Reproducibility of task activation using the Addenbrooke's cognitive examination in healthy controls: A functional Transcranial Doppler ultrasonography study

L Beishon[a], C.A.L Williams[a], R.B Panerai[a,b], T.G Robinson[a,b], V.J Haunton[a,b]

Author Affiliations

[a]University of Leicester, Department of Cardiovascular Sciences, Leicester, UK

[b]NIHR Leicester Biomedical Research Centre, University of Leicester, Leicester, UK

Corresponding author:

Lucy Beishon

Lb330@le.ac.uk

Full Address: College of Medicine, Biological Sciences and Psychology

Department of Cardiovascular Sciences

University of Leicester

Level 2, Clinical Sciences Building

Leicester Royal Infirmary

Leicester

LE2 7LX

Telephone: 0116 204 4752

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Highlights: (3-5 points, 85 characters)

- First study to examine the reproducibility of a complete cognitive assessment
- Reproducibility of both peak CBFv responses and area under the curve reported
- Intra-observer reliability of CBFv responses with different operators reported
- Reproducibility of CBFv responses demonstrated over a longer time interval

Abstract (246 words)

Introduction

Cerebral blood flow velocity (CBFv) changes occurring with cognitive stimulation can be measured by Transcranial Doppler ultrasonography (TCD). The aim of this study was to assess the reproducibility of CBFv changes to the Addenbrooke's cognitive examination (ACE-III).

New Method

13 volunteers underwent bilateral TCD (middle cerebral artery), continuous heart rate (HR, 3-lead ECG, Finometer), beat-to-beat mean arterial pressure (MAP, Finometer), and end-tidal CO_2 ($ETCO_2$, capnography). After 5 minutes baseline, all ACE-III tasks were performed in 3 domains (A/B/C). Data presented are population CBFv peak normalised changes and area under the curve (AUC). Statistical analysis was by 2-way repeated measures (ANOVA), intra-class correlation coefficient (ICC), standard error of measurement (SEM) and coefficient of variation (CV).

Results

12 bilateral data sets were obtained (10 right hand dominant, 6 female). Baseline parameters (MAP, HR, $ETCO_2$) did not differ between visits. All tasks increased CBFv. Only domain A on AUC analysis differed significantly on ANOVA, and one task on post hoc testing ($p<0.05$). ICC values were poor (<0.4) for most tasks, but 3 tasks produced more consistent results on AUC and peak CBFv analysis (range ICC: 0.15-0.73, peak CV: 16.2-56.1(%), AUC CV: 23.2-60.2(%), peak SEM: 2.5–6.0 (%), AUC SEM: 21.8-135.8 (%*s).

Comparison with existing methods

This is the first study to examine reproducibility of CBFv changes to a complete cognitive assessment tool.

Conclusions

Reproducibility of CBFv measurements to the ACE-III was variable. AUC may provide more reliable estimates than peak CBFv responses. These data need validating in patient populations.

1. Introduction

Transcranial Doppler ultrasonography (TCD) is a non-invasive technique which measures cerebral blood flow velocity (CBFv) in the intracranial arteries (middle, anterior and posterior cerebral arteries) (1, 2). There is increasing interest in both the clinical and research capacities of TCD to assess cognitive function through measurement of CBFv responses to cognitive task activation (3). Unlike available alternatives (functional magnetic resonance imaging (fMRI), positron emission tomography (PET)), it provides excellent temporal resolution of dynamic changes in CBFv, and can be used in patients with contraindications, such as: pacemakers, claustrophobia and metal implants (1, 2). Furthermore, it does not utilise ionising radiation and assessments can be carried out in a clinic-like setting which is more acceptable to patients (2). In a recent publication by this group, we demonstrated that the Addenbrooke's cognitive examination (ACE-III) was a feasible cognitive testing battery to elicit peak and sustained CBFv responses in a group of healthy volunteers (4). The ACE-III has excellent sensitivity and specificity for the diagnosis of dementia and mild cognitive impairment (5, 6).

The functional relationship between the neuronal unit (neurones, astrocytes and inter-neurones) and the cerebral vascular bed (7), allows for tight coupling between nutrient supply and demand at times of increasing cognitive load (8). This is known as neurovascular coupling (NVC) and is achieved through metabolic, myogenic and neurogenic components (8, 9). The metabolic component is mediated through the production of metabolites (prostaglandins, ATP and nitric oxide) as a result of rising cortical metabolic demands leading to cerebral vasodilation (7). Myogenic and neurogenic components result from smooth vessel constriction and autonomic nervous control, respectively (8, 9). NVC can be investigated by continuous TCD, in response to cognitive stimulation (3, 8).

In addition to being technically easy to train operators, the reproducibility of resting TCD has been demonstrated in a number of studies (10-13). Several fMRI and PET studies have demonstrated good

intra-/inter-rater and cross-site reliability for task activation protocols (14-17). However, analogous studies for TCD are fewer, and have largely focussed on the reproducibility of the laterality index (LI) (18-21). To date, no study has investigated the reproducibility of CBF activation using TCD to a complete cognitive testing battery. Reproducibility is an important concept in the development of diagnostic tests, where measurements need to be made consistently between and within subjects, particularly for diagnosis and to inform decision making for invasive procedures (22). The importance of functional imaging is gaining increasing recognition for the diagnosis, investigation and prognostication of cognitive disorders (23). Therefore, studies examining the reproducibility of cognitive testing protocols with simultaneous functional imaging are imperative to establish a standardised procedure for eliciting CBFv responses. Therefore, the aim of this study was to assess the inter-rater reproducibility of CBFv responses to the ACE-III using TCD in a healthy volunteer population.

2. Methods

Thirteen healthy volunteers were recruited from staff and students at the University of Leicester for the first measurement between February and May 2016. The same volunteers were then invited to participate in the reproducibility study between January and February 2017. The study had University of Leicester ethical approval (ref: 10102) and all volunteers provided written informed consent. Only healthy adults aged over 18 years were included, with volunteers who provided a first measurement with good quality data invited to return for a repeat measurement (24). Pregnant or lactating volunteers were excluded. At assessment, the medical history and current medication list were checked to ensure that no volunteer had a significant change in medication or health status that would affect cerebral autoregulation or NVC. All volunteers had previously completed the Edinburgh handedness inventory and both right- and left-handed individuals were included in the analysis. Volunteers were requested to abstain from nicotine, alcohol, caffeine, large meals or strenuous exercise four hours prior to the study.

Experiments were carried out in a temperature controlled (24^{o}C) and quiet laboratory (University of Leicester Cerebral Haemodynamics in Ageing and Stroke Medicine (CHIASM)). Bilateral CBFv were recorded by insonating the middle cerebral arteries (MCA) with TCD probes (Vyasis Companion III) secured using a head frame. In addition, beat-to-beat mean arterial pressure (MAP) (arterial volume clamping on non-dominant hand, Finometer, Finapres Medical Systems; Amsterdam, Netherlands), end-tidal CO_2 ($ETCO_2$) (capnography Capnocheck Plus), and heart rate (HR) (3-lead ECG, Finometer, Finapres Medical Systems; Amsterdam, Netherlands) were recorded continuously. Signals were sampled at 500 samples/s.

A similar protocol was performed to that undertaken on the first visit (24), though the observer changed (1st assessment: CALW; 2nd: LB). In brief, a 5-minute baseline recording was obtained where participants were instructed to rest. This was followed by all tasks from the ACE-III, undertaken in

the order it would be performed clinically. The ACE-III was divided into three sections: A (attention, fluency and memory tasks), B (language tasks), and C (visuospatial and memory tasks). Table 1 summarises the cognitive tasks from the ACE-III, and the components of the A, B, and C sections. There was a minimum of 1-minute rest between sections of the ACE-III. At first assessment, there was 30 seconds between tasks, but this was extended to one minute between tasks at repeat assessment to allow complete normalisation of signals to baseline. Data were stored using PHYSIDAS acquisition system, and analysed offline using software previously developed by this group. Brachial BP was measured prior to each recording in order to calibrate the Finometer readings.

Large, non-physiological spikes were removed using linear-interpolation, smaller spikes in the CBFv signal were removed by a median filter and all signals were lowpass filtered with a zero-phase, eight-order Butterworth filter. 3-lead ECG recordings determined the R-R interval in order to calculate: beat-to-beat mean ABP, mean CBFv, HR and $ETCO_2$. Data underwent standard polynomial interpolation and then re-sampling at 5 Hz in order to generate a uniform time base (25).

Peak CBFv responses were calculated as population mean averages for each task. The percentage change in CBFv response at 25-30 seconds was normalised to the 20-second baseline prior to task initiation. Area under the curve (AUC_{CBFv}) was determined for each CBFv response by determining the time interval from 20 seconds (task initiation) to the point at which the CBFv response normalised to baseline, and taking the mean of the positive values for this interval.

Task	Domain	Detail
A section		
A1	Attention	Orientation to time (day/date/month/year/season)
A2	Attention	Orientation to space (floor/hospital/city /county/country)
A3	Attention	Repeat and remember 3 words (lemon/key/ball)
A4	Attention	Subtract serial sevens from 100
A5	Memory	Recall the 3 words learnt earlier (A3: lemon/key/ball)
A6	Fluency	Naming as many words beginning with “P” in 1 minute
A7	Fluency	Naming as many animals in 1 minute
A8	Memory	Learn and remember a name and address
A9	Memory	Names of current and previous UK prime ministers and US presidents
B section		
B1	Language	Following verbal instructions
B2	Language	Writing 2 sentences
B3	Language	Repeating words and phrases aloud
B4	Language	Naming objects
B5	Language	Linking objects with statements
B6	Language	Reading words aloud
C section		
C1	Visuospatial	Drawing an infinity diagram and 3-dimensional cube
C2	Visuospatial	Drawing a clock face and correctly positioning the hands to a given time
C3	Visuospatial	Counting number of dots
C4	Visuospatial	Recognising obscured words
C5	Memory	Recalling the previously learnt name and address (A8)

Table 1. Tasks from the ACE III used to elicit CBFv responses.

2.1 Statistical analysis

Sample size was determined based on recent, previous TCD reproducibility studies (12, 26). Data were tested for normality using the Shapiro-Wilks test; 18% of the AUC_{CBFv} data were not normally distributed therefore parametric analyses were applied. Data are continuous and therefore presented as mean and standard deviation (SD), or median [IQR] for non-parametric data. A 2-way repeated measures analysis of variance (ANOVA) was used to assess for significant differences in normalised peak population CBFv change (%), and AUC_{CBFv} following task initiation at 20 seconds.

Individual intra-class correlation (ICC) was performed for inter-rater reliability for each task for peak CBFv (%) response and AUC_{CBFv}. ICC were calculated using a (2,2) model (27). The Cicchetti criteria (28) were used to determine cut offs for poor (<0.4), fair (0.4-0.59), good (0.6-0.74), and excellent (0.75-1.00) correlation on ICC. In addition, values for standard error of measurement (SEM) and coefficient of variation (CV) are reported. SEM represents the amount of error in the measurement (27), where:

$$SEM = \frac{SD}{\sqrt{n}} \qquad \text{Equation 1.}$$

It is expected that 68% of individuals will fall within +/- one SEM of the mean value (27). CV represents the standardised dispersion of measurements around the mean (29), where:

$$CV = \frac{SEM}{Subject\ mean} \qquad \text{Equation 2.}$$

2-way repeated measures ANOVA were calculated using *Statistica* software for Windows, Version 13, and ICC, SEM and CV using SPSS Version 22 for Windows. Statistical significance was set at $p<0.05$.

3. Results

Thirteen bilateral data sets were obtained, but one data set was excluded due to poor quality, leaving 12 data sets suitable for analysis. There was an equal split between male and female participants (6:6), and two volunteers were left-handed. The median age of participants at repeat assessment was 35.5 years [IQR: 26.8-50]. Mean ACE III scores were 98.0 (2.0) at initial assessment, and 98.7 (1.2) at follow-up assessment. Median time to follow-up was 343 days [IQR: 338-349]. No participants were below the established cut-off score for dementia (88) and no participant developed cognitive impairment during the interval between studies.

3.1 Baseline parameters

HR, MAP and $ETCO_2$ did not differ at baseline between visits (Table 2). CBFv in the dominant, but not non-dominant, hemisphere differed significantly between visits ($p<0.005$) (Table 2).

Parameter	n	Visit 1 Mean (SD)	Visit 2 Mean (SD)	P value
HR	12	69.6 (9.2)	75.0 (10.2)	0.5
MAP	12	94.1 (11.4)	93.5 (13.8)	1.0
$ETCO_2$	12	37.7 (2.4)	37.8 (2.5)	1.0
CBFv ND	12	49.9 (8.1)	55.5 (8.9)	0.13
CBFv D	12	48.3 (6.9)	55.3 (9.2)	**<0.005**

Table 2. Baseline demographics between the 2 visits. No baseline differences in cardiovascular parameters or $ETCO_2$. P values for paired t-testing with Bonferroni correction for multiple comparisons.

HR: heart rate; MAP: mean arterial pressure; $ETCO_2$: end tidal CO_2; CBFv: cerebral blood flow velocity; D: dominant hemisphere; ND: non-dominant hemisphere; SD: standard deviation

3.2 Peak population CBFv response reproducibility

All tasks resulted in an increase in CBFv from baseline in the dominant (range: Visit 1 (V1): 4.1-12.8%, Visit 2 (V2): 5.08-13.8%) and non-dominant hemispheres (range: V1: 4.9-11.6%, V2: 5.5-15.5%) (Table 3). There were no differences in peak population CBFv change from baseline on 2-way repeated ANOVA for each of the three categories of tasks (A/B/C) (Table 3, Figure 1). Furthermore, there were no significant differences between individual tasks on post hoc testing, or significant effects of paradigm or interaction between time and paradigm (Table 3, Figure 1).

On ICC, dominant hemisphere A1-4, A8, A9, and all B and C tasks had poor scores (<0.4) (Table 4). However, tasks A5- A7 had fair correlation (0.4-0.59) (Table 4). All tasks (dominant hemisphere) had a CV within the range: 18.0-56.1 (4.9-46.1)%, and tasks with higher CV (greater dispersion) were: A7 (56.1 (46.1)%) and B5 (49.0 (19.9)%) (Table 4). In the dominant hemisphere, SEM was comparable across tasks and ranged between 2.53 and 6.11 (1.9-5.7)%, indicating that 68% of individuals would be expected to fall within this SD range of the mean. A6 had the smallest SEM (2.53 (2.5)%), and A9 had the largest SEM (6.11 (5.7)%) (Table 4).

In the non-dominant hemisphere, the A6 task had fair intra-class correlation (0.53), and A7 had good correlation (0.73); which were lower and higher than values in the dominant hemisphere, respectively (Table 3). All other tasks (A, B and C) had poor values on ICC (<0.4) (Table 4). Values for CV were generally higher in the dominant than non-dominant hemisphere (range: 16.2-42.0 (10.2-28.0)%), where B2 had the lowest CV (16.2 (10.6)%, n=8), and A7 had the highest (42.0 (25.7)%, n=10) (Table 4). In addition, SEM had lower variation in the non-dominant hemisphere, where 68% of individuals could be expected to fall within 2.52-5.33 (1.5-5.1)% of the mean. B3 had the smallest and A1 the largest SEM in the non-dominant hemisphere (Table 4).

Paradigm	n	Dominant V1	Dominant V2	P value	Non -dominant V1	Non dominant V2	P value
A1	12	7.28 (6.05)	13.81 (8.65)	0.88	6.17 (5.81)	15.52 (9.04)	0.29
A2	12	10.63 (8.58)	10.02 (4.82)	1.00	9.4 (10.02)	8.93 (5.1)	1.00
A3	12	12.72 (12.91)	11.87 (7.51)	1.00	11.38 (11.84)	12.22 (8.83)	1.00
A4	12	11.03 (6.39)	6.6 (6.19)	1.00	10.2 (5.93)	6.23 (5.35)	1.00
A5	12	4.1 (13.03)	9.56 (6.96)	0.97	4.87 (14.15)	10.06 (6.46)	0.98
A6	12	6.83 (8.74)	7.88 (7.05)	1.00	6.21 (8.66)	8.84 (7.62)	1.00
A7	12	6.97 (9.29)	9.54 (8.54)	1.00	7.12 (12.22)	8.84 (9.9)	1.00
A8	12	8.03 (12.07)	5.08 (7.11)	1.00	7.06 (13.11)	5.53 (9.34)	1.00
A9	12	8.55 (9.52)	7.86 (10.55)	1.00	7.9 (8.21)	8.27 (11.16)	1.00
			P value for Time	**0.52**		P value for Time	**0.13**
			P value for Paradigm	**0.32**		P value for Paradigm	**0.60**
			P value for interaction effect	**0.32**		P value for interaction effect	**0.20**
B1	12	10.5 (7.58)	8.83 (7.01)	1.00	9.02 (8.25)	6.22 (8.59)	1.00
B2	12	12.52 (10.94)	7.08 (8.27)	0.81	11.59 (11.1)	7.46 (8.47)	0.97
B3	12	13.9 (9.68)	10.97 (3.18)	1.00	13.37 (9.6)	10.18 (5.02)	1.00
B4	12	12.77 (7.13)	9.79 (7.06)	1.00	11.62 (6.83)	10.88 (6.36)	1.00
B5	12	13.83 (9.84)	9.56 (6.96)	0.96	10.74 (10.05)	10.06 (6.46)	1.00
B6	12	8.65 (9.58)	11.67 (8.74)	1.00	8.65 (9.57)	14.74 (8.34)	0.70
			P value for Time	**0.22**		P value for Time	**0.62**
			P value for Paradigm	**0.78**		P value for Paradigm	**0.37**
			P value for interaction	**0.46**		P value for interaction	**0.22**
C1	12	9.95 (10.63)	12.46 (8.65)	1.00	8.85 (9.27)	12.39 (9.77)	1.00
C2	12	7.04 (8.78)	13.71 (8.5)	1.00	6.4 (9.26)	13.59 (8.12)	0.69
C3	12	8.57 (8.58)	10.59 (6.14)	1.00	5.85 (11.94)	10.41 (7.88)	0.97
C4	12	9.76 (11.41)	11.1 (8.21)	1.00	10.94 (11.2)	13.14 (8.87)	1.00
C5	12	7.33 (11.33)	7.43 (6.95)	1.00	8.71 (12.5)	8.48 (8.22)	1.00
			P value for Time	0.20		P value for Time	0.07
			P value for Paradigm	0.55		P value for Paradigm	0.51
			P value for interaction	0.70		P value for interaction	0.73

Table 3. Population normalised mean (SD) peak percentage change from baseline in CBFv (dominant and non-dominant). V1= visit 1, V2 = visit 2. P values (for each hemisphere) by 2-way repeated measures ANOVA for effects of paradigm and time. P values at the end of each row are the significance for each paradigm between time points by post hoc Tukey testing. P values at the end of each column are for time, paradigm and the interaction between paradigm and time overall for each section of the ACE-III.

		Dominant				Non-dominant			
Paradigm	**n**	**ICC**	**95 %CI**	**SEM (%)**	**CV (%)***	**ICC**	**95% CI**	**SEM (%)**	**CV***
A1	12	0.00	-	4.8 (3.9)	28.4 (18.4)	0.38	-0.22, 0.77	5.3 (3.3)	42.0 (25.7)
A2	12	0.00	-	3.5 (3.7)	28.5 (24.9)	0.04	-0.52, 0.58	3.9 (3.7)	41.3 (37.0)
A3	12	0.00	-	6.0 (4.7)	39.4 (23.8)	0.09	-0.49, 0.61	5.1 (4.6)	31.7 (19.3)
A4	12	0.32	-0.28, 0.74	3.1 (2.9)	20.9 (13.9)	0.36	-0.24, 0.76	3.0 (2.2)	18.4 (11.3)
A5	12	0.54	-0.01, 0.84	4.6 (3.2)	25.8 (17.9)	0.39	-0.21, 0.78	5.0 (4.2)	27.6 (27.2)
A6	12	0.59	0.05, 0.86	2.5 (2.5)	23.6 (19.2)	0.53	-0.03, 0.84	3.0 (2.7)	21.5 (14.8)
A7	12	0.50	-0.08, 0.82	4.0 (2.2)	56.1 (46.1)	0.73	0.29, 0.91	3.7 (1.6)	35.2 (28.0)
A8	12	0.29	-0.32, 0.72	4.3 (4.2)	31.0 (24.1)	0.30	-0.31, 0.73	5.0 (4.3)	36.6 (25.7)
A9	12	0.00	-	6.1 (5.7)	37.5 (18.1)	0.00	-	5.4 (5.1)	36.3 (19.8)
B1	12	0.00	-	4.7 (2.7)	42.9 (24.0)	0.01	-0.55, 0.56	3.7 (1.9)	36.2 (22.7)
B2	12	0.04	-0.52, 0.58	5.7 (4.1)	29.7 (28.4)	0.10	-0.48, 0.62	3.7 (3.1)	16.2 (10.6)
B3	12	0.26	-0.34, 0.71	3.7 (2.6)	25.8 (14.4)	0.35	-0.25, 0.76	2.5 (2.0)	20.6 (12.5)
B4	12	0.00	-	4.5 (3.4)	26.3 (26.9)	0.00	-	3.1 (2.3)	24.2 (18.6)
B5	12	0.17	-0.42, 0.66	5.1 (2.6)	49.0 (19.9)	0.08	-0.49, 0.61	3.6 (1.6)	26.7 (13.6)
B6	12	0.01	-0.54, 0.56	5.4 (3.5)	44.6 (34.7)	0.09	-0.48, 0.62	3.8 (2.8)	28.5 (23.6)
C1	12	0.00	-	3.9 (2.9)	18.2 (13.2)	0.23	-0.37, 0.70	3.0 (3.0)	17.1 (11.3)
C2	12	0.36	-0.24, 0.76	3.4 (2.4)	18.0 (4.9)	0.00	-	3.8 (3.3)	18.1 (11.5)
C3	12	0.08	-0.49, 0.61	3.0 (1.9)	27.8 (22.2)	0.02	-0.54, 0.57	3.9 (3.3)	21.5 (22.6)
C4	12	0.07	-0.50, 0.60	3.0 (3.7)	18.6 (15.9)	0.21	-0.39, 0.68	3.5 (2.7)	21.7 (16.9)
C5	12	0.05	-0.52, 0.59	3.7 (2.5)	28.7 (25.7)	0.00	-	4.6 (2.6)	31.3 (24.6)

Table 4. Reliability measures for population normalised peak CBFv response. Measures are: intra-class correlation coefficient (ICC), with 95% confidence intervals (CI), standard error of measurement (SEM) (%), and coefficient of variation (CV) (%). *Participants were excluded from CV analysis if the mean of the two visits was low or approached zero, producing distorted results, sample size range: 6-11.

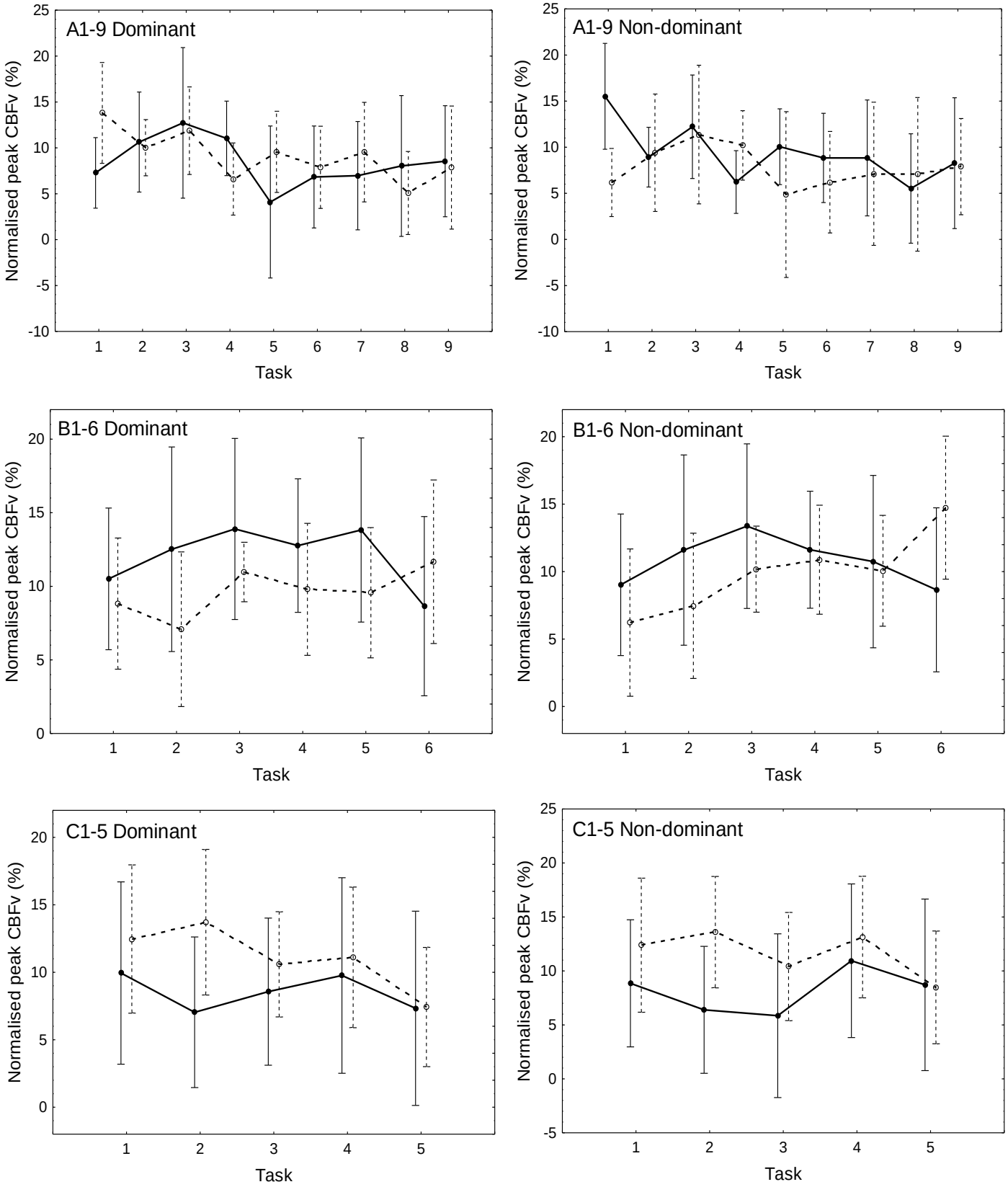

Figure 1. Repeated measures 2-way ANOVA interaction plots for population normalised peak CBFv change for each section of the ACE III. Visit 1 = solid line, Visit 2 = interrupted line. Dominant changes shown in the left hand panel and non-dominant changes in the right hand panel. Vertical lines represent 95% confidence interval error bars. There were no significant changes in any section between visits (p>0.05).

3.3 AUC_{CBFv} reproducibility

Mean AUC_{CBFv} between visits varied in both the dominant hemisphere (range: V1: 42.7-414.3(%*s), V2: 66.6-226.8(%*s)), and non-dominant hemisphere (range: V1: 49.7-405.0(%*s), V2: 72.1-261.0(%*s)) (Table 5).

On repeated measures 2-way ANOVA, only A1-9 in the dominant hemisphere was significantly different (Table 6, Figure 2). All other sections of the ACE-III produced non-significant results (Table 5, Figure 2). On post hoc testing, only the A8 AUC_{CBFv} was significantly larger on visit 1 compared to visit 2 (range: V1: 405.0-414.3 (%*s), V2: 190.9-208.8 (%*s), $P<0.05$); all other tasks being non-significant on post hoc testing (Table 5, Figure 2). The effect of paradigm was significant in all sections, in both hemispheres, with significant interaction between paradigm and time in both hemispheres in the A and B sections (Table 5).

There were a greater number of tasks on AUC_{CBFv} analysis demonstrating fair (dominant: A5, A7, C4), non-dominant: A1, A3, A6, A9, C4, B1) and good (dominant: B1 (0.6), non-dominant: A7 (0.66)) correlations, though all other tasks showed poor reproducibility on ICC (Table 6).

In the dominant hemisphere, SEM ranged between: 21.8-125.5 (13.9-101.0) (%*s), indicating that 68% of individuals would lie within this range of the mean. In the non-dominant hemisphere, this range was: 30.6-135.81 (22.7-105.5)(%*s) (Table 6). The smallest values were seen for B1 and A1 (dominant and non-dominant, respectively), and the largest for A8 (both hemispheres) (Table 6).

CV values were similar for both dominant (range: 26.5-53.7 (17.6-38.1)%) and non-dominant (range: 23.2-60.2 (20.9-37.0)%) hemispheres for AUC_{CBFv} compared to peak % CBFv changes, indicating similar dispersion for the two measures (Table 6). A6 had the lowest CV in the dominant hemisphere (26.5 (17.8)%) and non-dominant hemispheres (23.2 (22.8)%). The highest CV scores were for A7 and B3 (dominant and non-dominant, respectively) (Table 6).

Paradigm	n	Dominant T1	Dominant T2	P value	Non -dominant T1	Non dominant T2	P value
A1	12	93.21 (58.93)	105.37 (61.51)	1.00	86.34 (37.88)	135.72 (67.63)	1.00
A2	12	98.87 (62.09)	88.01 (51.18)	1.00	92.00 (74.87)	93.16 (53.81)	1.00
A3	12	181.89 (134.52)	142.52 (98.67)	1.00	168.16 (139.75)	157.84 (96.72)	1.00
A4	12	115.4 (50.63)	70.33 (38.09)	1.00	122.28 (63.44)	72.14 (32.25)	1.00
A5	12	42.69 (45.48)	85.31 (47.76)	**1.00**	49.74 (57.54)	105.88 (55.89)	0.99
A6	12	194.45 (117.32)	182.97 (108.52)	1.00	194.95 (102.33)	178.07 (87.66)	1.00
A7	12	64.82 (79.81)	127.1 (97.45)	0.95	70.62 (94.86)	121.64 (86.58)	1.00
A8	12	414.30 (244.27)	190.86 (126.76)	***<0.005**	404.98 (292.03)	208.8 (152.38)	***<0.005**
A9	12	136.29 (98.71)	134.79 (73.94)	1.00	136.11 (120.39)	141.41 (98.7)	1.00
			P value for Time	**0.03**		P value for Time	**0.35**
			P value for Paradigm	**<0.005**		P value for Paradigm	**<0.005**
			P value for Interaction	**<0.005**		P value for Interaction	**<0.005**
B1	12	201.68 (111.87)	162.33 (107.11)	1.00	184.40 (100.91)	138.36 (102.26)	0.99
B2	12	389.75 (279.84)	223.15 (146.91)	***0.008**	338.09 (276.53)	235.55 (139.33)	0.27
B3	12	213.15 (144.29)	158.90 (70.07)	0.97	210.00 (113.35)	154.35 (69.57)	0.95
B4	12	134.07 (84.02)	129.93 (85.96)	1.00	128.22 (85.88)	153.13 (90.88)	1.00
B5	12	215.78 (158.75)	94.16 (86.88)	0.15	154.80 (122.33)	86.73 (81.08)	0.83
B6	12	64.07 (40.27)	120.23 (71.09)	0.97	66.54 (47.05)	146.99 (84.38)	0.63
			P value for Time	0.07		P value for Time	0.3
			P value for Paradigm	**<0.005**		P value for Paradigm	**<0.005**
			P value Interaction Effect	**<0.005**		P value Interaction Effect	**<0.005**
C1	12	267.56 (144.06)	213.97 (159.77)	0.98	268.12 (160.96)	234.13 (174.07)	1.00
C2	12	143.44 (95.66)	226.75 (146.62)	0.75	137.96 (104.91)	260.97 (135.18)	0.37
C3	12	112.52 (83.52)	129.10 (85.12)	1.00	114.69 (109.22)	144.69 (109.22)	1.00
C4	12	126.05 (106.27)	109.21 (83.03)	1.00	120.43 (109.76)	129.95 (95.10)	1.00
C5	12	70.97 (64.53)	66.55 (37.06)	1.00	90.66 (75.91)	77.49 (46.61)	1.00
			P value for Time	**0.8**		P value for Time	**0.32**
			P value for Paradigm	**<0.005**		P value for Paradigm	**<0.005**
			P value for Interaction	**0.34**		P value for Interaction	**0.26**

Table 5. Area under the curve for changes in CBFv for all paradigms at visits 1 (T1) and 2 (T2) for both dominant and non-dominant hemispheres. P values from 2-way repeated measures ANOVA. * Statistically significant on post hoc testing by Tukey. P values at the end of each row are the significance for each paradigm between time points by post hoc Tukey testing. P values at the end of each column are for time, paradigm and the interaction between paradigm and time overall for each section of the ACE-III.

		Dominant				Non-dominant			
Paradigm	**n**	**ICC**	**95% CI**	**SEM (%*s)**	**CV (%)**	**ICC**	**95% CI**	**SEM (%*s)**	**CV (%)**
A1	12	0.12	-0.46, 0.63	27.5 (28.5)	32.3 (28.8)	0.42	-0.17, 0.79	30.6 (22.7)	34.3 (25.9)
A2	12	0.00	-	31.2 (28.8)	36.8 (33.1)	0.00	-	32.7 (33.9)	35.1 (32.3)
A3	12	0.10	-0.48, 0.62	56.5 (56.5)	39.4(33.0)	0.40	-0.19, 0.78	44.7 (46.6)	32.1 (25.8)
A4	12	0.00	-	36.2 (13.9)	42.4 (23.8)	0.00	-	40.5 (22.8)	42.2 (21.1)
A5	12	0.59	0.06, 0.86	21.8 (20.5)	43.2 (38.1)	0.15	-0.44, 0.65	35.1 (29.7)	51.2 (36.8)
A6	12	0.23	-0.37, 0.70	49.5 (47.7)	26.8 (20.9)	0.52	-0.047, 0.83	35.9 (29.3)	23.2 (22.8)
A7	12	0.51	-0.059, 0.83	40.4 (34.9)	53.7 (31.3)	0.66	0.16, 0.89	35.6 (27.2)	53.4 (28.4)
A8	12	0.36	-0.24, 0.76	125.5 (92.9)	44.8 (26.0)	0.24	-0.37, 0.70	135.8 (105.5)	52.3 (30.1)
A9	12	0.20	-0.40, 0.68	41.3 (34.5)	33.2 (23.3)	0.40	-0.19, 0.78	45.6 (36.7)	35.3 (22.0)
B1	12	0.60	0.07, 0.87	40.8 (31.7)	26.5 (17.8)	0.58	0.04, 0.86	41.8 (28.7)	31.9 (22.6)
B2	12	0.33	-0.28, 0.74	114.2 (101.0)	38.1 (29.3)	0.30	-0.30, 0.73	113.9 (73.7)	60.2 (37.0)
B3	12	0.04	-0.53, 0.58	65.0 (48.8)	34.6 (17.6)	0.03	-0.53, 0.57	53.3 (45.1)	30.4 (22.6)
B4	12	0.00	-	55.2 (34.5)	45.7 (26.3)	0.00	-	54.0 (46.9)	39.3 (33.0)
B5	12	0.16	-0.43, 0.66	74.7 (69.3)	43.2 (31.9)	0.37	-0.23, 0.77	48.2 (46.2)	48.1 (31.8)
B6	12	0.00	-	41.3 (31.2)	43.6 (29.2)	0.054	-0.51, 0.59	51.1 (33.5)	49.8 (24.8)
C1	12	0.00	-	90.8 (88.1)	35.7 (24.5)	0.00	-	94.5 (95.8)	33.8 (25.4)
C2	12	0.00	-	70.8 (67.7)	35.5 (27.2)	0.00	-	92.3 (71.9)	46.3 (31.8)
C3	12	0.00	-	48.9 (34.9)	47.6 (29.5)	0.00	-	61.9 (40.4)	54.6 (30.4)
C4	12	0.43	-0.16, 0.80	35.2 (36.1)	30.5 (20.3)	0.40	-0.20, 0.78	36.9 (41.2)	31.2 (20.9)
C5	12	0.00	-	28.3 (25.4)	42.0 (30.3)	0.00	-	38.6 (25.7)	50.9 (29.8)

Table 6. Reliability measures for population normalised AUC_{CBFv} response. Measures are: intra-class correlation coefficient (ICC), with 95% confidence intervals (CI), standard error of measurement (SEM) (%), and coefficient of variation (CV) (%).

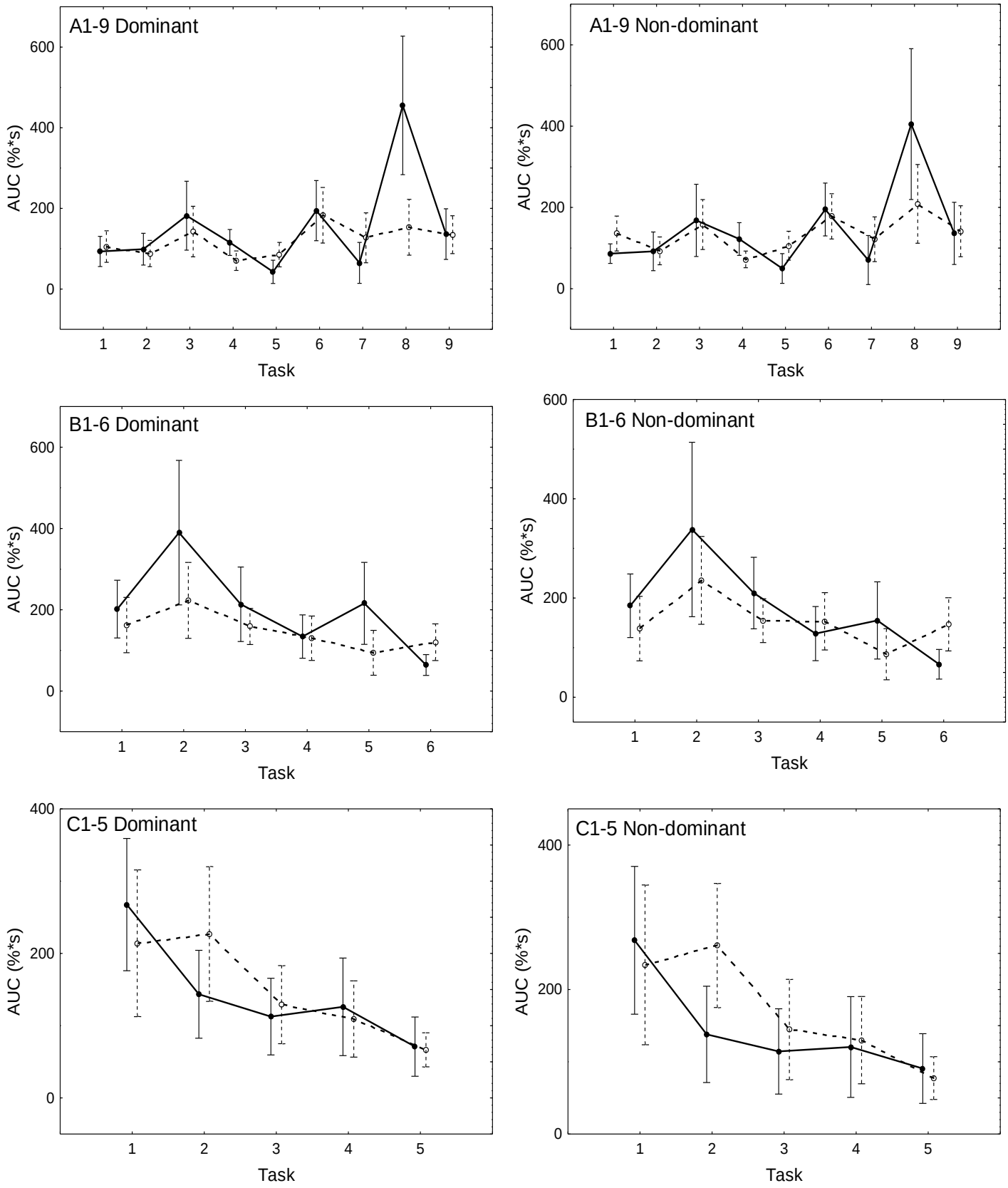

Figure 2. Repeated measures 2-way ANOVA for population normalised CBFv AUC for each section of the ACE-III. Visit 1 = solid line, Visit 2 = interrupted line. Dominant changes shown in the left hand panel and non-dominant changes in the right hand panel. Vertical lines represent 95% confidence interval error bars. Only A8 and B2 differed significantly between visits (p>0.05).

1. Discussion

4.1 Summary of main results

To our knowledge, this is the first study to determine the reproducibility of TCD measured peak, and AUC, CBFv responses to a complete cognitive assessment tool. Of the twenty paradigms studied, only one (A8) produced a significant difference between visits on ANOVA testing, indicating this paradigm may be less useful in studies of task activation. There were a greater number of tasks on AUC_{CBFv} analysis demonstrating fair to good ICC (n=11 vs. n=5), indicating AUC_{CBFv} may be a more reliable measure than peak CBFv response in task activation studies. A5, A6, and A7 tasks were associated with the most consistent peak and AUC_{CBFv} responses, having higher ICC values, indicating better reproducibility. In addition, the reproducibility of tasks varied between the dominant and non-dominant hemisphere, with more tasks in the dominant hemisphere producing better reproducibility in peak CBFv response, and conversely, more tasks in the non-dominant hemisphere for AUC_{CBFv} response.

4.2 Context of the literature

A number of TCD studies have previously investigated reproducibility (10, 12, 30). Brodie et al examined the within and between subject reproducibility of CBFv and derived parameters, demonstrating CBFv produced the most reproducible response over four recordings in a two-week period (12). Of note, Autoregulation Index (ARI) had the lowest ICC of the examined parameters (12). With regards to inter-rater reproducibility, in the study reported here, the two operators had a similar level of training and exposure. In a study by McMahon et al, intra-observer reproducibility was found to be much higher than inter-observer reproducibility suggesting clinical decisions should be based on multiple measures by the same operator (30). In addition, agreement was higher between more experienced operators (30). The higher intra-observer reliability is in agreement with

a study by McDonell et al, where intra-rater reliability performed better than inter-rater reliability in the assessment of cerebrovascular reactivity to CO_2 inhalation (13). Furthermore, a seated rather than supine position produced more reliable results, which is important for task activation studies, such as ours using a seated protocol (13).

Fewer studies are available examining the reproducibility of cognitive paradigms (18, 19, 31). Salinet et al investigated the reproducibility of passive, active and imagined motor paradigms in a group of healthy volunteers over a one-week period (26). ICC values were higher than those reported here (ICC range 0.5-0.8, SEM 2.4-5.5%), however relatively fewer paradigms were studied, measures were made by the same operator (intra-operator) and at a shorter time interval (26). Correlation was better at task initiation, however AUC values were not examined (26). The laterality index was investigated in three studies (18-20), where all demonstrated high test/re-test reproducibility (18-20). Stroobant et al examined 13 verbal and visuospatial tasks, and demonstrated reproducibility was task dependent (18), in agreement with the findings reported here. In a study by Whitehouse et al, a spatial working memory task produced good ICC scores for the laterality index, with no evidence of habituation to repeated assessment (19). Apart from one paradigm in this study, we also did not find habituation to be a significant factor in repeated CBFv responses. Vingerhoets et al examined the within subject reproducibility of an arithmetic task in one subject over 20 consecutive days (31). Variability remained stable over this time period, but measurements in the anterior cerebral artery had higher variability than those in the MCA, suggesting the MCA is a more reliable vessel to study (31). In particular, reliability was improved when averaged over three difficult conditions, leading the authors to conclude that short multiple activations within one session may be a more reliable protocol (31). Despite similarities in tasks between this study and that by Stroobant et al (i.e. word fluency, 3-D cube construction), we reported differences in the reproducibility of theses paradigms (18). However, here, we report the reproducibility of peak and AUC CBFv response rather than LI, and the time to follow up was notably significantly longer in this study. Furthermore, statistical comparisons were made here using ICC, SEM and CV, rather than

Pearson correlation, which may account for some of these discrepancies. Stroobant et al suggest that longer, more complex tasks with multiple within subject measures are more reproducible (18). We found mixed evidence to support this. Whilst complex tasks such as A4 (subtracting seven serially from 100), B2 (writing sentences), and C1 (constructing a cube and infinity diagram) were less reproducible, so were simpler tasks (A1 – orientation to time, A2 – orientation to space, C4-recognising obscured letters). A6, and A7 were relatively complex tasks, and were amongst the more reproducible paradigms in this study. This relationship becomes more complex, however when considering this respective to hemispheric dominance, where some paradigms have higher ICC and lower CV values in a specific hemisphere. Interestingly, tasks which demonstrated higher ICC scores (i.e. A7), do not necessarily have lower CV or SEM measures, indicating important differences in measurements of reproducibility. Furthermore, Stroobant suggests that longer tasks may also introduce lesser variability (18), which was demonstrated by A6 and A7, comprising some of the longest tasks in the ACE-III.

4.3 Sources of variability

A number of factors could potentially have introduced variation into the results reported here. The time elapsed between the original and repeat assessment was relatively long (median time to follow up: 343 days [11]). During this time, there was an increase in the mean ACE-III score, and there were no significant changes in medical history or medication for participants. In a previous study by Bay-Hansen et al, correlation coefficients on measures repeated at two hours were higher than those performed at 60 days, suggesting the longer the time interval, the greater the variability introduced into measurements (29). Furthermore, LI reproducibility studies by Knecht et al and Stroobant et al produced differing results which they attribute to different time intervals and population heterogeneity (18, 20). However, the stability of measurements over longer time intervals are of relevance to clinical practice, where follow-up times can range from three months to one year. The

learning or practice effect from repeated cognitive testing (32) is an important consideration, which could potentially habituate changes in CBFv between assessments (18, 20). Certainly, this could provide some explanation as to why the A8 task was less reliable, given that a number of participants could recall parts of the name and address. This was likely encoded from their last assessment, thus providing a smaller peak and AUC_{CBFv} response. Furthermore, the autonomic response contributes to a proportion of the CBFv change (33), but can attenuate between visits as participants are more comfortable having previously experienced the protocol (18, 20). Variability can also be introduced by the different operators, which can have a significant effect on the TCD procedure, and in terms of interaction and style during the cognitive assessment with participant (18, 20). The conditions were broadly similar between assessments, where both were undertaken in the same setting and participants were requested to abstain from nicotine, alcohol, large meals and strenuous exercise for the same time periods prior to study commencement. The rest period between tasks was increased from 30 seconds to one minute between visits, as a number of participants did not return to baseline in the initial assessment. This could impact on the AUC_{CBFv} analysis, although not for tasks with a shorter CBFv response.

4.4 Limitations

There are a number of limitations to consider in interpreting the results of this study. Firstly, the use of TCD relies on the assumption that the vessel diameter remains relatively constant, despite changes in MAP and $ETCO_2$, (25). In this study, we focused on inter-, rather than intra-rater reliability, reflecting the reality of multiple operators performing follow up assessments in clinical practice. This limits the inferences that can be made on the within subject, or intra-rater variability of this testing protocol. In addition, we cannot eliminate the potential intra-subject physiological variation that will have contributed to the differences in measurements. Furthermore, the variation occurring within a visit was not assessed. The protocol utilised in our study was relatively long

(approximately 90 minutes), which may therefore affect the reproducibility of responses (31). The major limitation to this study is the long time between the first and second measurements, likely introducing the greatest amount of variability. This has the advantage of limiting learning and practice effects that would occur in a shorter time interval, but perhaps gives less information on reproducibility at shorter intervals. Despite the longer time interval, accommodation was potentially a source of variation introduced with one of the paradigms investigated (A8). The second major limiting factor in this study is the relatively small sample size. The sample size used in this study was based on previous TCD studies (12, 26), but none the less a small, relatively young sample of healthy adults was studied. At the extremes of age, the reproducibility of TCD parameters decline (12) and this warrants a further investigation in a healthy older population. Whilst the peripheral parameters (MAP, HR and $ETCO_2$) did not differ between visits, variation in these parameters occurring across tasks could affect the reproducibility of the measurements reported. However, Maeda et al demonstrated $ETCO_2$ did not affect reproducibility of CBFv responses (10). The angle of insonation was a more important source of variation, however we eliminated this here by studying peak changes normalised to a baseline period, thus not entering in variation in raw CBFv data as a result of inter-observer technique (10, 20). In addition, spontaneous fluctuations in CBFv, HR, MAP, and $ETCO_2$ can occur and introduce noise or artefact into the data. However, all data were visually inspected and noisy data sets were excluded from this analysis; this affecting only one individual. The majority of the AUC_{CBFv} data were normally distributed (82%), therefore parametric analyses were used, however it must be considered that some of the data were skewed and this may affect the results. Only the mean of the positive values were taken to calculate the AUC_{CBFv} in order to estimate the percentage rise in CBFv from baseline. However, the AUC_{CBFv} will vary in duration between individuals, with some curves returning to baseline faster, and therefore negative values have been excluded from these individuals to estimate the total positive change in response to task activation. Furthermore, a 30 second time interval was used in the first visit, and a 1 minute interval between tasks in the second visit. This was due to not all responses returning to baseline in the first

measurement. This requires consideration in interpreting the results of this analysis. CV is highly dependent on the mean values, and if the mean of the two visits is low or approaching zero, this can significantly distort the results. Therefore, a number of participants were excluded, and this reduced the total number of measurements for the CV analysis.

4.5 Future work

Investigating the intra-observer reproducibility of this protocol would give greater insight into whether the variation demonstrated here was the result of differences in operator technique, or natural physiological variation. Furthermore, the reproducibility at varying time intervals would give an indication of the effect of habituation to cognitive testing on CBFv response. The reproducibility of this protocol requires further validation in healthy older adults, and those with cognitive impairment, to evaluate its utility as a reliable diagnostic protocol. Future improvements in reproducibility could follow from adopting multivariate modelling to adjust for concomitant individual changes in co-factors of CBFv variability such as pCO_2 and MAP.

5. Conclusions

In summary, a number of tasks from the ACE-III demonstrate fair-to-good correlation across peak and AUC_{CBFv} responses using TCD. The results from this study can inform future studies where task reproducibility is of importance, particularly in the setting of repeated follow-up. The reproducibility of these parameters may vary further in disease states. Further investigations in patient populations are therefore required.

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