# An Exploration Into Short-Interval Maintenance of Adult Hemispheric Cortical Thickness at an Individual Brain Level

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**ABSTRACT:** Adult cerebral cortical structure is thought to be statically maintained over short intervals. This view is based on group average findings but has never been studied at the individual level. This issue was examined with an unconventional longitudinal magnetic resonance imaging design which measured hemispheric mean cortical thickness of an adult man repeatedly at week intervals over 6 months. These measures were compared with measurement error estimates to test the current prediction that thickness measures would be statically maintained within measurement error variation. The results did not support this prediction. Thickness underwent incremental and decremental fluctuations which ranged up to 0.12 mm and 5.83% over week and multiweek intervals and which differed from measurement error variation. These exploratory analyses suggest a working hypothesis that short-interval cortical structural maintenance in an individual can involve fluctuations in thickness. If confirmed, this hypothesis has potential implications for cortical maintenance mechanisms and precision medicine approaches.

**KEYWORDS:** Allostasis/homeostasis, cortical maintenance, MRI morphometry, N-of-1, precision medicine, remodeling/turnover plasticity

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## Introduction

The cerebral cortex, the largest part of a human brain, is structurally assembled during development over prenatal to early adult years. The resulting mature adult structure is a 2- to 4-mm thick cortical covering of the brain. The structural complexity of adult human cortex is a topic of frequent amazement and discussion. Equally interesting and important is the issue of how this complex structure is continuously maintained in an individual person.

Cortical thickness, the distance between the outer cortical surface and underlying white matter, is a useful structural index of adult brain health and disease. For example, thickness reductions with diseases that critically affect cognitive/mental behaviors suggest that maintenance of cognitive/mental health is dependent on maintenance of cortical thickness.[1–3]

Normal steadiness in cognitive/mental behavior over short intervals of weeks is currently attributed, in part, to the capacity of adult cortical structure, including thickness, to be statically maintained over these times. Static maintenance is widely thought to result from sustained preservation of previously developed and constructed, mature cortical substrates. This view gets its support from mean thickness data from normal adult groups that were assessed in age-related and aging-related studies over long year-decade periods which, when extrapolated to short intervals of weeks, indicate a virtual absence of short interval thickness change.[4–6] This work is based entirely on group average data that do not directly address how cortical thickness is maintained in an individual.

Aligned with interests to increase understanding of brain organization at an individual brain level,[7] and to develop basic knowledge that may contribute to precision medicine approaches,[8–10] this study was based on the rationale that understanding short-interval cortical thickness maintenance of an individual brain may require unconventional longitudinal analysis, ie, an analysis that systematically examines regularly spaced, prospective thickness measurements from a relatively large sampling of short time intervals in the same brain. With this rationale, this study tested the static concept of thickness maintenance with a longitudinal analysis that used a structural magnetic resonance imaging (MRI), N-of-1 design to assess cortical thickness in an adult individual repeatedly at week intervals over 6 months.

As a baseline necessity for cortical structural survival, maintenance is a ubiquitous process that is ongoing across all cortices in a continuous fashion. This is evident from the fact that loss of continuous blood and related glucose and oxygen flow to any cortical location at any time results in rapid deterioration of structure that begins within minutes. Given the ubiquitous nature of maintenance, and the fact that the static view is largely based on hemispheric mean thickness measures, this study focused on hemispheric mean cortical thickness.

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Incorporated into the study design were simultaneous assessments of measurement error associated with each thickness measure. This permitted testing of the current prediction that maintenance variations in cortical thickness over short week and multiweek intervals would statically remain within measurement error variability. Despite the high experimental risks associated with (1) this prediction and the related very high likelihood of negative results (ie, no thickness variation outside measurement error) and (2) the unconventional resource-intensive need for extensive cortical measurements from an individual, we felt the issue of continuous cortical structural maintenance at an individual level was of sufficient general interest and significance to justify an initial exploratory study.

As reported below, the results provide initial evidence for a working hypothesis that, at an individual level, maintenance of cortical thickness may not be as static as currently thought.


**Figure 1.** Longitudinal N-of-1 study design. A pair of magnetic resonance imaging scans was made on each of 22 days over the 25-week period. Except for missed scans at weeks 2, 6, and 7, scans were taken at 1-week intervals on the same weekday. On each day, the 2 scans were completed in 1 session, with ≈5 minutes between scans for removal from the scanner, head repositioning, and scan setup. This provided 2 scan series, ie, series A and series B. Series B measures served as a replicate test for series A measures.

## Methods

### *Studied individual*

The studied individual is a 66-year-old left-handed man who does not smoke or use alcohol. He has been physically active across life and has no history of psychiatric problems, substance abuse, concussion, or head trauma. The MRI scans indicated no brain abnormalities. During the study, he underwent no illnesses or trauma, and day-to-day experiences involved usual work and home life routines with no travel, training, medical, or other unusual interventions. These experiences were considered consistent with usual daily maintenance of the brain and body.

Health status was regularly monitored during the study. This included daily measures of pulse, blood pressure, blood glucose, oral temperature, and weight. Physical activity based on steps/day was recorded each evening, and sleep duration was recorded each morning. Waist circumference, hemoglobin $A_{1c}$, and lipid measures were determined once at the end of the study period. Metabolic syndrome risk was assessed according to accepted standard criteria (ie, ≥ 3 measures above cut-point criteria for waist circumference, triglycerides, high-density lipoprotein, blood pressure, and fasting glucose).[11]

In accordance with the Declaration of Helsinki and National Ethical Guidelines, the study was done with informed and written consent of the subject and review and certification by the University of Toledo Institutional Review Board.

### *Longitudinal N-of-1 design*

The MRI brain scans were made on 22 dates across a 25-week period. Except for missed scans at weeks 2, 6, and 7, scans were taken at 1-week intervals on the same day of the week. On each date, 2 scans were completed in 1 session, with removal from the scanner between the first (scan A) and second (scan B) scans. Across dates, this provided 2 parallel scan series, ie, series A and B (Figure 1). Scanning on each date required ≈27.4 minutes, ie, 11.2 minutes for each scan with ≈5 minutes between scans for removal from the scanner, repositioning, and scan setup. This provided a total scan sample time of 8.2 hours.

### *MRI scanning and scan processing*

Scans were made with a 3T GE Signa scanner, 8 channel head coil, and T1-weighted scan protocol (fast spoiled gradient-recalled echo, repetition time = 7.8 ms, echo time = 3 ms, inversion time = 650 ms, flip angle = 9º, bandwidth = 31.25 kHz, field of view = 256 mm × 256 mm, voxel size = 1 mm × 1 mm × 1 mm, 164 continuous axial slices bracketing entire brain with no gaps between slices).

Image processing was done using automated FreeSurfer procedures which provided reliable measures of cortical thickness and intracranial volume (ICV) (http://surfer.nmr.mgh.harvard.edu).[12–14] As part of the design to treat data from each date as equal and independent measures, thickness and ICV measures were taken in native space without transformation to a template. The FreeSurfer longitudinal pipeline was not used and, to preserve variation at each time point, all scans were processed independently without across scan registration or averaging.

*Thickness measures.* FreeSurfer defined cortical thicknesses at ≈150 000 vertex locations per hemisphere. For each scan of each scan series, mean cortical thickness (mm) was determined for each hemisphere using all vertex measures from that hemisphere (Figure 2A to C).

*Thickness variation measures.* Hemispheric mean thickness measurements from a given pair of scans were used to calculate thickness variation over the intervening time interval. Thickness at the earlier time was subtracted from thickness at the later time, to indicate thickness increments over time as positive variations and thickness decrements over time as negative variations, which were expressed as percent changes [(later-earlier)/earlier × 100].

Because maintenance is continuous, thickness change between any pair of scans was considered a valid measure of variation in thickness maintenance. From the 22 scans in each

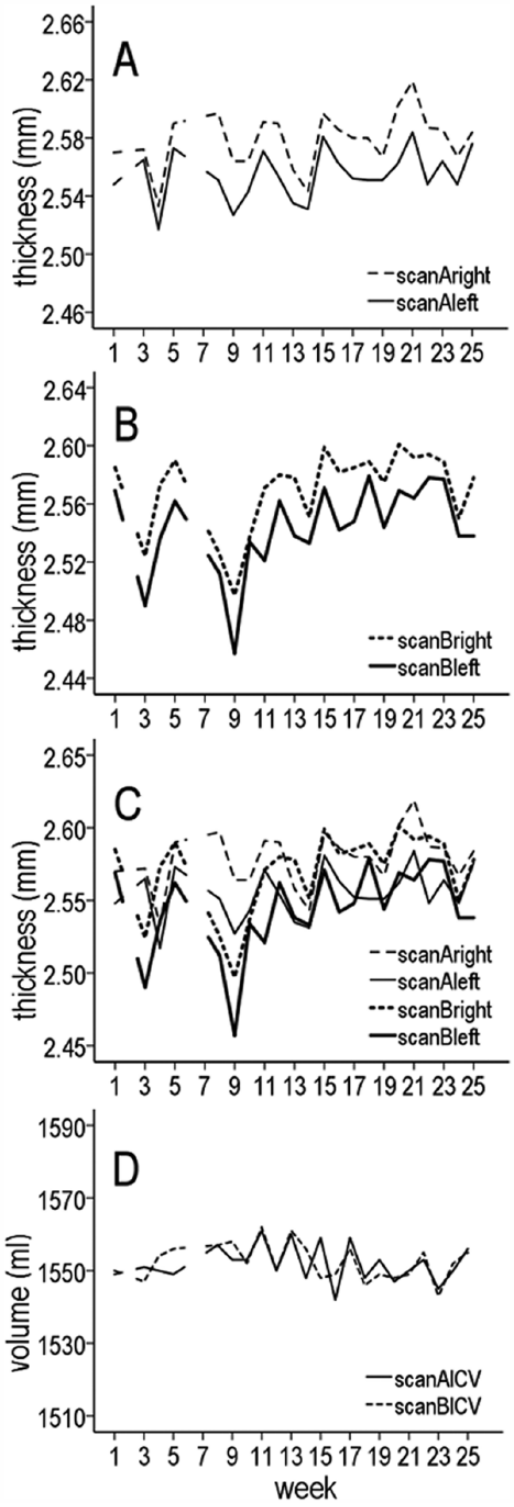


**Figure 2.** Left and right hemisphere thicknesses for the (A) scan A and (B) scan B series over the 25-week study period. For each series, neither hemisphere underwent significant progressive runs of thickness change; however, both hemispheres underwent continuously reversing incremental and decremental fluctuations over week and longer intervals. (C) Scan A and B thicknesses are graphed together for comparison. (D) Scan A and B ICV measures over the study period. ICV indicates intracranial volume.

scan series, mean hemisphere thickness changes were calculated for the 21 successive intervals, which varied by 1, 2, or 3 weeks. In addition, thickness changes were calculated for the more comprehensive total 231 intervals between all scan pairs of each scan series, which varied by 1 to 24 weeks.

### *Measurement error*

*Error factors.* Factors that potentially contributed to measurement error in this study were as follows: (1) variability in scanner function and scan quality, (2) head positioning, (3) movement (eg, head, body, respiration, heart/blood flow pulsation), and (4) workstation, operating system, and FreeSurfer processing factors. The MRI studies of intentional dehydration or large fluid ingestion suggest that hydration is also a potential source of measurement error in cross-sectional thickness analyses where hydration levels may differ across groups.[15] However, consistent with recent work on normal short-period cortical fluid dynamics[16,17] and normal fluid intake in the studied individual prior to scans (see section "Control procedures"), potential variability in cortical fluid substrates was considered a valid contributor to thickness maintenance in this study.

*Control procedures.* Several controls were used throughout the study to reduce measurement error. (1) All scans were made with the same scanner, head coil, and protocol. (2) Regular scanner quality assurance tests identified no scanner problems, and upgrades were not done during the study. (3) Scanning was done during similar midday times (start time mean ± SD: 1:55 PM ± 2.1 hour). (4) For each scan, the body was comfortably supine, and the head was positioned with consistent orthogonal laser beam alignment on the outer canthus of each eye and face midline. (5) Snug insertion of earplugs and padding around the head were used to minimize scanner sound and thickness biasing due to movement. To further minimize movement, the individual visually focused on a point in the scanner and remained attentive by counting seconds of scan time. Continuous attentiveness was affirmed by reaching an appropriate total count at scan end for each scan. (6) Processing of all scans was done with one workstation, operating system, and FreeSurfer program. (7) Interscan registration error and asymmetry bias were precluded by processing scans independently without registration to other scans and by treating all scans equally. (8) To insure uniform processing and as a blind control during the study, all scans were processed at one time after completion of all scans. (9) Scan A and B images were visually checked at scanning to rule out motion and other artifacts and to assure that continuous bilaterally symmetric axial slices were taken of the entire medulla to cortex neuraxis. Following processing, cortical borders for all slices of all scans were visually checked by an experienced FreeSurfer imaging specialist and were judged to be accurate and to not require manual correction. (10) Stimulants/diuretics, for example, caffeine containing beverages and chocolate, were not taken 12 hours before scanning, otherwise, drinking, eating, and physical activity prior to scans were within day-to-day normal ranges.

*Error measures.* Supplementing the above error reduction controls, 2 approaches were used to quantitatively measure measurement error. These error measures were taken simultaneously with thickness measures.

*Error defined from ICV variation.* Concurrent with each thickness measure, FreeSurfer provided an automated measure of ICV. Based on the following considerations, ICV variations were used as one estimate of measurement error. (1) Measurement error can be assessed with use of a structure that remains steady over the studied time intervals because any variations in measures of that structure reflect measurement error. Intracranial volume has been shown to be a steady structural characteristic in an individual.[14,18,19] From this, variation in ICV measures over short intervals in an individual arguably reflects measurement error. (2) Consistent with (1), prefatory tests in the studied individual showed that ICV measures from each scan series did not undergo nonrandom unidirectional change over the 22 scans (runs test: scan A series, $P = .126$; scan B series, $P = .535$). This indicated that ICV variations did not systematically drift over the study (Figure 2D). (3) Importantly, further prefatory tests demonstrated that ICV and hemispheric mean cortical thickness were measured with comparable reliability. Specifically, tests in the studied individual in which ICV and mean hemispheric thickness measurements were simultaneously taken and repeated in a test-retest manner from a given same scan, indicated that ICV and thickness measures had test-retest percent variations that did not significantly differ (Mann-Whitney; ICV vs thickness; scan A: left, $P = .514$; right, $P = .755$ and scan B: left, $P = .178$; right, $P = .514$). This indicated that despite differences in processing that were involved in defining ICV and thickness, both measures were derived with comparable reliability in this individual. This indicated that percent variations in ICV and thickness from the same interval between 2 scans could be validly compared. (4) Measures of ICV and mean cortical thickness were both affected by our study-specific error factors. Because ICV and thickness variations were measured simultaneously and compared for exactly matching intervals, common influences of all these factors on ICV and thickness measures were likely for a given interval. This, again, supported the validity of comparing thickness and ICV variations for the exact same interval. Intracranial volume measurement error variations were expressed as percent changes [(later − earlier)/earlier × 100] that were compared with analogously calculated mean thickness percent changes for exactly matched intervals.

*Error defined from intrasession thickness variation.* This approach was adopted from other longitudinal cortical thickness analyses in individuals[20] and provided a second way to account for measurement error. Given the premise that cortical thickness underwent no or minimal change during the scan session time (≈27.4 minutes), measurement error was defined for each hemisphere each week of the study from the intrasession percent difference between scan B and scan A thickness measures ((B − A)/A × 100). These intrasession variations were then compared with intersession, ie, across scan date, percent variations to test whether intersession fluctuations differed from and exceeded intrasession measurement error. The same thickness measures, together with their associated measurement error factors, were used to define both intrasession and intersession thickness variations, thus providing internal control of these factors.

### *Thickness analyses*

Five tests examining different aspects of the data addressed whether cortical thickness was statically maintained. Nonparametric statistical analyses were used for several reasons, including that data came from one subject. SPSS 21 was used to perform runs (test 1) and Kolmogorov-Smirnov (K-S; tests 2-5) analyses. Kolmogorov-Smirnov analysis provided the advantage of making no assumptions about compared distributions and having sensitivity to differences in multiple distribution properties including variation, skewness, kurtosis, modes, and central tendency.[21] For comparisons of the distributions of thickness vs measurement error measures, the most conservative metrics of measurement error distributions, ie, minimal and maximal extents, were used as indices.

*Test 1—analysis of randomness of thickness variation over the 6-month period.* Runs analysis was used to test whether hemisphere thickness measures over the 6-month period had nonrandom runs of unidirectional progressive variations relative to the median thickness. This might occur if thickness underwent, for example, progressive longitudinal decrease over the study period. A significant outcome would suggest that thickness underwent nonrandom progressive runs over the 6 months. A nonsignificant outcome would suggest that thickness did not undergo progressive runs and would indicate the need for further analyses to examine other potential changes. Runs analyses were done separately for the 2 hemispheres and 2 scan series, and a conservative Bonferroni-corrected $P < .012$ (.05/4) was applied to define significant results.

In addition, a correlation analysis was done to test whether, for corresponding weeks, left and right thicknesses from scan A were related to left and right thicknesses from scan B (Spearman correlation). This served as an initial analysis of the consistency of scan A and scan B thickness measures.

*Test 2—comparison of cumulative thickness vs measurement error absolute variations for successive intervals.* Thickness and ICV changes for each of the 21 successively occurring intervals were expressed as absolute percent variations that defined cumulative distribution functions for these variations over the study period. These distributions were then compared using K-S analysis. If thickness variations resulted from measurement error only, the 2 distributions should not differ. Alternatively, thickness variations that exceed and differ from measurement

error would suggest that thickness was not statically maintained within measurement error variability. Kolmogorov-Smirnov analyses were done separately for the 2 hemispheres and 2 scan series, and a conservative Bonferroni-corrected $P < .012$ (.05/4) was applied to define significant results.

Further analyses of measures from the 21 successive intervals were done to test whether percent fluctuations in left and right thicknesses were correlated with percent variations in ICV measurement error for corresponding intervals (Spearman correlation). These analyses provided a further comparison of thickness and ICV for these intervals. Separate analyses were done for scan A and scan B, and a Bonferroni-corrected $P <$ .025 (.05/2) was applied to define significant results.

*Test 3—comparison of the distributions of thickness vs measurement error incremental and decremental variations for all intervals.* The above test 2 focused on variations for only the 21 successive intervals, which comprised a small subsample of the 231 total intervals between all pairs of the 22 scans for each scan series. Because thickness maintenance occurred during all time intervals, this larger sample more comprehensively represented maintenance across all intervals. Test 3 compared the distributions of the percent variations in thickness vs ICV measurement error for the 231 intervals to test whether these distributions differed and whether thickness variations exceeded maximal error variations. If thickness was static, the distribution of thickness variations should not exceed or differ from the distribution of measurement error. Kolmogorov-Smirnov analyses were done for the 2 hemispheres and 2 scan series, and a conservative Bonferroni-corrected $P < .012$ (.05/4) was applied to define significant results.

*Test 4—residual thickness variations after removal of measurement error for each interval.* Each of the 231 intervals had a thickness measure and a corresponding ICV error variation measure for that exact interval. In this test, the ICV measurement error percent variation was subtracted from the hemisphere thickness percent fluctuation for that respective interval to test whether a residual thickness fluctuation remained following removal of measurement error. The hypothesis was that if thickness fluctuation was due to measurement error only, the residual percent fluctuation for each interval would approximate 0 and, for all intervals, the distribution of residual percent fluctuations would not differ from a horizontal null distribution. Test 4 K-S analyses were done for the 2 hemispheres and 2 scan series, and a conservative Bonferroni-corrected $P < .012$ (.05/4) was applied to define significant results.

*Test 5—comparison of intersession thickness variations vs intrasession measurement error.* Intrasession percent thickness variations reflecting measurement error were compared with the intersession percent thickness fluctuations. The hypothesis was that intersession fluctuations would not differ from or exceed intrasession measurement error. Percent variations were calculated for all pairs of later minus earlier intrasession and intersession A and B scans and compared using K-S analysis. Separate analyses were done for the 2 hemispheres, and a conservative Bonferroni-corrected $P < .025$ (.05/2) was applied to define significant results.

## Results

### *Health of the studied individual*

Table 1 summarizes indicators of the health status of the individual during the study period. Three physicians independently rated these indicators to be within, or approximate (marginally low pulse, marginally high systolic pressure), healthy ranges. Metabolic syndrome indicators ruled out this condition (see section "Methods" and Table 1).

### *Thickness analyses*

*Test 1.* Test 1 tested whether hemisphere mean thickness (mm) underwent nonrandom, unidirectional progressive variations over the 6 months. Scan A left hemisphere thickness did not undergo significant runs of change (Table 2, *test 1*). Similarly, no significant runs were seen for scan A right hemisphere or for either hemisphere from scan B (Table 2, *test 1*). However, for both scan series, each hemisphere did undergo reversing incremental and decremental fluctuations over week and longer intervals (Figure 2A to C). The fluctuations between minimal and maximal thicknesses for scan A left and right hemispheres and scan B left and right hemispheres were, respectively, 0.07, 0.09, 0.12, and 0.10 mm. Thicknesses of the left and right hemispheres from scan A were significantly positively correlated with respective corresponding time thicknesses of the left and right hemispheres from scan B (Figure 3, $R^2 = 0.264$, Spearman $\rho = .549$, $P < .001$). These results suggest, first, that each hemisphere underwent reversing incremental and decremental thickness fluctuations over week and multiweek intervals that concurrently resulted in no progressive unidirectional runs over the 6-month study period and, second, that scan B thickness measures were consistent with scan A thickness measures.

*Test 2.* Test 2 examined whether the distributions of cumulative absolute percent measures of thickness fluctuations differed from distributions of cumulative absolute percent ICV measurement error variations for the 21 successive intervals of the study. Kolmogorov-Smirnov analysis indicated the scan A left thickness distribution significantly differed from the scan A ICV distribution (Table 2, *test 2*). Further consistent with this difference, over successive weeks scan A left thickness fluctuations diverged from, and remained above, scan A ICV measurement error and reached a maximum (18.03%) that was 1.9 times larger than the measurement error maximum (9.57%) at 25 weeks (Figure 4A). Similarly, K-S analyses indicated the scan A right thickness distribution, and the scan B left and right thickness distributions significantly differed from their

respective scan A and scan B ICV distributions (Table 2, *test 2*). Further consistent with these differences, over successive weeks, scan A right hemisphere fluctuations and scan B left and right hemisphere fluctuations reached cumulative maximums that were, respectively, 1.7, 3.4, and 2.8 times larger than the corresponding scan measurement error maximum at 25 weeks (Figure 4A and B). These results suggest that thickness fluctuations in each hemisphere from both scan series significantly differed from and exceeded measurement error.

Further comparisons of thickness vs ICV measurement error for the 21 successive intervals indicated left and right thickness percent fluctuations were not significantly correlated with their corresponding ICV error percent variations. This applied to both scan A (Figure 5A, $R^2$ = 0.021, Spearman ρ = .113, $P$ = .475) and scan B (Figure 5B, $R^2$ = 0.037, Spearman ρ = −.148, $P$ = .349). These results provided further suggestions that thickness fluctuations were not dictated entirely by measurement error variations.

*Test 3.* In test 3, the distributions of thickness variations for all 231 intervals were compared with the distributions of ICV measurement error variations for these intervals to test whether these more comprehensive sample distributions differed and whether thickness variations exceeded maximal limits of error variations.

Scan A left thickness fluctuations significantly differed from scan A ICV measurement error variations (Table 2, *test 3*). Partly reflecting this difference, for example, the maximal limits of measurement error were +1.05% and −1.17% with a related range of 2.22%, whereas maximal thickness fluctuations were +2.66% and −1.87%, with a range of 4.53% that was 2.0 times wider than the maximal measurement error range (Figure 6A).

An analogous significant difference in thickness vs error variations was seen for scan A right hemisphere (Table 2, *test 3*). Partly reflecting this difference, for example, scan A ICV maximal measurement error limits (+1.05%, −1.17%) contrasted with scan A right hemisphere maximal thickness fluctuations of, respectively, +3.40% and −2.08% that had a related range of 5.48% and that was 2.5 times wider than the maximal measurement error range (2.22%) (Figure 6B).

For scan B, ICV maximal measurement error limits were +0.92% and −1.16% with a related range of 2.08% (Figure 7A and B). Thickness vs error variation distributions significantly differed for scan B left and right hemispheres (Table 2, *test 3*). Partly reflecting these differences, for example, scan B left maximal

**Table 1.** Health markers.

| MARKER | MEASUREMENT |
|---|---|
| Pulse, bpm[a] | 57 (±3.3) |
| Systolic BP, mm Hg[a,e] | 124 (±7.4) |
| Diastolic BP, mm Hg[a,e] | 79 (±3.6) |
| Oral temperature, °C[a] | 36.5 (±0.2) |
| Weight, lbs[a] | 138.9 (±1.4) |
| Body mass index, kg/m$^2$ | 21-22 |
| Waist, cm[e] | 81 |
| Blood glucose, mg/dL[a,b,e] | 96 (±4.4) |
| $HbA_{1c}$, %[c] | 5.4 |
| Lipids, mg/dL[b,c] | |
| Cholesterol | 174 |
| HDL[e] | 53 |
| LDL | 107 |
| Triglycerides[e] | 72 |
| Activity (steps)[d] | 10 714 (±3260) |

Abbreviations: BP, blood pressure; HDL, high-density lipoprotein; LDL, lipoprotein.
[a]Daily on awakening over 25 weeks (mean ± SD).
[b]Overnight fasted.
[c]Taken at end of 25 weeks.
[d]Daily end of day over 24 weeks (mean ± SD).
[e]Metabolic syndrome factor.

**Table 2.** Hemisphere thickness analyses.

| | SCAN A | | SCAN B | |
|---|---|---|---|---|
| | LEFT | RIGHT | LEFT | RIGHT |
| *Test 1*: Unidirectional variation[a,b] | $P$ = .512 | $P$ = .512 | $P$ = .512 | $P$ = .512 |
| *Test 2*: Cumulative absolute % thickness vs measurement error variation[b,c] | $P$ < .001 | $P$ < .008 | $P$ < .001 | $P$ < .001 |
| *Test 3*: Incremental and decremental % thickness vs measurement error variation[b,c] | $P$ < .001 | $P$ < .001 | $P$ < .001 | $P$ < .001 |
| *Test 4*: Residual thickness vs error baseline[b,c] | $P$ < .001 | $P$ < .001 | $P$ < .001 | $P$ < .001 |
| | LEFT | | RIGHT | |
| *Test 5*: Intersession % thickness vs measurement error variation[c,d] | $P$ < .047 | | $P$ < .012 | |

[a]Runs analyses.
[b]Bonferroni-corrected significance level: $P$ < .012.
[c]K-S analyses.
[d]Bonferroni-corrected significance level: $P$ < .025.

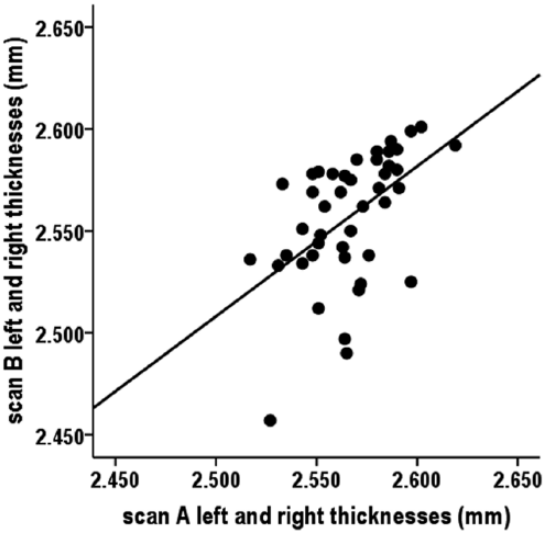


**Figure 3.** Scatterplot and linear regression line for the relationship between left and right cortical thickness measures from scan A vs corresponding left and right cortical thickness measures from scan B for the 22 scan dates. Scan A and scan B measures were significantly positively correlated.

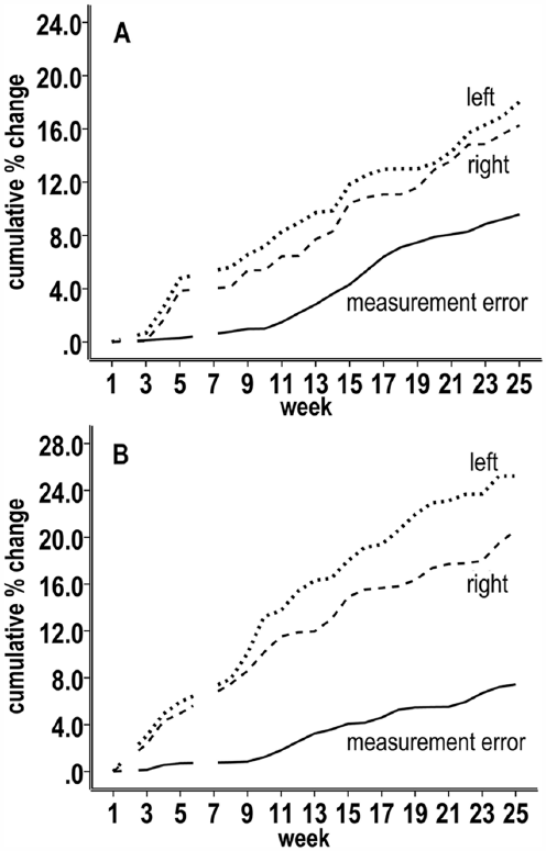


**Figure 4.** Test 2 examinations of cumulative absolute percent changes in left and right hemisphere thicknesses for (A) scan A and (B) scan B series over the 25-week study period. For each scan series, thickness fluctuations in each hemisphere significantly differed from the corresponding measurement error variations.

thickness fluctuations were, respectively, +4.97% and −4.36%, with a related range of 9.33%, and scan B right maximal thickness fluctuations were, respectively, +4.17% and −3.59% with a related range of 7.76% (Figure 7A and B). For scan B left and right hemispheres, thickness fluctuations had, respectively, 4.5 and 3.7 times wider ranges than the maximal measurement error range.

Scan A left and right hemispheres and scan B left and right hemispheres, respectively, had 27.7%, 30.7%, 51.5%, and 42.8% of thickness fluctuations that exceeded maximal measurement error (Figure 6A and B and Figure 7A and B). In each case, incremental fluctuations were a larger percent of the fluctuations that were outside maximal measurement error. Finally, for both scan series, thickness fluctuations that were outside maximal error occurred for 1-week and multiweek intervals without progressive drifts in percent variations for different length intervals (Figure 6C and D and Figure 7C and D). Taken together, test 3 results indicate thickness fluctuations in each hemisphere and scan series differed from and exceeded measurement error.

*Test 4.* This test examined, for each of the 231 intervals, whether a residual thickness fluctuation remained when ICV measurement error percent variation was subtracted from the thickness percent variation for the corresponding interval.

Scan A left residual thickness fluctuations significantly differed from the predicted horizontal null distribution (Table 2, *test 4*). Reflecting this, for example, maximal incremental and decremental residual fluctuations were, respectively, +2.65% and −2.20%, with an intervening continuous gradient of more incremental than decremental thickness changes and with only 4/231 intervals having a 0 (≤±0.009%) residual fluctuation (Figure 8A).

Similarly, scan A right and scan B left and right residual thickness fluctuations each significantly differed from the predicted horizontal null distribution (Table 2, *test 4*). Maximal incremental and decremental residual fluctuations for scan A right hemisphere were, respectively, +3.39% and −1.98%, and for scan B left and right hemispheres were, respectively, +5.83% and −4.92% and +4.80% and −3.97% (Figure 8C and Figure 9A and C). For each hemisphere, there was a continuous gradient of residual fluctuations that reflected more incremental than decremental changes, with 0 (≤±0.009%) residual fluctuations seen for only 0 or 1 interval (Figure 8C and Figure 9A and C).

The continuous gradients seen for each scan and hemisphere suggest that, at the individual interval level, residual thickness fluctuations occurred within the maximal error limits identified, at the distribution level, in test 3. This increases the fraction of fluctuations that were outside error as estimated in test 3. For both hemispheres and scans, residual incremental and decremental fluctuations were apparent for 1-week and multiweek intervals (Figures 8B and D and 9B and D). Test 4 results did not support the prediction that variations in thickness and measurement error would be comparable.

*Test 5.* The distributions of intrasession percent thickness variations reflecting measurement error were compared with the distributions of intersession percent thickness fluctuations for

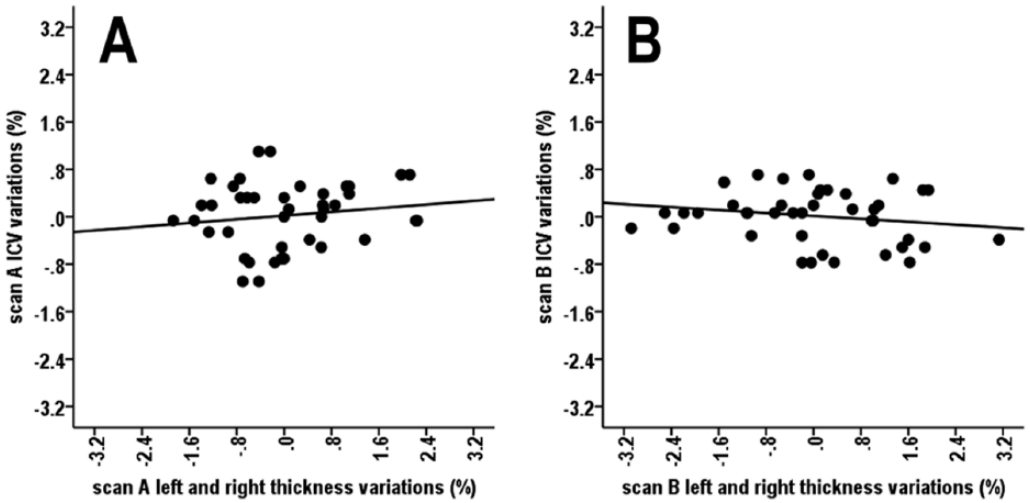


**Figure 5.** Scatterplots and linear regression lines for relationships between left and right percent fluctuations in thickness vs corresponding scan ICV percent variations for successive intervals for (A) scan A and (B) scan B. For both scan series, thickness fluctuations were not significantly related to ICV variations. ICV indicates intracranial volume.

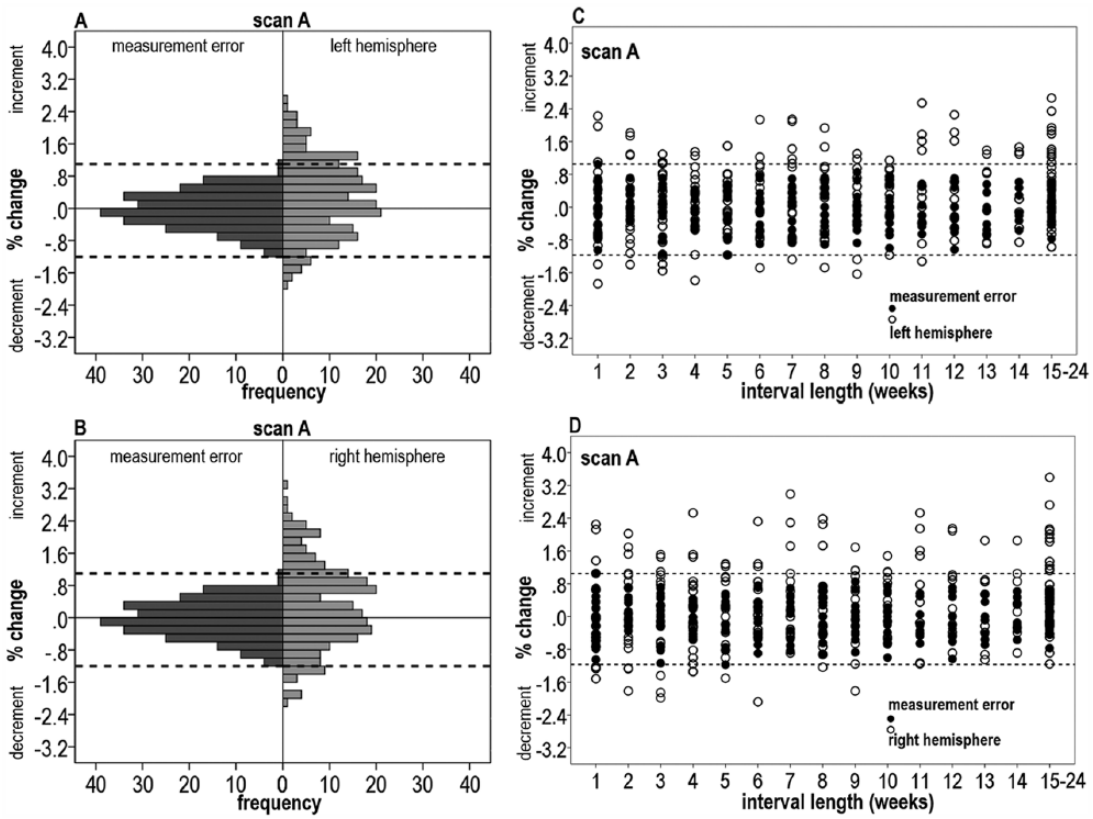


**Figure 6.** Scan A test 3 comparisons of the distributions of incremental and decremental percent changes in intracranial volume measurement error and (A) left and (B) right hemisphere thicknesses for all intervals. In addition, incremental and decremental percent changes in measurement error and (C) left and (D) right hemisphere thicknesses are shown for different interval lengths. Dashed lines again indicate maximal measurement error variations.

the left and right hemispheres. Left hemisphere intersession thickness fluctuations differed from left hemisphere intrasession error variations at an uncorrected significance level of $P = .05$ but were above a Bonferroni-corrected significance level of $P = .025$ (Table 2, *test 5*). Maximal intersession incremental and decremental fluctuations were +5.17% and −4.51% (+0.12 and −0.11 mm), as compared with maximal intrasession error variations of +1.18% and −2.92% (+0.03 and −0.07 mm). This reflected a 2.4 times wider left hemisphere intersession vs intrasession range between maximal variations (Figure 10A; intersession, 9.68%; intrasession, 4.10%). Right hemisphere intersession thickness fluctuations significantly differed from right hemisphere intrasession error variations at the Bonferroni-corrected significance level (Table 2, *test 5*). Maximal right

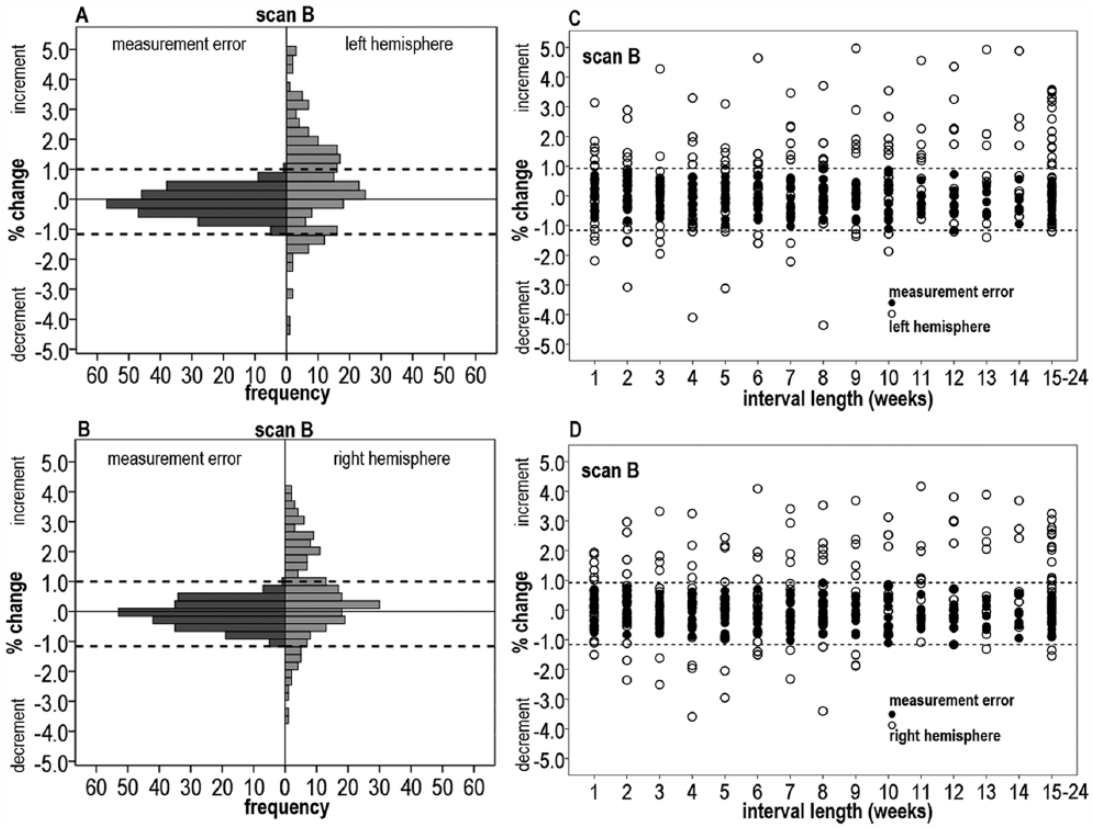


**Figure 7.** Scan B test 3 comparisons of the distributions of incremental and decremental percent changes in intracranial volume measurement error and thicknesses of each hemisphere for all intervals. Dashed lines again indicate maximal measurement error variations.

hemisphere intersession fluctuations were +4.89% and −3.85% (+0.12 and −0.10 mm) as compared with maximal error variations of +1.58% and −2.77% (+0.04 and −0.07 mm). This reflected a 2.0 times wider intersession vs intrasession range between maximal variations (Figure 10B; intersession, 8.74%; intrasession, 4.35%). These results indicate that thickness fluctuations in the right hemisphere exceeded and significantly differed from measurement error, whereas fluctuations in the left hemisphere also exceeded but did not significantly differ from measurement error at the conservative Bonferroni-corrected significance level.

## Discussion

### *Present findings*

This is a first exploratory study of short interval cortical thickness maintenance in an individual brain. Overall, the results do not support the static maintenance prediction that short-interval thickness variations would remain statically within measurement error variation. Comparisons of the distributions of thickness and measurement error measures indicate thickness measures underwent reversing incremental and decremental fluctuations which, although passing through levels of measurement error variability, significantly differed in distribution and extended outside maximal error limits (tests 2-5) over 28% to 52% (test 3) or more (test 4) of intervals, with magnitudes of up to 0.12 mm (tests 1 and 5) and 5.83% (tests 3-5) for week and multiweek times. These fluctuations resulted in no unidirectional thickness progression across the broader 6-month study period (test 1). From these findings, we suggest the working hypothesis that short-interval maintenance of cortical thickness in an individual can involve reversing incremental and decremental fluctuation.

### *The static maintenance view*

The starting prediction was based on current thinking that adult human cortical thickness is statically maintained. This view is supported by existing elegant lines of MRI studies that define group average rates of thickness variability over long time periods.

First, age studies that defined cortical thicknesses of adults whose ages fell within different decades of life show that hemispheric/regional thicknesses almost exclusively decrease across successive decades at group average rates of 0.01 to 0.20 mm or 2% to 4% per decade.[4,22–25] When these rates are extrapolated to short periods of a week, ie, 0.00001 to 0.0003 mm or 0.004 to 0.008% per week, thickness is predicted to be virtually static over short periods.

In addition, aging work has defined thickness changes of adults who were studied with initial and follow-up scans over long intervals of 0.5 to 12 years. This work indicates hemispheric/regional cortical thicknesses almost exclusively decrease at group average rates of 0.003 to 0.06 mm or 0.01% to 4.9% per year.[5,6,25–30]

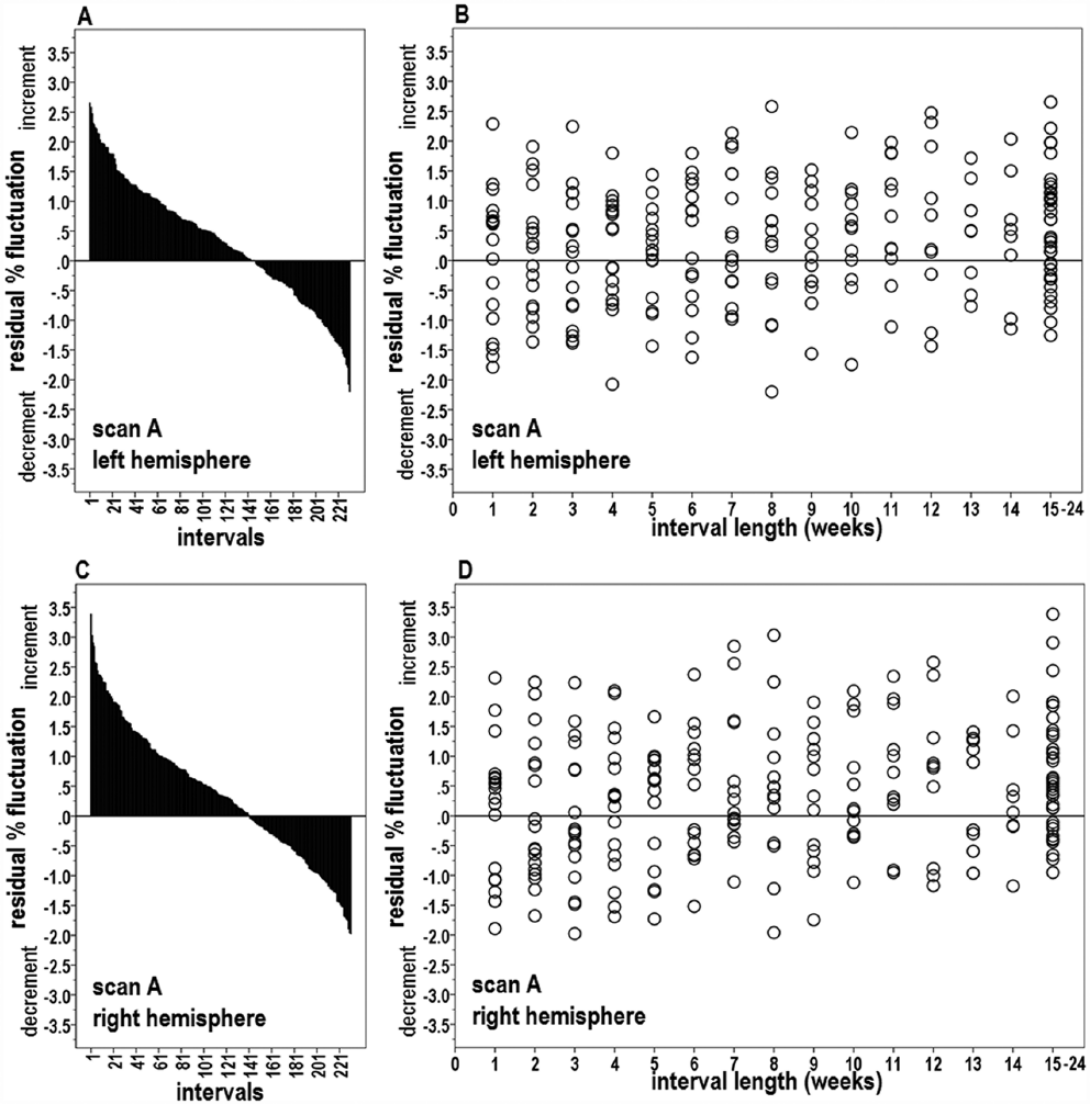


**Figure 8.** Scan A test 4 residual percent thickness fluctuations after subtraction of the corresponding measurement error for each interval for (A) left and (C) right hemispheres. Residual fluctuations across intervals are arranged from maximal incremental to maximal decremental fluctuations to illustrate the continuousness of residual fluctuation gradients. (B) Left and (D) right hemisphere incremental and decremental residual percent fluctuations for different length intervals.

Extrapolation to a week indicates group average changes of 0.00005 to 0.001 mm and 0.0001% to 0.09% per week, which again predict thickness is virtually static over short periods.

Existing studies do not directly address the issue of thickness variation over short periods in an individual person. Reflecting this, the quantity of data from any subject in these studies is many times smaller than that from the present individual (>8 imaging hours). The present fluctuations of up to 0.12 mm or 5.83% over week and multiweek periods are bidirectional, and reach magnitudes that equal or are larger than decremental group average ranges for year-decade periods, and are an order of magnitude or larger than group average extrapolations to week periods.

Just as group average findings are not necessarily representative of an individual, findings in an individual are not necessarily representative of group average results. With respect to the generality of the present findings, it appears unlikely that the present exploratory investigation serendipitously studied the only individual with maintenance fluctuations in thickness. It also appears possible the present fluctuations at a single subject level, and static maintenance findings at a group average level, are entirely consistent if fluctuations in different individuals have different temporal or other properties and become canceled with group averaging. This draws attention to a need for further detailed individual analyses.

### *Implications*

The present exploratory study provides original data on short-interval cortical maintenance which suggests the proposed fluctuation hypothesis. If correct, this hypothesis has interesting implications.

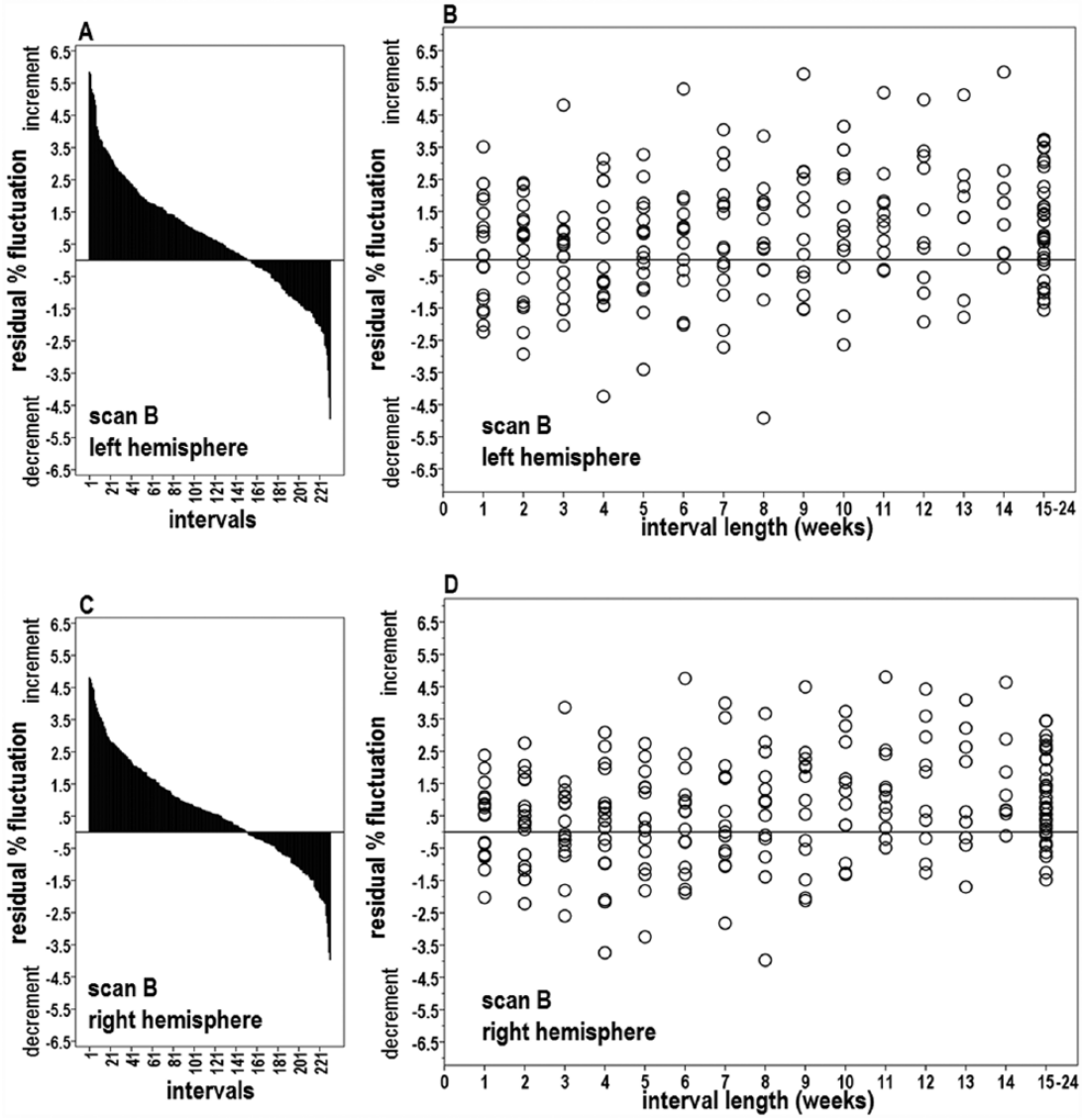


**Figure 9.** Scan B test 4 residual percent thickness fluctuations.

*Implications for mechanisms of cortical structural maintenance.* From a static view, cortical thickness maintenance entails mechanisms that preserve postdevelopmental, mature structural substrates which, together, make up thickness. Specifically, these substrates are neurons with their neuropil, glia and associated process specializations, arterial-capillary-venous cells, and extracellular-glymphatic-vascular fluid spaces. In contrast, from a fluctuation hypothesis, maintenance appears to involve more than preservation of mature structural status quo; that is, it appears to entail mechanisms that cause reversing incremental and decremental fluctuations in these cortical substrates over short intervals.

Do mechanisms exist that are consistent with a fluctuation hypothesis? The above cortical substrates in adult animals have capacities that cause some substrate properties to undergo little or no variation, and other properties to reversibly remodel or turnover, within short periods. Over days to weeks, remodeling/turnover effects involve virtually all the above substrates that contribute to thickness. Documented effects include, for example, extensions and retractions of: axonal branches and boutons,[31–33] spines and dendrites,[34–37] and glial processes[38–40] over distances of, for example, 1 to 3 μm for spines,[41] and several, tens, or more microns for cell processes.[31,39,42,43] Turnover includes cell loss as well as angiogenesis,[44,45] gliogenesis,[46,47] and hippocampal cortex neurogenesis.[48] Also ongoing are fluxes in volumes of cells[49,50] and intravascular and extracellular spaces.[17,51] These remodeling/turnover effects have been shown to operate broadly across cortex.

The above substrate remodeling/turnover in animals can contribute to thickness changes over short intervals.[52–55] Group average thickness changes in adult animals over weeks to months have been linked to cortical plasticity due to learning, environmental enrichment, and exercise. Similarly, group average thickness changes in adult humans over short periods have been linked to plasticity due to training.[56] If the proposed fluctuation hypothesis is correct, substrate remodeling/turnover plasticity

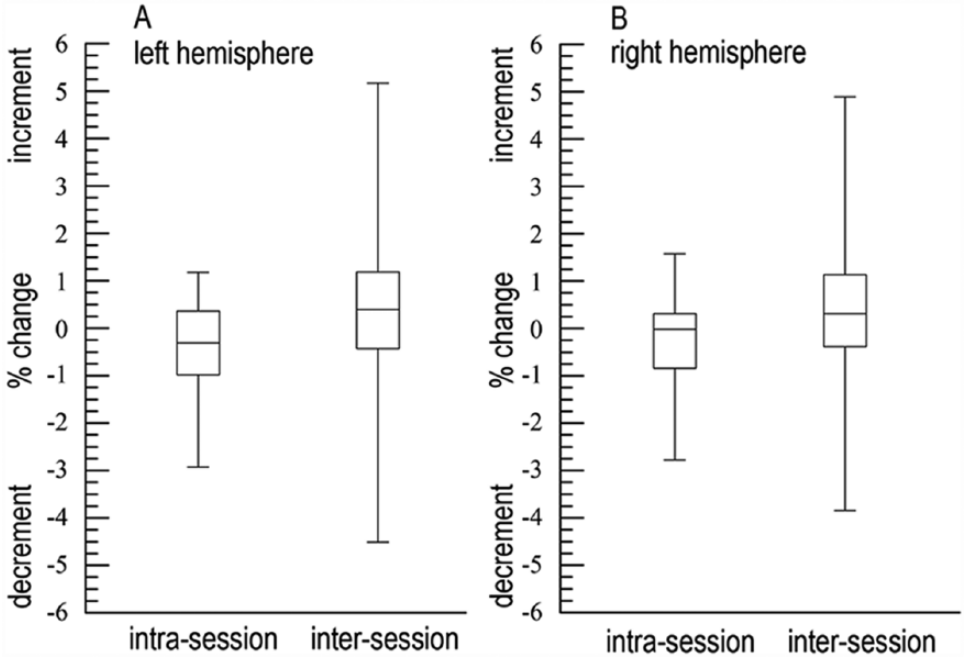


**Figure 10.** Test 5 comparisons of incremental and decremental changes for intersession thickness fluctuations vs intrasession measurement error variations for the (A) left and (B) right hemispheres. Box plots indicate maximum, median, and quartile changes. For the (A) left hemisphere, maximum intrasession changes were +1.18% and –2.92% (corresponding to changes of +0.03 and –0.07 mm) and maximum intersession changes were +5.17% and –4.51% (+0.12 and –0.11 mm). For the (B) right hemisphere, maximum intrasession changes were +1.58% and –2.77% (+0.04 and –0.07 mm) and maximum intersession changes were +4.89% and –3.85% (+0.12 and –0.10 mm).

may further contribute to fluctuations in thickness of cortex in an individual brain as a consequence of ongoing maintenance.

*Implications for development of a precision medicine knowledge base.* The present results suggest that cortical thickness underwent bidirectional maintenance fluctuations over weeks, which contributed to relative maintenance stability over the 6-month period. This view of maintenance, ie, where stability is produced through fluctuation, potentially resembles stability that is produced through systemic homeostatic and allostatic fluctuations that underlie broader maintenance of a person's body and cognitive/mental health.[57,58] There currently is no reason to expect relationships between short-term cortical structural staticness as defined by group average findings and homeostatic or allostatic fluctuations in an individual. The fluctuation hypothesis raises the possibility that, at the individual person level, short-term maintenance fluctuations of cortical thickness, and fluctuations in broader maintenance of the body and cognitive/mental health, may be related. Recent precision medicine discussions propose to use extended longitudinal measures of an individual's body maintenance dynamics to optimize her or his health.[8–10] A main focus has been on individual-based genome, proteome, metabolome, and microbiome dynamics. If the proposed fluctuation hypothesis is correct, an individual's cortical structural maintenance and its potential interactions with body maintenance may be a further avenue of investigation for precision medicine, N-of-1, approaches.

### *Study limitation*

Given the (1) starting prediction that thickness maintenance would be static and the resultant likelihood of negative findings (ie, no thickness variation difference from measurement error variation) and (2) resource-intensive requirement for extensive systematic sampling in one subject, this study was exploratory and not definitive. It provides initial findings and a working hypothesis from one individual and a starting, rather than finishing, line for addressing continuous cortical structural maintenance in an individual.

### *Future directions*

Further studies of ongoing cortical maintenance using an N-of-1 design are needed in other individuals. Also needed are additional conventions for defining measurement error. Analyses of cortical structure at global and regional levels would be useful for evaluating how adult maintenance fluctuations are related to known developmental and aging changes at these levels.

## Conclusions

This exploratory study provides initial evidence for a working hypothesis that, at an individual level, maintenance of cortical thickness over short intervals can involve reversing incremental and decremental thickness fluctuations. This hypothesis requires further testing at the individual level. It merits interest because it has potential implications for cortical maintenance mechanisms and for understanding brain/body maintenance

interactions that may be an avenue of development for precision medicine, N-of-1, approaches.

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## Author Contributions

JW, HX, and XW conceived and designed the experiment; agree with manuscript results and conclusions; made critical revisions; and approved final version. JW and XW contributed to data acquisition. JW and HX analyzed the data, wrote the manuscript, and jointly developed the structure and arguments for the paper. All authors reviewed and approved the final manuscript.

## REFERENCES

1. Nygaard GO, Walhovd KB, Sowa P, et al. Cortical thickness and surface area relate to specific symptoms in early relapsing-remitting multiple sclerosis. *Mult Scler*. 2015;21:402-414.
2. Moran C, Beare R, Phan TG, Bruce DG, Callisaya ML, Srikanth V. Type 2 diabetes mellitus and biomarkers of neurodegeneration. *Neurology*. 2015;85:1123-1130.
3. Pereira JB, Ibarretxe-Bilbao N, Marti MJ, et al. Assessment of cortical degeneration in patients with Parkinson's disease by voxel-based morphometry, cortical folding, and cortical thickness. *Hum Brain Mapp*. 2012;33:2521-2534.
4. van Velsen EF, Vernooij MW, Vrooman HA, et al. Brain cortical thickness in the general elderly population: the Rotterdam Scan Study. *Neurosci Lett*. 2013;550:189-194.
5. Pacheco J, Goh JO, Kraut MA, Ferrucci L, Resnick SM. Greater cortical thinning in normal older adults predicts later cognitive impairment. *Neurobiol Aging*. 2015;36:903-908.
6. Shaw ME, Sachdev PS, Anstey KJ, Cherbuin N. Age-related cortical thinning in cognitively healthy individuals in their 60s: the PATH through life study. *Neurobiol Aging*. 2016;39:202-209.
7. Van Essen DC, Barch DM. The human connectome in health and psychopathology. *World Psychiatry*. 2015;14:154-157.
8. Kuntz TM, Gilbert JA. Introducing the microbiome into precision medicine. *Trends Pharmacol Sci*. 2017;38:81-91.
9. Insel TR, Cuthbert BN. Medicine. Brain disorders? Precisely. *Science*. 2015;348:499-500.
10. Schork NJ. Personalized medicine: time for one-person trials. *Nature*. 2015;520:609-611.
11. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120:1640-1645.
12. Jovicich J, Czanner S, Han X, et al. MRI-derived measurements of human subcortical, ventricular and intracranial brain volumes: reliability effects of scan sessions, acquisition sequences, data analyses, scanner upgrade, scanner vendors and field strengths. *Neuroimage*. 2009;46:177-192.
13. Fischl B. FreeSurfer. *Neuroimage*. 2012;62:774-781.
14. Nordenskjold R, Malmberg F, Larsson EM, et al. Intracranial volume estimated with commonly used methods could introduce bias in studies including brain volume measurements. *Neuroimage*. 2013;83:355-360.
15. Biller A, Reuter M, Patenaude B, et al. Responses of the human brain to mild dehydration and rehydration explored in vivo by 1H-MR imaging and spectroscopy. *Am J Neuroradiol*. 2015;36:2277-2284.
16. Nedergaard M, Goldman SA. Brain drain. *Sci Am*. 2016;314:44-49.
17. Ding F, O'Donnell J, Xu Q, Kang N, Goldman N, Nedergaard M. Changes in the composition of brain interstitial ions control the sleep-wake cycle. *Science*. 2016;352:550-555.
18. Whitwell JL, Crum WR, Watt HC, Fox NC. Normalization of cerebral volumes by use of intracranial volume: implications for longitudinal quantitative MR imaging. *Am J Neuroradiol*. 2001;22:1483-1489.
19. Buckner RL, Head D, Parker J, et al. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *Neuroimage*. 2004;23:724-738.
20. Wang X, Gerken M, Dennis M, et al. Profiles of precentral and postcentral cortical mean thicknesses in individual subjects over acute and subacute time-scales. *Cereb Cortex*. 2010;20:1513-1522.
21. Sokal RR, Rohlf FJ. *Biometry: The Principles and Practice of Statistics in Biological Research*. 4th ed. New York, NY: W.H. Freeman; 2012.
22. Salat DH, Buckner RL, Snyder AZ, et al. Thinning of the cerebral cortex in aging. *Cereb Cortex*. 2004;14:721-730.
23. Hutton C, Draganski B, Ashburner J, Weiskopf N. A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. *Neuroimage*. 2009;48:371-380.
24. Lemaitre H, Goldman AL, Sambataro F, et al. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and grey matter volume? *Neurobiol Aging*. 2012;33:617.e611-617.e619.
25. Fjell AM, Westlye LT, Grydeland H, et al. Accelerating cortical thinning: unique to dementia or universal in aging? *Cereb Cortex*. 2014;24: 919-934.
26. Murphy EA, Holland D, Donohue M, et al. Six-month atrophy in MTL structures is associated with subsequent memory decline in elderly controls. *Neuroimage*. 2010;53:1310-1317.
27. Thambisetty M, Wan J, Carass A, An Y, Prince JL, Resnick SM. Longitudinal changes in cortical thickness associated with normal aging. *Neuroimage*. 2010;52:1215-1223.
28. Knight WD, Kim LG, Douiri A, Frost C, Rossor MN, Fox NC. Acceleration of cortical thinning in familial Alzheimer's disease. *Neurobiol Aging*. 2011;32:1765-1773.
29. Storsve AB, Fjell AM, Tamnes CK, et al. Differential longitudinal changes in cortical thickness, surface area and volume across the adult life span: regions of accelerating and decelerating change. *J Neurosci*. 2014;34:8488-8498.
30. Jiang J, Sachdev P, Lipnicki DM, et al. A longitudinal study of brain atrophy over two years in community-dwelling older individuals. *Neuroimage*. 2014;86:203-211.
31. De Paola V, Holtmaat A, Knott G, et al. Cell type-specific structural plasticity of axonal branches and boutons in the adult neocortex. *Neuron*. 2006;49:861-875.
32. Stettler DD, Yamahachi H, Li W, Denk W, Gilbert CD. Axons and synaptic boutons are highly dynamic in adult visual cortex. *Neuron*. 2006;49:877-887.
33. Wimmer VC, Broser PJ, Kuner T, Bruno RM. Experience-induced plasticity of thalamocortical axons in both juveniles and adults. *J Comp Neurol*. 2010;518:4629-4648.
34. Lee W-CA, Chen JL, Huang H, et al. A dynamic zone defines interneuron remodeling in the adult neocortex. *Proc Natl Acad Sci U S A*. 2008;105:19968-19973.
35. Kozorovitskiy Y, Gross CG, Kopil C, et al. Experience induces structural and biochemical changes in the adult primate brain. *Proc Natl Acad Sci U S A*. 2005;102:17478-17482.
36. Holtmaat A, Randall J, Cane M. Optical imaging of structural and functional synaptic plasticity in vivo. *Eur J Pharmacol*. 2013;719:128-136.
37. Perez-Cruz C, Simon M, Flugge G, Fuchs E, Czeh B. Diurnal rhythm and stress regulate dendritic architecture and spine density of pyramidal neurons in the rat infralimbic cortex. *Behav Brain Res*. 2009;205:406-413.
38. Theodosis DT, Poulain DA, Oliet SH. Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. *Physiol Rev*. 2008;88:983-1008.
39. Hughes EG, Kang SH, Fukaya M, Bergles DE. Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. *Nat Neurosci*. 2013;16:668-676.
40. Liu J, Dietz K, DeLoyht JM, et al. Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nat Neurosci*. 2012;15:1621-1623.
41. Villa KL, Berry KP, Subramanian J, et al. Inhibitory synapses are repeatedly assembled and removed at persistent sites in vivo. *Neuron*. 2016;89:756-769.
42. Lee W-CA, Huang H, Feng G, et al. Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex. *PLoS Biol*. 2006;4:e29.
43. Bloss EB, Janssen WG, McEwen BS, Morrison JH. Interactive effects of stress and aging on structural plasticity in the prefrontal cortex. *J Neurosci*. 2010;30:6726-6731.
44. Huang CX, Qiu X, Wang S, et al. Exercise-induced changes of the capillaries in the cortex of middle-aged rats. *Neuroscience*. 2013;233:139-145.
45. Ekstrand J, Hellsten J, Tingstrom A. Environmental enrichment, exercise and corticosterone affect endothelial cell proliferation in adult rat hippocampus and prefrontal cortex. *Neurosci Lett*. 2008;442:203-207.
46. Emsley JG, Macklis JD. Astroglial heterogeneity closely reflects the neuronal-defined anatomy of the adult murine CNS. *Neuron Glia Biol*. 2006;2:175-186.
47. Young Kaylene M, Psachoulia K, Tripathi Richa B, et al. Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. *Neuron*. 2013;77:873-885.
48. Jessberger S, Gage FH. Adult neurogenesis: bridging the gap between mice and humans. *Trends Cell Biol*. 2014;24:558-563.

49. Risher WC, Andrew RD, Kirov SA. Real-time passive volume responses of astrocytes to acute osmotic and ischemic stress in cortical slices and in vivo revealed by two-photon microscopy. *Glia*. 2009;57:207-221.
50. Xie L, Kang H, Xu Q_, et al. Sleep drives metabolite clearance from the adult brain. *Science*. 2013;342:373-377.
51. Cho ZH, Kang CK, Han JY, et al. Functional MR angiography with 7.0 T Is direct observation of arterial response during neural activity possible? *Neuroimage*. 2008;42:70-75.
52. Diamond MC. *Enriching Heredity: The Impact of the Environment on the Anatomy of the Brain*. New York, NY: Free Press, 1988.
53. Anderson BJ, Eckburg PB, Relucio KI. Alterations in the thickness of motor cortical subregions after motor-skill learning and exercise. *Learn Mem*. 2002;9:1-9.
54. Kleim JA, Barbay S, Cooper NR, et al. Motor learning-dependent synaptogenesis is localized to functionally reorganized motor cortex. *Neurobiol Learn Mem*. 2002;77:63-77.
55. Fares RP, Belmeguenai A, Sanchez PE, et al. Standardized environmental enrichment supports enhanced brain plasticity in healthy rats and prevents cognitive impairment in epileptic rats. *PLoS ONE*. 2013;8:e53888.
56. Valkanova V, Eguia Rodriguez R, Ebmeier KP. Mind over matter: what do we know about neuroplasticity in adults? *Int Psychogeriatr*. 2014;26:891-909.
57. Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neurosci Biobehav Rev*. 2010;35:2-16.
58. McEwen BS, Getz L. Lifetime experiences, the brain and personalized medicine: an integrative perspective. *Metabolism*. 2013;62:S20-S26.