SUPPLEMENTARY MATERIAL

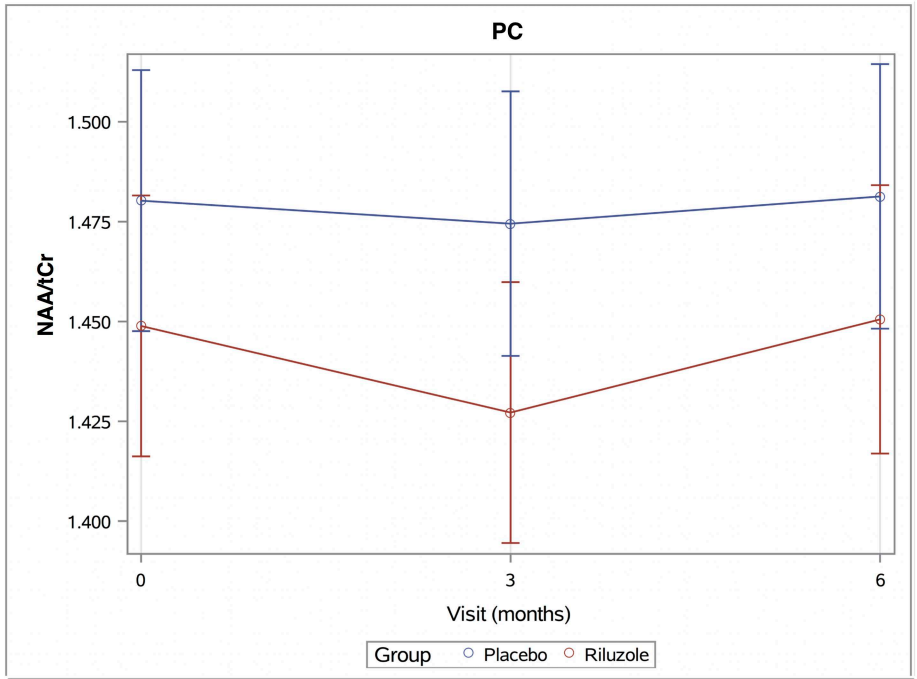
Statistical Powering

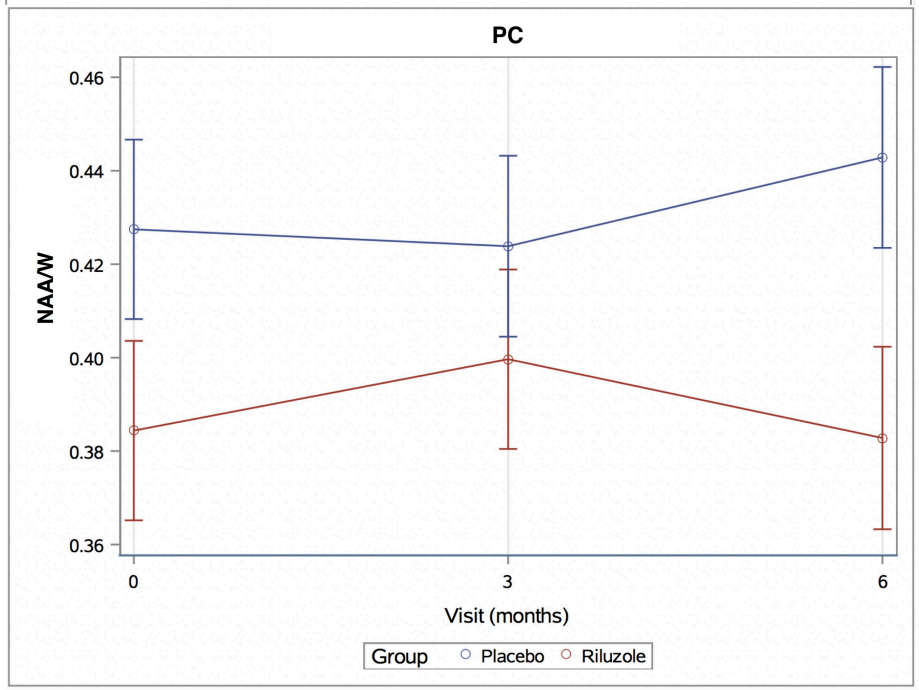
A previous study of memantine, which affects the glutamatergic system through a different mechanism, suggested that significant treatment-related differences in glucose metabolism measured using FDG PET in posterior cingulate and precuneus could be detected in Alzheimer's disease patients at 80% power, 2-tailed t-test, significance at $P<0.05$ (uncorrected for multiple comparisons), with as few as 16 participants per arm [1]. In a previous study of AD patients, the mean change in NAA concentration over 12 months was 12% (standard deviation (SD) 15%) compared to no change in healthy controls, SD 8% [2]. Based upon this finding, we expected a change of approximately 6% (SD 10%) over 6 months in placebo treated patients and projected an 8% standard deviation if disease progression were halted by riluzole. Based on these FDG and MRS studies, this pilot study of riluzole targeted at least 42 completing subjects, 21 per arm.

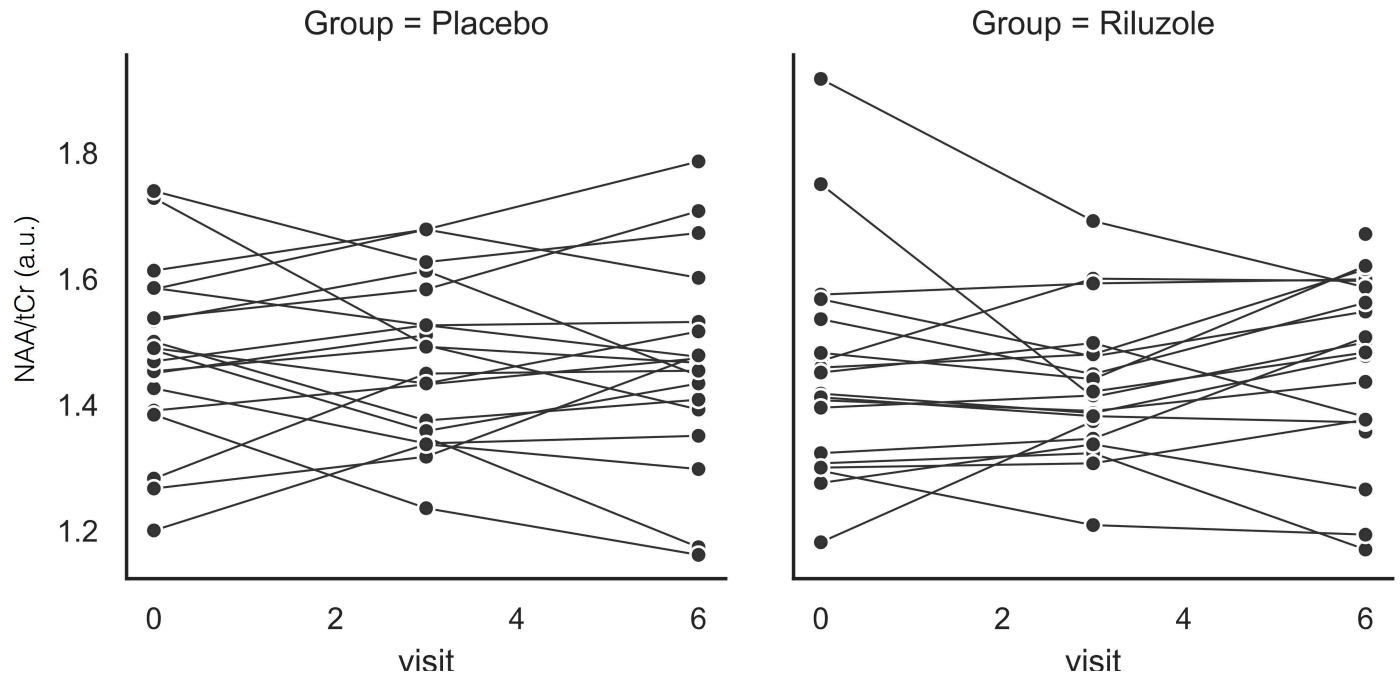
Methods

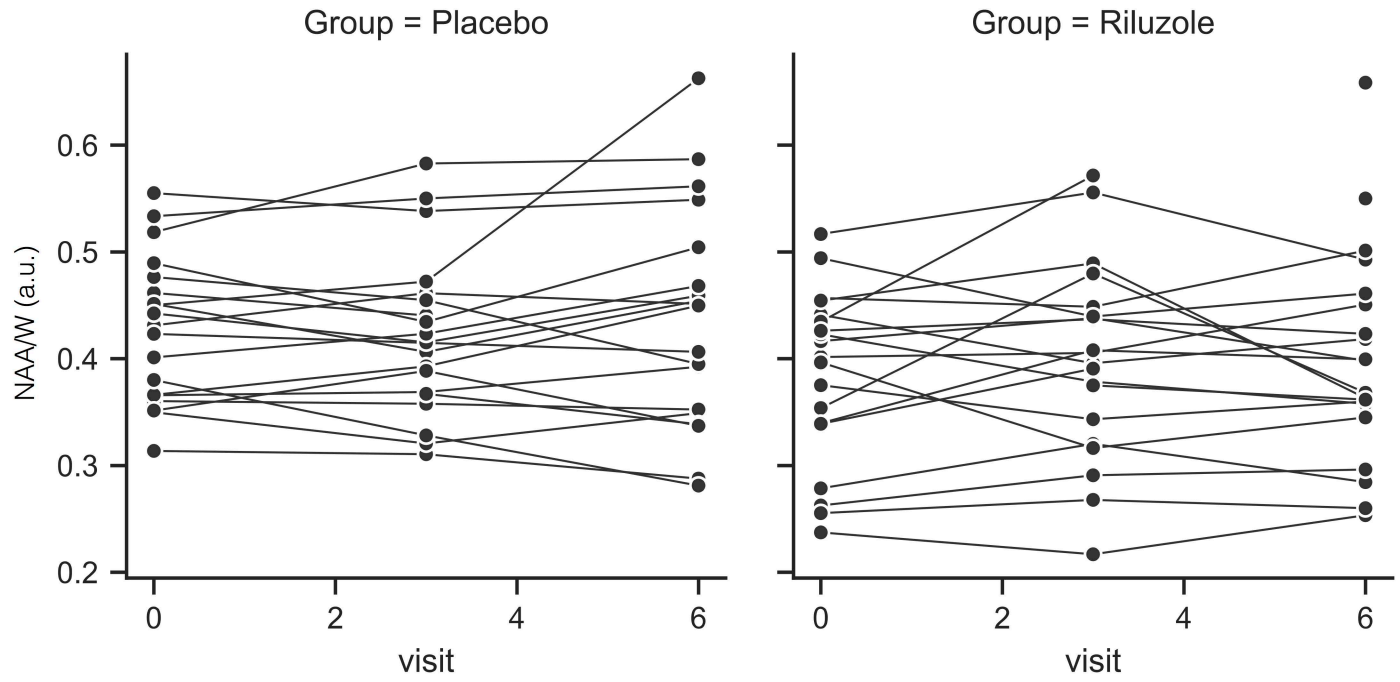
^{1}H MRS of Hippocampi was obtained for exploratory analysis. Levels of hippocampal NAA, GABA and combined glutamate and glutamine, referred to as Glx, were obtained from a 4.5 cm x 2.0 cm x 1.5 cm-voxel oriented along the long axis of the right or left hippocampus (**Supplementary Figure 2A [a-c]),** using the standard J-edited spin echo difference MRS method [3-5] with TE/TR 68/1500ms and a receive-only 8-channel phased-array head coil, as we previously described [4]. Spectra were acquired in 15 minutes using 290 interleaved excitations (580 total) with the editing pulses on or off. **Supplementary Figure 2A[d]** illustrates the acquisition and processing of a sample J-edited GABA and Glx spectrum. Levels of NAA and tCr were derived from the processing subspectra acquired with the editing pulse turned off. We reported a high test-retest reliability for detection of GABA and Glx with this ^{1}H MRS technique on the same 3.0T GE instrument as used in this study [4].

Supplementary Figure 1: ^{1}H MRS measures of NAA/tCr (top) and NAA/W (bottom) levels changes in Posterior Cingulate (PC) over 6 months in riluzole and placebo groups.


PC
1.500
1.475
1.450
1.425
1.400
NAA/tCr
0
3
6
Visit (months)
Group
Placebo
Riluzole

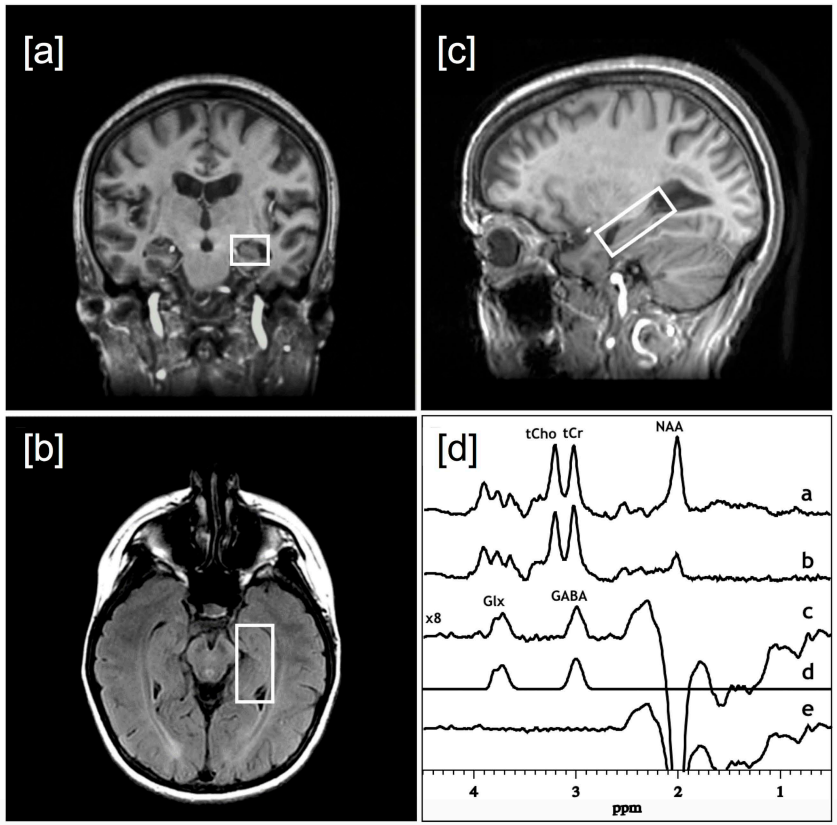

PC
0.46
0.44
0.42
0.40
0.38
0.36
NAA/W
0
3
6
Visit (months)
Group
Placebo
Riluzole


Group = Placebo
Group = Riluzole
1.8
1.6
1.4
1.2
NAA/tCr (a.u.)
0
2
4
6
visit

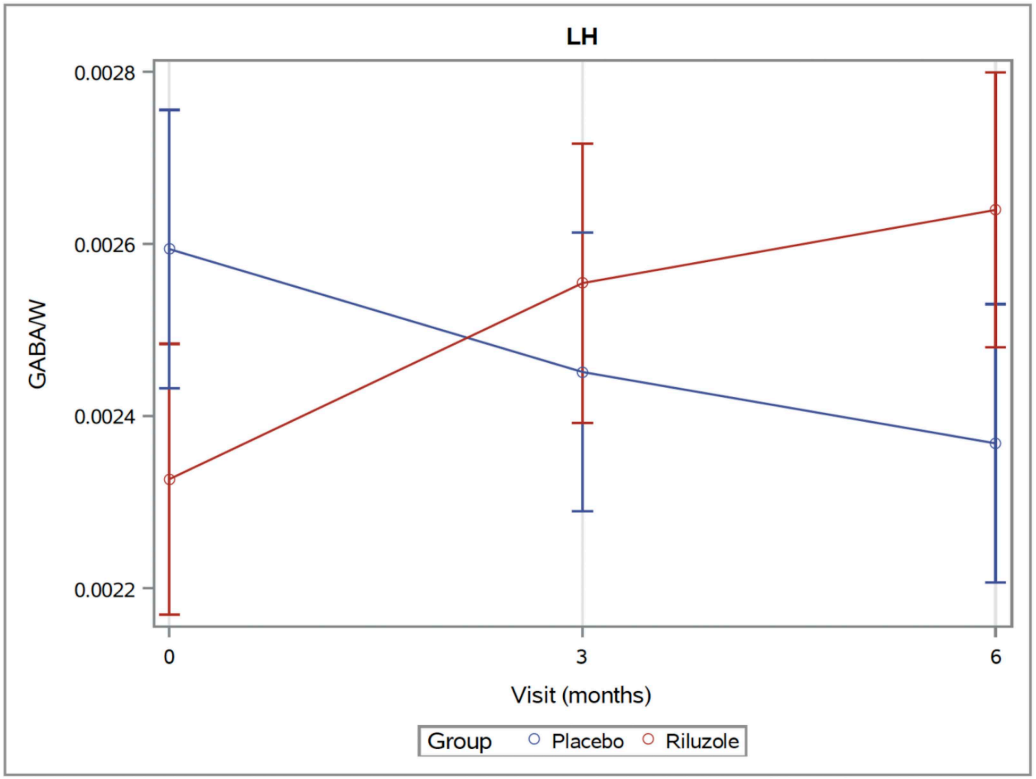

Group = Placebo
Group = Riluzole
0.6
0.5
0.4
0.3
0.2
NAA/W (a.u.)
0
2
4
6
visit

Supplementary Figure 2. A. [a] Coronal, **[b]** axial oblique and **[c]** sagittal MR images of a human brain, with depiction of the size, placement and angulation the voxel of interest, selected to completely contain the left hippocampus. Voxel dimensions: 4.5 cm (anterior-posterior) x 2.0 cm (left-right) x 1.5 cm (superior-inferior), or 13.5 cm^3. **[d]** Demonstration of hippocampal GABA and glutamate+glutamine (Glx) detection with J-edited ^1H MRS and TE/TR 68/1500ms: (a) and (b), single-voxel subspectra acquired with the editing pulses on and off; (c), difference between spectra in (a) and in (b) showing the edited brain GABA and Glx resonances; (d), selective model fitting of the GABA and Glx resonances in spectrum in (c) to obtain the GSH peak area; (e), residual of the difference between spectra in (c) and in (d). Also detected are the resonances for N-acetyl-L-aspartate (NAA), total creatine (tCr), total choline (tCho). **B.** GABA/W levels changes in L hippocampus over 6 months in riluzole and placebo groups. **C.** Correlation at baseline between GABA/W and Logical Memory 1 (left) and GABA/W and Logical Memory 2 (right) across subjects.

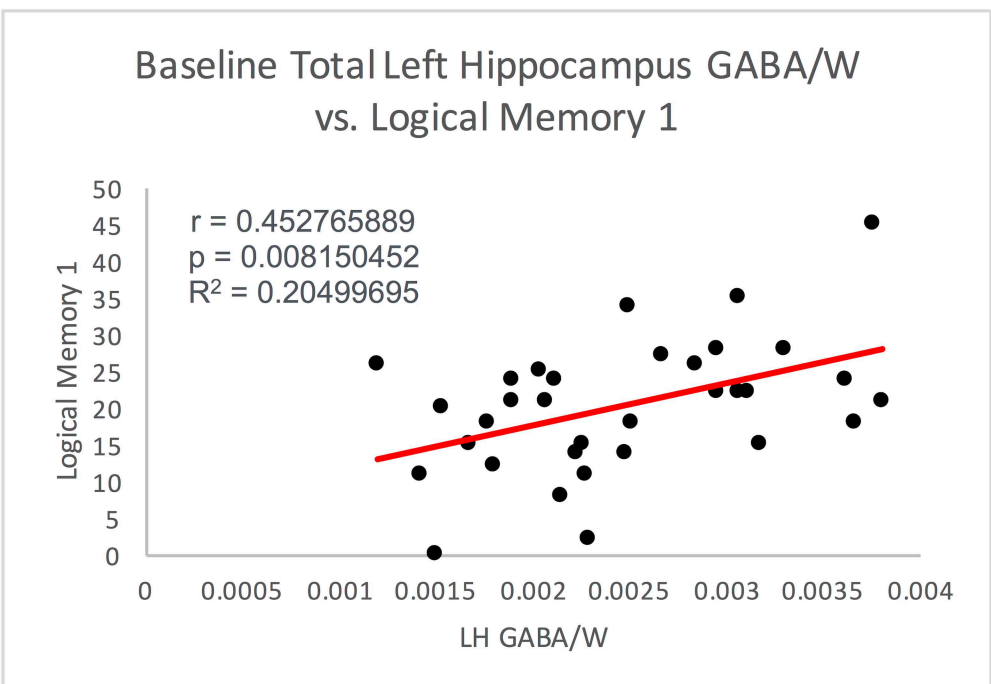
A

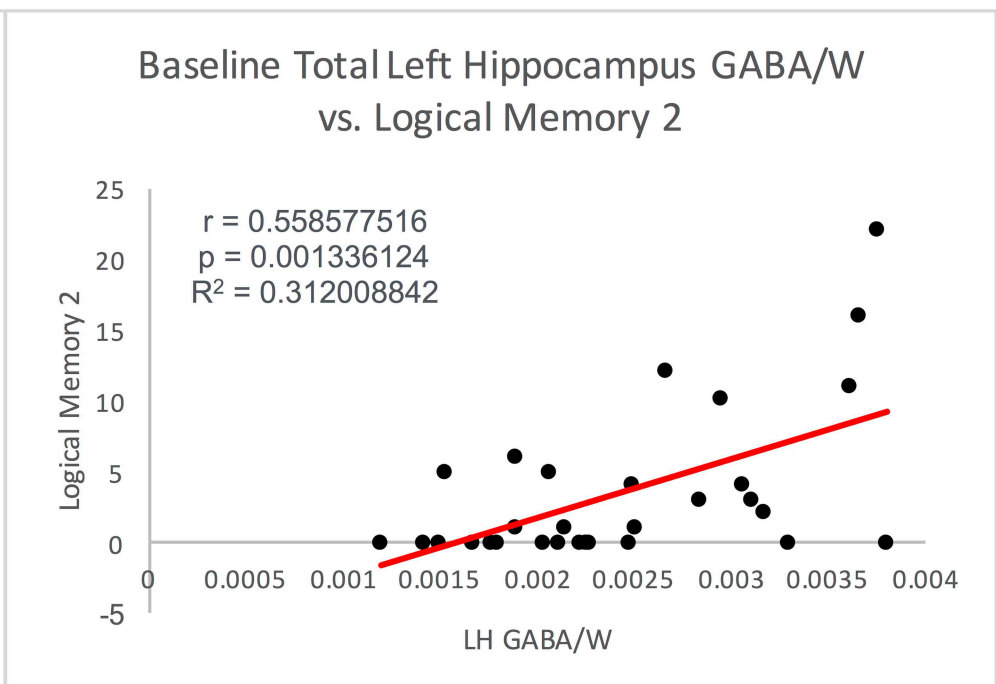

[a]
[c]
[b]
[d]
tCho tCr
NAA
Glx
GABA
x8
a
b
c
d
e
4
3
2
1
ppm

B


LH
0.0028
0.0026
0.0024
0.0022
GABA/W
0
3
6
Visit (months)
Group
Placebo
Riluzole

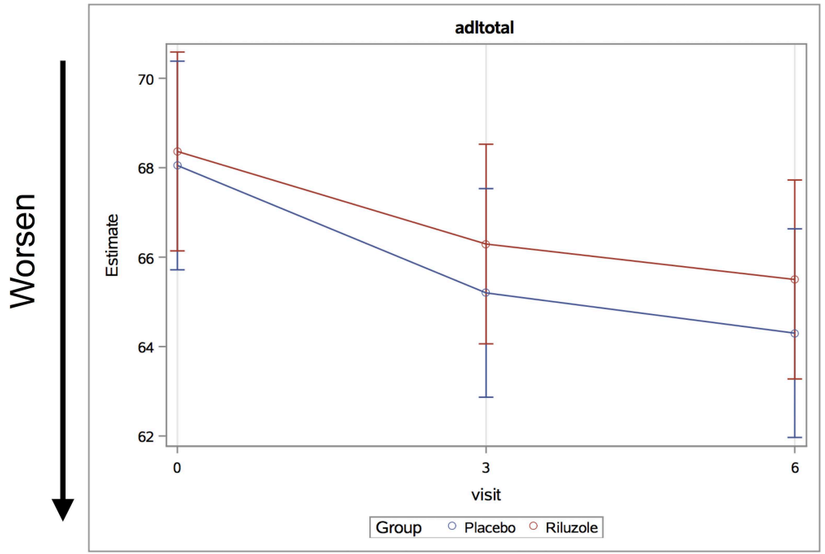
C


Baseline Total Left Hippocampus GABA/W vs. Logical Memory 1
r = 0.452765889
p = 0.008150452
R² = 0.20499695
Logical Memory 1
50
45
40
35
30
25
20
15
10
5
0
0
0.0005
0.001
0.0015
0.002
0.0025
0.003
0.0035
0.004
LH GABA/W

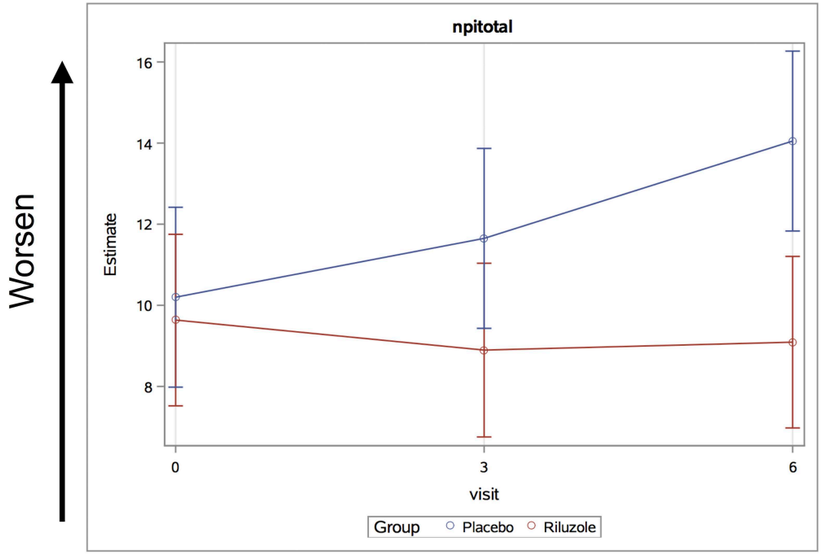

Baseline Total Left Hippocampus GABA/W vs. Logical Memory 2
r = 0.558577516
p = 0.001336124
R² = 0.312008842
Logical Memory 2
25
20
15
10
5
0
-5
0
0.0005
0.001
0.0015
0.002
0.0025
0.003
0.0035
0.004
LH GABA/W

Supplementary Figure 3: Neuropsychological changes over 6 months for (A) ADCS Activities of Daily Living – ADL Inventory, (B) Neuropsychiatry Inventory, NPI and (C) Alzheimer's Disease Assessment Scale- Cognitive Subscale – ADAScog. Means and standard error are plotted at each time point by group. ADL Inventory had a significant overall effect ($P<0.001$). Post-hoc tests for comparing the two groups showed no significant differences at baseline ($P=0.92$) and 6 months ($P=0.71$). NPI had a trend group effect ($P=0.44$). Post-hoc tests for showed no significant differences between the groups at baseline ($P=0.85$) and 6 months ($P=0.11$). ADAS-cog did not have a significant group effect ($P=0.73$). Post-hoc tests comparing the two groups showed no significant differences at baseline ($P=0.13$) and 6 months ($P=0.33$).

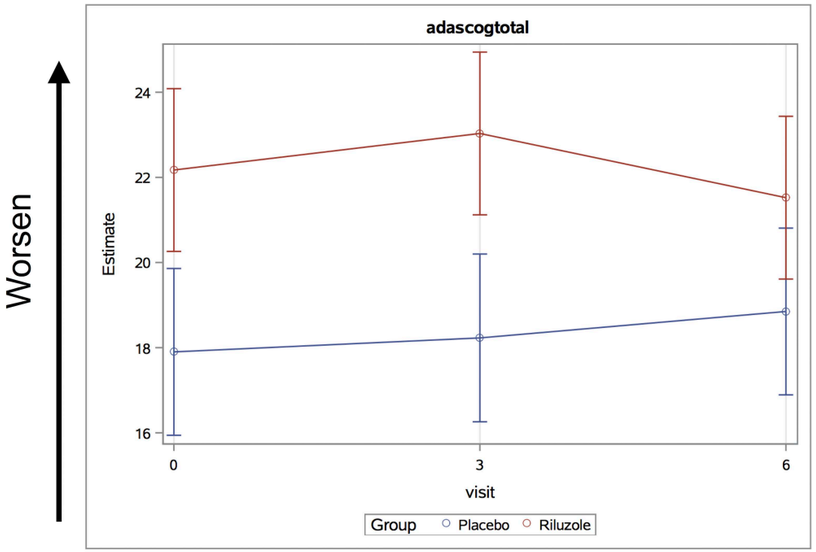
A


Worsen
adltotal
Estimate
70
68
66
64
62
0
3
6
visit
Group
Placebo
Riluzole

B


Worsen
npitotal
Estimate
16
14
12
10
8
0
3
6
visit
Group
Placebo
Riluzole

C


Worsen
adascogtotal
Estimate
24
22
20
18
16
0
3
6
visit
Group
Placebo
Riluzole

Supplementary Table 1: Summary of adverse events

Reasons for discontinuation in the riluzole group were: gastrointestinal hemorrhage with hospitalization and study article discontinuation (1), laboratory abnormalities (thrombocytopenia) (1), increased anxiety and agitation requiring other medication adjustments (1) and diarrhea (1). In the placebo group, the subjects discontinued due to: bradycardia and pacemaker placement with study article discontinuation (1); increased paranoia, agitation and treatment non-compliance (1), consent withdrawal after newly hypertensive event after starting study article (1), consent withdrawal due to long distance travel and new delusion onset (1).

Supplementary Table 1: Number (Percent) of Adverse Events

Event	Riluzole (n=26)	Placebo (n=24)
Any adverse event	23 (88.5)	22 (91.7)
Any serious adverse event	2 (7.7)	1 (4.2)
Cough	5 (19.2)	3 (12.5)
Abdominal discomfort	4 (15.4)	0 (0.0)
Diarrhea	4 (15.4)	2 (8.3)
Dizziness	4 (15.4)	1 (4.2)
Urinary frequency	3 (11.5)	0 (0.0)
Nausea	2 (7.7)	0 (0.0)
Back pain	2 (7.7)	2 (8.3)
Anxiety	2 (7.7)	4 (16.7)
Elevated liver enzymes	2 (7.7)	1 (4.2)
Paranoia	1 (3.9)	3 (12.5)
Rash	1 (3.9)	2 (8.3)
Fatigue	1 (3.9)	2 (8.3)
Somnolence	0 (0.0)	3 (12.5)

Supplementary Table 2: Mean (SD) tissue composition and spectral quality parameters for the ^{1}H MRS voxels of interest

Supplementary Table 2. Mean (SD) Tissue Composition and Spectral Quality Parameters for the Two MRS Voxels of Interest

	Baseline		At 6 Months	
	Placebo	Riluzole	Placebo	Riluzole
Posterior Cingulate Cortex (PC)				
Internal Water (W), Signal Intensity	2.05 (1.01) $\times 10^{+12}$	1.81 (1.02) $\times 10^{+12}$	2.00 (1.01) $\times 10^{+12}$	1.93 (0.97) $\times 10^{+12}$
Gray Matter (GM), %	65.9 (2.2)	63.9 (2.4)	64.4 (2.4)	64.6 (2.8)
White Matter (WM), %	17.6 (2.7)	15.6 (3.2)	17.8 (3.4)	16.3 (3.1)
Cerebrospinal Fluid (CSF), %	16.0 (3.9)	16.0 (4.5)	17.4 (4.0)	18.5 (4.5)
Full Width at Half Maximum (FWHM), Hz	4.9 (2.1)	4.6 (1.9)[a]	5.5 (2.3)	6.0 (2.2)[a]
Left Hippocampus				
Internal Water (W), Signal Intensity	5.73 (2.59) $\times 10^{+12}$	4.64 (2.7) $\times 10^{+12}$	5.28 (2.56) $\times 10^{+12}$	4.91 (2.34) $\times 10^{+12}$
Gray Matter (GM), %	56.7 (4.8)	58.4 (6.4)	55.3 (6.6)	58.5 (5.3)
White Matter (WM), %	31.3 (7.4)	28.9 (7.3)	32.4 (6.6)	28.4 (7.6)
Cerebrospinal Fluid (CSF), %	11.3 (3.4)	12.1 (3.5)	11.4 (3.7)	12.1 (3.5)
Full Width at Half Maximum (FWHM), Hz	13.0 (6.5)	11.9 (5.6)	11.6 (5.6)	10.8 (3.3)

a Comparisons of all listed parameters between and within the placebo and riluzole groups at baseline and 6 months revealed only a single significant (p=0.04) but inconsequential difference, in mean FWHM in the PC for the riluzole group at baseline vs. 6 months.

Supplementary Table 3: FDG PET Longitudinal changes over 6 months (Mean, p value and effect size)

	FDG PET Longitudinal change over 6 months					
	Placebo		Riluzole		Comparison	
	Mean (SD)	95% CI Mean	Mean (SD)	95% CI Mean	p-value	Effect size (d)
Posterior cingulate	-0.048 (0.035)	(0.065, -0.031)	-0.005 (0.035)	(-0.021, 0.011)	0.0002	1.31
Precuneus	-0.032 (0.028)	(-0.045, -0.018)	(-0.007, 0.032)	(-0.021, 0.008)	0.007	0.84
Temporal	-0.023 (0.033)	(-0.038, -0.007)	0.002 (0.029)	(-0.010, 0.015)	0.014	0.80
Frontal	-0.129 (0.066)	(-0.159, -0.098)	-0.077 (0.072)	(-0.109, -0.045)	0.031	0.70
Parietal	-0.020 (0.027)	(-0.032, -0.007)	-0.005 (0.024)	(-0.016, 0.005)	0.09	0.54
Hippocampus	-0.018 (0.034)	(-0.034, -0.002)	-0.002 (0.029)	(-0.015, 0.011)	0.11	0.50
Right Hippocampus	-0.021 (0.036)	(-0.038, -0.004)	0.002 (0.027)	(-0.010, 0.014)	0.025	0.72
AD Progression score	0.579 (0.607)	(0.294, 0.863)	0.245 (0.558)	(-0.003, 0.493)	0.07	0.57
Post Cing - Precuneus	-0.041 (0.042)	(-0.061, -0.021)	-0.006 (0.038)	(-0.023, 0.011)	0.007	0.88
Orbitofrontal	-0.019 (0.044)	(-0.039, 0.002)	0.014 (0.036)	(-0.002, 0.030)	0.008	0.86

1. Wang T, Huang Q, Reiman EM, et al. Effects of memantine on clinical ratings, fluorodeoxyglucose positron emission tomography measurements, and cerebrospinal fluid assays in patients with moderate to severe Alzheimer dementia: a 24-week, randomized, clinical trial. *J Clin Psychopharmacol*. Oct 2013;33(5):636-42. doi:10.1097/JCP.0b013e31829a876a
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5. Pereira AC, Mao X, Jiang CS, et al. Dorsolateral prefrontal cortex GABA deficit in older adults with sleep-disordered breathing. *Proc Natl Acad Sci U S A*. Sep 19 2017;114(38):10250-10255. doi:10.1073/pnas.1700177114