Abstract

Background: We tested the hypothesis that paradigms from the Addenbrooke's Cognitive Examination (ACE-III), including those that had not been studied using TCD previously (novel) versus those which had been (established), would elicit changes in CBF velocity (CBFv).

New Method: Healthy subjects were studied with bilateral transcranial Doppler (TCD), beat-to-beat blood pressure (Finapres), continuous electrocardiogram (ECG), and end-tidal CO_2 (nasal capnography). After a 5-minute baseline recording, cognitive tests of the ACE-III were presented to subjects, covering *attention* (SUB7, subtracting 7 from 100 sequentially), *language* (REP, repeating words and phrases), *fluency* (N-P, naming words), *visuospatial* (DRAW, clock-drawing), and *memory* (MEM, recalling name and address). An event marker noted question timing.

Results: Forty bilateral data sets were obtained (13 males, 37 right-hand dominant) with a median age of 31 years (IQR 22-52). Population normalised mean peak CBFv % in the dominant and non-dominant hemispheres, respectively, were: SUB7 (11.3±9.6%, 11.2±10.5%), N-P (12.7±11.7%, 11.5±12.0%), REP (12.9±11.7%, 11.6±11.6%), DRAW (13.3±11.7%, 13.2±15.4%) and MEM (13.2±10.3%, 12.0±10.1%). There was a significant difference between the dominant and non-dominant CBFv responses ($p<0.008$), but no difference between the amplitude of responses.

Comparison with Existing Methods: For established paradigms, our results are in excellent agreement to what has been found previously in the middle cerebral artery.

Conclusions: Cognitive paradigms derived from the ACE-III led to significant lateralised changes in CBFv that were not distinct for novel paradigms. Further work is needed to assess the potential of paradigms to improve the interpretation of cognitive assessments in patients at risk of mild cognitive impairment.

1. Introduction

By 2050, 135.5 million people are predicted to be living with dementia worldwide (Robinson et al. 2015); cognitive impairment having a great impact not only on the patient, but also on their relatives, carers and health care providers. Previous studies have shown an association between cerebral haemodynamic abnormalities and cognitive decline (den Abeelen et al. 2014, Keage et al. 2012).

Neurovascular coupling (NVC) refers to the changes in cerebral blood flow (CBF) in response to brain activation, and is carried out by the components of the neurovascular unit; endothelial cells, neurons, pericytes and astrocytes. Transcranial Doppler ultrasound (TCD) is a non-invasive tool used to continuously monitor CBF velocity (CBFv) in major vessels such as the middle cerebral artery. The technique has a good temporal, but poorer spatial, resolution in comparison with other methods used to measure CBF, including functional magnetic resonance imaging (fMRI). However, advantages of using TCD are its ability to be used at the bed-side, and the ability of subjects to be in the seated position whilst carrying out tasks such as writing.

Cognitive batteries are widely used clinically in the diagnosis of cognitive impairment, including dementia and mild cognitive impairment (MCI). CBFv and NVC responses to questions commonly used in the diagnosis of cognitive impairment have not been studied in detail to date; previous studies having largely investigated the responses to individual cognitive tasks which are not widely used to aid the diagnosis of dementia (Keage et al. 2012).

The Addenbrooke's Cognitive Examination-III (ACE-III) is a validated tool, with an excellent sensitivity and specificity for diagnosing dementia, which is routinely used in clinical practice (Hodges 2011, Hsieh S et al. 2013). The examination takes approximately 25 minutes to complete and comprises questions from five cognitive domains (attention, fluency, language, visuospatial and memory).

The possibility of matching the ACE-III results to corresponding NVC responses has considerable potential to improve assessment of individuals at risk of MCI, particularly in the identification of cognitive losses of vascular origin. However, whilst some of the ACE-III questions can be regarded as equivalent to cognitive paradigms that have been previously adopted in NVC studies, such as the N-back and naming words, the majority of the questions involved in its five domains have not been validated regarding their ability to elicit an equivalent NVC response as quantified from the CBFv change following neural activation (Moody et al. 2005, Vermeij et al. 2014). As a first step towards creating a NVC-based 'mapping' to the ACE-III battery, we tested the hypothesis that it is feasible to use TCD to assess CBFv responses in the MCA to paradigms contained within the ACE-III. We also tested the hypothesis that paradigms which had not been studied previously (novel), such as simply drawing a clock, would be able to elicit a detectable change in CBFv similarly to more established protocols. For these purposes, we selected five representative questions, one for each of the ACE-III's main domains.

2. Methods

This observational, cross-sectional study took place over a four-month period. Ethical approval was obtained from the University of Leicester (Reference: 5355). Healthy controls were recruited via the University of Leicester by means of poster and email invitation and formally consented. Inclusion criteria were age ≥18 years and willingness to comply with all study requirements. The exclusion criteria included females who were pregnant, lactating or planning a pregnancy during the course of the study as well as being unable to comply with the study requirements. The

experiment took place in the Cerebral Haemodynamics in Ageing and Stroke Medicine (CHIASM) laboratory at the Leicester Royal Infirmary, which is of controlled temperature and free from distraction.

Volunteers were asked to abstain from large meals, strenuous exercise, nicotine, caffeine and alcohol for four hours prior to the recordings. CBFv was recorded bilaterally with TCD (Viasys Companion III) in the MCA, together with heart rate (3-lead ECG), beat-to-beat blood pressure(BP) using arterial volume-clamping of the digital artery on the non-dominant hand (Finometer, Finapres Medical Systems; Amsterdam, the Netherlands) and end-tidal CO_2 via nasal cannula , (Salter Labs, ref. 4000; Capnocheck Plus). TCD probes were held in place using a head-frame. Signals were sampled at 500 samples/second and stored in the PHYSIDAS data acquisition system

Four separate recordings were performed in each subject, comprising a five-minute baseline recording during which participants sat quietly with their eyes open, followed by three separate recordings which divided the ACE-III questions into the following sections: 'A' (4 attention, 3 memory and 2 fluency paradigms), 'B' (6 language paradigms) and 'C' (4 visuospatial and 1 memory paradigm). Each recording began and ended with a one-minute baseline recording and there was a thirty-second rest between individual questions to enable CBFv to decrease following previous stimulation. An event-marker was used to note question timings and the brachial BP (UA767 BP monitor) of the dominant hand was measured prior to each recording for calibration of the Finometer device. The entire protocol took ninety minutes from set up to completion, but individual paradigm durations varied according to how long each subject took to complete each of the tasks. There were no requirements for any follow up sessions.

Following these measurements, data editing software (RBP) was used to determine which recordings were suitable for further analysis. Firstly, the raw signals were visually inspected and linear interpolation was used to remove non-physiological spikes (<100ms) in the individual CBFv waves. A median filter was used to remove smaller spikes and all signals were low-pass filtered (20 Hz) with a zero-phase Butterworth filter. The beginning and end of each cardiac cycle were detected on the ECG and mean values of CBFv and BP, as well as heart rate and end-tidal CO_2, were obtained for each heart beat. Standard polynomial interpolation, followed by re-sampling at 5 Hz, was performed to obtain signals with a uniform time base (Moody et al. 2005).

After this analysis of the four recordings from each volunteer, files containing the groups of participants with good-quality data were created from which population average responses could be determined using the saved events marker timings as the point of synchronism. The population average responses were assessed bilaterally, in the dominant and non-dominant hemispheres, and were normalised to the fifteen-seconds immediately preceding that particular question. In addition to the population averages, the standard error of the mean (SEM) was also calculated at each sample point.

One question from each domain was analysed in this feasibility study; subtracting seven from one hundred and subsequent answers repeatedly (attention, SUB7), naming words beginning with 'P' (fluency, N-P), repeating words and phrases (language, REP), clock-drawing (visuospatial, DRAW) and recalling a previously learned name and address (memory, MEM).

3. Statistical analysis

For each question, a graph of the population average bilateral CBFv changes with time was plotted and the peak CBFv responses to questioning were determined, as well as the largest SEM at the point of occurrence. In addition to the calculation of population mean and SEM at each sample point, that is every 0.2 s within a 60s window

of observation, the peak value of each normalised CBFv response was extracted for both right and left MCA as the averaged CBFv value from the 10s surrounding these peaks. A repeated measures 2-way ANOVA was performed (see Appendix A) on the averaged peak values to test for differences amongst paradigms as well as the influence of hemispheric dominance. The peak cross-correlation value between each individual response and the population average was used to assess the extent to which a response could be identified in each subject. Statistical significance was assumed for $p<0.05$.

4. Results

Forty-eight healthy volunteers were approached and subsequently recruited to the study between February and May 2016. All tolerated the study protocol but,because limited information is available about the effect of handedness on CBFv in response to cognitive stimuli, only those participants with full bilateral data sets were analysed. This excluded participants with bilateral window failure (n=1), unilateral poor quality data (n=6) and equipment failure (n=1). Therefore, 40 good quality bilateral data sets were obtained (13 males, 37 right-hand dominant) with a median age of 31 years (IQR 22-52).

Participants had an average ACE-III score of 98 ±2.03 with a lowest score of 93 out of 100, suggesting that no participants were cognitively impaired (Hodges 2011). Mean individual paradigm scores were; SUB7 (4.73 out of 5 points), N-P (6.18 out of 7), REP (4 out of 4), DRAW (4.93 out of 5) and MEM (11.73 out of 12). Population average paradigm durations were: SUB7 (27.76 ± 11.68s), N-P (96.75 ± 9.10s), REP (27.17 ± 3.73s), DRAW (41.57 ± 10.82s) and MEM (20.00 ± 13.28s).

Figs. 1 (established tasks) and 2 (novel tasks) show the population averaged normalized CBFv changes with time for all five questions in both the dominant and non-dominant hemispheres. Despite marked differences in temporal patterns, all CBFv responses had an initial sharp rise upon paradigm commencement (at t=0), followed by a second peak and then a slow decrease to baseline level.

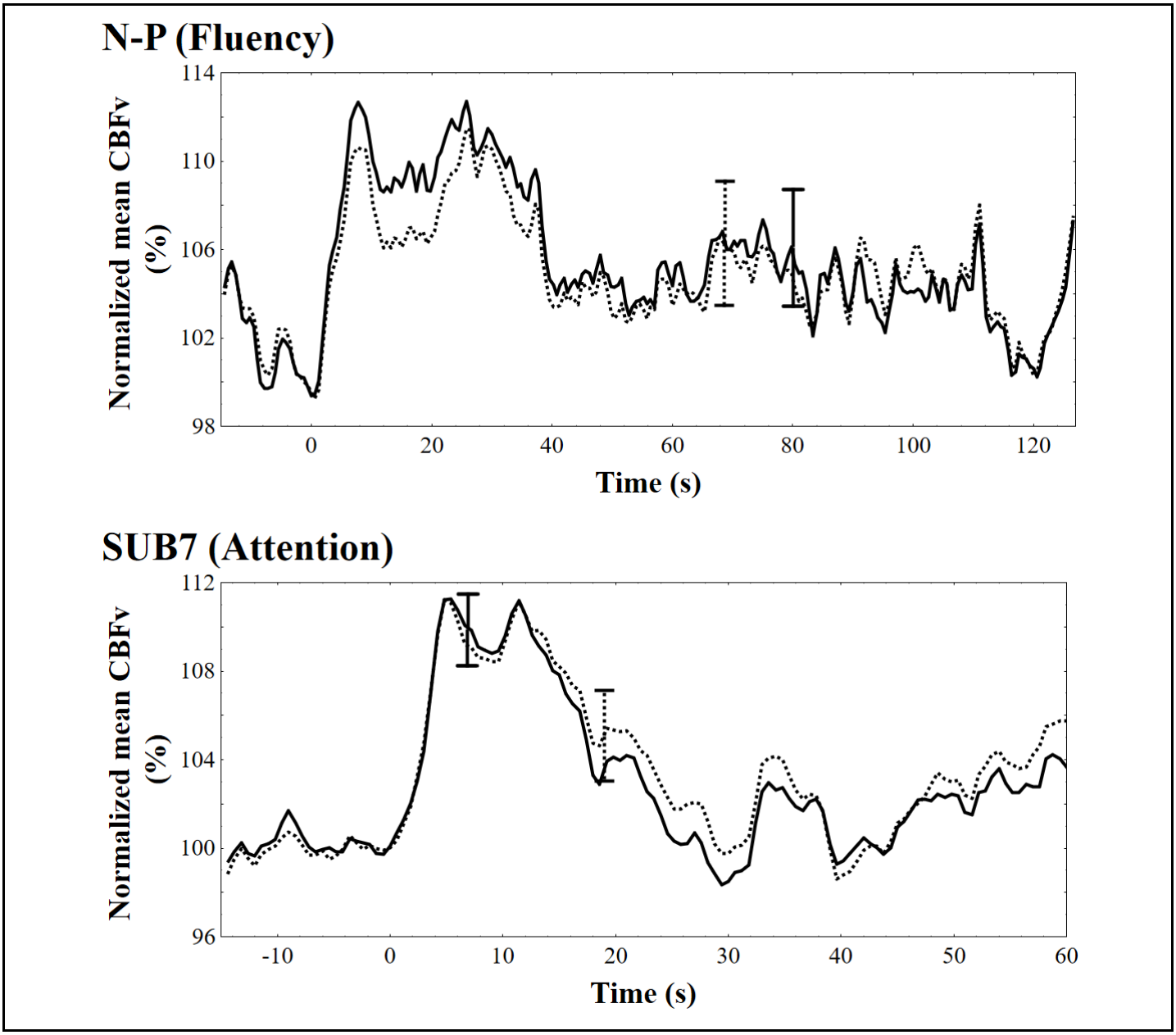

Fig. 1. Dominant (solid line) and non-dominant (dotted line) CBFv population averages (normalized by 15s preceding rest) during the established (N-P and SUB7) paradigms. Task initiation is at t=0s. Fifteen seconds of rest preceding and 30s following each paradigm are shown. Error bars represent the largest +/- 1 SE at the point of occurrence (timing of the greatest SE) bilaterally.

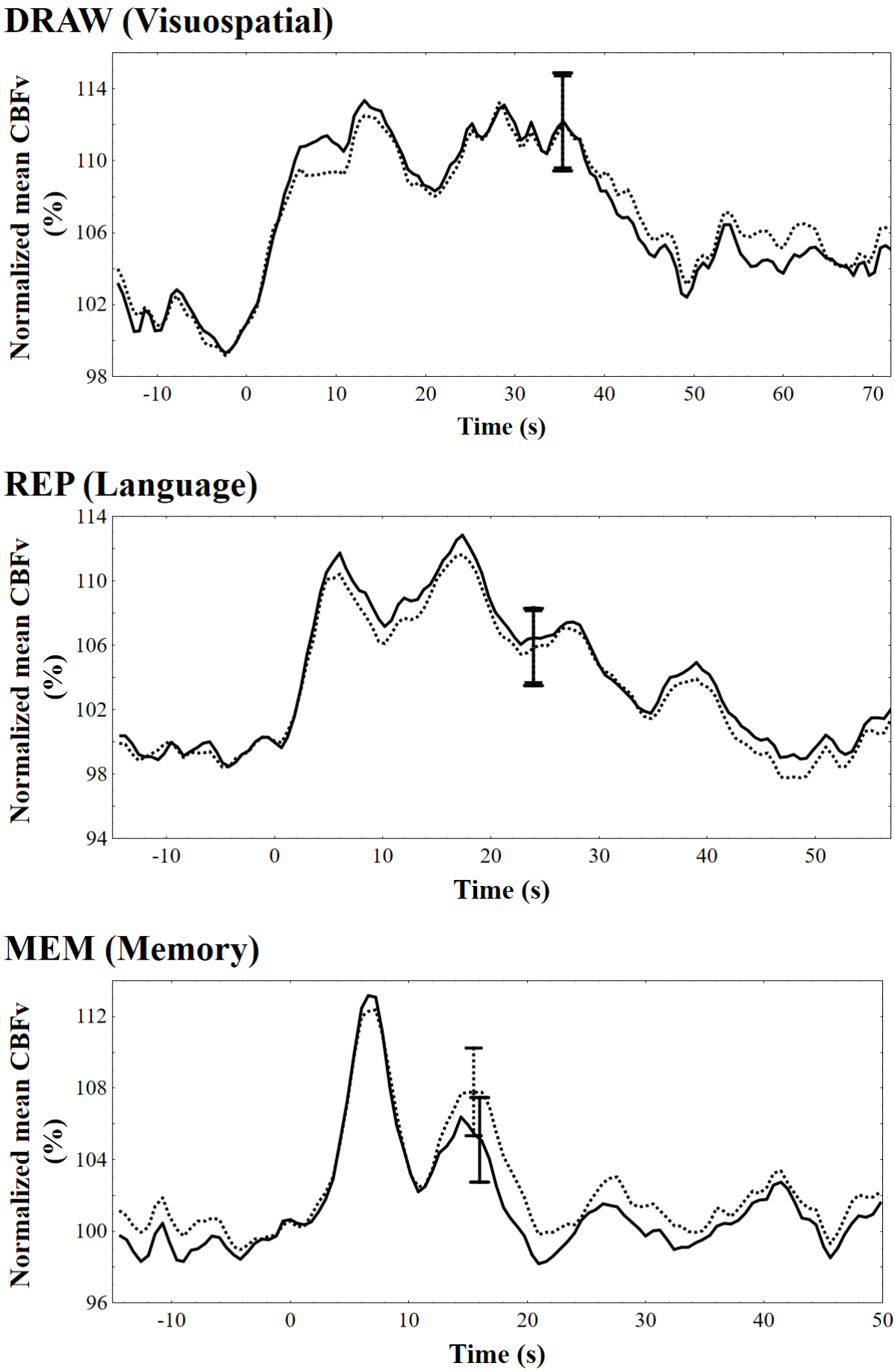
DRAW (Visuospatial)
Normalized mean CBFv (%)
114
110
106
102
98
-10
0
10
20
30
40
50
60
70
Time (s)
REP (Language)
Normalized mean CBFv (%)
114
110
106
102
98
94
-10
0
10
20
30
40
50
Time (s)
MEM (Memory)
Normalized mean CBFv (%)
112
108
104
100
96
-10
0
10
20
30
40
50
Time (s)

Fig. 2. Dominant (solid line) and non-dominant (dotted line) CBFv population averages (normalized by 15s preceding rest) during the novel (DRAW, MEM and REP) paradigms. Task initiation is at t=0s. Fifteen seconds of rest preceding and 30s following each paradigm are shown. Error bars represent the largest +/- 1 SE at the point of occurrence (timing of the greatest SE) bilaterally.

SUB7 5.4s and 4.8s, N-P 25.8s and 25.8s, REP 17.4s and 17.4s, DRAW 13.2s and 28.2s, and MEM 6.6s and 6.0s (ANOVA, p=0.03). Peak population average normalized CBFv changes in the dominant and non-dominant hemispheres were, respectively, SUB7 (11.3 ± 9.6% at 5.4s 11.2 ± 10.5% at 4.8s), N-P (12.7 ± 11.7% at 25.8s, 11.5 ± 12.0% at 25.8s), REP (12.9 ±11.7% at 17.4s, 11.6 ± 11.6% at 17.4s), DRAW (13.3 ± 11.7% at 13.2s, 13.2 ± 15.4% at 28.2s) and MEM (13.2 ± 10.3% at 6.6s, 12.0 ± 10.1% at 6.0s). No significant differences in averaged peak CBFv response were observed between the five paradigms (ANOVA p=0.12), but the effect of hemispherical dominance was highly significant (ANOVA, p<0.008).

No significant differences were found between paradigms regarding peak cross-correlation (CCmax) values for individual responses in relation to population averages. For each paradigm, the CCmax [median (IQR)] for individuals compared to the population averages in the dominant and non-dominant hemispheres, respectively, were: SUB7 [0.53 (0.36-0.64), 0.52 (0.33-0.62)], N-P [0.54 (0.30-0.72), 0.51 (0.29-0.69)], REP [0.64 (0.39-0.73), 0.63 (0.37-0.71)], DRAW [0.58 (0.27-0.78), 0.56 (0.25-0.77], and MEM [0.51 (0.40-0.63), 0.49 (0.30-0.64)]. In all cases the lower IQR was higher than the 95% confidence limit for the cross-correlation peak, estimated as 0.113. In general, in each of the paradigms, the number of subjects with non-significant cross-correlation peaks (CCmax<0.113) ranged from two to three.

5. Discussion

The key finding from this work is that the bilateral MCA CBFv can be measured using TCD in real-time during performance of the ACE-III. Although some of the tests representing each of the five domains of the ACE-III would be expected to elicit a significant CBFv response (SUB7, N-P), the other three tests (REP, DRAW, MEM) have not been previously assessed in their ability to reflect increases in metabolic demand associated with transient neural activation.

There was a significant increase in CBFv during all five paradigms investigated, with each question eliciting an immediate sharp and biphasic increase in blood flow in both the dominant and non-dominant hemisphere. Time to peak response varied significantly between paradigms, suggesting important differences in temporal patterns of neuro-activation. Visually, the DRAW paradigm demonstrated the greatest peak CBFv bilaterally out of all five paradigms and lowest CBFv responses were observed for the SUB7 (established) and MEM (novel) paradigms.

We are not aware of previous studies using TCD to perform a complete set of NVC assessments for a well-known battery of cognitive tests such as the ACE-III. For isolated paradigms, usually of the type we have labelled as 'established, our results are in excellent agreement regarding temporal pattern and amplitude values to what has been found by other investigators (Boban et al. 2014, Droste et al. 1989, Matteis et al. 1998, Moody et al. 2005, Smirl et al. 2016, Sorond et al. 2008, Stroobant, Vingerhoets 2000, Vingerhoets, Stroobant 1999). Nevertheless, we did not find a statistically significant difference in the amplitude of responses as reported by Sorond and colleagues for a different set of cognitive tasks (Sorond et al. 2008). Relevant to our aims, a highly influential review has suggested ways of standardising cognitive TCD protocols to improve inter-centre comparisons (Stroobant, Vingerhoets 2000).

5.1 Limitations

We chose to use the MCA as the artery for measuring CBFv. As different arteries supply different cortical regions of the brain, and these regions are involved in different aspects of cognition, for different questions it may have been appropriate to have monitored different arteries, for example the anterior or posterior cerebral arteries. Note that by using TCD we monitored CBFv as opposed to CBF, but as studies have shown MCA diameter to remain almost constant at normocapnia, we can use this as an acceptable surrogate for CBF (Ainslie, Hoiland 2014).

Leaving just 30s between questions meant that the CBFv did not always return to resting baseline levels in all participants for all questions. It is important to note that these "resting" periods may not have been the same in all volunteers. Some participants stated that they had used that time to think about previous questions, such as remembering the address which they were told they would have to later recall.

This paper investigated the measurement of CBFv during the ACE-III with the objective of demonstrating the feasibility of assessing NVC based on standard battery tests involving novel cognitive paradigms. Considerable work will be needed for detailed analysis of similar responses for all 20 questions. We must bear in mind that the ACE-III was designed for use in a patient population. The feasibility demonstrated in this piece of work is therefore limited to the relatively young and healthy population investigated and can therefore not be immediately assumed for an older, cognitively impaired patient group.

6. Conclusion

This study has shown that it is feasible to use TCD to monitor changes in CBFv during performance of cognitive tasks from all 5 domains of the ACE-III in healthy controls. CBFv increased with all 5 paradigms analysed, and there was a correlation between this bilaterally in all participants for all questions. Additionally, no significant differences in CBFv responses were noted when comparing conventional and non-conventional paradigms. Lastly, a significant effect of dominance on the elicited CBFv response was noted. Having demonstrated that TCD can be used to record the CBFv responses of healthy controls to detailed cognitive testing in real-time, this technique could now be used to study CBFv responses in patient populations, including those with cognitive impairment.

7. Author Contributions

Study conceptualization and design (CALW, VJH, RBP, TGR), application for ethical approval (VJH), data collection and analysis (CALW), software design and data analysis supervision (RBP), drafted manuscript (CALW), revised and approved final version of manuscript (CALW, VJH, RBP, TGR).

8. Disclosure/Conflict of Interest

The authors have no conflicts of interest to declare.

9. Acknowledgements

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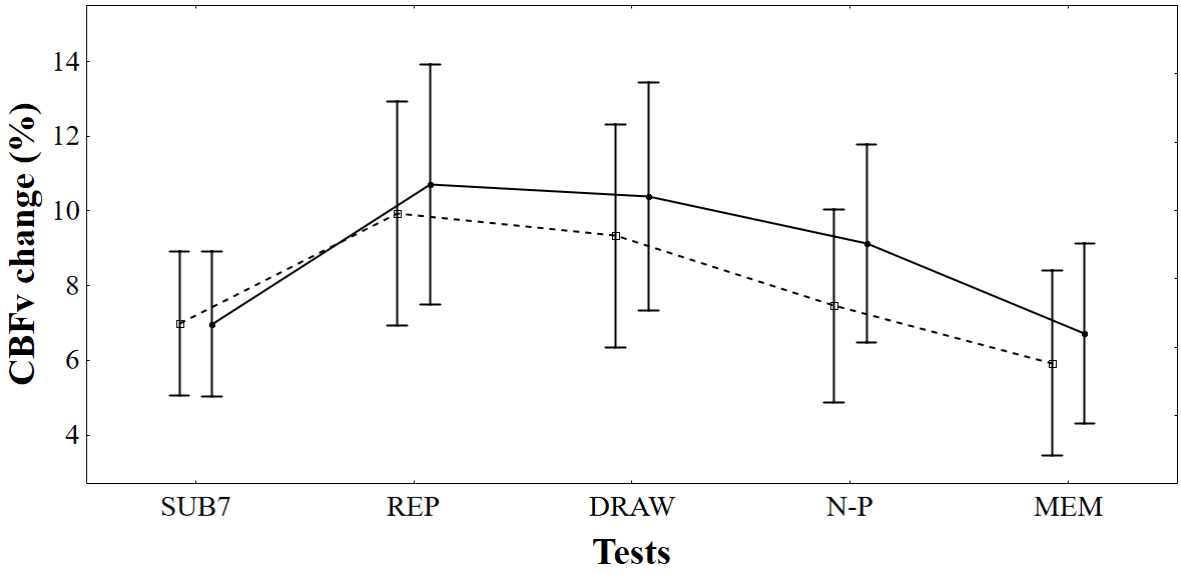
10. Appendices



Appendix A. Two-way ANOVA results comparing the CBFv changes for the 5 paradigms (SUB7, REP, DRAW, N-P and MEM) bilaterally (dominant (solid line) and non-dominant (dotted line)). The vertical bars represent 95% confidence intervals.

Paradigm	Parameter	Mean (SD)
SUB7	**ABP**	91.62(±15.02)
	HR	80.18(±12.95)
N-P	**ABP**	93.30(±15.45)
	HR	80.72(±12.72)
REP	**ABP**	95.68(±15.95)
	HR	73.64(±9.88)
DRAW	**ABP**	96.14(±13.94)
	HR	72.62(±9.24)
MEM	**ABP**	94.54(±12.92)
	HR	71.84(±9.55)

Appendix B: Mean ABP (mmHg) and HR (beats per minute) during each task

Task	Parameter	Area Under the Curve (SD)
SUB7	**BP**	132.26 (±81.65)
	HR	1216.85 (±142.02)
N-P	**BP**	225.76 (±151.81)
	HR	1863.27 (±190.87)
REP	**BP**	194.18 (±135.42)
	HR	1872.10 (±223.12)
DRAW	**BP**	214.28 (±202.69)
	HR	1853.84 (±220.97)
MEM	**BP**	74.83 (±48.47)
	HR	762.70 (±77.77)

Appendix C. Area under the curve for population average changes in BP and HR for each task. Data are mean (SD).

Paradigm	Hemisphere	Area Under the Curve (SD)
SUB7	**Dominant**	110.86(±67.53)
	Non-dominant	122.12 (±75.64)
N-P	**Dominant**	173.28 (±99.21)
	Non-dominant	143.46 (±88.48)
REP	**Dominant**	202.62 (±161.94)
	Non-dominant	183.58 (±137.36)
DRAW	**Dominant**	233.57 (±171.15)
	Non-dominant	230.45 (±168.35)
MEM	**Dominant**	75.31 (±50.86)
	Non-dominant	72.34 (±50.72)

Appendix D. Area under the curve for population average dominant (ANOVA F=11.715, p<0.001) and non-dominant (ANOVA F=11.408, p<0.001) changes in CBFv for each task. Data are mean (SD).

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