**Supplemental Material**

**Appendix e-1**

**Gradient non-linearity correction**

Gradient non-linearities, due to imperfect gradient coil hardware, can cause image distortion. The effect increases with distance from the scanner isocenter and can cause severe geometric and intensity inaccuracies for structures at the periphery of the imaging FOV. This particularly applies to the spinal cord area, when the FOV is centered over the subject’s head, as is the case for the HCP structural images. Distortion levels depend on the gradient system, and the gradient coil insert of the HCP Skyra scanner exhibits strong non-linearities that cause displacements on the order of millimeters in the area of the spinal cord (Papinutto et al., 2017). These non-linearities can further lead to signal rarefaction or pile up in the voxels. Hence, images need to be undistorted to guarantee a reliable segmentation.

To correct the images for gradient non-linearity induced distortion we applied the *gradient\_unwarp.py* script which is part of the HCP’s *minimal processing pipeline* (Glasser et al., 2013),version 3.25.0, downloaded from the HCP github repository. This algorithm uses spherical harmonics to approximate the magnetic fields created by the gradient coils, given a vendor-provided gradient coefficient file for the specific system(Jovicich et al., 2006). A transformation warp file is then generated and applied to shift voxels and further adjust their intensity values. Of note, the gradient corrected structural MRI data made available by HCP is truncated and does not encompass the full FOV.

**Impact of gradient field nonlinearity correction**

To evaluate the effects of correcting gradient field non-linearity, we segmented the spinal cord using identical methods in 240 subjects before and after applying the correction. The correction led to an average increase in C2 CSA of 13.6 mm2 (range -0.4 to 27.4 mm2), an average increase in APW of 0.8 mm (range -1.6 to 1.5 mm), and an average increase in LRW of 1.1 mm (range 0.1 to 2.4 mm).

The correction led to an increase in test-retest reliability, from 0.70 to 0.75 for CSA, and, notably, to an increase in the estimate of broad-sense heritability, from 0.40 to 0.91 for CSA; from 0.66 to 0.86 for APW, and from 0.59 to 0.85 for LRW.

**Test – Retest data**

Initially, the measurements of C2 CSA, LRW and APW from both sessions were tested for normality with a D’Agostino & Pearson normality test. Any significant differences in C2 CSA, LRW and APW between the test and the retest sample were assessed using a paired samples t-test. An intraclass correlation (ICC) analysis was carried out as a measure of reproducibility. A Pearson correlation was also conducted to examine how closely the two datasets correlated with each other in each of the measurements.

**Results**

**Test - Retest data**

The test-retest C2 CSA data measurements had an ICC score of 0.75 (CI: 0.56 – 0.86), which is considered a good to excellent level of reproducibility. APW had an ICC of 0.92 (CI: 0.86 – 0.96), and the LRW had an ICC of 0.89 (CI: 0.80 – 0.94).

Paired t-tests revealed no significant difference between the test and the retest measurements of C2 CSA. Pearson correlation tests showed that all test-retest measurements were significantly correlated (see Supplementary Table e-3 and Supplemental Figure e-3 for an illustration.)

**References**

Glasser, M. F., Sotiropoulos, S. N., Wilson, J. A., Coalson, T. S., Fischl, B., Andersson, J. L., … Jenkinson, M. (2013). The minimal preprocessing pipelines for the Human Connectome Project. *NeuroImage*, *80*, 105–124. https://doi.org/10.1016/j.neuroimage.2013.04.127

Jovicich, J., Czanner, S., Greve, D., Haley, E., Van Der Kouwe, A., Gollub, R., … Dale, A. (2006). Reliability in multi-site structural MRI studies: Effects of gradient non-linearity correction on phantom and human data. *NeuroImage*, *30*(2), 436–443. https://doi.org/10.1016/j.neuroimage.2005.09.046

Papinutto, N., Bakshi, R., Bischof, A., Calabresi, P. A., Caverzasi, E., Constable, R. T., … Henry, R. G. (2017). Gradient nonlinearity effects on upper cervical spinal cord area measurement from 3D T 1 -weighted brain MRI acquisitions . *Magnetic Resonance in Medicine*, *79*(3), 1595–1601. https://doi.org/10.1002/mrm.26776

**Supplementary material**

**Figures**



**Figure e-1**: **Relationship between CSA, LRW and APW**. A Pearson correlation analysis found C2 CSA values significantly correlated with the spinal cord diameter in the A-P and R-L directions. CSA, cross sectional area; A-P, anterior – posterior; R-L, right – left.



**Figure e-2: Relationship between LRW and APW**. A Pearson correlation analyses established no significant correlation between LRW and APW, indicating that the two measures are independent from each other. A-P, anterior – posterior; R-L, right – left.

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**Figure e-3: Graphical representation of test-retest measurements.** A paired sample t-test was carried out to assess significant differences between measurements in C2 CSA, APW and LRW , shown in panels **a)**, **b)** and **c)** respectively. A Pearson correlation analysis was conducted to examine correlations of measurements of C2 thickness (shown in panel **d)**), APW (panel **e)**), and LRW (panel **f)**). CSA, cross sectional area; A-P, anterior – posterior; R-L, right – left.

**Supplementary material**

**Tables**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Paired t-test | | Pearson correlation | |
|  | Test (sd) | Retest (sd) | p-value | Δ | r | p-value |
| C2 CSA | 69.98 (±4.00) | 69.15 (±4.53) | 0.106 | -0.824 | 0.763 | <0.0001\* |
| APW | 7.92 (±0.42) | 7.90 (±0.41) | 0.322 | -0.027 | 0.924 | <0.0001\* |
| LRW | 11.31 (±0.59) | 11.24 (±0.57) | 0.165 | -0.064 | 0.892 | <0.0001\* |

**Table e-1:** Spinal cord metrics, paired t-test and Pearson correlation of the test and retest dataset. CSA, cross sectional area; APW anterior – posterior width; LRW, left– right width.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | CSA | | | APW | | | LRW | | |
|  | **R square** | **** | **p-value** | **R square** | **** | **p-value** | **R square** | **** | **p-value** |
| **General Physical Measures** | | |  |  |  |  |  |  |  |
| Height | 0.068 | 0.307 | 0.022704819 | 0.066 | -0.001 | 0.946365335 | 0.048 | 0.054 | 0.000268234 |
| Within pair  Height |  | -0.036 | 0.85643513 |  | -0.006 | 0.723802057 |  | 0.005 | 0.825905257 |
| Gender |  | -1.299 | 0.159990398 |  | -0.271 | 0.0008761 |  | 0.195 | 0.051789553 |
| Weight | 0.052 | 0.001 | 0.891955973 | 0.068 | -0.001 | 0.459451556 | 0.008 | 0.001 | 0.238393413 |
| Within pair  Weight |  | -0.008 | 0.514604959 |  | 0.000 | 0.706330959 |  | -0.001 | 0.487905139 |
| Gender |  | -2.662 | 0.00033426 |  | -0.282 | 1.31048E-05 |  | -0.020 | 0.806890097 |
| BMI | 0.054 | -0.058 | 0.382279845 | 0.067 | -0.004 | 0.453925085 | 0.004 | -0.003 | 0.71857795 |
| Within pair  BMI |  | -0.049 | 0.517399456 |  | -0.002 | 0.77733211 |  | -0.007 | 0.40996353 |
| Gender |  | -2.679 | 0.00015678 |  | -0.267 | 1.53866E-05 |  | -0.047 | 0.539362749 |
| Brain Volume | 0.212 | 0.000 | 1.72152E-12 | 0.150 | 0.000 | 3.3893E-07 | 0.066 | 0.000 | 4.20367E-05 |
| Within pair  Brain V. |  | 0.000 | 0.094242544 |  | 0.000 | 0.422605085 |  | 0.000 | 0.16623337 |
| Gender |  | 0.924 | 0.251330281 |  | -0.036 | 0.624371721 |  | 0.190 | 0.043934926 |
| **Motor Behaviors** | |  |  |  |  |  |  |  |  |
| Grip strength | 0.019 | 0.045 | 0.020487631 | 0.006 | 0.002 | 0.227860055 | 0.016 | 0.004 | 0.037803413 |
| Within pair  Grip strength |  | -0.001 | 0.971729334 |  | -0.001 | 0.640586034 |  | 0.002 | 0.571838912 |
| Dexterity | 0.000 | -0.001 | 0.988066062 | 0.001 | -0.002 | 0.550861173 | 0.002 | 0.003 | 0.493501751 |
| Within pair  Dexterity |  | 0.001 | 0.971320746 |  | -0.001 | 0.836794808 |  | 0.001 | 0.800058695 |
| Gait speed | 0.004 | -2.383 | 0.270420266 | 0.002 | -0.135 | 0.473678782 | 0.004 | -0.220 | 0.343274562 |
| Within pair  Gait speed |  | -0.068 | 0.967741957 |  | -0.053 | 0.719093258 |  | 0.074 | 0.685038748 |
| Reaction Time | 0.006 | 0.004 | 0.231126797 | 0.014 | 0.001 | 0.053244639 | 0.001 | 0.000 | 0.732146404 |
| Within pair  Reaction Time |  | -0.001 | 0.76627387 |  | 0.000 | 0.864625483 |  | 0.000 | 0.644634853 |
| Endurance | 0.007 | 0.036 | 0.182652702 | 0.007 | 0.003 | 0.160092825 | 0.001 | 0.001 | 0.855016152 |
| Within pair  Enducrance |  | -0.014 | 0.667991282 |  | 0.000 | 0.91189027 |  | -0.001 | 0.66868442 |
| **Regional brain metrics** | |  |  |  |  |  |  |  |  |
| Precentral Area. | 0.229 | -0.002 | 0.053700117 | 0.154 | 0.000 | 0.510636733 | 0.078 | 0.000 | 0.083174722 |
| Within pair  Precentral A. |  | -0.002 | 0.045711931 |  | 0.000 | 0.180418061 |  | 0.000 | 0.285959778 |
| Whole brain volume |  | 0.000 | 4.73449E-11 |  | 0.000 | 3.26444E-05 |  | 0.000 | 1.61705E-05 |
| Gender |  | 0.651 | 0.413861942 |  | -0.050 | 0.496989091 |  | 0.168 | 0.072712166 |
| Postcentral Area | 0.228 | -0.003 | 0.034477596 | 0.158 | 0.000 | 0.228576188 | 0.072 | 0.000 | 0.213299477 |
| Within pair  Postcentral A. |  | -0.002 | 0.075627936 |  | 0.000 | 0.095923021 |  | 0.000 | 0.539200248 |
| Whole brain volume |  | 0.000 | 1.76412E-11 |  | 0.000 | 3.84799E-06 |  | 0.000 | 7.8863E-05 |
| Gender |  | 0.710 | 0.371394606 |  | -0.048 | 0.507439849 |  | 0.179 | 0.056579808 |
| Thalamus Volume | 0.237 | 0.002 | 0.005114386 | 0.169 | 0.000 | 0.014962135 | 0.070 | 0.000 | 0.304107068 |
| Within pair  Thalamus V. |  | 0.001 | 0.05836178 |  | 0.000 | 0.104690428 |  | 0.000 | 0.479316326 |
| Whole brain volume |  | 0.000 | 0.00426729 |  | 0.000 | 0.115361459 |  | 0.000 | 0.034559319 |
| Gender |  | 0.563 | 0.472463176 |  | -0.067 | 0.34585753 |  | 0.179 | 0.054417664 |
| Cerebellum Volume | 0.211 | 0.000 | 0.955201337 | 0.149 | 0.000 | 0.570028077 | 0.067 | 0.000 | 0.622242852 |
| Within pair  Cerebellum V. |  | 0.000 | 0.740809784 |  | 0.000 | 0.835502356 |  | 0.000 | 0.989874994 |
| Whole brain volume |  | 0.000 | 3.9985E-10 |  | 0.000 | 3.68716E-06 |  | 0.000 | 0.000410702 |
| Gender |  | 0.746 | 0.369744165 |  | -0.064 | 0.394704975 |  | 0.203 | 0.036789757 |
| Occipital Area | 0.240 | -0.003 | 0.001488242 | 0.229 | -0.002 | 0.053700117 | 0.228 | -0.003 | 0.034477596 |
| Within pair  Occipital A. |  | -0.001 | 0.240287365 |  | -0.002 | 0.045711931 |  | -0.002 | 0.075627936 |
| Whole brain volume |  | 0.000 | 2.93439E-14 |  | 0.000 | 4.73449E-11 |  | 0.000 | 1.76412E-11 |
| Gender |  | 0.534 | 0.497141138 |  | 0.651 | 0.413861942 |  | 0.710 | 0.371394606 |

**Table e-2:** Results of the multiple regression analysis investigating the relationship between spinal cord metrics and measures of general physical measures, motor function and regional brain metrics. Results marked in red are significant after Bonferroni correction. CSA, cross sectional area; APW anterior – posterior width; LRW, left– right width.