**Supplementary Materials**

**Title:** Linking Neuroinflammation and Extracellular Free Water in HIV Infection: A Longitudinal Study

**Authors:** Md Nasir Uddin1,\*, Abrar Faiyaz2, Lu Wang3, Yuchuan Zhuang2, Kyle D Murray4, Maxime Descoteaux5, Madalina E. Tivarus6,7, Miriam T. Weber1, Jianhui Zhong4,6, Xing Qiu3, and Giovanni Schifitto1,6

**Affiliations:**

1Department of Neurology, University of Rochester, Rochester, NY, USA

2Department of Electrical and Computer Engineering, University of Rochester, Rochester, NY, USA

3Department of Biostatistics and Computational Biology, University of Rochester, Rochester, NY, USA

4Department of Physics and Astronomy, University of Rochester, Rochester, NY, USA

5Department of Computer Science, Université de Sherbrooke, QC, Canada

6Department of Imaging Sciences, University of Rochester, Rochester, NY, USA

7Department of Neuroscience, University of Rochester, Rochester, NY, USA

***Table S1: Mean and standard error (SE) of FW index (%) for 25 brain structures in HIV+ and HIV- participants at baseline***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Structure** | **HIV+**  | **HIV-**  | **t-value** | **p-value** | **p-value****(Adjusted)** |
| **mean** | **SE** | **mean** | **SE** |
| FMaj | 7.61 | 0.35 | 7.48 | 0.34 | 0.276 | 0.784 | 0.948 |
| FMin | 4.11 | 0.17 | 3.79 | 0.12 | 1.548 | 0.126 | 0.788 |
| GCC | 11.34 | 0.50 | 10.44 | 0.39 | 1.411 | 0.162 | 0.812 |
| SCC | 9.92 | 0.30 | 9.95 | 0.27 | -0.082 | 0.935 | 0.948 |
| TH | 14.73 | 0.50 | 13.27 | 0.49 | 2.093 | **0.039** | 0.328 |
| CN | 11.46 | 0.87 | 10.5 | 0.77 | 0.826 | 0.412 | 0.928 |
| PUT | 0.65 | 0.06 | 0.68 | 0.05 | -0.376 | 0.708 | 0.948 |
| GP | 0.48 | 0.07 | 0.61 | 0.14 | -0.835 | 0.406 | 0.928 |
| Amyg | 18.30 | 0.50 | 15.29 | 0.5 | 4.239 | **<0.001** | **0.002** |
| AccN | 1.87 | 0.29 | 1.79 | 0.23 | 0.226 | 0.822 | 0.948 |
| ATR | 2.83 | 0.14 | 2.75 | 0.13 | 0.394 | 0.695 | 0.948 |
| CST | 2.26 | 0.10 | 2.34 | 0.09 | -0.652 | 0.516 | 0.928 |
| SLF | 1.48 | 0.14 | 1.46 | 0.12 | 0.127 | 0.900 | 0.948 |
| ALIC | 0.14 | 0.02 | 0.16 | 0.02 | -0.661 | 0.511 | 0.928 |
| PLIC | 0.07 | 0.01 | 0.05 | 0.01 | 0.906 | 0.369 | 0.928 |
| PTR | 3.86 | 0.22 | 3.83 | 0.21 | 0.104 | 0.917 | 0.948 |
| EC | 0.37 | 0.04 | 0.35 | 0.04 | 0.213 | 0.832 | 0.948 |
| HC | 21.5 | 0.75 | 19.14 | 0.61 | 2.444 | **0.017** | 0.211 |
| CP | 6.97 | 0.30 | 6.81 | 0.27 | 0.376 | 0.708 | 0.948 |
| ACR | 0.11 | 0.03 | 0.11 | 0.03 | 0.066 | 0.948 | 0.948 |
| SCR | 0.03 | 0.01 | 0.04 | 0.02 | -0.232 | 0.817 | 0.948 |
| RLIC | 0.60 | 0.08 | 0.51 | 0.04 | 1.119 | 0.268 | 0.928 |
| SFOF | 0.23 | 0.08 | 0.17 | 0.05 | 0.648 | 0.519 | 0.928 |
| CG | 0.76 | 0.05 | 0.69 | 0.05 | 1.117 | 0.267 | 0.928 |
| FX | 46.46 | 1.55 | 44.68 | 1.35 | 0.867 | 0.389 | 0.928 |

Note: Significant p-values are shown as bold. GP: Globus Pallidus; PUT: Putamen; CN: Caudate Nucleus; TH: Thalamus; Hippo: Hippocampus; Amyg: Amygdala; AccN: Accumbens Nucleus; SCC: Splenium of Corpus Callosum; GCC: Genu of Corpus Callosum; CST: Corticospinal Tract; ATR: Anterior Thalamic Radiation; PTR: Posterior Thalamic Radiation; ALIC: Anterior Limbic Internal Capsule; PLIC: Posterior Limbic Internal Capsule; EC: External Capsule; SLF: Superior Longitudinal Fasciculus; FMaj: Forceps Major; FMin: Forceps Minor; CP: Cerebral Peduncle; ACR: Anterior Corona Radiata; SCR: Superior Corona Radiata; RLIC: Retrolenticular part of Internal Capsule; SFOF: Superior Fronto-Occipital Fasciculus; CG: Cingulum and FX: Fornix. FW values were averaged over bilateral ROIs (except some WM tracts) for each participant.

***Table S2: Mean and standard error (SE) of FW index (%) for 25 brain structures at baseline and 12 weeks after the cART treatment in HIV+ participants***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Structure** | **BSL** | **W12**  | **t-value** | **p-value** | **p-value****(Adjusted)** |
| **mean** | **SE** | **mean** | **SE** |
| FMaj | 7.40 | 0.36 | 7.00 | 0.28 | 1.771 | 0.087 | 0.241 |
| Fmin | 4.14 | 0.19 | 3.80 | 0.22 | 2.328 | **0.027** | 0.096 |
| GCC | 11.37 | 0.56 | 9.96 | 0.55 | 3.417 | **0.002** | **0.015** |
| SCC | 9.83 | 0.33 | 9.79 | 0.43 | 0.155 | 0.878 | 0.878 |
| TH | 14.9 | 0.57 | 13.2 | 0.68 | 3.767 | **0.001** | **0.009** |
| CN | 11.58 | 1.00 | 10.31 | 0.91 | 2.776 | **0.009** | **0.039** |
| PUT | 0.67 | 0.07 | 0.70 | 0.09 | -0.442 | 0.662 | 0.790 |
| GP | 0.49 | 0.08 | 0.52 | 0.12 | -0.248 | 0.806 | 0.876 |
| Amyg | 18.54 | 0.56 | 15.42 | 0.67 | 4.865 | **<0.001** | **0.001** |
| AccN | 1.95 | 0.35 | 1.64 | 0.25 | 1.594 | 0.121 | 0.304 |
| ATR | 2.86 | 0.16 | 2.74 | 0.18 | 1.388 | 0.175 | 0.365 |
| CST | 2.26 | 0.12 | 2.21 | 0.13 | 0.620 | 0.540 | 0.790 |
| SLF | 1.53 | 0.16 | 1.48 | 0.18 | 0.672 | 0.507 | 0.790 |
| ALIC | 0.13 | 0.02 | 0.14 | 0.02 | -0.545 | 0.590 | 0.790 |
| PLIC | 0.06 | 0.01 | 0.04 | 0.01 | 1.455 | 0.156 | 0.355 |
| PTR | 3.73 | 0.23 | 3.28 | 0.23 | 2.971 | **0.006** | **0.034** |
| EC | 0.37 | 0.05 | 0.36 | 0.05 | 0.191 | 0.849 | 0.878 |
| HC | 22.01 | 0.85 | 20.62 | 0.84 | 2.902 | **0.007** | **0.034** |
| CP | 6.91 | 0.34 | 6.84 | 0.39 | 0.254 | 0.801 | 0.876 |
| ACR | 0.11 | 0.03 | 0.10 | 0.02 | 0.439 | 0.664 | 0.790 |
| SCR | 0.03 | 0.01 | 0.03 | 0.01 | 0.458 | 0.650 | 0.790 |
| RLIC | 0.60 | 0.09 | 0.44 | 0.04 | 2.031 | 0.051 | 0.160 |
| SFOF | 0.24 | 0.1 | 0.21 | 0.05 | 0.525 | 0.603 | 0.790 |
| CG | 0.74 | 0.05 | 0.68 | 0.06 | 1.159 | 0.256 | 0.491 |
| FX | 46.72 | 1.73 | 46.2 | 1.84 | 0.610 | 0.547 | 0.790 |

Note: Significant p-values are shown as bold. GP: Globus Pallidus; PUT: Putamen; CN: Caudate Nucleus; TH: Thalamus; Hippo: Hippocampus; Amyg: Amygdala; AccN: Accumbens Nucleus; SCC: Splenium of Corpus Callosum; GCC: Genu of Corpus Callosum; CST: Corticospinal Tract; ATR: Anterior Thalamic Radiation; PTR: Posterior Thalamic Radiation; ALIC: Anterior Limbic Internal Capsule; PLIC: Posterior Limbic Internal Capsule; EC: External Capsule; SLF: Superior Longitudinal Fasciculus; FMaj: Forceps Major; FMin: Forceps Minor; CP: Cerebral Peduncle; ACR: Anterior Corona Radiata; SCR: Superior Corona Radiata; RLIC: Retrolenticular part of Internal Capsule; SFOF: Superior Fronto-Occipital Fasciculus; CG: Cingulum and FX: Fornix. FW values were averaged over bilateral ROIs (except some WM tracts) for each participant.

***Table S3: Cohort and 12 weeks of cART treatment effects in free water (FW) based on the short-term model (STM) for 25 brain structures.***

|  |  |  |
| --- | --- | --- |
| **Structure** | **ß ×10-3** | **p-value** |
| **Cohort** | **Visit** | **Age** | **Cohort** | **Visit** | **Age** |
| FMaj | 0.30 | -4.50 | -0.10 | 0.946 | 0.052 | 0.730 |
| Fmin | 4.10 | -3.70 | 0.30 | 0.054 | **0.012** | **0.001** |
| GCC | 10.90 | -15.20 | 0.70 | 0.067 | **<0.001** | **0.002** |
| SCC | 1.40 | -0.60 | 0.30 | 0.738 | 0.807 | **0.048** |
| TH | 20.90 | -17.10 | 1.40 | **0.001** | **<0.001** | **<0.001** |
| CN | 22.40 | -12.50 | 2.40 | **0.020** | **0.009** | **<0.001** |
| PUT | 0.40 | 0.20 | 0.20 | 0.631 | 0.660 | **<0.001** |
| GP | -0.40 | 0.00 | 0.30 | 0.815 | 0.997 | **<0.001** |
| Amyg | 37.80 | -27.60 | 1.30 | **<0.001** | **<0.001** | **<0.001** |
| AccN | 3.00 | -3.40 | 0.60 | 0.318 | 0.085 | **<0.001** |
| ATR | 2.50 | -1.30 | 0.40 | 0.114 | 0.140 | **<0.001** |
| CST | 1.70 | 0.00 | 0.20 | 0.326 | 0.987 | **0.005** |
| SLF | 2.20 | -0.50 | 0.40 | 0.155 | 0.532 | **<0.001** |
| ALIC | 0.30 | 0.10 | 0.10 | 0.406 | 0.493 | **<0.001** |
| PLIC | 0.30 | -0.10 | 0.00 | 0.063 | 0.194 | **0.047** |
| PTR | 1.80 | -4.40 | 0.10 | 0.562 | **0.006** | 0.467 |
| EC | 0.80 | 0.00 | 0.10 | 0.125 | 0.896 | **<0.001** |
| HC | 30.00 | -13.60 | 1.60 | **0.001** | **0.007** | **<0.001** |
| CP | 10.00 | 0.70 | 0.40 | 0.083 | 0.780 | 0.088 |
| ACR | 0.20 | -0.20 | 0.00 | 0.483 | 0.506 | **<0.001** |
| SCR | 0.00 | -0.10 | 0.00 | 0.960 | 0.555 | **0.015** |
| RLIC | 1.60 | -1.00 | 0.10 | **0.047** | 0.174 | **0.004** |
| SFOF | 1.00 | -0.40 | 0.10 | 0.178 | 0.528 | **0.001** |
| CG | 3.40 | -0.30 | 0.20 | **0.008** | 0.561 | **<0.001** |
| FX | 33.00 | -6.30 | 3.90 | 0.060 | 0.462 | **<0.001** |

Note: Significant p-values are shown as bold. GP: Globus Pallidus; PUT: Putamen; CN: Caudate Nucleus; TH: Thalamus; Hippo: Hippocampus; Amyg: Amygdala; AccN: Accumbens Nucleus; SCC: Splenium of Corpus Callosum; GCC: Genu of Corpus Callosum; CST: Corticospinal Tract; ATR: Anterior Thalamic Radiation; PTR: Posterior Thalamic Radiation; ALIC: Anterior Limbic Internal Capsule; PLIC: Posterior Limbic Internal Capsule; EC: External Capsule; SLF: Superior Longitudinal Fasciculus; FMaj: Forceps Major; FMin: Forceps Minor; CP: Cerebral Peduncle; ACR: Anterior Corona Radiata; SCR: Superior Corona Radiata; RLIC: Retrolenticular part of Internal Capsule; SFOF: Superior Fronto-Occipital Fasciculus; CG: Cingulum and FX: Fornix. FW values were averaged over bilateral ROIs (except some WM tracts) for each participant

***Table S4: Cohort and long-term cART treatment effects in free water (FW) based on the long-term model (LTM) for 25 brain structures.***

|  |  |  |  |
| --- | --- | --- | --- |
| **Structure** | **ß ×10-3** |  | **p-value** |
| **Cohort** | **Visit** | **Age** | **Cohort.Visit** | **Cohort** | **Visit** | **Age** | **Cohort.Visit** |  |
| FMaj | -5.80 | 1.60 | 0.00 | 3.30 | 0.215 | 0.080 | 0.911 | **0.038** |
| FMin | 0.20 | 1.00 | 0.20 | -0.80 | 0.919 | 0.067 | **0.025** | 0.426 |
| GCC | -4.20 | 4.90 | 0.50 | 1.70 | 0.468 | **0.003** | **0.014** | 0.533 |
| SCC | 0.10 | 2.10 | 0.30 | -0.40 | 0.973 | 0.054 | 0.071 | 0.820 |
| TH | 3.00 | 7.90 | 1.30 | -0.20 | 0.628 | **<0.001** | **<0.001** | 0.952 |
| CN | 9.50 | 9.00 | 2.30 | -0.70 | 0.352 | **<0.001** | **<0.001** | 0.833 |
| PUT | 0.50 | 0.00 | 0.10 | 0.00 | 0.591 | 0.817 | **<0.001** | 0.899 |
| GP | -0.70 | -0.10 | 0.20 | -0.10 | 0.622 | 0.894 | **<0.001** | 0.917 |
| Amyg | 7.60 | 16.60 | 1.10 | -0.80 | 0.231 | **<0.001** | **<0.001** | 0.853 |
| AccN | 0.00 | -1.00 | 0.50 | 3.90 | 0.999 | 0.373 | **<0.001** | **0.034** |
| ATR | 1.00 | 0.30 | 0.40 | -0.50 | 0.529 | 0.423 | **<0.001** | 0.432 |
| CST | 1.50 | 1.30 | 0.20 | -0.70 | 0.413 | **0.001** | **0.015** | 0.273 |
| SLF | 1.80 | 0.80 | 0.40 | -1.20 | 0.296 | **0.009** | **<0.001** | **0.029** |
| ALIC | 0.40 | 0.50 | 0.10 | -0.30 | 0.315 | **0.001** | **<0.001** | 0.349 |
| PLIC | 0.20 | 0.20 | 0.00 | 0.00 | 0.317 | **0.008** | **0.044** | 0.721 |
| PTR | -2.90 | 2.10 | 0.10 | 1.90 | 0.375 | **0.010** | 0.659 | 0.180 |
| EC | 0.70 | 0.50 | 0.10 | -0.30 | 0.198 | **0.002** | **<0.001** | 0.233 |
| HC | 16.60 | 5.50 | 1.50 | 0.40 | 0.077 | **<0.001** | **<0.001** | 0.862 |
| CP | 9.60 | 3.90 | 0.20 | -4.90 | 0.086 | **0.036** | 0.220 | 0.119 |
| ACR | 0.00 | 0.10 | 0.00 | 0.10 | 0.889 | **0.045** | **0.006** | 0.593 |
| SCR | -0.10 | 0.00 | 0.00 | 0.00 | 0.772 | 0.072 | **0.045** | 0.991 |
| RLIC | 0.90 | 1.10 | 0.10 | -0.10 | 0.295 | **<0.001** | **0.022** | 0.836 |
| SFOF | 0.70 | 0.30 | 0.10 | -0.40 | 0.278 | **0.022** | **0.003** | 0.110 |
| CG | 3.10 | 1.50 | 0.20 | -1.50 | **0.015** | **<0.001** | **0.001** | **0.029** |
| FX | 23.40 | 2.70 | 3.90 | -8.60 | 0.230 | 0.603 | **<0.001** | 0.329 |

Note: Significant p-values are shown as bold. GP: Globus Pallidus; PUT: Putamen; CN: Caudate Nucleus; TH: Thalamus; Hippo: Hippocampus; Amyg: Amygdala; AccN: Accumbens Nucleus; SCC: Splenium of Corpus Callosum; GCC: Genu of Corpus Callosum; CST: Corticospinal Tract; ATR: Anterior Thalamic Radiation; PTR: Posterior Thalamic Radiation; ALIC: Anterior Limbic Internal Capsule; PLIC: Posterior Limbic Internal Capsule; EC: External Capsule; SLF: Superior Longitudinal Fasciculus; FMaj: Forceps Major; FMin: Forceps Minor; CP: Cerebral Peduncle; ACR: Anterior Corona Radiata; SCR: Superior Corona Radiata; RLIC: Retrolenticular part of Internal Capsule; SFOF: Superior Fronto-Occipital Fasciculus; CG: Cingulum and FX: Fornix. FW values were averaged over bilateral ROIs (except some WM tracts) for each participant.

***Table S5: Pearson Correlations for FW vs. NfL, FW vs. CD4 counts and FW vs. VL at baseline for 25 brain structures in HIV+ cohort***

|  |  |  |  |
| --- | --- | --- | --- |
| **Structure** | **NfL** | **CD4 counts**  | **VL** |
| **ß** | **p-value** | **ß** | **p-value** | **ß** | **p-value** |
| FMaj | 0.045 | 0.797 | -0.204 | 0.227 | -0.072 | 0.688 |
| FMin | 0.479 | **0.004** | -0.314 | 0.058 | 0.183 | 0.301 |
| GCC | 0.462 | **0.005** | -0.256 | 0.127 | 0.100 | 0.575 |
| SCC | 0.438 | **0.008** | -0.334 | **0.043** | 0.029 | 0.872 |
| TH | 0.621 | **<0.001** | -0.351 | **0.033** | -0.042 | 0.812 |
| CN | 0.503 | **0.002** | -0.372 | **0.023** | -0.104 | 0.558 |
| PUT | 0.415 | **0.013** | -0.434 | **0.007** | 0.022 | 0.902 |
| GP | 0.366 | **0.031** | -0.428 | **0.008** | -0.058 | 0.746 |
| Amyg | 0.656 | **<0.001** | -0.318 | 0.055 | 0.056 | 0.751 |
| AccN | 0.564 | **<0.001** | -0.390 | **0.017** | -0.032 | 0.857 |
| ATR | 0.627 | **<0.001** | -0.339 | **0.040** | -0.075 | 0.673 |
| CST | 0.455 | **0.006** | -0.464 | **0.004** | -0.105 | 0.555 |
| SLF | 0.474 | **0.004** | -0.356 | **0.031** | 0.057 | 0.748 |
| ALIC | 0.536 | **0.001** | -0.368 | **0.025** | -0.210 | 0.233 |
| PLIC | 0.023 | 0.894 | -0.076 | 0.653 | -0.198 | 0.262 |
| PTR | 0.174 | 0.318 | -0.101 | 0.553 | -0.004 | 0.984 |
| EC | 0.325 | 0.057 | -0.206 | 0.222 | -0.189 | 0.285 |
| HC | 0.696 | **<0.001** | -0.378 | **0.021** | 0.025 | 0.890 |
| CP | 0.062 | 0.722 | -0.182 | 0.282 | -0.002 | 0.993 |
| ACR | 0.567 | **<0.001** | -0.358 | **0.030** | 0.009 | 0.959 |
| SCR | 0.595 | **<0.001** | -0.357 | **0.030** | -0.085 | 0.634 |
| RLIC | 0.572 | **<0.001** | -0.251 | 0.134 | -0.117 | 0.510 |
| SFOF | 0.495 | **0.002** | -0.253 | 0.130 | 0.037 | 0.837 |
| CG | 0.214 | 0.217 | -0.234 | 0.164 | -0.184 | 0.297 |
| FX | 0.491 | **0.003** | -0.407 | **0.012** | -0.104 | 0.559 |

Note: Significant p-values are shown as bold. NfL: Neurofilament llight chain; CD4: Cluster of differentiation 4; VL Viral Loads. GP: Globus Pallidus; PUT: Putamen; CN: Caudate Nucleus; TH: Thalamus; Hippo: Hippocampus; Amyg: Amygdala; AccN: Accumbens Nucleus; SCC: Splenium of Corpus Callosum; GCC: Genu of Corpus Callosum; CST: Corticospinal Tract; ATR: Anterior Thalamic Radiation; PTR: Posterior Thalamic Radiation; ALIC: Anterior Limbic Internal Capsule; PLIC: Posterior Limbic Internal Capsule; EC: External Capsule; SLF: Superior Longitudinal Fasciculus; FMaj: Forceps Major; FMin: Forceps Minor; CP: Cerebral Peduncle; ACR: Anterior Corona Radiata; SCR: Superior Corona Radiata; RLIC: Retrolenticular part of Internal Capsule; SFOF: Superior Fronto-Occipital Fasciculus; CG: Cingulum and FX: Fornix. FW values were averaged over bilateral ROIs (except some WM tracts) for each participant.