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Supplementary Data

Efficacy of Souvenaid in Mild Alzheimer's Disease: Results from a Randomized, Controlled Trial

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SUPPLEMENTARY METHODS

Electroencephalography (EEG)

Resting state EEGs were recorded with a standard protocol at the study centers for all patients. Data were recorded on digital EEG systems from 21 electrodes at the positions of the 10–20 system: Fp2, Fp1, F8, F7, F4, F3, A2, A1, T4, T3, C4, C3, T6, T5, P4, P3, O2, O1, Fz, Cz, Pz. Not all sites were able to measure A2 and A1, thus these two electrodes were not included in the analyses. A common or average reference (including all electrodes except Fp2/1 and A2/1) was used. Sample frequency varied between study sites (200, 256, 400, 500, 512, or 1000 Hz) and were downsampled for the analyses. On-line filter settings were high pass 0.16 Hz, and low pass 70 Hz. Four 4096-sample epochs of artifact-free data (containing no eye blinks, muscle artifacts, slow eye movements or ECG-artifacts) were selected from each EEG. Relative and absolute power of all frequency bands (delta 0.5–4 Hz, theta 4–8 Hz, alpha 8–13 Hz, beta 13–25 Hz, and gamma 30–48 Hz) were calculated for all EEG channels (except A2 and A1) using Fast Fourier Transformation (FFT) (with open access Brainwave

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Supplementary Table 1
Descriptive statistics for individual NTB item scores (intent-to-treat population)

	Control ($n = 129$)	Active ($n = 130$)	p-value
RAVLT immediate recall score [0−75]			
Baseline	27.12 (10.04) [129]	26.19 (9.53) [129]	
Change baseline – Week 12	1.73 (6.88) [120]	1.21 (5.97) [121]	
Change baseline – Week 24	2.16 (7.01) [119]	2.03 (7.67) [117]	0.861[1]
24-week trajectory			0.655[1]
RAVLT delayed recall score [0−15]			
Baseline	3.57 (3.17) [129]	3.19 (3.20) [129]	
Median [range]	3.0 [(0.0)–(12.0)] [129]	2.0 [(0.0)–(13.0)] [129]	
Change baseline – Week 12	0.58 (2.02) [119]	0.40 (1.96) [122]	
Median [range]	0.0 [(−5.0)–(6.0)] [119]	0.0 [(−5.0)–(6.0)] [122]	
Change baseline – Week 24	0.70 (2.23) [120]	0.50 (2.04) [118]	
Median [range]	0.0 [(−9.0)–(7.0)] [120]	0.0 [(−3.0)–(8.0)] [118]	0.338[2]
RAVLT recognition performance score [−15–15]			
Baseline	8.09 (4.62) [129]	7.73 (4.52) [126]	
Median [range]	9.0 [(−.0)–(15.0)] [129]	8.0 [(−7.0)–(15.0)] [126]	
Change baseline – Week 12	0.64 (2.85) [118]	0.12 (3.83) [119]	
Median [range]	0.0 [(−6.0)–(12.0)] [118]	0.0 [(−13.0)–(11.0)] [119]	
Change baseline – Week 24	−0.20 (3.04) [119]	0.86 (3.97) [116]	
Median [range]	0.0 [(−8.0)–(9.0)] [119]	1.0 [(−13.0)–(12.0)] [116]	0.010[2]
WMS-VPA immediate recall score [0−24]			
Baseline	11.24 (4.57)[121]	10.33 (4.78) [119]	
Change baseline – Week 12	0.64 (3.34) [106]	0.55 (3.10) [111]	
Change baseline – Week 24	0.80 (3.54) [106]	1.34 (3.07) [104]	0.232[1]
24-week trajectory			0.207[1]
WMS-VPA delayed recall score [0−8]			
Baseline	4.33 (2.30) [119]	4.04 (2.06) [118]	
Median [range]	4.0 [(0.0)–(8.0)] [119]	4.0 [(0.0)–(8.0)] [118]	
Change baseline – Week 12	0.11 (1.38) [104]	0.07 (1.43) [108]	
Median [range]	0.0 [(−4.0)–(4.0)] [104]	0.0 [(−6.0)–(4.0)] [108]	
Change baseline – Week 24	−0.07 (1.49) [104]	0.24 (1.50) [105]	
Median [range]	0.0 [(−5.0)–(4.0)] [104]	0.0 [(−5.0)–(4.0)] [105]	0.176[2]
WMS digit span score [0−24]			
Baseline	10.54 (3.18) [125]	10.78 (3.66) [130]	
Change baseline – Week 12	−0.15 (2.33) [116]	0.46 (2.48) [121]	
Change baseline – Week 24	−0.02 (2.17) [117]	0.22 (2.34) [118]	0.339[1]
24-week trajectory			0.104[1]
TMT condition A, s [max. 150 s]			
Baseline	90.04 (38.46) [124]	92.46 (37.58) [127]	
Median [range]	79.5 [(30.0)–(150.0)] [124]	88.0 [(26.0)–(150.0)] [127]	
Change baseline – Week 12	−1.62 (29.68) [116]	−3.76 (29.63) [118]	
Median [95% CI] [range]	−2.0 [(−90.0)–(85.0)] [116]	−1.5 [(−105.0)–(79.0)] [118]	
Change baseline – Week 24	−0.84 (27.15) [114]	−2.36 (28.35) [113]	
Median [95% CI] [range]	0.0 [(−89.0)–(108.0)] [114]	−3.0 [(−108.0)–(106.0)] [113]	0.606[2]
TMT condition B, s [max. 240 s]			
Baseline (95% CI)	193.15 (60.64) [116]	195.64 (55.81) [115]	
Median [95% CI]	240 [(63.0)–(240.0)] [116]	234.0 [(64.0)–(240.0)] [115]	
Change baseline – Week 12	0.12 (43.21) [99]	−0.10 (40.56) [102]	
Median [range]	0.0 [(−140.0)–(128.0)] [99]	0.0 [(−116.0)–(166.0)] [102]	
Change baseline – Week 24	1.48 (40.35) [101]	−9.15 (41.70) [95]	
Median [range]	0.0 [(−96.0)–(165.0)] [101]	0.0 [(−120.0)–(160.0)] [95]	0.023[2]
Category fluency score			
Baseline	12.42 (5.55) [129]	12.16 (5.42) [128]	
Change baseline – Week 12	−0.12 (3.75) [120]	−0.62 (3.48) [121]	
Change baseline – Week 24	−0.38 (3.83) [120]	−0.73 (3.51) [116]	0.438[1]
24 week trajectory			0.459[1]
COWAT score			
Baseline	23.92 (10.99) [129]	25.90 (10.33) [128]	
Change baseline – Week 12	1.61 (5.92) [120]	0.60 (6.11) [121]	
Change baseline – Week 24	2.10 (5.98) [120]	0.01 (6.29) [117]	0.031[1]
24-week trajectory			0.087[1]

Supplementary Table 1
(*Continued*)

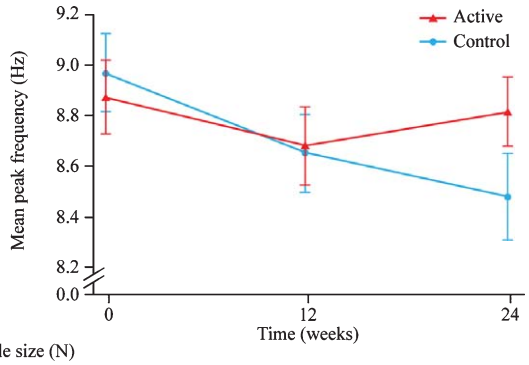
	Control ($n=129$)	Active ($n=130$)	p-value
ADAS-cog orientation task score [0–8]			
Baseline	1.16 (1.51) [129]	1.58 (1.68) [129]	
Median [range]	1.0 [(0.0)–(8.0)] [129]	1.0 [(0.0)–(8.0)] [129]	
Change baseline – Week 12	0.12 (1.27) [119]	−0.02 (1.55) [122]	
Median [range]	0.0 [(−6.0)–(3.0)] [119]	0.0 [(−8.0)–(4.0)] [122]	
Change baseline – Week 24	0.25 (1.42) [120]	0.15 (1.64) [117]	
Median [range]	0.0 [(−6.0)–(6.0)] [120]	0.0 [(−8.0)–(4.0)] [117]	0.724[2]
LDST score [0–125]			
Baseline	18.59 (8.45) [127]	18.38 (8.08) [125]	
Change baseline – Week 12	0.13 (3.89) [113]	0.25 (3.95) [114]	
Change baseline – Week 24	0.37 (4.10) [113]	−0.22 (4.92) [112]	0.235[1]
24-week trajectory			0.285[1]

NTB, Neuropsychological Test Battery; RAVLT, Rey Auditory Verbal Learning Test; WMS-VPA, Wechsler Memory Scale-Verbal Paired Associates Test; TMT, Trail-Making Test; COWAT, Controlled Oral Word Association Test; ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; LDST, Letter Digit Substitution Test; Data are presented as mean (standard deviation) [N], unless stated otherwise. [1]Mixed model for repeated measures with change from baseline as outcome, baseline as covariate. [2]Mann-Whitney U test.

Supplementary Table 2
Baseline demographics and characteristics of the subset of subjects for whom EEG data were available (intent-to-treat population)

	Control ($n=93$)	Active ($n=86$)
Male, *n* (%)	42 (54.5)	34 (51.5)
Age, y [range]	72.1 (7.8) [52–85]	73.8 (7.2) [55–87]
Body mass index, kg/m^2	26.5 (4.3)	25.8 (4.1)
Years of education beyond primary school	6.5 (4.6)	6.6 (4.9)
Duration of Alzheimer's disease since diagnosis, months, Median [range]	2.0 [0.0–88.0]	1.0 [0.0–38.0]
Apolipoprotein E ε4 carrier, *n* (%)		
No	31 (44.3)	29 (46.8)
Yes	39 (55.7)	33 (53.2)
Unknown	7	4

Data are presented as mean (standard deviation) unless stated otherwise.



Supplementary Figure 1. Mean Peak frequency during 24 weeks. Error bars represent standard errors. The difference in trajectories over time between active and control groups during the 24-week intervention period: $p=0.019$ (mixed model for repeated measures, 2 degrees of freedom contrast).

software, version 0.9.20, developed by Professor C.J. Stam (http://home.kpn.nl/stam7883/brainwave.html). Subsequently, relative and absolute power values were averaged over the four epochs (Brainwave software version 0.9.37) and over electrodes for six different brain areas (i.e., fronto-central, temporal, and parieto-occipital areas on the left and right side). Gamma band was not included in the analyses because of the possible admixture of muscle artifacts in this frequency band [1, 2]. The absolute power spectrum was used to appreciate whether differences in relative power between the groups were based on changes in absolute power or due to a shift of power between relative frequency bands. Peak frequency (i.e., the dominant frequency of the power spectrum) was determined for the parieto-occipital electrodes [3] as the median frequency between 4 and 13 Hz. Subsequently, peak frequency was averaged over the four epochs and over electrodes. Phase Lag Index (PLI) in the four frequency bands was calculated to measure functional connectivity between measurement points (EEG electrodes). PLI is a measure of the asymmetry of the distribution of phase differences between two signals [4]. For every electrode the PLI between that electrode and all other electrodes was measured and averaged. Mean PLI values were calculated for six brain areas (i.e., fronto-central, temporal, and parieto-occipital areas on the left and right side).

Supplementary Table 3
Descriptive statistics for peak frequency and Phase Lag Index (PLI) (intent-to-treat population)

	Control ($n = 93$)	Active ($n = 86$)	p-value[†]
Peak frequency (in Hz)			
Baseline	8.970 (1.348) [77]	8.876 (1.188) [66]	
Week 12	8.655 (1.327) [76]	8.685 (1.311) [73]	
Week 24	8.485 (1.461) [75]	8.818 (1.149) [70]	0.005
24-week trajectory			0.019
PLI*			
Delta (0.5–4 Hz)			
Baseline	0.131 (0.027) [77]	0.134 (0.031) [66]	
Week 12	0.127 (0.030) [76]	0.142 (0.036) [73]	
Week 24	0.131 (0.034) [75]	0.138 (0.036) [70]	0.776
24-week trajectory			0.011
Theta (4–8 Hz)			
Baseline	0.126 (0.046) [77]	0.131 (0.051) [66]	
Week 12	0.129 (0.051) [76]	0.132 (0.052) [73]	
Week 24	0.135 (0.051) [75]	0.132 (0.046) [70]	0.832
24-week trajectory			0.852
Alpha (8–13 Hz)			
Baseline	0.167 (0.085) [77]	0.179 (0.071) [66]	
Week 12	0.175 (0.084) [76]	0.169 (0.076) [73]	
Week 24	0.170 (0.084) [75]	0.171 (0.082) [70]	0.781
24-week trajectory			0.735
Beta (13–25 Hz)			
Baseline	0.069 (0.017) [77]	0.073 (0.017) [66]	
Week 12	0.069 (0.016) [76]	0.072 (0.018) [73]	
Week 24	0.070 (0.020) [75]	0.075 (0.018) [70]	0.099
24-week trajectory			0.203

Data are means (standard deviation) [N]. [†]EEG data at baseline were missing for 20% of the patients with Alzheimer's disease. Therefore, p-values were based on a mixed model for repeated measures (2 degrees of freedom contrast) with post-baseline, instead of change from baseline, measurements as an outcome. Baseline was still included as a covariate and the center's mean baseline value was imputed in case of missing baseline. All analyses included baseline and sample frequency as covariates. *For the statistical analysis, PLI was transformed using log(x/(1-x)). PLI values range from 0 to 1. Higher PLI values indicate increased connectivity.

SUPPLEMENTARY RESULTS

Neuropsychological Test Battery (NTB)

The results of all NTB individual items are summarized in supplementary Table 1.

EEG

Baseline demographics and characteristics of the subpopulation studied in the EEG analysis are presented in supplementary Table 2. The study groups were well matched with regard to all characteristics.

Descriptive statistics for peak frequency and PLI are given in supplementary Table 3. As shown in supplementary Figure 1, peak frequency slowed in the control group (indicative of cognitive deterioration[3]) and remained relatively stable in the active group.

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