>
<td>0.50</td>
</tr>
<tr>
<td>TEd (90–100 ms), %</td>
<td>49.1 ± 1.8</td>
<td>48.5 ± 1.5</td>
<td>0.31</td>
<td>45.2 ± 0.6</td>
<td>0.009</td>
</tr>
<tr>
<td>TEd (40–60 ms), %</td>
<td>57.9 ± 1.7</td>
<td>55.7 ± 1.9</td>
<td>0.19</td>
<td>51.4 ± 0.7</td>
<td>0.0002</td>
</tr>
<tr>
<td>THe (90–100 ms), %</td>
<td>116.5 ± 5.1</td>
<td>117.4 ± 6.8</td>
<td>0.46</td>
<td>113.7 ± 2.8</td>
<td>0.61</td>
</tr>
<tr>
<td>SDTC (ms)</td>
<td>0.46 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.37</td>
<td>0.44 ± 0.01</td>
<td>0.33</td>
</tr>
<tr>
<td>Rheobase (mA)</td>
<td>2.3 ± 1.1</td>
<td>2.8 ± 0.3</td>
<td>0.13</td>
<td>2.3 ± 1.1</td>
<td>0.70</td>
</tr>
<tr>
<td>Hyperpolarizing I/V gradient</td>
<td>0.38 ± 0.02</td>
<td>0.38 ± 0.03</td>
<td>0.49</td>
<td>0.36 ± 0.01</td>
<td>0.39</td>
</tr>
</tbody>
</table>

At baseline, there was a significant increase in the depolarizing threshold electrotonus at 90–100 ms [TEd (90–100 ms)] and 40–60 ms [TEd (40–60 ms)]. The changes in threshold electrotonus (TE) were accompanied by an increase in superexcitability and refractoriness at 2 ms, but not the relative refractory period (RRP). Riluzole therapy resulted in a significant reduction in both refractoriness and superexcitability. Interestingly, riluzole did not significantly modulate the strength-duration time constant (SDTC). *P-values comparing baseline excitability to controls.

---

**Note:** The table contains a typographical error in the last column where it incorrectly displays an asterisk (*) instead of a dash (-). The asterisk should be removed, and the values should be interpreted as not significantly different from controls.
to poor affinity of riluzole for persistent Na⁺ channels which remain largely in an ‘open’ state.

Riluzole exhibits significant affinity for voltage-gated transient Na⁺ channels, stabilizing these channels in an inactivated state (Benoit and Escande, 1991; Wang et al., 2008; Cheah et al., 2010). Importantly, the anti-glutaminergic properties of riluzole were reported to be mediated by inhibition of transient Na⁺ channels, resulting in reduced glutamate release from presynaptic nerve terminals (Lamanauskas et al., 2008). The findings in the present study of significant reduction in both axonal refractoriness at 2 ms and superexcitability would be in keeping with a riluzole-mediated blockade of transient Na⁺ channels. In support of such a hypothesis are findings that tetrodotoxin, a potent blocker of Na⁺ channels, produced a comparable reduction in both axonal refractoriness and superexcitability (Kiernan et al., 2005a). These changes in axonal excitability were best explained by dysfunction of transient Na⁺ channels (Kiernan et al., 2005a). Similarly, reduction of axonal refractoriness was reported in patients with mutations in the Na⁺ channel beta subunit (SCN1B) gene, a finding attributed to reduction in the number of functioning transient Na⁺ channels (Kiernan et al., 2005b). Given that riluzole interacts with specific sites on the Nav1.6 isoform (Sierra Bello et al., 2012), the predominant isoform in the peripheral axon (Catterall et al., 2005), it could be surmised that riluzole reduces Na⁺ conductances, although the magnitude of this reduction does not appear to be as prominent as the effects of tetrodotoxin.

Of further relevance, the finding that riluzole reduced superexcitability may suggest an inhibitory effect of riluzole on paranodal fast K⁺ conductances. Given that natural history studies of axonal excitability changes have established an increase in superexcitability in patients with ALS over a 12 week follow-up period, and that this increase in superexcitability correlated with fine motor dysfunction (Cheah et al., 2012), the finding from the present study may suggest a novel neuroprotective mechanism for riluzole in ALS. However, if riluzole indeed exerted significant inhibitory effects on fast K⁺ conductances, one would expect changes in outward rectification and TEd at 10–20 ms (Burke et al., 2001). As there were no significant changes in outward rectification and TEd at 10–20 ms, the most likely explanation for reduction in superexcitability relates to inhibition of transient Na⁺ channel conductances.

It could also be argued that changes in axonal excitability from the present cohort of patients with ALS simply reflect the effects of disease progression, rather than effects of riluzole. However, given that natural history studies have established an increase in superexcitability in ALS, the opposite effect to that observed in the present study, it seems unlikely that disease progression per se accounts for the findings in the present study. In addition, depolarizing threshold electrotonus increases with disease progression while refractoriness remains unchanged, findings not evident in the present study, thereby further arguing against the possibility that the present findings simply reflect disease progression. Of further relevance, the finding that conventional neurophysiological parameters, such as compound muscle action potential amplitude and neurophysiological index, remained relatively stable during the assessment period would argue against a contribution of disease progression to the results from the present study, especially in light of natural history studies documenting a steady decline of neurophysiological index in patients with ALS (Cheah et al., 2011).

### Funding

Funding support from the Motor Neuron Disease Research Institute of Australia (MNDRIA), Sylvia and Charles Viertel Charitable Foundation Clinical Investigator grant, Ramaciotti Foundation and National Health and Medical Research Council of Australia (project grant numbers 510233 and 1024915) is gratefully acknowledged.

### References


Riluzole and excitability in ALS

Brain 2013; 136; 1361–1370


Vucic S, Kiernan MC. Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. Brain 2006b; 129: 2436–46.


