Supplementary Material

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ALL** | | | **CLAD** | | | **ALEM** | | |
|  | **η²** | **p\_value** | **n** | **η²** | **p\_value** | **n** | **η²** | **p\_value** | **n** |
| **Accumbens** | 0,00222 | 0,65559 | 97 | 0,00002 | 0,97580 | 60 | 0,00795 | 0,62163 | 37 |
| **Amygdala** | 0,15657 | 0,00009 | 97 | 0,05963 | 0,06972 | 60 | 0,24923 | 0,00310 | 37 |
| **Pallidum** | 0,05384 | 0,02605 | 97 | 0,05413 | 0,08444 | 60 | 0,06511 | 0,15180 | 37 |
| **Cerebellar WM** | 0,02681 | 0,11885 | 97 | 0,06295 | 0,06217 | 60 | 0,00496 | 0,69702 | 37 |
| **Thalamus** | 0,02326 | 0,14665 | 97 | 0,07203 | 0,04551 | 60 | 0,00371 | 0,73616 | 37 |
| **Putamen** | 0,01765 | 0,20680 | 97 | 0,02659 | 0,22980 | 60 | 0,02001 | 0,43235 | 37 |
| **CC** | 0,01051 | 0,33081 | 97 | 0,00784 | 0,51651 | 60 | 0,03162 | 0,32213 | 37 |
| **VentralDC** | 0,00562 | 0,47722 | 97 | 0,02117 | 0,28463 | 60 | 0,00213 | 0,79883 | 37 |
| **CSF** | 0,00089 | 0,77800 | 97 | 0,00460 | 0,61950 | 60 | 0,00001 | 0,98697 | 37 |
| **Cortex** | 0,00050 | 0,83296 | 97 | 0,00011 | 0,93958 | 60 | 0,00004 | 0,97189 | 37 |
| **Total GM** | 1,59727 | 0,99046 | 97 | 8,24809 | 0,98324 | 60 | 0,00160 | 0,82523 | 37 |
| **DGM** | 0,00352 | 0,57433 | 97 | 0,06859 | 0,05120 | 60 | 0,11350 | 0,05522 | 37 |
| **Caudate** | 0,01297 | 0,27965 | 97 | 0,02688 | 0,22728 | 60 | 0,10269 | 0,06904 | 37 |
| **Cerebral WM** | 0,00182 | 0,68606 | 97 | 0,00047 | 0,87424 | 60 | 0,00586 | 0,67190 | 37 |
| **Total WM** | 0,00118 | 0,74537 | 97 | 0,00010 | 0,94142 | 60 | 0,00500 | 0,69579 | 37 |
| **Cerebrum** | 0,00408 | 0,54534 | 97 | 0,00083 | 0,83326 | 60 | 0,02289 | 0,40068 | 37 |
| **Hippocampus** | 0,00683 | 0,43340 | 97 | 0,05590 | 0,07937 | 60 | 0,08110 | 0,10819 | 37 |
| **Cerebellum** | 0,00027 | 0,87599 | 97 | 0,00810 | 0,50938 | 60 | 0,02633 | 0,36694 | 37 |

**Supplementary Table S1. Year-to-year correlations between Expanded Disability Status Scale (EDSS) changes and volume changes.**P-value and eta-squared (η²) were obtained from a linear model and are adjusted by sex, age, baseline EDSS, and number of previous disease modifying therapies (DMTs).  
Cerebellar WM - volume of cerebellar white matter, CC- corpus callosum, Ventral DC - ventral diencephalon, CSF - cerebrospinal fluid, Total GM - total volume of gray matter, DGM - volume of subcortical gray matter, Cerebral WM – volume of white matter of brain, Total WM – total white matter volume, η² - eta- squared

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| --- | --- | --- | --- |
|  | **p\_value** | **η²** | **levene** |
| **Accumbens** | 0,54995 | 0,01335 | 0,24379 |
| **Amygdala** | 0,00640 | 0,10733 | 0,64920 |
| **Pallidum** | 0,02904 | 0,07645 | 0,38506 |
| **Cerebellar WM** | 0,43801 | 0,01838 | 0,79384 |
| **Thalamus** | 0,04390 | 0,06783 | 0,09508 |
| **Putamen** | 0,32098 | 0,02521 | 0,10031 |
| **CC** | 0,08925 | 0,05285 | 0,06377 |
| **VentralDC** | 0,74349 | 0,00664 | 0,27336 |
| **CSF** | 0,96608 | 0,00076 | 0,66417 |
| **Cortex** | 0,92504 | 0,00175 | 0,54318 |
| **Total GM** | 0,83954 | 0,00392 | 0,34426 |
| **DGM** | 0,60995 | 0,01105 | 0,22322 |
| **Caudate** | 0,07896 | 0,05546 | 0,16519 |
| **Cerebral WM** | 0,60995 | 0,01105 | 0,22322 |
| **Total WM** | 0,92618 | 0,00172 | 0,48398 |
| **Cerebrum** | 0,90956 | 0,00213 | 0,21654 |
| **Hippocampus** | 0,35255 | 0,02316 | 0,04545 |
| **Cerebellum** | 0,78143 | 0,00553 | 0,24742 |

**Supplementary Table S2. Comparison of annual percentage volume changes in the amygdala, thalamus, and pallidum, grouped by annual Expanded Disability Status Scale (EDSS) change: regression(n = 10), progression (n = 11), and stabilization (n = 74).**The division into groups with regression or progression of EDSS was defined as for PIRA. P-values and effect sizes (eta-squared (η²)) were obtained from an ANCOVA-like linear model and are adjusted by sex, age, baseline EDSS, and number of previous disease modifying therapies (DMTs). The final column reports Levene’s test for equality of variances.Cerebellar WM - volume of cerebellar white matter, CC- corpus callosum, Ventral DC - ventral diencephalon, CSF - cerebrospinal fluid, Total GM - total volume of gray matter, DGM - volume of subcortical gray matter, Cerebral WM – volume of white matter of brain, Total WM – total white matter volume, η² - eta- squared

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|  | **ALL** | | | **CLAD** | | | **ALEM** | | |
|  | **slope** | **p\_value** | **n** | **slope** | **p\_value** | **n** | **slope** | **p\_value** | **n** |
| **Accumbens** | -0.004496 | 0.004729 | 16 | -0.003546 | 0.183391 | 9 | -0.004344 | 0.164171 | 7 |
| **Amygdala** | 0.007408 | 0.100005 | 16 | 0.004935 | 0.446728 | 9 | -0.001331 | 0.867344 | 7 |
| **Pallidum** | 0.014928 | 0.167143 | 16 | 0.023624 | 0.139916 | 9 | 0.013639 | 0.595771 | 7 |
| **Cerebellar WM** | -0.058299 | 0.125633 | 16 | -0.160321 | 0.016256 | 9 | -0.063693 | 0.335631 | 7 |
| **Thalamus** | -0.013744 | 0.536467 | 16 | -0.043677 | 0.214945 | 9 | 0.002264 | 0.964312 | 7 |
| **Putamen** | -0.011155 | 0.363053 | 16 | -0.034699 | 0.099525 | 9 | -0.006033 | 0.811994 | 7 |
| **CC** | -0.009781 | 0.553158 | 16 | -0.015668 | 0.540845 | 9 | -0.045309 | 0.165980 | 7 |
| **VentralDC** | 0.000305 | 0.948883 | 16 | -0.001881 | 0.854199 | 9 | -0.001860 | 0.806616 | 7 |
| **CSF** | 0.002572 | 0.662606 | 16 | 0.001493 | 0.843625 | 9 | -0.010955 | 0.373611 | 7 |
| **Cortex** | -0.855879 | 0.179017 | 16 | -0.842764 | 0.279751 | 9 | 0.633971 | 0.619418 | 7 |
| **Total GM** | -0.761140 | 0.247719 | 16 | -0.728934 | 0.426676 | 9 | 0.721831 | 0.570328 | 7 |
| **DGM** | 0.016306 | 0.668102 | 16 | -0.051790 | 0.347340 | 9 | 0.002519 | 0.975433 | 7 |
| **Caudate** | 0.011170 | 0.164590 | 16 | -0.001609 | 0.921645 | 9 | 0.025978 | 0.040177 | 7 |
| **Cerebral WM** | 1.251780 | 0.193973 | 16 | 1.088100 | 0.356498 | 9 | -0.367101 | 0.864465 | 7 |
| **Total WM** | 1.193481 | 0.223531 | 16 | 0.927779 | 0.439702 | 9 | -0.430793 | 0.843745 | 7 |
| **Cerebrum** | 0.501961 | 0.296687 | 16 | 0.193800 | 0.842137 | 9 | 0.467369 | 0.579103 | 7 |
| **Hippocampus** | 0.006905 | 0.454676 | 16 | 0.002023 | 0.698946 | 9 | -0.021714 | 0.219378 | 7 |
| **Cerebellum** | 0.025326 | 0.845889 | 16 | -0.063687 | 0.798938 | 9 | 0.043806 | 0.864135 | 7 |

**Supplementary Table S3.** Correlations between three-year changes in volume and changes in the Expanded Disability Status Scale (EDSS). Cerebellar WM - volume of cerebellar white matter, CC- corpus callosum, Ventral DC - ventral diencephalon, CSF - cerebrospinal fluid, Total GM - total volume of gray matter, DGM - volume of subcortical gray matter, Cerebral WM – volume of white matter of brain, Total WM – total white matter volume

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|  | **ALL** | | | **CLAD** | | | **ALEM** | | |
|  | **slope** | **p\_value** | **n** | **Slope** | **p\_value** | **n** | **slope** | **p\_value** | **n** |
| **Accumbens** | -0.002466 | 0.046096 | 28 | -0.001029 | 0.421473 | 18 | -0.003473 | 0.120290 | 10 |
| **Amygdala** | 0.005465 | 0.090801 | 28 | 0.004602 | 0.168752 | 18 | 0.005525 | 0.391266 | 10 |
| **Pallidum** | 0.012103 | 0.132616 | 28 | 0.007902 | 0.204525 | 18 | 0.017502 | 0.346771 | 10 |
| **Cerebellar WM** | -0.036641 | 0.380991 | 28 | 0.019432 | 0.748592 | 18 | -0.092244 | 0.141272 | 10 |
| **Thalamus** | -0.042300 | 0.001176 | 28 | -0.024319 | 0.108546 | 18 | -0.061922 | 0.011247 | 10 |
| **Putamen** | -0.014940 | 0.070088 | 28 | -0.011484 | 0.153036 | 18 | -0.019177 | 0.286711 | 10 |
| **CC** | -0.004993 | 0.640091 | 28 | 0.000116 | 0.992915 | 18 | -0.011209 | 0.578975 | 10 |
| **VentralDC** | -0.004301 | 0.330427 | 28 | -0.004213 | 0.423155 | 18 | -0.004070 | 0.638763 | 10 |
| **CSF** | -0.006114 | 0.122665 | 28 | 0.002865 | 0.499203 | 18 | -0.014801 | 0.051152 | 10 |
| **Cortex** | -0.235637 | 0.642249 | 28 | -0.001838 | 0.997374 | 18 | -0.288283 | 0.746902 | 10 |
| **Total GM** | -0.366282 | 0.472723 | 28 | -0.201192 | 0.726369 | 18 | -0.339244 | 0.692870 | 10 |
| **DGM** | -0.051146 | 0.000779 | 28 | -0.050201 | 0.010769 | 18 | -0.056734 | 0.019775 | 10 |
| **Caudate** | -0.004661 | 0.513289 | 28 | -0.019162 | 0.048056 | 18 | 0.008805 | 0.418455 | 10 |
| **Cerebral WM** | -0.125400 | 0.871434 | 28 | -0.290134 | 0.778805 | 18 | -0.157075 | 0.899350 | 10 |
| **Total WM** | -0.162041 | 0.837789 | 28 | -0.270702 | 0.795900 | 18 | -0.249320 | 0.846743 | 10 |
| **Cerebrum** | -0.331412 | 0.371918 | 28 | -0.391761 | 0.509458 | 18 | -0.268982 | 0.561471 | 10 |
| **Hippocampus** | -0.000274 | 0.954762 | 28 | 0.000146 | 0.979445 | 18 | -0.002600 | 0.732540 | 10 |
| **Cerebellum** | -0.108668 | 0.128709 | 28 | -0.142391 | 0.149708 | 18 | -0.061487 | 0.584041 | 10 |

**Supplementary Table S4.** Correlations between volume and Expanded Disability Status Scale (EDSS) changes in the first two years of observation. Cerebellar WM - volume of cerebellar white matter, CC- corpus callosum, Ventral DC - ventral diencephalon, CSF - cerebrospinal fluid, Total GM - total volume of gray matter, DGM - volume of subcortical gray matter, Cerebral WM – volume of white matter of brain, Total WM – total white matter volume

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| --- | --- | --- |
|  | **Sample size** | **η²** |
| **Accumbens** | 238 | 0.0133 |
| **Amygdala** | 28 | 0.107 |
| **Pallidum** | 40 | 0.076 |
| **Cerebellar WM** | 173 | 0.018 |
| **Thalamus** | 45 | 0.068 |
| **Putamen** | 125 | 0.025 |
| **CC** | 59 | 0.053 |
| **VentralDC** | 482 | 0.007 |
| **CSF** | 4141 | 0.001 |
| **Cortex** | 1833 | 0.002 |
| **Total GM** | 817 | 0.004 |
| **DGM** | 288 | 0.011 |
| **Caudate** | 55 | 0.055 |
| **Cerebral WM** | 2301 | 0.001 |
| **Total WM** | 1863 | 0.001 |
| **Cerebrum** | 1507 | 0.002 |
| **Hippocampus** | 136 | 0.023 |
| **Cerebellum** | 579 | 0.006 |

**Supplementary Table S5. Estimated number of study participants per group necessary to detect statistically significant differences, assuming the effect sizes observed in our study, using one-way analysis of variance with α = 0.05 and a power of 80%.**Cerebellar WM - volume of cerebellar white matter, CC- corpus callosum, Ventral DC - ventral diencephalon, CSF - cerebrospinal fluid, Total GM - total volume of gray matter, DGM - volume of subcortical gray matter, Cerebral WM – volume of white matter of brain, Total WM – total white matter volume, η² - eta- squared