The Natural History of the Cerebral Blood Flow Regulation after Acute Ischaemic Stroke

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Abstract

Acute stroke is known to lead to impairment of cerebral blood flow (CBF) regulation, but its natural history and techniques for its comprehensive assessment have not been previously reported. Noninvasive measurements of blood pressure (BP), end-tidal CO_2 and CBF velocity (CBFv, using transcranial Doppler ultrasound) during active, passive and motor imagery paradigms were performed in healthy older controls (n=27) and in stroke patients (n=27). Two innovative analytical techniques were firstly used in stroke studies: subcomponent analysis and multivariate dynamic modeling. In controls, significant increase in CBFv during the paradigms with no significant difference in the response amplitude was found. A reproducibility study, not previously reported, was also performed. Following acute stroke, subcomponents analysis revealed a decrease of CBFv response to the passive paradigm and impairment of the myogenic pathways of CBF regulation. Multivariate dynamic modeling removed the influences of BP and $PaCO_2$ showing that the reduced CBFv response to neural activation was directly related and better expressed by the contribution of the stimulation component, instead of the CBFv raw change. The contribution of motor imagery in the CBFv increase was lower compared to the other two paradigms. Impairment of cerebrovascular reactivity to CO_2 was also detected by the model, without the need of performing specific tests for this purpose. The natural history of CBF regulation revealed a deterioration of control mechanisms in both the acute (< 72h) and subacute (2 weeks) phases, reaching the controls' levels in the chronic phases (1 and 3 months). It has been demonstrated in this thesis that CBF regulation changes significantly over time after stroke (particularly in the first weeks after onset), having potential impact not only immediately post ictus but also during the subsequent rehabilitation phase.

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Dedicated to my family,

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PANERAI, R. B., SALINET, A. SM. & ROBINSON, T. G. 2012a. Contribution of arterial blood pressure and $PaCO_2$ to the cerebrovascular responses to motor stimulation. *American Journal of Physiology - Heart and Circulatory Physiology*, 302**,** H459-H466.

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Conference abstracts

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SALINET, A.SM., HAUNTON, V., PANERAI, R. & ROBINSON, T. Correlation of Cerebral Blood Flow Response to Brain Activation Paradigms and Recovery after Ischaemic Stroke in Adults: A Systematic Review. 8th World Stroke Congress. Brasilia, Brazil. October 10-13, 2012.

SALINET, A. SM., PANERAI, R. B. & ROBINSON, T. G. Is the Neurovascular Coupling Response Impaired in the Acute Phase of Stroke? A Transcranial Doppler study. 7th UK Stroke Forum Conference. Harrogate, UK. December 4 to 6, 2012.

SALINET, A. SM., ROBINSON, T. G. & PANERAI, R. B. Can Subcomponents Analysis Help With the Interpretation of the CBFv Response to Neural Activation After Acute Ischaemic Stroke? 18th Meeting of European Society of Neurosonology and Cerebral Hemodynamics and 3rd Meeting of Cerebral Autoregulation Network. Porto, Portugal. May 24-27, 2013.

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Figure 10.16 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.....190

Fig 10.17 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 191

Fig 10.18 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 192

Fig 10.19 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 193

Fig 10.20 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 194

Fig 10.21 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 195

Fig 10.22 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 196

Fig 10.23 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 197

Fig 10.24 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 198

Fig 10.25 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 199

Fig 10.26 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 200

Fig 10.27 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence. 201

List of Abbreviations

ACA	anterior cerebral artery
AF	atrial fibrillation
AH	affected hemisphere
AR	autoregressive
ARI	autoregulatory index
ARMA	autoregressive moving average
ATP	adenosine triphosphate
AUC	area under the curve
AVERT	a very early rehabilitation trial
BMS	between-subject mean sum of squares
BP	blood pressure
CA	cerebral autoregulation
CBF	cerebral blood flow
CBFv	cerebral blood flow velocity
CBV	cerebral blood volume
$CMRO_2$	cerebral metabolic rate of oxygen
CO_2	carbon dioxide
CPP	cerebral perfusion pressure
CrCP	critical closing pressure
CSF	cerebrospinal fluid
CT	cranial tomography

CVR	cerebrovascular reactivity
dCA	dynamic cerebral autoregulation
ECG	electrocardiogram
$EtCO_2$	end-tidal carbon dioxide
EMG	electromyography
FFT	fast Fourier transform
fMRI	functional magnetic resonance imaging
FPE	final prediction error
HR	heart rate
ICA	internal carotid artery
ICC	intraclass correlation coefficient
ICP	intracranial pressure
MA	moving average
MCA	middle cerebral artery
MI	motor imagery
NIHSS	National Institutes of Health Stroke Scale
mmHg	millimeter of mercury
mRS	modified Rankin Scale
NO	nitric oxide
NIRS	near-infrared spectroscopy
NVC	neurovascular coupling
O_2	oxygen
$PaCO_2$	partial pressure of CO_2
PCA	posterior cerebral artery

PET	positron emission tomography
RAP	resistance area product
RMS	residual error mean sum
RoR	rate of return
sCA	static cerebral autoregulation
SD	standard deviation
SE	standard error
SPECT	single-photon emission computed tomography
SS	stimuli signal
TCD	transcranial Doppler ultrasound
TFA	transfer function analysis
TIA	transient ischaemic attack
TICA	terminal internal carotid artery
UH	unaffected hemisphere
Xe_{133}	$Xenon_{133}$
WSQ	within-subjects sum of squares

1 Research Introduction

Ischaemic stroke is typically associated with impaired cerebral blood flow (CBF) regulation and metabolism, leading to secondary brain injury and consequently worsening clinical outcome. Assessment of both cerebral haemodynamics and metabolism in all stroke phases could contribute to more effective planning of therapeutic strategies for reducing secondary brain injury following ischaemic stroke. This introductory chapter concentrates on the clinical aspects of stroke, focusing on stroke pathophysiology and its effects on cerebral haemodynamic regulation. Moreover, it also briefly introduces the relevance of the two main CBF regulatory mechanisms in the healthy brain that will be described in more detail in the subsequent chapter.

1.1 Stroke

The traditional definition of stroke, devised by the World Health Organization, is a neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours.

1.1.1 Epidemiology

Stroke is the second largest cause of death (around 9%) and the leading cause of adult disability worldwide (Donnan *et al.*, 2008). There is a 25% mortality rate, and of those that survive, 65% will require some degree of assistance with activities of daily living (Donnan *et al.*, 2008). Moreover, stroke consumes about 2 to 4% of total health-care resources worldwide and a total cost of £7.6 billion was estimated in the United Kingdom (Saka *et al.*,

2009). Risk factors for stroke include smoking, hypertension, obesity, high cholesterol, atrial fibrillation (AF) and transient ischaemic attack (TIA) (Table 1.1). However, these risk factors explain only about 60% of the attributable risk (Donnan *et al*., 2008).

Table 1.1: Risk factors for stroke

Risk factors for Stroke	
Fixed	Increasing age, male gender, family history of stroke/TIA, previous stroke/TIA
Modifiable	Hypertension, diabetes, smoking, AF, hypercholesterolaemia, carotid artery disease, coronary artery disease

1.1.2 Pathophysiology

Stroke can be classified as either ischaemic or haemorrhagic, although about 80% of all cases are ischaemic (Donnan *et al*., 2008). Due to its greater incidence and the difference in underlying pathophysiological mechanisms, only the ischaemic type will be considered further. There are essentially 4 groups of ischaemic stroke: atherothromboembolic (the commonest), intracranial small vessel disease, cardioembolic and rare causes, such as arterial dissection.

Ischaemic stroke is a dynamic and highly unstable process. There is an initial vascular, haematological, or cardiac event which leads to formation of a local thrombus, which halts the blood supply to the brain distal to this point. After transient or permanent focal ischaemia, a series of neurochemical processes are unleashed and are referred to as the *ischaemic cascade* which can be summarized as cellular energetic failure due to focal cerebral

hypoperfusion, followed by excitotoxicity, oxidative stress, blood-brain barrier dysfunction, microvascular injury, haemostatic activation, post-ischaemic inflammation and finally death of neurons, glia and endothelial cells (Brouns and De Deyn, 2009, Olsen *et al*., 1983). These processes are typically associated with impaired substrate delivery to active brain cells and removal of potentially deleterious by-products of cerebral anaerobic metabolism (lactic acid and free radicals) contributing to cerebral dysfunction (Bor-Seng-Shu *et al*., 2012, Girouard and Iadecola, 2006, Kernagis and Laskowitz, 2012).

The extent of damage is dependent on the blood flow that is able to get through to the distal tissue, either through the damaged vessel because of incomplete occlusion or reperfusion, or from collaterals. Following infarction, a central area of necrosis develops where irreversible cell damage has occurred, called the *ischaemic core*. Surrounding this, an area of tissue exists that retains some collateral perfusion and remains viable for some time, known as the *ischaemic penumbra* (Paciaroni *et al*., 2009). Although an electrical failure (flat electroencephalogram signal) is present in this area, ion pump failure has not yet occurred (Paciaroni *et al*., 2009), and therefore the tissue has the potential to recover (Liu *et al*., 2010). With this in mind, these surrounding areas have been target of many brain plasticity, neuroprotective and neurorepair research studies (Liu *et al*., 2010, Liu *et al*., 2012, Ramos-Cabrer *et al*., 2011).

1.1.3 Stroke Prognosis

Stroke incidence in the United Kingdom has decreased and survival after stroke has improved in the past 10 years (Lee *et al*., 2011). However, improved management is needed to help prevent poor outcome and recurrence, particularly in light of the ageing population (Donnan *et al*., 2008). The classical predictors of stroke recovery are initial neurological deficit, lesion

size and age, followed by other factors including high blood glucose, body temperature and previous stroke. Since only up to 50% of the variance in recovery can be explained by the reported predictors (Marshall *et al*., 2009), it has been suggested that studies of brain activity-related CBF response may give information about final neurological outcome in the acute phase of stroke (Matteis *et al*., 2003, Rehme *et al*., 2011).

The next section gives a brief introduction of the CBF regulatory mechanisms in a healthy brain and these are expanded further in the next chapter. The relationship between stroke and the regulation of cerebral haemodynamics is described in the following sections.

1.2 Cerebral Haemodynamic Regulatory Mechanisms

More than 100 years ago, Roy and Sherrington (1890) came to the conclusion that the vascular supply of the brain can be adjusted locally in association with local variations in functional activity. In the early 1960s, Lassen and Ingvar (1961) introduced a method for the measurement of regional CBF in humans and during the 1970s, once it was more widely acknowledged that CBF increases in response to cerebral activation, these methods were applied to functional brain mapping (Lassen *et al*., 1978). The close spatial and temporal relationship between CBF and neuronal activity was described as the *neurovascular coupling mechanism* - NVC (Lassen *et al*., 1978, Girouard and Iadecola, 2006). This integrated mechanism ensures that the active neurons can be supplied with adequate amounts of oxygen (O_2) and metabolic substrates. In conditions leading to increased neuronal activity, a feedback mechanism is triggered leading to a localized and restricted CBF increase through vasodilatation.

Another very important mechanism that regulates the homeostatic balance in the human brain is called *cerebral autoregulation* (CA). The early work of Lassen (Lassen, 1959) described

CA as a mechanism which maintains CBF relatively constant over a wide range of blood pressure (BP). CA may be assessed using *static* (sCA) and *dynamic* (dCA) methods. As the name says, sCA reflects the relationship between changes in CBF and BP in a steady-state process (Lassen, 1959). On the other hand, the term dCA refers to relatively fast, coherent changes of BP, either evoked or spontaneous, inducing the response of CBF observed over time (Aaslid *et al*., 1989). Independent of the basic mechanisms (described in the next chapter), changes in BP trigger vasomotor adjustments in *cerebrovascular resistance*, by changing arteriolar diameter, in an attempt to maintain CBF constant. Therefore, in the case of a reduction in BP, dilatation of the arteriolar bed occurs and cerebral perfusion is maintained. In addition to providing protection against cerebral ischaemia due to arterial hypotension, the autoregulatory mechanism also protects cerebral vessels against excessive flow during transient or chronic arterial hypertension, which could damage capillaries or lead to intracranial hypertension because of the corresponding increase in cerebral blood volume (Paulson *et al*., 1990).

1.3 Cerebral Haemodynamic Regulation and Ischaemic Stroke

Following cerebral ischaemia, when a blood vessel is cleared of occluding intraluminal thrombus (either spontaneously or therapeutically), after a brief period of hyperaemia there is a decrease in CBF lasting several hours (Hauck *et al*., 2004, Leffler *et al*., 1989). A failure of the cerebrovascular resistance mechanism after stroke has been described (Gur *et al*., 2007, Krainik *et al*., 2005, Troisi *et al*., 2012), which may underlie the long-lasting decrease in CBF (Leffler *et al*., 1989).

It is well known that ischaemic lesions of the brain may also cause functional effects in the contralateral hemisphere. The term *diaschisis* has been used to describe a loss of facilitating

or excitatory input from the area of the injury to other regions of the nervous system, including contralateral areas (Andrews, 1991). Acute infarcts could therefore lead to a global decrease in CBF, which tends to recover with time, as described by previous studies (Chollet et al., 1991, Silvestrini et al., 1993b, Weiller et al., 1992a).

1.3.1 Neurovascular Coupling and Ischaemic Stroke

The inadequate matching of CBF to neural activity may produce damage to neurons or glial cells beyond that caused by the initial ischaemia (Attwell *et al*., 2010). Recent evidence indicates an important role for astrocyte end-feet and capillary pericytes in the CBF decline after ischaemia. The decreased flow was attributed to a reduction in capillary diameter as a result of astrocyte end-feet swelling (Hauck *et al*., 2004). Moreover, some pericytes constrict at the start of ischaemia (Peppiatt *et al*., 2006) and, because there is no adenosine triphosphate (ATP) to pump calcium (Ca^{2+}) out of the cell, they stay in rigor causing the capillaries to remain too small for the passage of the red blood cells (Attwell *et al*., 2010).

After stroke, NVC studies have been used to assess changes in CBF response as an index of synaptic activity (Jueptner and Weiller, 1995), and consequently as an indicator of functional brain recovery. Though abnormalities in cerebral haemodynamics, such as changes in local perfusion pressure and vascular integrity, are important pathophysiological elements in ischaemic stroke, the influence of impaired haemodynamics on the improvement of clinical brain function and stroke prognosis is still unclear (Marshall, 2004). Although functional neuroimaging has contributed greatly to our understanding of brain reorganization after an injury, these techniques rely on the local haemodynamic response, which itself can be altered either by arterial vasoparalysis (Iadecola, 1998) or reduction of the neural activity driving the haemodynamic response (Bundo *et al*., 2002).

1.3.2 Cerebral Autoregulation and Ischaemic Stroke

CA is particularly challenged during acute ischaemic stroke. Working CA is important both during the acute vessel occlusion and during the reperfusion phase. Though impairment of CA in the acute phase of stroke is generally accepted (Aries *et al*., 2010), there is no consistent information to date about whether CA in the middle cerebral artery (MCA) is associated with clinical factors during acute phase and clinical outcome (Eames *et al*., 2002, Reinhard *et al*., 2012).

There have been suggestions that dCA is more sensitive to ischaemic injury than sCA (Dawson *et al*., 2000, Dawson *et al*., 2003). This may be explained by differences in underlying mechanisms studied by sCA and dCA investigation techniques. The clinical relevance of such changes remain unclear (Aries *et al*., 2010). It has been described that acute ischaemic stroke impairs dCA in the affected hemisphere (AH) (Reinhard *et al*., 2005), called *local dysautoregulation* and bilaterally, called *global autoregulatory dysfunction* (Reinhard *et al*., 2012). Within the ischaemic core, CA seems to be impaired to a considerable degree in the early stages. Tissue lactate acidosis leads to local vasoparalysis in both the core and the penumbral region (Dohmen *et al*., 2007). Thus, this secondary global impairment does not seem to be the direct effect of infarction size, but rather reflects a response to endothelial dysfunction triggered by inflammation or autonomic changes after ischaemia (Reinhard *et al*., 2012). Moreover, a milder and more global CA dysfunction has also been described which probably evolves during the first days after ischaemic stroke. Studies in which autoregulation in the MCA was measured once within days of stroke onset found a bilateral reduction in dCA independent of infarct type and vascular risk factors (Atkins *et al*., 2010, Dawson *et al*., 2000, Dawson *et al*., 2003, Eames *et al*., 2002, Reinhard *et al*., 2012).

However, one main methodological problem of the studies reported above is the low spatial resolution of the transcranial Doppler ultrasound (TCD). A small infarct within the MCA territory could also lead to severe focal CA impairment without a clear autoregulatory dysregulation in the main stem of the MCA. To better understand the spatial characteristics of impaired CA in ischaemic stroke (focal versus global dysautoregulation), more studies are needed using haemodynamic monitoring techniques with a high spatial resolution, such as functional magnetic resonance imaging (fMRI).

The course of CA over time still requires further study. Previous studies have found CA impairment bilaterally in both the subacute and chronic phases of ischaemic stroke (Dawson *et al*., 2003, Gommer *et al*., 2008). However, only one study has described CA time course in all phases of stroke (Kwan *et al*., 2004). Kwan *et al.* (2004), using rhythmic handgrip, have found a bilateral CA impairment in the acute phase (< 7 days) and an improvement over time (6 weeks and 3 months).

A recent systematic review of TCD in CA studies (Aries *et al*., 2010) has revealed considerable heterogeneity in autoregulation methods and time points of measurement which limits further comparisons. Though some general conclusions can be drawn, the relationship between stroke and CA requires further studies.

1.4 Clinical Importance of Cerebral Haemodynamics after Stroke

As stated in section 1.1.1, outcome in stroke is poor. Around 30% of patients die within a year in the United Kingdom, whilst half of those who survive are left dependent from permanent neurological deficit (Lee *et al*., 2011). This may be improved with better management; at present there is much debate around optimal treatment of stroke patients.

1.4.1 Blood Pressure after Stroke

BP is known to be acutely elevated following stroke in around 70% of patients, returning to baseline gradually over the first week (Britton *et al*., 1986, Jansen *et al*., 1987, Wallace and Levy, 1981). Furthermore, 40% of acute stroke patients are already receiving antihypertensive therapy (Britton *et al*., 1986). It was hypothesised that the BP rise may represent a physiological response to brain ischaemia intended to counteract the O_2 reduction and maintain neuronal activity (Wallace and Levy, 1981). The possible physiological benefit of increased BP (improved perfusion to the ischaemic penumbra) creates debate over the use of antihypertensive therapy acutely following infarction.

1.4.2 Blood Pressure Management after Stroke

With the impairment of CA acutely following infarction, it renders CBF totally reliant on systemic BP. Therefore, any reduction in the latter may have potential adverse consequences for the viability of the ischaemic penumbra. On the other hand, raised BP may result in increased cerebral oedema or haemorrhagic transformation of the infarct. Because of these concerns, current guidelines from the American Stroke Association and the European Stroke Initiative only recommend lowering BP in the acute stages of infarction if repeatedly >220/120 mmHg. They recommend a target BP of 180/100 mmHg for those previously hypertensive and 160/90 mmHg for normotensive patients (Klijn and Hankey, 2003). Until the state of cerebral haemodynamics regulation acutely following infarction is clarified, it is difficult to fully assess the risks of acute BP manipulation in patients.

1.4.3 Rehabilitation Therapy

Only 25% of patients return to the level of everyday participation and physical functioning of community-matched persons who have not had a stroke (Lai *et al*., 2002). Together with

medication therapy, rehabilitation is an important issue for optimizing outcomes for people with stroke (Ilett *et al*., 2010). Evidence for the benefit of starting mobilization as early as possible after stroke (preferably < 24 hours of stroke onset) has been accumulating over the last decade through a multicentre randomized controlled trial called *A Very Early Rehabilitation Trial* (AVERT) (Bernhardt *et al*., 2006). Despite very good results (Bernhardt *et al*., 2007), the impact of early exercise on brain injury and reorganization is still very poorly investigated.

The physiological impact of very early mobilization in patients with moderate or severe stroke within 24 h of stroke onset was presented by a study (Indredavik *et al*., 2007). These patients experienced a transient increase in BP and heart rate during sitting out of the bed and gait training. However, very few studies have examined the impact of these changes during mobilization in particular or exercise, in general, on brain reorganization or injury and most of them are in animals. A number of animal studies published in the late 90s suggested that early activity after stroke was associated with significant harm (Humm *et al*., 1998, Kozlowski *et al*., 1996, Risedal *et al*., 1999). The results showed that early forced activation over 7 to 15 days post-lesion led to a greater increase in lesion volume as compared to that in animals that commenced training later. While the state of cerebral haemodynamics regulation after stroke is not yet fully understood, very early mobilization should be undertaken with caution, as an increase in BP in the presence of impaired CA and NVC may bring potential harm to the recovering brain.

2 Background and Literature Review

This chapter begins with a description of the concept of CBF regulatory mechanisms and their physiological control. This is followed by a brief summary of previous studies looking at conditions that affect cerebral haemodynamics. Finally, the chapter finishes with a systematic literature review covering the work undertaken in the field of CBF response to neural activation after ischaemic stroke.

2.1 Background

2.1.1 Cerebral Circulation

The majority of blood directed to the brain is delivered by the two internal carotid and the two vertebral arteries. A small amount is brought by the anterior spinal artery to the brainstem. In their final course, the vertebral arteries converge to form the basilar artery. The conjunction of the internal carotid arteries and basilar artery forms the *circle of Willis* (Fig. 2.1). It represents the origin of six great vessels that perfuse the cerebral cortex: right and left anterior, middle and posterior cerebral arteries. More importantly, this circle represents a connection system among the main vessels of the cerebral circulation, providing a protection against occlusion of a single artery.

Intracranial arteries are characterized by a small component of elastic tissue in the media layer, a layer of smooth muscle cells, and by the absence of an external elastic lamina. The penetrating arteries and arterioles are formed by an endothelial cell layer, and adventitia containing fibroblast, collagen, and perivascular nerves. As they progress through the grey

matter, arterioles become progressively smaller and, losing the smooth muscle cells, become capillaries. They are formed by a compact stratum of endothelial cells, surrounded by pericytes and by a basal lamina on which astrocytes attach their termination (astrocytes' end-feet).

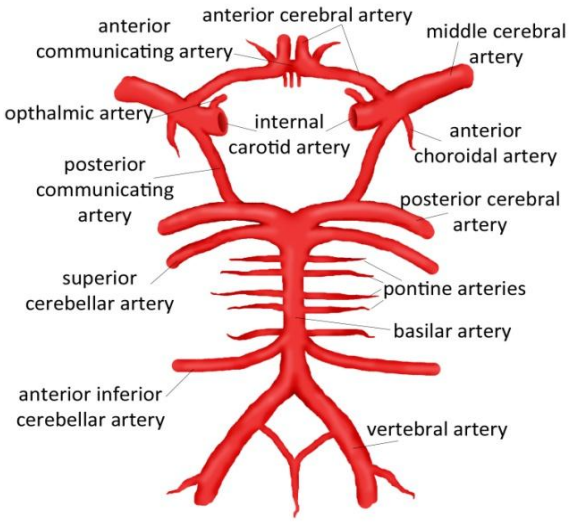

Fig 2.1: Detail of the circle of Willis

CBF is influenced by the cerebral perfusion pressure (CPP), which is usually defined as the difference between blood pressure (BP) and intracranial pressure (ICP), and inversely with cerebrovascular resistance (the sum of vascular resistance to flow, particularly at the level of the small pial arteries and penetrating pre-capillary arterioles). In normal subjects, and patients in whom intracranial hypertension is unlikely, it is often assumed that ICP remains below 10 millimetres of mercury (mmHg) and for this reason its contribution has usually been neglected. Therefore, BP becomes the principal determinant of CPP. From Poiseuille's law, it is possible to express the relationship between flow F_0, and pressure, P_0 at equilibrium as:

$$F_0 = \frac{P_0}{R_0} \qquad Eq.2.1$$

Where R_0 is the cerebrovascular resistance which is directly proportional to vessel length and blood viscosity and inversely proportional to vessel diameter elevated to the 4^{th} power.

First described by Burton (1951), critical closing pressure (CrCP) is defined as a value to explain the influence of active wall tension on collapsible vessels. CrCP refers to the minimum CPP required to keep a blood vessel patent against vascular tone and tissue pressure. Below this pressure, the transmural pressure will not be sufficient to counteract the active tension imposed by the smooth muscle layer, and the vessel will collapse and CBF cease. There is evidence that CrCP is influenced by ICP, partial pressure of carbon dioxide ($PaCO_2$), hyperaemia and BP since they have a direct effect on active wall tension and transmural pressure (Panerai *et al*., 1995, Panerai *et al*., 2012a, Reinhard *et al*., 2000, Weyland *et al*., 2000). The inverse of the slope of the CBFV-BP scatter plot for each cardiac cycle has been termed the resistance area product (RAP). RAP represents the relationship between estimated BP and CBF and it has been used as an indicator of cerebrovascular resistance as it also takes account of the cross-sectional area of the vessel (Evans *et al*., 1988).

The brain cannot tolerate significant increases in volume due to the rigid enclosure represented by the skull. Moreover, the brain's ability to store energy is very limited (so small that the stores of glycogen in the brain would be exhausted in less than three minutes at normal rates of ATP production (Fitch, 1999)) making it uniquely dependent on a continuing, and adequate, supply of substrate. Therefore, impairment in the supply of nutrients and oxygen to the brain can rapidly lead to cellular damage.

2.2 Regulation of Cerebral Haemodynamics

Homeostatic mechanisms and physiological compensations for pathological disturbances essentially safeguard the function of the central nervous system. This includes supplying it with O_2, glucose and nutrients, removing carbon dioxide (CO_2) and other products of metabolism, and regulating its internal ionic environment (Harper A and Jennett S, 1990). Regulation of blood flow in the human brain is exceedingly complex. There exist multiple overlapping regulatory mechanisms and key structural components. The interaction of these components, as well as their detailed structure, is still not fully understood. Nonetheless, a great deal of progress has been made in this important field. Fig. 2.2 provides a simplified block diagram of the main CBF regulatory variables and the relationships involved. The following sections explain what these variables are and how they affect CBF.

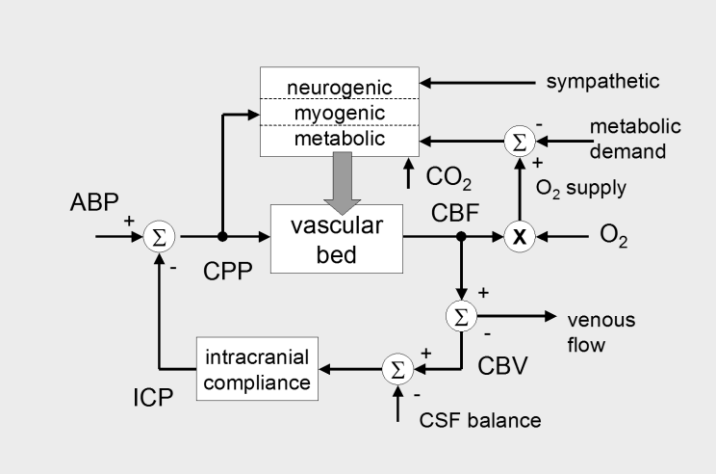

Fig 2.2 Simplified block diagram of the main determinants of cerebral blood flow (CBF) and its regulatory mechanisms. The vascular bed includes small arteries and arterioles that control vascular resistance due to changes in arterial diameter exerted by smooth muscle. BP, arterial blood pressure; CPP, cerebral perfusion pressure; ICP, intracranial pressure; CBV, cerebral blood volume; CSF, cerebrospinal fluid (Panerai, 2004).

2.2.1 Neurovascular Coupling

In recent years, there have been conceptual shifts in the understanding of how NVC is regulated (Attwell *et al*., 2010). It is now thought that neural activation generates the CBF response rather than the increase in metabolic rate; that the astrocytes mediate a large part of the CBF control; that O_2 concentration regulates the relative importance of the signalling pathways involved; and the capillaries and arterioles are responsible for the control of CBF.

Increases in local neuronal activity lead to dynamic increases in CBF that tend to exceed the increased demands of oxidative metabolism. This response provides an increase of O_2 gradient between cerebral arteries and tissue assuring adequate O_2 diffusion to the mitochondria when O_2 demand fluctuates (Attwell *et al*., 2010, Attwell and Iadecola, 2002).

Initially, the local accumulation of metabolic products, such as carbon monoxide and lactate, was proposed as an important factor for CBF regulation. However, it became clear that the time course of this process was not consistent with the rapid response observed in the cerebral arteries upon neuronal activation (Lou *et al*., 1987). Indeed, results obtained over the years provide support for the view that the haemodynamic changes are induced by the release of vasodilatory mediators (such as nitric oxide (NO) and Ca^{2+}) triggered by neurotransmitter molecules released during neuronal activity (such as glutamate), leading to an increase in CBF (Attwell and Iadecola, 2002, Donahue *et al*., 2009, Paulson, 2002).

The NVC mechanism is modulated by the contribution of many cellular and molecular signalling pathways (Zonta, 2003). A growing body of evidence indicates that neurons, glia (astrocytes, microglia, oligodentrocytes), and vascular cells (endothelium, smooth muscle cells and pericytes) are closely related developmentally, structurally and functionally (Attwell *et al*., 2010, del Zoppo, 2009, del Zoppo, 2010, Girouard and Iadecola, 2006, Koehler *et al*.,

2009) (Fig 2.3) . Due to the close proximity of endothelial cells to astrocyte end-feet within microvessels and the support of astrocytes for neurons, it has been suggested that communication could also be directed from microvessels to the neurons they supply (del Zoppo, 2009). This neuron-microvascular functional interaction can be termed the *neurovascular unit* (del Zoppo, 2009, del Zoppo, 2010, Girouard and Iadecola, 2006, Iadecola, 1993)

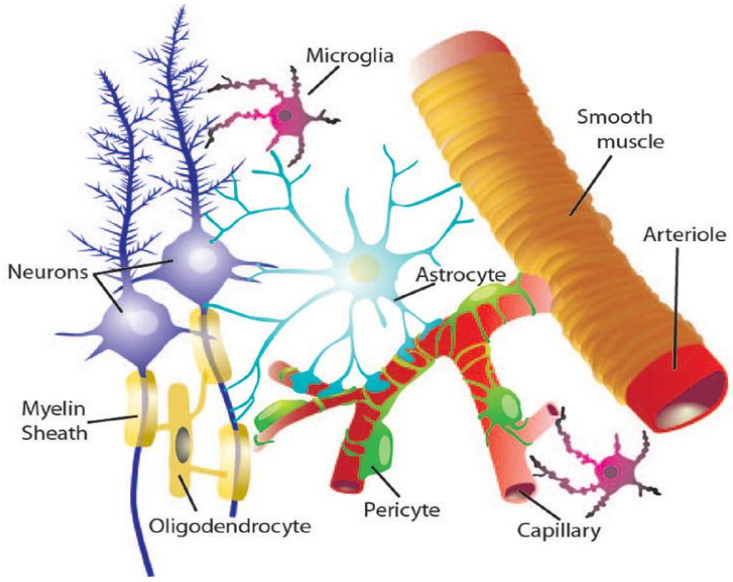

Fig 2.3 Neurovascular coupling components (http:// www.grutzendler-laboratory.com/)

Astrocytes exert many functions in the brain, such as the modulation of neuronal excitability, pH homeostasis, trophic influences on neuronal processes, and stabilization of synaptic connections (Koehler *et al*., 2009). However, particularly important is their central position in the neurovascular unit. There is evidence that they modulate local microvessel tone as the astrocyte end-feet encase the smooth muscle cells of parenchymal arterioles and capillaries inducing a localized increase in CBF (Carmignoto and Gomez-Gonzalo, 2010, Koehler *et al*., 2009). In brain slice preparations *in vitro*, glutamate-mediated intracellular Ca^{2+} elevations triggered an increase in diameter of the cerebral vessel in contact with the activated end-feet

(Zonta, 2003). Thus, the release of active substances from astrocytes in response to neuronal activation requires the elevation of end-feet Ca^{2+} concentration. Moreover, this rise in Ca^{2+} in the end-foot activates potassium (K^+) channels that release K^+ ions into the perivascular space resulting in membrane hyperpolarization and consequently leading to vasodilatation of the arterioles (Dunn and Nelson, 2010). It is noteworthy that pericytes are also able to induce changes in capillary diameter by means of their own contractile properties (Hamel, 2006, Peppiatt *et al*., 2006).

2.2.2 Cerebral Autoregulation

Due to the temporal heterogeneity in which physiological adjustments of CBF occur, a distinction between static and dynamic autoregulation has been made (Aaslid *et al*., 1989, Tiecks *et al*., 1995). Static cerebral autoregulation (sCA) can be described as the CBF response condition reflecting the efficiency of the CA mechanism during steady-state changes in BP (Panerai, 2008, Tiecks *et al*., 1995). This steady state approach is the basis for the classical autoregulatory curve presented in Fig. 2.4. The origin of the autoregulation curve is usually attributed to Lassen (1959). He plotted the results of multiple studies in a single graph and the interpolated curve shows a plateau region that is flat, corresponding to an absolutely constant CBF for changes in mean BP in the range 50–170 mmHg. This occurs due to changes in BP leading to cerebral adjustments in cerebrovascular resistance (Fig. 2.4). Thus, sCA is considered effective when CBF remains approximately constant and cerebrovascular resistance changes proportionally to changes in mean BP (Paulson *et al*., 1990). On the other hand, outside these BP limits, cerebrovascular resistance adjustments cannot compensate for changes in BP any longer, and CBF becomes dependent on BP with impaired or abolished CA. Drawbacks of single steady state evaluations are the vulnerability to confounding by spontaneous non-BP-related variability, such as CO_2 changes called *CO_2*

cerebrovascular reactivity (CVR) (Aaslid, 2006, Panerai, 1998), the time-consuming nature of procedures, the need for invasive pharmacological interventions, and the lack of information on any period of hypoperfusion possibly preceding the eventual return to stable perfusion (Panerai, 2009, van Beek *et al*., 2008). Following initial observations in animals, Greenfield *et al* (1984) first described the time-variant response of CBF to acute BP fluctuations in humans, later termed the dynamic autoregulation mechanism (Aaslid *et al*., 1989, Panerai *et al*., 1998b). Dynamic CA (dCA) reflects the latency of the cerebral vasoregulatory system after a sudden change of BP. With a normal dCA, mean CBF or mean CBF velocity (CBFv), as usually measured in these studies, restores faster than BP. With impaired dCA though, CBF will follow the same time course as mean BP. The information about the latency and magnitude of the CBF response has a particular importance in a clinical setting, as progressive dCA impairment might affect firstly the latency and then the efficacy of the autoregulatory response (Aaslid *et al*., 1989, Birch *et al*., 2002, Tiecks *et al*., 1995).

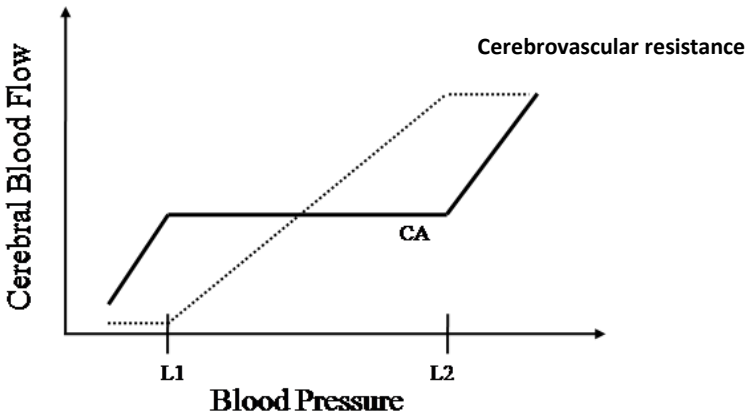

Fig 2.4 Schematic representation of the characteristic of a normal CA curve (continuous line). Points L1 and L2 represent the limits of autoregulation. Also represented is the CVR modulation curve (dashed line).

2.2.2.1 Mechanisms of Cerebral Autoregulation

As in other vascular beds, myogenic, neurogenic and metabolic mechanisms have been proposed to explain CA responses, although the relative contribution of these mechanisms and their integration are still debated. Further details of these mechanisms are presented below.

2.2.2.1.1 Myogenic

Although the mechanoreceptor proprieties of smooth muscle cells have been described since the 1900s (Bayliss, 1902), its demonstration and analysis in isolated blood vessels is more recent (Johansson and Ljung, 1967). Similar to other tissues, the increase in transmural pressure stretches smooth muscle cells leading to alteration in the resting tone of the cerebral resistance vessels, producing vasoconstriction or vasodilatation. Despite considerable progress in understanding the molecular biology of vascular regulation, the exact mechanisms whereby stretch induces changes in the resting tone of vascular smooth muscle remain unclear (Peterson *et al*., 2011).

Many studies have focused on the transduction mechanisms between myogenic stretch and subsequent vasoconstriction, particularly the role of stretch-activated ion channels. It is generally accepted that stretching alters the configuration of membrane channels affecting the ionic permeability to Ca^{2+} and K^{+}.

In response to BP increasing, the increased arterial wall tension evokes membrane potential depolarization leading to a Ca^{2+} influx via voltage-gated Ca^{2+} channels (Bevan and Hwa, 1985). The Ca^{2+} influx activates a chain of molecular mechanisms that regulate contraction and relaxation of the resistance vessels (Walsh and Cole, 2013). In an *in vitro* experiment, the myogenic response is abolished in the presence of inhibitors to voltage-gated Ca^{2+} channels

(Davis and Hill, 1999). Inside the autoregulatory range, the depolarization and intensity of contraction are proportional to the pressure inside the vessels (Bevan and Hwa, 1985). On the other hand, variation in pressure outside the autoregulatory range overwhelms the capacity of the smooth muscle cells to generate changes in resistance (Walsh and Cole, 2013).

Although the cerebral myogenic response is intrinsic to vascular smooth muscle, it can be modulated by extrinsic factors from the endothelium. The endothelium, both in the systemic and cerebral circulation, represents a complex organ that integrates many fundamental functions for local regulation of circulation and homeostasis of the vessels themselves (Andresen *et al*., 2006). Acetylcholine, adenosine triphosphate and other substances induce vasodilatation by activation of G-protein-coupled receptors present in the endothelium, and through the production of endothelium-derived hyperpolarization factor and nitric oxide (Andresen *et al*., 2006, You *et al*., 1997). Nitric oxide (NO) is continuously produced by the endothelium and it is thought to be the most important mediator for maintenance of resting cerebral vascular tone. In addition, it has been proposed that the endothelium has mechanoreceptor properties that respond to stretch allowing it to contribute to CA (Peterson *et al*., 2011). Shear stress increases activity of endothelium nitric synthase leading to a release of NO, which diffuses to adjacent smooth muscle cells and induces vasodilatation through activation of K^+ channels and/or closure of voltage-gated Ca^{2+} channels in those cells (Faraci, 2006, Kuschinsky and Wahl, 1978, McCarron *et al*., 1989). On the contrary, other factors produced by the endothelium, such as endothelin-1 and prostaglandin F2α, can induce vasoconstriction (Andresen *et al*., 2006).

2.2.2.1.2 Metabolic

Some authors refer to the metabolic supply or demand as 'tests of autoregulation', while others make the distinction between *flow-metabolic coupling* (NVC) and *metabolic autoregulation*. Although the basic mechanisms are rather different, the integrated approach usually adopted to assess CA cannot differentiate between the two. In metabolic regulation, arterial resistance is modified by waste products of energy metabolism, partial pressure of O_2, and release of specific vasoactive substances such as adenosine from neurons in response to insufficient blood supply (Rudziński et al., 2007).

The effect of CO_2 on CBF is one of the most pronounced and most easily demonstrable influences, a concept known as *CO_2 cerebrovascular reactivity* (CVR) (Ringelstein et al., 1988). CO_2 reactivity is mediated through the action of H^+ on cerebral arteries, rather than CO_2 itself (Peterson et al., 2011). The effect of pH changes on cerebral vascular tone is complex, there is evidence that it is mediated by NO, intracellular Ca^{2+} and adenosine due to activation of K^+ channels (Brian, 1998). Aaslid *et al.* (1989) demonstrated that in normal subjects, changes in CBFv in response to sudden changes in BP were faster during hypocapnia, associated with an increase of resting arteriolar tone, representing an improvement in autoregulatory capacity. The opposite effect was observed in hypercapnia. Studies of the effect of CO_2 on dCA have been performed by several investigators, showing a similar trend towards a weaker autoregulatory response with increases in arterial CO_2 (Aaslid *et al*., 1989, Dineen *et al*., 2011, Panerai *et al*., 1999, Panerai *et al*., 1996). $PaCO_2$ increases CBF by acting on arterial smooth muscle to bring about vasodilatation (Lassen, 1959, Paulson *et al*., 1990) and a positive inotropic effect on the heart causing an increase in BP (Garnham *et al*., 1999). On the other hand, reduction of $PaCO_2$ causes a shift of periarteriolar pH that induces vasoconstriction (Huber and Handa, 1967), leading to an increase in

cerebrovascular resistance that is, in turn responsible for the reduction in CBF (Malatino *et al*., 1992).

2.2.2.1.3 Neurogenic

During the last 20 to 30 years, much research has focussed on the role of the autonomic nervous system in CBF regulation. However, the exact mechanism remains uncertain and is a topic of much debate (Gierthmuhlen *et al*., 2010, van Lieshout and Secher, 2008). The neurogenic mechanism is based on the presence of a rich invervation of cerebral vessels by both parasympathetic and sympathetic nerves (Goadsby and Edvinsson, 2002). The latter is believed to influence more the CBF regulation by exerting vasoconstriction mediated by neurotransmitters. Sympathetic stimulation is tonically active maintaining constant resting CBF, as confirmed by a CBF decrease after ganglion blockade and by a less pronounced decrease in resistance upon neural activation in patients with Horner's syndrome (which impairs the sympathetic nervous system) (Gierthmuhlen *et al*., 2010, Zhang *et al*., 2002). However, in some situations, characterized by a decrease in cardiac output (such as cardiac failure), it seems that vasoconstriction of cerebral vessels can occur similarly to what happens to the peripheral circulation (Hellstrom and Wahlgren, 1993, Ide *et al*., 1998). On the contrary, the parasympathetic regulation of CBF is far from clear (Hamner *et al*., 2012). Some studies have stated that the cholinergic system does not play a relevant role (Goadsby and Edvinsson, 2002, Kontos, 1981). However, Hamner *et al*. (2012) have shown an impairment of CA during cholinergic blockade, suggesting that parasympathetic system tonically balances sympathetic vasoconstriction with active vasodilatation.

2.3 Factors affecting Cerebral Haemodynamics

Cerebral haemodynamics and its regulatory mechanisms may be altered in several conditions. Stroke risk factors and co-morbidities need to be taken into account in any study of CBF regulation following stroke, and will be outlined below.

2.3.1 Age

It is thought that age is a potent risk factor for cardiovascular disease, as it can lead to degenerative changes in the vasculature, such as fibrohyaline thickening of the vessel wall, necrosis of smooth muscle cells and thickening of the basal membrane (Najjar *et al*., 2005, Vaitkevicius *et al*., 1993). In the past few years, evidence has accumulated that normal ageing is associated with a decline in resting CBFv (Calautti *et al*., 2001b, Carey *et al*., 2003, Dineen *et al*., 2011, Sorond *et al*., 2008, Tiecks *et al*., 1995). It is, therefore, likely that ageing influences NVC and CA as well.

Neuroimaging techniques have shown decreased activation in some regions of the brain in the elderly compared to young adults, and it is often accompanied by increased activation in other areas, such as the prefrontal cortex (Calautti *et al*., 2001b) and contralateral areas (Nielson *et al*., 2002). These observations have led to the hypothesis that older adults compensate for age-related neural changes by recruiting either additional or alternative neural circuitry. In agreement with this, a TCD study has found a greater bilateral CBFv increase in response to cognitive paradigms in an elderly group (Sorond *et al*., 2008). However, age-related changes of the vascular system (described above) as well as a high incidence of risk factors for atherosclerosis in the elderly (such as hypertension and/or diabetes mellitus) could diminish the distensibility of the cerebral vessels which could also be responsible for a decreased CBF response to focal brain activation. Groschel *et al.* (2007) have demonstrated that ageing by itself did not affect NVC, but in the presence of cardiovascular risk factors, the

increase caused by neural activation is reduced. Therefore, the effects of ageing on cerebral haemodynamics during neuronal activation have to be differentiated from the effects of other vascular risk factors.

Surprisingly, given the effects of ageing on other physiological parameters, CA does not alter with increasing age (Brodie *et al*., 2009b, Carey *et al*., 2000, Carey *et al*., 2003, Dineen *et al*., 2011, Lipsitz *et al*., 2000). It has been hypothesized that no difference in CA between young and old participants is due to a degree of physiological reserve within the autoregulatory system (Dineen *et al*., 2011). In respect of CVR, there is little evidence in human populations of the effects of age, lately most of the studies have shown no correlation between reduced CVR and ageing (Dineen *et al*., 2011, Groschel *et al*., 2007, Lipsitz *et al*., 2000).

Nonetheless, in any study involving cerebral haemodynamic assessment, an age-matched population is needed to make meaningful conclusions between group differences.

2.3.2 Diabetes Mellitus

Diabetes induces chronic vascular complications, not only atherosclerotic macrovascular disease affecting arteries that supply the heart, brain and lower extremities, but also microvascular disorders in the retina, renal glomerulus and peripheral nerves. (e.g. nephropathy, retinopathy and neuropathy) (Brownlee, 2001). Evidence from animal studies demonstrates an altered function of the blood–brain barrier that could be a potential contributing cause of the loss of homeostasis of the cerebral microenvironment (Vetri *et al*., 2012). As a consequence, intracellular hyperglycaemia causes abnormalities in CBF (impairment in vasodilatatory function, and endothelium and astrocytic dysfunction) and/or autonomic neuropathy early in the course of diabetes (Brownlee, 2001, Mogi and Horiuchi,

2011, Vetri *et al*., 2012). Therefore, the loss of homeostasis of the cerebral circulation may lead to CA and NVC impairment.

Impairment of dCA associated with diabetes may be related to autonomic neuropathy (Mankovsky *et al*., 2003), although the literature remains inconclusive (Huq *et al*., 2012, Kim *et al*., 2008, Last *et al*., 2007, Mankovsky *et al*., 2003, Novak *et al*., 2006). On the other hand, diabetes-related cognitive dysfunction has been recognized in humans (Biessels *et al*., 2006), suggesting that it should be viewed as a NVC disorder. Moreover, in experimental animals with chemically induced diabetes (type I), there is increasing evidence of a profound impairment in NVC (Vetri *et al*., 2012).

2.3.3 Hypertension

Hypertension modifies the structure of cerebral blood vessels by producing vascular hypertrophy and remodelling with luminal narrowing, and by promoting atherosclerosis in large cerebral arteries and in penetrating arterioles (Faraci *et al*., 1990). Moreover, endothelium-dependent relaxation is impaired during chronic hypertension (Faraci *et al*., 1990).

It is still unclear the extent to which CBF is changed in hypertension. A prospective study showed that uncontrolled hypertension presented similar baseline CBF levels compared to controlled and healthy volunteers (Olsen *et al*., 1995). This is in line with hypertensive rat studies (Hoffman *et al*., 1981, Kazama *et al*., 2003, Tamaki *et al*., 1995). The main role of CA in hypertensive patients is to protect against an excessive increase in BP. Although CA is thought to remain intact, the limits of CA are shifted to the right compared to normotensive individuals (Strandgaard *et al*., 1973). Modification of CA limits is probably related to functional and structural modifications described above. As a consequence, cerebrovascular

resistance is elevated to maintain a resting CBF of the same magnitude as that in normotensive subjects (Malatino *et al*., 1992, Strandgaard *et al*., 1973).

Recent evidence suggests that NVC is also altered in hypertension. Attenuation of the CBF increase in the somatosensory cortex was seen after administration of angiotensin II (which increases BP around 20 to 30 mmHg) in mice (Kazama *et al*., 2003). There are also indications that hypertension alters CBF to neural activation in humans (Jennings *et al*., 2005). Increases of CBF in hypertensive subjects were less than in normotensive subjects in the posterior parietal area when they were engaged in memory tasks.

2.3.4 Stenosis or Occlusion of the External Arteries

Over the past several years, evidence has been accumulating that CBF is compromised in patients with moderate carotid artery stenosis or occlusion by the exhaustion of cerebral compensatory mechanisms thus leading to maximum vasodilatation (Bokkers *et al*., 2010, Derdeyn *et al*., 2002, Silvestrini *et al*., 2000). Many previous studies have also demonstrated a diminished vasodilatory response to CO_2 ipsilateral to asymptomatic and symptomatic carotid artery stenosis and occlusive disease (Markus and Cullinane, 2001, Reinhard *et al*., 2003a, Reinhard *et al*., 2003b, Silvestrini *et al*., 1996, Bokkers *et al*., 2010). The severity of the impairment of CVR has correlated positively with the degree of stenosis, the number of extracranial arteries affected, and the clinical condition (e.g. post stroke, history of TIA) (Ringelstein *et al*., 1988). Moreover, it appears that symptomatic patients usually have the most severely impaired CVR (Silvestrini *et al*., 1996, Derdeyn *et al*., 2002). Endarterectomy does appear to be beneficial in terms of changes in CBF and CVR, despite improvements in risk of stroke (Klijn *et al*., 1997, Reinhard *et al*., 2003a, Silvestrini *et al*., 1996).

There is evidence that CA is impaired in severe and moderate carotid stenosis (Reinhard *et al*., 2003a, Reinhard *et al*., 2003b, White and Markus, 1997). Previous studies have also shown impairment of the CBF response to neural activation in carotid stenosis and occlusion (Lin *et al*., 2011, Yamauchi *et al*., 2005). Although it is not well understood how carotid disease influences cerebral haemodynamics, a chronic hypoperfusion state (during slowly progressive narrowing of the large cerebral artery) that leads the arterioles and capillaries to maximally dilate may be the cause of altered CVR and CBF control (Derdeyn *et al*., 1999, Rudziński *et al*., 2007).

2.3.5 Transient Ischaemic Attack

Little is known about the effect of TIA on cerebral haemodynamics. A recent study analysed dCA in TIA patients, at 36 and 96 hours from symptom onset (Atkins *et al*., 2010). To our knowledge, this is the only study that has been conducted investigating dCA following TIA. No autoregulatory disturbances were reported but these results may be attributable to the small sample size.

2.4 Literature Review

Cerebral haemodynamic impairment is an important pathophysiological element in stroke. However, the influence of abnormal CBF regulation on the disruption and improvement of clinical brain function after ictus is largely unknown. We therefore conducted a systematic review about the quantitative and temporal pattern of the CBF response to neural activation after cerebral ischaemia, associated factors and clinical outcomes (Salinet *et al*., 2013a).

2.4.1 Materials and Methods

A literature search in the bibliographic databases MEDLINE, Web of Science, EMBASE and CINAHL was undertaken by an independent researcher and myself. The combinations of the

search terms used are shown in Table 2.1; different MeSH terms or subcategories available on the search database were truncated to increase the sensitivity of the electronic search. The references of selected articles were hand-searched for additional relevant articles.

Table 2.1 Search strategy

"Stroke" OR "cerebr vascular disease" OR "cerebral isch*" OR "brain isch*" AND "cerebral blood flow" OR "cerebral hemodynamics" OR "cerebral haemodynamics" OR cerebral blood flow velocit* OR "cerebral autoregulation" AND "neurovascular coupling" OR "cerebral activation" OR "neur* activation" OR "movement*"*

Peer-reviewed studies of the quantification of CBF (or CBFv) responses to neural activation paradigms after ischaemic stroke were included. Eligibility was assessed by reading abstracts and, if necessary, whole articles. Excluded were case reports, abstracts, dissertations, paediatric studies, non-English language articles and studies that did not specify the type of stroke. Studies including patients with other cerebrovascular diseases without a separate analysis for patients with ischaemic stroke were also excluded.

The following data were extracted: study design; inclusion and exclusion criteria; stroke population; stroke phase and severity; co-morbidities; method of CBF recording; peripheral haemodynamic recordings; description of intervention; recording protocol; number of patients and controls; neurological outcome; rehabilitation treatment; CBF response in the affected (AH) and unaffected hemispheres (UH); peripheral haemodynamic responses; correlation between CBF response and stroke outcome; conclusions and study limitations.

Two researchers, the independent researcher and I, evaluated the selected studies in terms of quality using a checklist adapted from authors, editors, and reviews of meta-analyses of observational studies (Stroup DF, 2000) which has been used previously (Aries *et al.*, 2010).

The checklist was adapted to critically appraise CBF studies using 15 relevant items, as shown in Table 2.2.

Table 2.2 Study quality assessed using a checklist proposed by PRISMA.

EVALUATION OF	CRITERIA (1 if)	CRITERION
Background and Methods		
Aims/hypothesis statement in introduction or method section	the aims/ hypothesis of the research is described either in introduction or methods	A
Description of the study population	the population of the study is detailed described (age, co-morbidities)	B
Medical ethics review with informed consent	informed consent is obtained from the patients/controls in accordance with the approval by the Ethical Committee	C
Sample size calculation before start of experiment	sample size is calculated	D
Sample size (n) adequate in relation to the number of determinants (K)	ratio n:K exceeds 10:1	E
Clear inclusion and exclusion criteria	the inclusion and exclusion criteria are clearly described	F
Statistical validation of relationship between dependent and independent variables	relationship between dependent and independent variable is tested for statistical significance	G
Type of brain activation task described sufficiently (or with literature reference)	brain activation task was clearly described	H
CBF measurements clearly presented and consistent	CBF(v) calculation is presented	I
Results		
Specification of relevant patient characteristics? (i.e. age, type, number and localization)	age, type, localization as well as number of strokes are specified in the cohort	J
Graph/table summarizing results	main outcome is presented in graph and/or table	K
Proven reproducibility of data	the study tested the reliability and validity of used measurements or referred to other studies which established reliability and validity	L
Discussion		
Considerations of alternative explanation for observed results in discussion	the strength of evidence for each main outcome was discussed	M
Discussion of limitations	limitation of the study is presented and discussed	N
Guideline for future research	any suggestion for future research is made	O

2.4.2 Results

A total of 1521 citations were identified. After dismissing duplicates, non-relevant topics, and studies where CBF was not quantified, thirty abstracts remained. Thirteen of these studies were subsequently excluded because TIA, abscess, haemorrhagic and cerebellar stroke were grouped together with ischaemic stroke. A further two were conference abstracts and were also excluded, leaving a total of fifteen articles (please see Table 2.3 for the complete list of studies). These were supplemented with one more study found through a reference search. The characteristics of these sixteen studies are described in Table 2.3.

2.4.2.1 Study Quality

There were no disagreements in quality assessment between reviewers. The overall methodological quality of each study is presented in Table 2.4. The median score on the proposed quality checklist was nine out of fifteen (range 6-11), reflecting incomplete reporting of key methodological criteria in the majority of studies.

2.4.2.2 Study Characteristics

Study samples varied from two to forty-five patients. Only one study included patients in the acute phase (Kessler *et al*., 2000), whereas patients were studied in the subacute and chronic phases in five (Bragoni et al., 2000, Honda et al., 1997, Matteis et al., 2003, Silvestrini et al., 1998b, Treger et al., 2007) and six (Caramia et al., 2000, Seitz et al., 1998, Silvestrini et al., 1993a, Silvestrini et al., 1995b, Weder and Seitz, 1994a, Weder et al., 1994b) studies, respectively. Four studies (Dettmers et al., 1997, Lin et al., 2011, Weiller et al., 1992b, Weiller et al., 1993) included both subacute and chronic phases in the same stroke group. Five (Bragoni et al., 2000, Kessler et al., 2000, Matteis et al., 2003, Silvestrini et al., 1998b, Treger et al., 2007) studies had follow up measurements between two weeks and two months post ictus, but none had more than two assessments. In ten studies, information about other

clinical conditions (such as diabetes mellitus, hypertension) was not reported. Although twelve studies included only right-handed subjects, the handiness was only assessed quantitatively in four studies (Edinburgh Inventory (Matteis et al., 2003, Silvestrini et al., 1993a, Silvestrini et al., 1995b, Silvestrini et al., 1998b)). Rehabilitative therapy was part of five studies (Bragoni *et al*., 2000, Caramia *et al*., 2000, Kessler *et al*., 2000, Silvestrini *et al*., 1998b, Treger *et al*., 2007).

2.4.2.3 Measurement Techniques and Paradigms

An overview of selected studies is reported in Table 2.3. Two different techniques were used to assess cerebral haemodynamics: PET (Dettmers et al., 1997, Honda et al., 1997, Kessler et al., 2000, Seitz et al., 1998, Weder et al., 1994b, Weder and Seitz, 1994a, Weiller et al., 1992b, Weiller et al., 1993) and TCD (Bragoni et al., 2000, Caramia et al., 2000, Lin et al., 2011, Matteis et al., 2003, Silvestrini et al., 1993a, Silvestrini et al., 1995b, Silvestrini et al., 1998b, Treger et al., 2007). The selection of paradigms to stimulate CBF differed significantly across studies. Eleven studies chose a sensorimotor paradigm, (Caramia et al., 2000, Dettmers et al., 1997, Honda et al., 1997, Matteis et al., 2003, Seitz et al., 1998, Silvestrini et al., 1993a, Silvestrini et al., 1995b, Weiller et al., 1992b, Weiller et al., 1993, Weder et al., 1994b, Weder and Seitz, 1994a), whereas word finding (Silvestrini et al., 1995b, Silvestrini et al., 1998b) and object recognition (Bragoni *et al*., 2000, Treger *et al*., 2007) were used in two studies each. Word repetition and reading were used by one study each. Systemic haemodynamics (beat-to-beat blood pressure, heart rate and end tidal CO_2) were recorded in five studies (Bragoni et al., 2000, Matteis et al., 2003, Silvestrini et al., 1993a, Silvestrini et al., 1995b, Silvestrini et al., 1998b). Three studies reported variations in blood pressure and heart rate (Silvestrini et al., 1993a, Silvestrini et al., 1995b, Silvestrini et al., 1998b).

Table 2.3 Characteristics of identified publications examining CBF response to neural activation after ischaemic stroke

Study	Patient Numbers	Age, years ± SD	Activation paradigm	Side of stimulation	Timing of CBF assessment
TCD					
Matteis *et al.* 2003	30	69.0 ±8.0	passive movement of the elbow	Bilateral	14 (8-14) days
Caramia *et al.* 2000	14	57.7 ±9.0	thumb finger opposition	Bilateral	6 months
Silvestrini *et al.* 1993	12	58.1 ±6.9	thumb finger opposition	Bilateral	5-13 months
Silvestrini *et al.* 1995	45	right infarction: 63.0 ±11.0 left infarction: 59.0 ±16.0	thumb finger opposition/ word finding	Bilateral	6-10 months
Silvestrini *et al.* 1998b	26	61.3 ±5.7	word finding	Left	2 to 3 weeks
Treger *et al.* 2007	37	61.3 ±11.9	naming and comprehension/ recognition task	Left	15.8 ±7.3 days
Lin *et al.* 2011	13 LIAS 21 SVD	LIAS: 58 (-) SVD: 64 (-)	reading paradigm	AH	LIAS: 7 (2-19) months SVD: 18 (2-85) months
Bragoni *et al.* 2000	29	group 1: 69.6 ± 3.5 group 2: 67.0 ± 5.3 group 3: 66.8 ± 4.6 group 4: 66.5 ± 4.7	object recognition	Simultaneous Bilateral	3 to 4 weeks
PET					
Dettmers *et al.* 1997	6	64.0 ±10.0	exertion of 5, 10, 20, 40, 46.7±1.8% of maximal contraction	AH	15.0 ±28.0 months
Honda *et al.* 1997	2 (1 considered)	60.0	brisk extension of wrist	Bilateral	3 weeks
Kessler *et al.* 2000	24	placebo: 56.3 ±9.9 treatment: 57.4 ±13.5	word repetition	Simultaneous Bilateral	2 weeks
Seitz *et al.* 1998	7	53.9 ±8.0	sequential finger opposition/ tactile exploration	Bilateral	6 months
Weder *et al.* 1994a	5	47.2 ±11.5	somatosensory discrimination	AH	3 to 8 months
Weder *et al.* 1994b	5	47.2 ±11.5	somatosensory discrimination	AH	4 to 8 months
Weiller *et al.* 1992	10	41 (range 21-62)	sequential finger opposition	Bilateral	14.6 (±21.1) months
Weiller *et al.* 1993	8	43 (range 21-67)	sequential finger opposition	Bilateral	7 weeks to 6 years

TCD, Transcranial Doppler ultrasound; PET, positron emission tomography; LIAS, large intracranial artery stenosis; SVD, small vessel disease; AH, affected hemisphere; UH, unaffected hemisphere. Activation paradigm refers to the neuro activation paradigm used in the study. Side of stimulation refers to the hemisphere stimulated by the paradigm. Timing of CBF assessment refers to the duration after stroke onset that the study was undertaken.

Table 2.4 Scores for quality assessment

Study	CRITERION															TOTAL
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
Bragoni *et al.* 2000	1					1	1	1	1	1	1		1	1	1	10
Caramia *et al.* 2000	1	1	1		1	1	1	1		1	1		1		1	11
Dettmers *et al.* 1997	1		1			1	1	1	1	1	1		1	1	1	11
Honda *et al.* 1997	1	1	1					1	1	1	1		1		1	9
Kessler *et al.* 2000	1	1	1		1	1	1	1	1		1		1		1	11
Lin *et al.* 2011	1	1	1	1	1	1	1	1			1		1		1	11
Matteis *et al.* 2003	1	1	1		1	1	1	1		1	1		1		1	11
Seitz *et al.* 1998	1		1			1	1	1	1	1	1		1			9
Silvestrini *et al.* 1993					1		1	1		1	1		1		1	7
Silvestrini *et al.* 1995	1				1	1	1	1			1		1			7
Silvestrini *et al.* 1998b	1	1	1		1	1	1	1			1	1	1			10
Treger *et al.* 2007	1					1	1	1			1		1	1	1	8
Weder *et al.* 1994a	1		1					1	1	1	1		1			7
Weder *et al.* 1994b	1							1	1		1	1	1			6
Weiller *et al.* 1992	1	1	1					1	1	1	1		1			8
Weiller *et al.* 1993	1		1					1	1	1	1		1			7
TOTAL	15	7	11	1	7	10	11	16	9	10	16	2	16	3	9	

2.4.2.4 Cerebral Haemodynamic Responses

All paradigms led to a significant CBF increase in patients, with the exception of the naming, comprehension and recognition tasks (Treger *et al.*, 2007). Twelve studies compared the quantitative CBF response between stroke patients and healthy controls (Bragoni et al., 2000, Caramia et al., 2000, Dettmers et al., 1997, Lin et al., 2011, Matteis et al., 2003, Silvestrini et al., 1993a, Silvestrini et al., 1995b, Silvestrini et al., 1998b, Treger et al., 2007, Weder and Seitz, 1994a, Weiller et al., 1992b, Weiller et al., 1993) (Table 2.5). No agreement was found as to whether the AH CBF (or CBFv) response was impaired after ischaemic stroke. In sensorimotor activation studies, either a lack of contralateral dominance (Caramia et al., 2000, Silvestrini et al., 1993a, Silvestrini et al., 1995b, Weder and Seitz, 1994a, Weiller et al., 1992b, Weiller et al., 1993), or a greater CBF (or CBFv) contribution of the unaffected hemisphere (UH) (Caramia et al., 2000, Seitz et al., 1998, Silvestrini et al., 1993a, Silvestrini

et al., 1995b, Weiller et al., 1992b, Weiller et al., 1993), and/ or a lower CBF (or CBFv) increase in the AH (Matteis *et al*., 2003, Seitz *et al*., 1998) during activation of the AH was variably reported. In cognitive activation studies, no significant bilateral CBFv increase (Treger *et al.*, 2007), a decreased bilateral gain (Lin *et al.*, 2011) and a greater UH CBFv response was reported (Silvestrini *et al*., 1995b) (Table 2.5). When the UH was stimulated, a normal lateralized response was found in seven studies (Caramia et al., 2000, Honda et al., 1997, Matteis et al., 2003, Seitz et al., 1998, Silvestrini et al., 1993a, Weiller et al., 1992b, Weiller et al., 1993). Only five studies reported significant differences between patients and controls during activation of the AH (Bragoni *et al*., 2000, Lin *et al*., 2011, Matteis *et al*., 2003, Silvestrini *et al*., 1998b, Treger *et al*., 2007) (Table 2.5). Correlation between CBF and aphasia was studied in four articles (Kessler *et al*., 2000, Silvestrini *et al*., 1995b, Silvestrini *et al*., 1998b, Treger *et al*., 2007) showing that UH plays an important role in aphasia recovery in patients with left lesions (Table 2.5). Although longitudinal changes after stroke were studied in five articles, longitudinal assessment of the cerebral haemodynamics was only reported in two studies (Kessler *et al*., 2000, Silvestrini *et al*., 1998b). Following Piracetam treatment (a drug which enhances verbal memory), Kessler *et al.* (2000) found a significant CBF increase during word repetition at the end of treatment only in the AH (Kessler *et al*., 2000). After two months aphasia rehabilitation, Silvestrini *et al.* (1998) demonstrated a loss of hemisphere dominance in the CBFv responses over time. CBF responses were associated directly with stroke recovery in six studies (Table 2.6), suggesting that greater recovery was associated with an UH CBF increase (Caramia *et al*., 2000, Silvestrini *et al*., 1993a, Silvestrini *et al*., 1998b, Treger *et al*., 2007), an AH CBF increase (Matteis *et al*., 2003) and a bilateral increase (Bragoni *et al*., 2000).

Table 2.5 Results Overview of Identified Studies

Studies	AH Activation		UH Activation		Patient vs. Control CBF responses
	AH CBF Response	UH CBF Response	AH CBF Response	UH CBF Response	
Sensorimotor studies					
Caramia *et al.* 2000	↑	↑	NS	↑	↑ UH
Dettmers *et al.* 1997	↑	NA			↑ patient group
Honda *et al.* 1997	↑↑	↑	↑	↑	
Matteis *et al.* 2003	↑↑	↑	↑	↑↑	↓ patient group
Seitz *et al.* 1998	↑	↑↑	NS	↑↑	
Silvestrini *et al.* 1993	↑	↑	NS	↑	↑ UH
Silvestrini *et al.* 1995	↑	↑	NS	↑↑	↑ UH
Weder *et al.* 1994	↑↑	↑			
Weder *et al.* 1994	↑↑	↑			↑ patient group
Weiller *et al.* 1992	↑	↑↑	↑	↑↑	↑ UH
Weiller *et al.* 1993	↑	↑↑	↑	↑↑	↑ UH
Cognitive studies					
Bragoni *et al.* 2000	Good recovery:↑ Poor recovery:↑	↑ ↑↑	Good recovery:↑ Poor recovery:↑	↑ ↑↑	↓ patient group
Kessler *et al.* 2000	↑↑	NS			
Lin *et al.* 2011	↑	↑			↓ patient group
Silvestrini *et al.* 1995	↑	↑	↑	↑	↑ UH
Silvestrini *et al.* 1998	↑	↑			↑ UH
Treger *et al.* 2007	NS	NS			↓ patient group

Affected Hemisphere (AH) vs Unaffected Hemisphere (UH): For CBF changes in AH and UH in response to AH or UH stimulation - ↑↑, indicates significant (greater) CBF increase compared to the opposite hemisphere; ↑, indicates CBF increase compared to baseline; NS, indicates non-significant CBF increase; **Patient vs. control:** ↑ UH, greater increase in the unaffected hemisphere in patients when compared with the ipsilateral CBF response in controls; ↓ patient group, reduced CBFv increase in patients when compared to controls; ↑ patient group, percentage CBF increases were higher in patients compared with controls.

Table 2.6 Details of the relationship between CBF responses and clinical recovery following stroke

Studies	Relationship between CBF and Recovery
Sensorimotor studies	
Caramia *et al.* 2000	Greater UH CBFv increase during movement of the recovered hand was related to good recovery
Matteis *et al.* 2003	For each additional point of CBFv increase in the AH, the relative probability of good clinical recovery increased 5.68 times
Silvestrini *et al.* 1993	UH may play an important role in motor functional recovery
Cognitive studies	
Bragoni *et al.* 2000	Patients with recovery - bilateral CBFv increase Patients without recovery - CBFv increase limited to the UH
Silvestrini *et al.* 1998	Good recovery: no differences between AH CBFv increases between visits/ higher UH CBFv after treatment Poor recovery: no differences between before and after treatment
Treger *et al.* 2007	Poor language ability: bilateral increase Good language ability: greater UH increase

2.1 Discussion

2.1.1 Summary of Findings

Even though the biological basis for, and precise mechanisms of recovery remain largely unknown, cerebral haemodynamic studies may provide important information about the adaptive mechanisms after ischaemic stroke. Improvement of brain function was shown to be accompanied by increased CBF in impaired brain regions surrounding focal infarcts, as well as in contralateral regions of the unaffected hemisphere. Moreover, CBF responses have been shown to change over time, which was assumed to be related to functional reorganization. Despite the consensus in sensorimotor and cognitive studies that the CBF responses after stroke have some degree of impairment, no agreement was found about the clinical significance of such impairment.

2.1.2 Areas of Agreement vs. Contradictory Results

Functional involvement of the UH after stroke has been consistently reported in the literature (Carey *et al*., 2005, Cuadrado *et al*., 1999, Marshall *et al*., 2000, Nelles *et al*., 1999b, Rehme *et al*., 2011, Silvestrini *et al*., 1998a), suggesting that this hemisphere may adaptively compensate for the associated brain damage. This is in line with the results obtained from the cognitive activation studies in aphasic patients. Indeed, mirror movements (Wittenberg *et al*., 2000), disinhibition of the intact motor cortex due to reduced transcallosal influences (Gerloff *et al*., 1998), and reflection of increased attention to movement (Dennis *et al*., 2011, Thiel *et al*., 2007) were previously correlated to UH activation. Sensorimotor activation studies have described changes of significant AH CBF increases, bilateral changes, as well as specific to UH. No agreement was found regarding CBF responses and the relationship with recovery. In keeping with other stroke recovery studies (Carey *et al*., 2006, Marshall *et al*., 2009, Nelles *et al*., 2011), Matteis *et al.* (2003) found that the probability of good clinical recovery increased 5.7 times for each additional point of AH CBFv increase. In contrast, Caramia *et al.* (2000) has related a good clinical recovery with a greater UH CBFv increase during thumb finger opposition in the affected side. No consensus has yet been reached regarding the type, cue, movement rate and complexity of the paradigms. It is possible that less demanding paradigms will lead to smaller CBF responses as well as smaller changes in systemic haemodynamic parameters. Therefore, generalization of the results needs to be undertaken with caution, even within the cognitive or sensorimotor paradigms.

2.1.3 Gaps in Knowledge

Limited data are available on the evolution of CBF responses to activation for different levels of motor impairment and recovery post stroke, especially severe impairment and poor recovery. Further studies of the functional changes will be useful for investigating its

prognostic significance, and in particular the ability to identify patterns that are predictive of good recovery and/ or specific therapeutic applications. Another point is the lack of knowledge regarding the temporal (beat-to-beat) pattern of CBF response and the potential influence of peripheral haemodynamics on such a response. It is well known in the literature that BP (Tiecks *et al*., 1995) and $PaCO_2$ (Poulin *et al*., 1996) may alter CBF responses, at least at the level of resistance vessels triggering other cerebral autonomic mechanisms (such as CA and CVR). In healthy controls, significant systemic haemodynamic changes during sensorimotor paradigms have recently been reported in studies where beat-to-beat of CBFv, BP and $EtCO_2$ were recorded (Moody *et al*., 2005, Salinet *et al*., 2012b, Salinet *et al*., 2012a).

2.1.4 Critique of Methodological Approaches

The interpretation of the cerebral haemodynamic responses to brain activation after ischaemic stroke is also hampered by some methodological issues. Firstly, little attention has been given to the consistency of findings. Study power and reproducibility assessment of the results are very important issues in clinical research because time-dependent changes in brain activity may be erroneously attributed to task-dependent effect rather than simply to random variation (Salinet *et al*., 2012a). This is further complicated by the potentially large number of other relevant covariates in stroke recovery, including age, infarct size, and infarct location. These data were missing, or insufficient, in several studies. Secondly, there are methodological issues related to differences in CBF techniques. For example, multiple studies within the same subject may be less practical with PET because of concerns related to ionizing radiation. Moreover, while PET studies give important insights about the local distribution pattern and regional quantification of CBF, more attention is attached to the dynamic component and global quantification of CBFv in fTCD studies. Another methodological issue

is that fTCD does not measure CBF directly, but relies on the assumption that changes in CBFv are directly proportional to changes in CBF (Lindegaard *et al*., 1987).

2.2 Conclusion

Overall, AH and UH CBF increases are reported in response to sensorimotor paradigm stimulation of the AH, though there was much inconsistency regarding the role of the UH. Furthermore, responses to neural activation seem to be impaired after ischaemic stroke. However, the literature on neural activation following stroke is limited in its scope and of uneven quality, lacking uniformity regarding the control of a large number of clinical and physiological co-variates that could influence results. Nevertheless, despite these limitations, there is suggestion from some studies that CBF responses can have prognostic value, and help to guide rehabilitation strategies. This should provide an impetus for further studies in this area with adequate design, appropriate statistical power calculations, and standardized procedures that take into account differences in and the potential effects of individual patient characteristics.

3 Aims and Outline of the Thesis

3.1 Aims

This PhD thesis aims to contribute new and unique evidence to the debate surrounding the longitudinal changes of CBFv regulation in ischaemic stroke population. Moreover, the relationship between recovery and the pattern observed in the CBF regulation will be also discussed. As mentioned in previous chapters, it is now well established that mechanisms of CBF regulation are impaired during the acute phase of stroke, and considerable uncertainty remains about the natural history of this impairment and its prognostic value (questions raised in the preceding literature review). This thesis also aims to highlight the potential importance of developing new methods of assessment that can optimise the accuracy of cerebral haemodynamic measurement. Better and more reliable methods of assessment will enable research findings to be translated into sustainable health care improvements, particularly in the acute phase of stroke.

Besides examining the CBF regulation over time in stroke patients, data were also gathered from healthy older controls aiming to better understand the pathophysiological changes that might happen after acute ischaemic stroke. It will be particularly important to improve knowledge of the neurovascular coupling mechanism into the data analysis proposed here and to test new techniques to assess CBF regulation.

3.2 Outline

This thesis uses evidence from the literature to investigate cerebral haemodynamics and its regulation. Chapter 1 focused on describing the physiopathology of ischaemic stroke and its

effects on CBF regulation. The background of CBF regulation in healthy and ischaemic brain and a systematic review of the CBF responses to neural activation after stroke were described in chapter 2. A brief description of the methodologies available to study CBF regulation is found in chapter 4. It also highlights some of the methodological elements used to record and analyse the data involved in this project, including the research protocol. After extensive search, active and passive movement of elbow, as well as, the imaginative performance of the elbow movement, were chosen to stimulate the brain and, therefore, assess NVC mechanism. Chapter 5 aims to investigate the cerebral and peripheral haemodynamic beat-to-beat responses to the three paradigms in healthy older volunteers. This chapter focused on the comparison of the CBFv response between paradigms. Chapter 6 analyses the extent of variability in CBF response to the paradigms in healthy older volunteers over two visits. An important outcome from this chapter is that it provides estimates of sample sizes for cross-sectional as well as longitudinal studies. Chapter 7 focused on separating the contribution of blood pressure, $PaCO_2$ and the stimulus itself to the CBF response during the paradigms' performance using an original model to improve the accuracy of NVC estimates.

Chapter 8 tests the hypothesis that the beat-to-beat CBF response to the passive paradigm differs between acute stroke patients and healthy control subjects. Moreover, a subcomponents analysis was performed adding to the interpretation of differences between CBF responses. Chapter 9 uses the same methodology as chapter 7 aiming to improve the sensitivity and overall diagnostic accuracy of the CBF studies in stroke. Chapter 10 describes the natural history of the CBF responses to the three paradigms over three months enabling a better understanding of the potential changes in CBF regulation and the potential clinical importance of CBF responses to neural activation in predicting patient recovery.

4 Research Methods

Accurate haemodynamic measurement is of fundamental importance in the investigation of cerebral physiology changes in healthy subjects under a variety of conditions and to understand alterations that occur in neurovascular disease. In the following sections, an overview of the measurements of parameters that have been used to assess cerebral haemodynamics is provided, with special attention to TCD and beat-to-beat BP monitoring. Later the different methods of CA and NVC measurement are presented, before detailing research methods in the third section.

4.1 Measurement of CBF

Cerebral blood flow, or CBF, is measured as the blood volume traveling through a specific point of the brain circulation per unit time. CBF is typically 750 millilitres per minute or 15% of the cardiac output. This equates to 50 to 54 millilitres of blood per 100 grams of brain tissue per minute.

The optimal method for measuring CBF has yet to be discovered. Because of the anatomical difficulties inherent in accessing the central nervous system, quantitative measurements of CBF are difficult. The first quantitative measurements of CBF using an inert tracer were performed by Kety and Schmidt (1948) using an inert gas inhalation technique. This technique is based on Fick's principle, whereby 'the quantity of any substance taken up in a given time by an organ from the blood which perfuses it is equal to the total amount of the substance carried to the organ by the arterial in flow less the amount removed by the venous drainage during the same time period'(Kety and Schmidt, 1948). In simple words, the arterio-

venous difference of a highly diffusible inert tracer is proportional to the flow going to the brain (Kety and Schmidt, 1948, Traystman, 2004).

Though the development of this technique for measurement of CBF in humans represents a major accomplishment in the field, it is not considered adequate for studying the dynamic changes in CBF due to its poor temporal resolution as several minutes are required to yield a single CBF value. The evolution of mathematical models and electronic technologies has led to sophisticated methods of CBF estimations, such as positron emission tomography (PET), single-photon emission computed tomography, xenon computed tomography (CT), magnetic resonance imaging (MRI), and perfusion CT. Each method presents specific characteristics that make it more appropriate to particular clinical situations. Given the relevance of this Thesis, I will describe in more detail the techniques more usually used to evaluate the regulation of cerebral haemodynamics.

4.1.1 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) has provided unprecedented image resolution for structural brain imaging and is now used widely in the assessment of cerebrovascular disorders, given its non-invasiveness, and also the option of using a range of different modalities (e.g., diffusion-weighted imaging, metabolite spectroscopy, tissue relaxometry). All these points together allow the combined longitudinal assessment of tissue perfusion, morphologic features, metabolism and function. Moreover, MRI can detect the changes in susceptibility during the passage of a compact bolus injection of contrast agents and so provide information about the pattern of brain perfusion (Calamante *et al.*, 1999, Detre *et al.*, 1999).

More recently, a method known as the functional MRI (fMRI) has been developed, which images actual activated regions of the brain. It does not measure CBF directly, fMRI detects changes in the concentration of deoxyhaemoglobin dependent on a complex interplay among blood flow, blood volume and cerebral oxygen consumption. When neurons increase their activity with respect to a baseline level, a modulation of the deoxyhaemoglobin concentration is induced, generating the so-called blood oxygen level-dependent (BOLD) contrast. BOLD dynamics are characterized by an initial transient small decrease below baseline due to initial oxygen consumption (negative dip), followed by a large increase above baseline, due to an oversupply of oxygenated blood only partially compensated for by an increase in the deoxygenated venous blood volume (Donahue *et al*., 2009, Rossini *et al*., 2004a). The same stimulus that increases CBF generally results in elevated regional relative proportion of oxy-haemoglobin (Rudziński *et al.*, 2007). Despite many studies using this technique to understand NVC in healthy and disease states, there are limited BOLD imaging studies demonstrating CA responses to vasomotor challenge (Saeed *et al.*, 2011).

4.1.2 Positron emission tomography

Positron emission tomography (PET) is a nuclear medicine technology that uses short-lived radionuclides (carbon-11, oxygen-15, nitrogen-13 and fluorine-18) attached to biological molecules to allow the measurement of local metabolism, blood flow and blood volume. The determination of presynaptic transmitter concentration, postsynaptic receptor density, and the site of action of drugs can also be obtained by means of specific tracers. The acquisition time is about 5 to 10 minutes, and the minimum interval between two examinations is approximately 10 minutes (Rudziński *et al.*, 2007). Though PET is a reliable method for measuring regional CBF (Hirano *et al.*, 1994, Ostergaard *et al.*, 1998), it is not widely used clinically because of the high cost and limited availability. Moreover, it often requires an

exposure to significant radiation doses. PET has been used to study CBF responses to neural activation, but has not been widely employed in CA studies.

4.2 Transcranial Doppler Ultrasound

TCD refers to a technique introduced by Aaslid *et al.* (1982) using ultrasound probes within the frequency range of 1-2 MHz making it possible to measure CBFv beat-to-beat changes through thinner regions of the skull, termed *acoustic windows*. From there on, it has turned out to be a useful tool in the entire field of neuroangiology for both scientific as well as practical clinical purposes. The major advantage of TCD is it very high temporal resolution making it a excellent method for quantification of rapid changes in CBF. Together with its relatively low cost, non-invasiveness and easy operation, it has become one of the main tools for studying flow and flow-metabolic coupling in the human cerebral circulation (Aries et al., 2010, Duschek and Schandry, 2003, Panerai, 2009, Willie et al., 2011a).

Before focusing on the study of NVC and CA mechanisms, it is opportune to illustrate the multiple applications that TCD can have in the clinical setting. TCD is a well-established technique in the diagnosis and assessment of intracranial occlusive disease (Hussain and Gupta, 2008, Ley-Pozo and Bernd Ringelstein, 1990, Lindegaard *et al.*, 1986) and stroke (Akopov and Whitman, 2002, Razumovsky *et al.*, 1999), as well as for the evaluation of collateral flow (Kaps *et al.*, 1992). TCD has a range of applications in detecting emboli in and out the perioperative settings, (Mackinnon *et al.*, 2004, Ringelstein *et al.*, 1998, Vuković-Cvetković, 2012). In addition to detecting vasospasm (Martin *et al.*, 1992, Soustiel *et al.*, 1998), TCD has several other applications in severe head injury as an indicator of severity (Martin *et al.*, 1992) and brain death (Petty *et al.*, 1990, Zurynski *et al.*, 1991).

4.2.1 Recording Principles

The physical basis of Doppler sonography is a frequency shift ("Doppler effect" first described in 1842 by the Viennese mathematician Christian Doppler) caused by the relative movement between the transmitter and the receiver. A piezoelectric material used to generate ultrasound waves that are focused into a beam by a lens. The difference in frequency between the emitted and reflected ultrasound signals is a measure of the velocity of the reflecting elements in blood, when normalised by the speed of sound in the propagation medium.

TCD follows the same principle of the Doppler effect to measure CBFv in the large vessels. A piezoelectric transducer generates an ultrasound beam, and the ultrasound pressure waves are reflected off of the insonated moving red blood cells with a frequency shift (Doppler effect) proportional to the velocity of the scattering elements (Aaslid, 1986a, Duschek and Schandry, 2003). In the presence of laminar flow, the velocity distribution across the vessel diameter will be approximately parabolic. Consequently, the reflected Doppler frequency shift will comprise a distribution of frequencies, rather than a single value. Different alternatives exist to extract meaningful velocity information. Typically, a fast Fourier transform (FFT) from segments of approximately 5ms is used to generate a three-dimensional presentation of the raw Doppler data. From the frequency distribution, either the maximum frequency (i.e. maximum velocity), or its intensity-weighted mean are extracted to represent the mean velocity for the time interval (Aaslid *et al.*, 1982). The velocity distribution, the maximum velocity envelope and the intensity-weighted mean are normally displayed by most TCD devices as a colour coded *sonogram* (Fig 4.1).

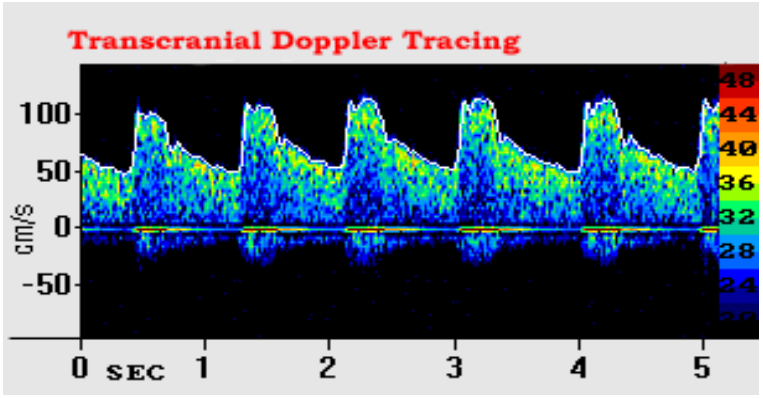

Fig 4.1 Sonogram showing the distribution of TCD ultrasound shift frequencies in the middle cerebral artery.

4.2.2 Identification of the Main Intracerebral Arteries

As described before, the presence of an acoustic window in the skull is necessary for adequate insonation of intracerebral arteries. TCD allows for insonation of the six major cerebral arteries via four main approaches based on the principal acoustic windows: transtemporal, transocular, suboccipital, and submandibular. For the purposes of this study, the middle cerebral arteries (MCA) were insonated through the temporal window.

The transtemporal window is located above the zygomatic arc, 1 to 5 cm in front of the ear where the temporal bone is thinner. Using that window, assessment of CBFv can (theoretically) be carried out in the terminal internal carotid artery (TICA), anterior, middle and posterior cerebral artery (ACA, MCA, PCA). Aaslid *et al.* (1982) have described in detail the insonation of the basal cerebral arteries constituting the Circle of Willis (Table 4.1). The TICA is the point just prior to the bifurcation into the MCA and ACA; the MCA is a direct continuation of the TICA and tends to lie lateral, superior, and at a shallower depth, whereas the ACA is more medial, crossing towards the mid-line of the brain. The ACA/MCA bifurcation is an anatomical landmark for signal location. Of note, the MCA provides the

highest velocity measurement of all vessels insonated through the transtemporal window, the average being 62±12 $cm.s^{-1}$(Aaslid *et al*., 1982).

Only the MCA, which supplies the premotor and primary sensoriomotor cortical areas responsible for movements of the arm, was insonated in this study. Bilateral MCA were identified according to their signal depth and velocity characteristics as described in the literature (Aaslid *et al.*, 1982, Ringelstein *et al.*, 1990). Traceability of the insonation depth is a key criterion, especially for MCA. By reducing the depth gradually (up to 35 mm), and then evenly increasing it again, the signal pattern and velocity amplitude should remain approximately constant. Correct identification of the MCA can also be confirmed by compression of the common carotid artery. With a normal circle of Willis, only MCA velocity will show an ipsilateral reduction whereas other arteries may increase or even reverse the flow (Fujioka and Douville, 1992). Nevertheless, carotid compression is contra indicated in subjects with unstable cerebral or peripheral circulations (e.g. stroke patients) and for this reason it was not used in this study.

There are, however, subjects who do not have adequate acoustic windows, thus making it impossible to obtain suitable recordings by TCD. This is particularly the case in older women and certain ethnic groups, including the Chinese and Afro-Caribbean populations (Halsey, 1990).

Table 4.1 TCD identification of the main intracranial arteries according to their signal depth, flow direction, relation to ACA/MCA bifurcation (an anatomical landmark for signal location) and mean velocity (Aaslid *et al.*, 1982).

Artery	**Depth (mm)**	**Flow direction**	**Relation to ACA/ MCA bifurcation**	**Mean CBFv ($cm.s^{-1}$)**
MCA	30-60	Towards the probe	same	62 ±12
ACA	60-80	Away from the probe	anterior and superior	50 ±11
PCA	60-70	Proximal (P1) toward, distal (P2) away from probe	posterior and inferior	40 ±10
MCA/ACA bifurcation	55-65	bidirectional	-	-

MCA, middle cerebral artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery; CBFv, cerebral blood flow velocity

4.2.3 Validation of TCD as a method of measuring CBF

TCD-derived arterial velocities have been used as a surrogate for CBF. Velocities and changes in velocity measured with TCD have been shown to correlate well with results from traditional techniques (e.g. correlation between SPECT and TCD r=0.63 (Lindegaard *et al.*, 1987), and between Xe and TCD during CVR testing r=0.85 (Bishop *et al.*, 1986). On the other hand, TCD does not lend itself well to the calculation of absolute values of CBF.

Theoretically CBF can be calculated from measurements of velocity using the following equation:

$$\text{Volume flow rate} = \sum_{i} v_i \Delta A_i \qquad \textit{Eq. 4.1}$$

where v_i is the velocity in velocity gate i and $\Delta A i$ is the area of the annulus within the vessel with that flow velocity.

However, this requires the assumption that there are no significant changes in the diameter of the insonated vessel. The angle of insonation is less than 30° to reduce error in the calculation of velocity to <15%. The angle of insonation is assumed to be zero, because it is impossible to measure it and it is generally believed that the diameter of the MCA changes minimally (Serrador *et al.*, 2000, Newell *et al.*, 1994, Giller *et al.*, 1993). Having said that, it remains possible that large cerebral vessel do, in fact, change diameter, and any TCD data, therefore, should be openly interpreted.

Cerebral haemodynamic function is clearly regulated by an array of functionally integrated processes (as described in chapter 2). Further exciting applications may be possible, if TCD measurements are combined with capnography (breath-by-breath $EtCO_2$) and parameters of peripheral haemodynamics (beat-to-beat BP and heart rate measurement); which may lead to a better understanding of the interaction between cerebral circulation and brain function.

4.3 Continuous Blood Pressure Monitoring

The reference method for measuring BP continuously is intra-arterial monitoring. This method is designed to measure the systemic pressure as close to the heart as possible, through the use of a central aortic pressure catheter, which can only be justified during surgery or during intensive care monitoring. Together with its invasiveness, intra-arterial monitoring can be distressing to subjects and put them at considerable risk of thromboembolism, arterial dissection or other complications (Mangano and Hickey, 1979).

Non-invasive BP continuous measurements are available at a peripheral location, such as tonometry and volume clamping techniques. The principles of tonometry are based on compressing and partially flattening an artery on to a bone surface (Drzewiecki *et al.*, 1983). The intra-arterial forces (forces exerted by arterial pressure), sensed by a transducer, are translated into arterial BP waveforms. Penaz (1973) described the volume clamp technique, which is a development enabling beat to beat non-invasive assessment of mean arterial pressure in a digit. With this method, finger arterial pressure is measured using a finger cuff of the appropriate size placed around the subject's finger and an inflatable bladder in combination with an infrared volume measuring device (called a *photoplethysmograph*) (Imholz *et al.*, 1998, Silke and McAuley, 1998). The photoplethysmograph consists of an infrared light source and detector, the blood absorbs the infrared light, and the pulsation of arterial diameter during a heartbeat causes a pulsation in the light detector signal.

The cuff is automatically inflated until it detects the maximal plethysmograph finger pulsation. Then, the arteries are clamped by varying the pressure of the finger cuff inflatable bladder using the fast cuff pressure control system, and the cuff is automatically calibrated against pre-determined criteria in order to adjust readings for greater accuracy (known as the Physiocal, described below). The cuff pressure provides an indirect measure of intra-arterial pressure. It is of note that the hand should be at atrial level for reliable BP measurement.

The Physiocal (abbreviation for Physiologic Calibration) algorithm eliminates the changes in tone of smooth muscles in the arterial wall, the haematocrit and other finger volume changes during measurement periods of constant pressure. By analysing the photoplethysmograph signal at two or more pressure levels (opening the vascular unloading feedback loop), the Physiocal algorithm performs a new pressure ramp search before the measurement starts again. This algorithm, which needs to interrupt periodically the finger blood pressure

measurement resulting in short data loss during that time, is further referred to as "*Physiocal*".

The volume clamp technique has been commercially produced by Ohmeda in devices called the Finapres™, Finometer® and Portapres, which have previously been validated with regard to their accuracy and precision (Sammons *et al*., 2007, Schutte *et al*., 2004). Overall, it was found that though the Finapres® tended to underestimate readings, it could accurately represent the temporal changes of BP (Imholz *et al.*, 1998, Parati *et al.*, 1989). Waveform filtering, level correction, and level calibration have been integrated in the Finometer® to correct these physiological brachial to finger differences.

Due to physiological and/or methodological causes, the finger arterial pressure may differ from the brachial pressure, both in pulse shape as well as in pressure levels. This might limit the use of finger arterial pressure in certain circumstances. Poor peripheral circulation, finger vasospasm (cold hands) and dysregulation in sympathetic innervation may affect BP recording accuracy. The methodological causes are thought to include inadequate positioning of the cuff, inappropriate cuff size, and differences in height between the transducer and left atrium. In our subjects, an appropriately sized cuff, as suggested by the manufacturers was used, and the hand and heart were kept at the same level.

4.4 Measurement of Carbon Dioxide Partial Pressure

As has previously been discussed, CO_2 can significantly alter resting CBF, as well as, NVC and CA mechanisms. It is consequently essential to monitor CO_2 levels in any cerebral haemodynamic studies to determine that either it remains stable during the recording or whether it may be influencing CBF changes.

As with BP, the gold standard method for measuring $PaCO_2$ is through an invasive arterial blood gas sampling. Together with its invasiveness, it does not give information of the temporal pattern of $PaCO_2$ variation, and therefore it is not used in cerebral haemodynamic studies. Continuous measurement of end-tidal carbon dioxide partial pressure ($EtCO_2$) is possible by either mass spectrometry, or Raman spectrometry or infrared capnometry. Both mass and Raman spectrometry have small representation in the cerebral haemodynamics literature. On the other hand, infrared capnometry has been used extensively and as it was the technique used in this Thesis, a more detailed explanation can be found in the following paragraphs.

Capnography refers to the measurement and display of $EtCO_2$ that can be examined on a breath-by-breath basis or for long-term trends. Most monitoring devices incorporate the transmission of infrared light to measure the concentration of exhaled CO_2 either as a percentage (%) or as partial pressure in mmHg. With this method, the absorption of CO_2 molecules exposed to various wavelengths of light within a sample chamber or cell is measured. A photo detector then compares the relative amount of light absorbed by the sample with the amount absorbed by a gas that is free of CO_2. The difference between the two represents the concentration of CO_2.

$EtCO_2$ is often used as a surrogate of $PaCO_2$. In previous studies, $EtCO_2$ measurement directly correlated with $PaCO_2$ concentration (Kerr *et al.*, 1996, McSwain *et al.*, 2010, Nunn and Hill, 1960), though $EtCO_2$ readings have also been shown to underestimate $PaCO_2$ (Husaini and Choy, 2008). The difference can be explained by the theories of dead space, shunt and ventilation-perfusion mismatch (V/Q mismatch) (Burrows, 1989, Husaini and Choy, 2008). The gas from lung units that are ventilated but not perfused (alveolar dead space or high ventilation-perfusion ratio units) contains little or no CO_2 and, when this gas

mixes with that from "normal" lung units, the resultant concentration of CO_2 is reduced in the expired gas.

4.5 Assessment of Cerebral Haemodynamic Function

4.5.1 Assessment of Neurovascular Coupling

4.5.1.1 Techniques for Measuring NVC

The NVC mechanism has been studied through many functional activation paradigms to stimulate different cortical areas in normal healthy brain, as well as in neurovascular disease as a predictor of neuronal activity. The most popular paradigms are visual (Azevedo *et al.*, 2007, Donahue *et al.*, 2009, Rosengarten *et al.*, 2001), language (Altamura *et al*., 2009, Groschel *et al*., 2007, Silvestrini *et al*., 1998b, Silvestrini *et al*., 2009), motor (Bene et al., 2009, Silvestrini et al., 1993b, Silvestrini et al., 1998b), and writing (Moody *et al*., 2005, Silvestrini *et al*., 1998b, Silvestrini *et al*., 2009, Stroobant and Vingerhoets, 2000).

Sensorimotor paradigms have become increasingly popular in the field of neuronal recovery studies, since impairment, preservation, and rehabilitation of sensorimotor function is a pivotal issue in many neurological disorders. Voluntary movement has been extensively used and has provided useful information regarding the mechanisms of cerebral haemodynamics and its recovery. However, some neurological disorders often preclude the use of an active motor paradigm; therefore non-voluntary-motor-control based paradigms have been proposed in the literature.

In neurological and sport rehabilitation, the use of passive and motor imagery (MI) exercise is very common practice in physiotherapy allowing patients to train motor function when voluntary movement is (partly) impaired. Passive exercise is a movement performed without

volitional control. It is accomplished by the therapist moving a patient's limbs, and is reported to be used commonly by physiotherapists in critically ill patients (Stockley *et al.*, 2010), whereas MI requires the mental representation of an overt action without any concomitant movement (Jeannerod, 1994). To correctly execute this exercise, the patient's level of consciousness needs to be taken into account.

In healthy volunteers, equivalence of brain activity between MI, active and passive motor performance has been reported both at the structural and functional level. These three paradigms have been shown to share common functional circuits that include primary sensorimotor, premotor, supplementary motor and somatosensory areas, basal ganglia and cerebellum (Guzzetta et al., 2007, Jeannerod, 1994, Roosink and Zijdewind, 2010, Sharma et al., 2009, Steuernagel et al., 2002, Stippich et al., 2002, Weiller et al., 1996). Noteworthy, cerebral haemodynamic responses induced by active, passive and MI paradigms have not been assessed in the same population using TCD method.

Because of the great importance of having early prognostic information about cerebral functional changes, active paradigms have been widely used to study NVC in acute stroke. However, it is possible that strong evidence of similarity of brain activation found in healthy controls encouraged other NVC studies to use other alternatives in stroke. The possibility of using passive movement to study NVC in stroke hemiplegic patients was suggested by preliminary PET investigations (Nelles *et al.*, 1999a, Nelles *et al.*, 1999b). After that, one fMRI and one TCD study have reported the cerebral haemodynamic responses to a passive paradigm in an attempt to find a functional pattern of cerebral recovery (Blatow *et al.*, 2011, Matteis *et al.*, 2003). MI has not to my knowledge been used to assess CBF in stroke patients.

4.5.1.2 Quantification of CBF Responses

Hitherto no classic method for assessing CBF (or CBFv) responses to neural activation has been defined. Most of the studies in the literature used CBF or CBFv responses to different stimulation paradigms as an index of NVC. Notably, for measurements in the PCA, Rosengarten *et al*. (2003a, 2003b) have used a second-order model to fit the CBFv response to on-off reading, with good results in terms of reproducibility and sensitivity.

The most common choice involved the maximum value of the CBF or CBFv response (Sitzer *et al*., 1994, Tiecks *et al*., 1998). Due to the poor temporal resolution, this method is the most used in PET studies. In other studies, CBFv values were either averaged over 30-60s (Silvestrini *et al*., 1995a, Silvestrini *et al*., 1996, Silvestrini *et al*., 1998b) or the mean of the complete response (Bragoni *et al.*, 2000, Matteis *et al.*, 2001) without clear synchronization of the beginning of the neural activation paradigm with the CBFv response. Only a few studies in the literature have presented the temporal pattern of beat-to-beat CBFv responses to neural activation (Duschek *et al.*, 2008, Moody *et al.*, 2005, Panerai *et al.*, 2005b).

The limitation of using raw CBF data to assess NVC is that it does not take into account the influences of covariates (e.g. BP and $PaCO_2$). As described in chapter 2, BP and $PaCO_2$ variations can significantly influence CBF, and it would, therefore, be important to assess CBF without the contribution of other variables.

4.5.2 Assessment of Cerebral Autoregulation

4.5.2.1 Techniques for Measuring CA

In classical studies of dCA, BP stimuli were induced using thigh cuff inflation and release producing a stepwise decrease in BP (Aaslid *et al.*, 1989).With this approach, large (20 cm) cuffs are placed around both thighs and are inflated 20–40 mmHg above systolic pressure for

at least 2 minutes. Following the rapid deflation of thigh cuffs, peripheral resistance is reduced leading to an acute drop in BP (the representative BP and CBFv response to such method is shown in Fig. 4.2). Undesirably, this technique can be fairly uncomfortable and some argue that it may partially exert its effects through activation of sympathetic pathways, thus confounding the interpretation of results. Moreover, a further problem with the thigh-cuff technique is that the induced BP change may not be recommended in some patient groups with altered cerebrovascular or systemic haemodynamics, such as heart failure, autonomic failure or significant carotid artery stenosis. These drawbacks limit the clinical application of the thigh cuff method.

Significant changes in mean BP can also be induced by the Valsava manoeuvre, tilting or change in posture, and isometric handgrip. However, these stimuli affect other subsystems (e.g. sympathetic activation) or parameters ($PaCO_2$, pH). The use of spontaneous BP fluctuations for the assessment of dCA was first suggested by Giller (1990) and later demonstrated by Panerai *et al.*(1996). A subsequent study compared the use of spontaneous BP transients with thigh cuff manoeuvres for assessing dCA. They found the two techniques produced significantly correlated results (Panerai *et al.*, 1998b). Moreover, this approach can also be applied with other manoeuvres that induce changes in BP, for instance, respiratory manoeuvres (Panerai *et al.*, 1999) and brain activation paradigms (Panerai *et al.*, 2005a, Panerai *et al.*, 2012a).

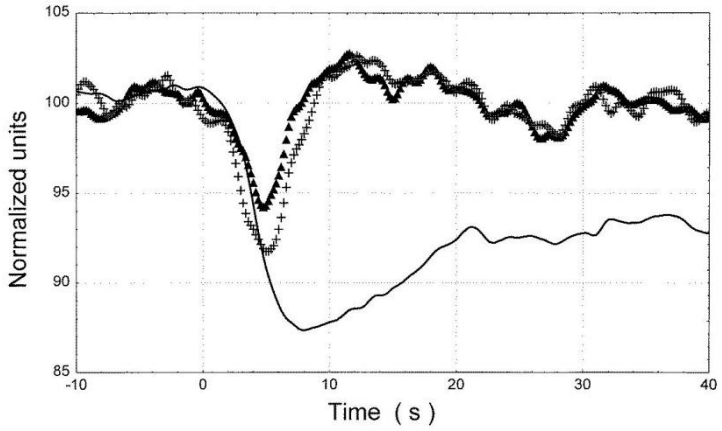

Fig 4.2 Representative average responses to thigh cuff deflation from 50 healthy volunteers. BP (continuous line), CBFv right MCA (crosses), CBFv left MCA (triangles) (Panerai, 1998).

4.5.2.2 CA Measurement

The majority of studies now measure dCA instead of sCA. This is because the dynamic test used is also non-invasive, less time consuming and more easily repeatable than conventional sCA tests (Panerai, 1998). Of equal importance is the fact that dCA can show interactions between pressure autoregulation and other variables such as $PaCO_2$ and pharmacological agents.

The interaction between many input variables makes it even more difficult to predict the CBF response to a BP change. The pressure-flow relationship has thus been termed ‘multivariate’. Accurately modelling the pressure-flow relationship to reflect these physiological phenomena has thus proved challenging. Up to date, there is no gold standard to assess CA making it difficult to compare results, as well as methods. Different methods have been suggested to calculate an index of CA including: rate of return (Aaslid *et al.*, 1989), coherence (Giller, 1990), phase (Diehl *et al.*, 1995), gain (Giller, 1990) and autoregulatory index (Tiecks *et al.*, 1995).

In this section, the most common methods to assess dCA will be discussed. The initial approach has been to describe the relationship between BP and CBF as a linear dynamic system in the frequency domain or time domain. Non-linear models presently await further validation and comparison to linear methods in a clinical setting (Panerai, 2008).

4.5.2.2.1 Frequency Domain Analysis

In the frequency domain, as a consequence of system linearity, variations of a particular frequency in the input signal are transformed to signals of the same frequency in the output signal. This coupling between input (BP) and output (CBF) can be quantified be means of transfer function analysis (TFA) (Zhang R *et al.*, 1998). The transfer function is calculated as the ratio of the smoothed cross-spectra [$G_{PV}(f)$] to the autospectra of BP [$G_{PP}(f)$]

$$H_{(f)} = \frac{G_{PV}(f)}{G_{PP}(f)} \qquad Eq.\,4.2$$

Welch´s method and FFT are used to decompose the input and output signals into sinusoidal waves of various frequencies (Giller, 1990, Panerai *et al.*, 1998a, Zhang R *et al.*, 1998). The parameter, which tests the linearity between these output and input waves at each frequency, is called *coherence,* and its value ranges between zero and 1. The squared coherence function is estimated as follows

$$y^2(f) = \frac{\left|G_{pv}(f)\right|^2}{G_{VV}(f)G_{PP}(f)} \qquad Eq.\,4.3$$

Values of coherence approaching 1 indicate a perfect linear relationship between CBFv and BP. On the other hand, when coherence tends to zero it suggests a non-linear relationship or different processes, such as excessive signal noise or no input-output relationship (Giller, 1990, Panerai *et al.*, 2006, Zhang R *et al.*, 1998). Many studies have adopted a lower

threshold of coherence = 0.5 to accept the relationship as significant (Panerai *et al.*, 1998a, Panerai *et al.*, 2006).

Also through TFA, two further parameters explore the BP and CBFv relationship: the *gain* and *phase* shift. Gain quantifies the amplification of the output signal (CBFv) in comparison to the input signal (BP) at a given frequency. In fact, the gain of this transfer function marks the efficiency of the regulator (response of CA). An increase in gain suggests that CA is impaired whereas a low gain indicates an efficient CA (Giller, 1990, van Beek *et al.*, 2008). Phase shift represents the displacement of the output waveform (CBFv) relative to input waveform with the same period. During steady-state conditions, phase is positive in the intact CA (CBFv recovers faster than changes in BP) and it tends to zero in the impaired CA when CBF tends to follow BP (Birch *et al.*, 2002, Diehl *et al.*, 1995). The gain and phase frequency response are obtained from the real and imaginary parts of *H(f)* as follows

$$|H(f)| = [H_R(f)^2 + H_i(f)^2]^{1/2} \qquad Eq.4.4$$

$$\phi(f) = \tan^{-1}\left[\frac{H_i(f)}{H_R(f)}\right] \qquad Eq.4.5$$

The dynamic regulation of CBFv is more effective at low frequencies of BP changes, gain tends to fall and the phase is positive at frequencies below 0.2 Hz (Panerai, 2004, Panerai, 2008, van Beek *et al.*, 2008). This may be due to the delay in initiating cerebrovascular resistance adaptations to fast frequency changes (Panerai, 2008, van Beek *et al.*, 2008). Therefore, the CA mechanism acts as a high-pass filter, damping effectively only very low frequency fluctuations of BP; in the high frequency range variations in BP transfer to variations on CBFv (Diehl *et al.*, 1995, Zhang R *et al.*, 1998).

4.5.2.2.2 Time Domain Analysis

Time domain approach is used to extract information about the CA mechanism from the analysis of mean BP and CBFv, heart rate, $EtCO_2$ with respect to time. Historically, dCA has been assessed using induced changes in BP while the rate of return (RoR) to baseline CBFv was measured (Aaslid *et al.*, 1989). Other time-domain methods have also been applied, including the impulse and step responses, moving average (MA) and autoregressive moving average (ARMA) (Panerai *et al.*, 2003).

Using alterations in CBFv in relation to change in BP, Tiecks *et al.* (1995) studied both dCA during thigh cuff manoeuvres and sCA responses with intact autoregulation during propofol anaesthesia (normal CA) and then again during high-dose isoflurane (impaired CA) anaesthesia. In this study, dCA yielded similar results to sCA in normal human subjects validating the thigh cuff manoeuvres method. Moreover, the concept of *autoregulatory index* (ARI) was also introduced in this study, which uses a second-order differential equation model based on three main parameters: a time-constant, gain and a damping factor to produce 10 hypothetical CBFv step-response curves (Fig. 4.3). Each of the 10 models, corresponding to ARI values from 0 (absent CA) to 9 (best CA) is fitted to the first 30 seconds of the CBFv step-response and the best fit is taken as the representative value of ARI for that segment of data. An ARI of 5 (4.8±1.0, mean±SD) is accepted as the mean ARI in "normal" individuals (Tiecks *et al.*, 1995).

From inverse Fourier transform of the gain and phase, it is possible to estimate the CBFv response to a sudden change in BP in the time domain, termed impulse response (Panerai *et al.*, 1998a, Ramos *et al.*, 2006). The CBFv step response can be calculated from the time-integral of the impulse response.

Nevertheless, it is possible to extract ARI values from the estimated step response, and equivalent impulse/step responses can also be obtained directly from the time domain, using ARMA, named ARMA-ARI (Panerai *et al.*, 2003). A step response is generated by the ARMA and matched to the most appropriate of the ten step responses given by Tiecks' model, taking into account only the first six seconds of the step response. ARMA-ARI showed greater stability and reduced variability in relation to ARI if fitted directly from Tiecks *et al.* (1995) equations (Panerai *et al.*, 2003).

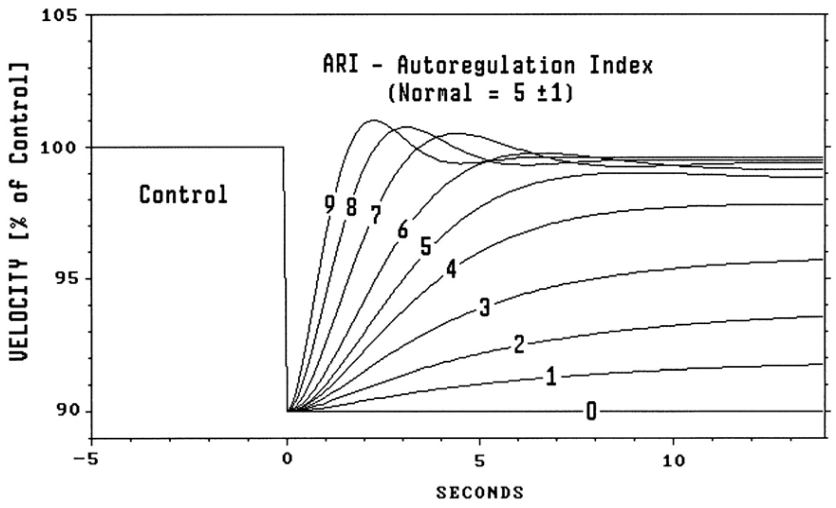

Fig 4.3 Tiecks'autoregulatory curves (Tiecks *et al*., 1995).

4.6 Research Methods

4.6.1 Subjects

Study participants were recruited from consecutive patients admitted to the Stroke Unit at the Leicester Royal Infirmary (University Hospitals of Leicester NHS Trust) after a first ever episode of ischaemic stroke within 72 hours of symptoms onset. For patients waking with a stroke, time of onset was taken to be the time when the patient was last asymptomatic. Patients suffering from other pre-existing neurological disorders, previous stroke or TIA,

atrial fibrillation and severe cognitive disturbance (making the patient unable to collaborate with the study protocol) were excluded. Neurological examinations were conducted immediately before TCD recording. Patients received pharmacological treatment including antihypertensive, antiarrhythmic, antithrombotic and statin therapy and/ or prophylactic low molecular weight heparin according to standard stroke unit protocols.

Age- and blood pressure-matched controls were also recruited. Only right-handed healthy controls were recruited in the study. In both patients and controls group, handedness was established by means of the Edinburgh Inventory (Oldfield, 1971).

4.6.2 Data Collection

The method was explained to each volunteer and patient. They were also given an information sheet to read (Appendix). The study was approved by the Nottingham Research Ethics Committee 1, United Kingdom (Ref: 11/EM/0016), and written informed consent was obtained from each participant (Appendix).

4.6.2.1 Research Protocol

Measurements took place in a dedicated cardiovascular research laboratory, which was of controlled temperature (20-24°C), free from distraction. As nicotine and caffeine may affect, either directly or indirectly, cerebral vascular smooth muscle tone, volunteers were asked not to smoke or drink coffee during the 12 h before the recording whereas patients had abstained for at least 2 hours before the measurement.

Beat-to-beat BP was recorded continuously using interchangeably the Finapres® (Ohmeda 2300; Finapres, Louisville, CO, USA) and the Finometer® devices (FMS, Finapres Measurement Systems, Arnhem, Netherlands). The finger cuff of the device was attached to

the middle finger of the left hand or non-paretic hand in control and stroke groups, respectively. The servo-correcting and *Physiocal* mechanism was switched on and then off, and for the Finapres®, BP calibration recorded.

Electrocardiogram (ECG) was continuously recorded using a 3-lead configuration. The respiratory rate and $EtCO_2$ were measured via nasal prongs by a capnograph (Capnocheck Plus). Bilateral insonation of the MCA was performed using a TCD (Viasys Companion III; Viasys Healthcare) with a 2MHz probe, which was secured in place using a head-frame (Fig 4.4). These parameters were simultaneously recorded onto a computer software system, providing data for subsequent analysis. The data collection protocol is described below.

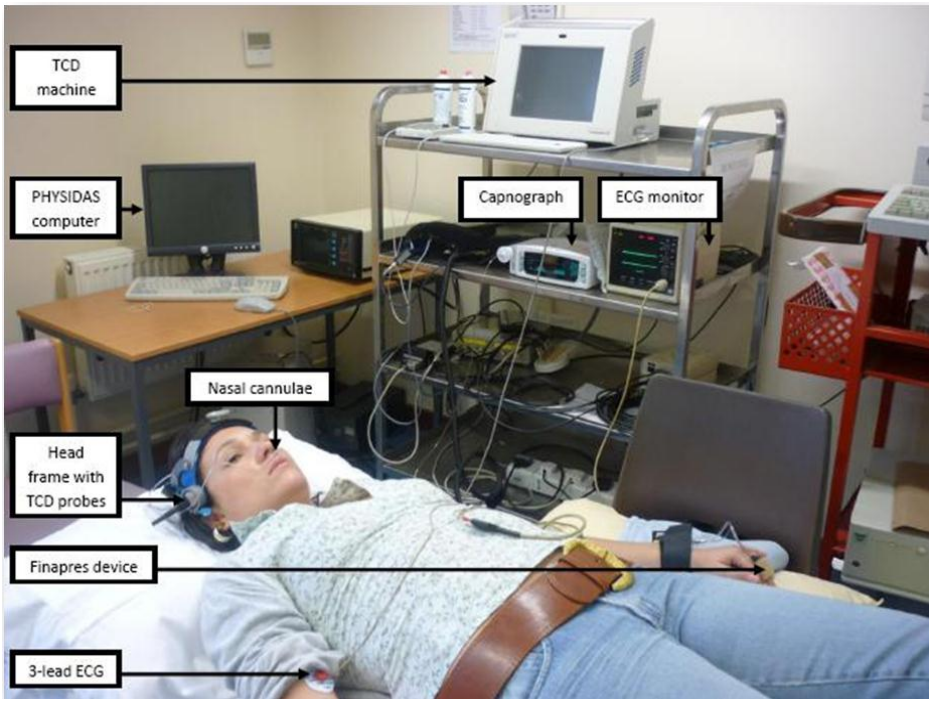

Fig 4.4 Continuous recordings of bilateral CBFv, blood pressure, heart rate and end-tidal CO_2 were recorded during neural activation paradigms.

4.6.2.2 Data Collection Protocol

During the entire procedure, subjects were in the supine position and detailed instructions were received before measurements. After a period of 15 minutes of stabilization, a 5 minutes baseline recording was taken. Then the participants performed three different neural activation paradigms (described next), each repeated twice, in random order. In the healthy group, the paradigms were performed only on the dominant side (right).

4.6.2.3 Neural Activation Paradigms

All paradigms recordings started with a 90-s baseline phase. Thereafter, the paradigm was performed over 60-s, with a 90-s recovery phase.

Active motor paradigm

Participants were asked to perform active repetitive flexion and extension of the elbow; the movements were driven by a metronome to ensure a standard frequency of 1 Hz.

Passive motor paradigm

Participants were asked to relax while the examiner undertook repetitive flexion and extension of their elbow. Movements were again driven by a metronome (1 Hz). During the rest and recovery periods, the examiner kept hold of the participant's arm.

Motor imagery paradigm

Participants imagined that they were actively moving their elbow. Again, this was controlled by a metronome.

4.6.3 Data Editing

Data were simultaneously recorded onto a data acquisition system (PHYSIDAS, Department of Medical Physics, University Hospitals of Leicester NHS Trust) for subsequent off-line analysis. ECG, $EtCO_2$, BP and stimulus marker signals were sampled at 500 samples/s.

Data editing was completed using in-house software designed by the Medical Physical Department at the University of Leicester. BP was calibrated at the start of each recording. All signals were visually inspected (Fig. 4.5) to identify artefacts and noise, and narrow spikes were removed by linear interpolation. The CBFv channels were subjected to a median filter and all signals were filtered by a low pass filter with a cut-off frequency of 20Hz. The R-R interval was then automatically marked from the ECG and continuous heart rate plotted against time. Ectopic beats caused spikes in the heart rate signal; these were manually removed by remarking the R-R intervals for the time points at which they occurred. If the heart rate could not be calculated from the ECG (due to a poor quality trace), beat-to-beat BP was used as an alternative. Mean BP, CBFv, systolic/diastolic values, RAP and CrCP were calculated for each cardiac cycle and linear interpolation was used to obtain estimates of $EtCO_2$ synchronized to the end of each cardiac cycle. Beat-to-beat data were spline interpolated and resampled at 5 samples/s to produce signals with a uniform time-base. The instantaneous relationship between BP and CBFv was used to estimate CrCP and RAP for each cardiac cycle using the first harmonic method (Panerai, 2003).

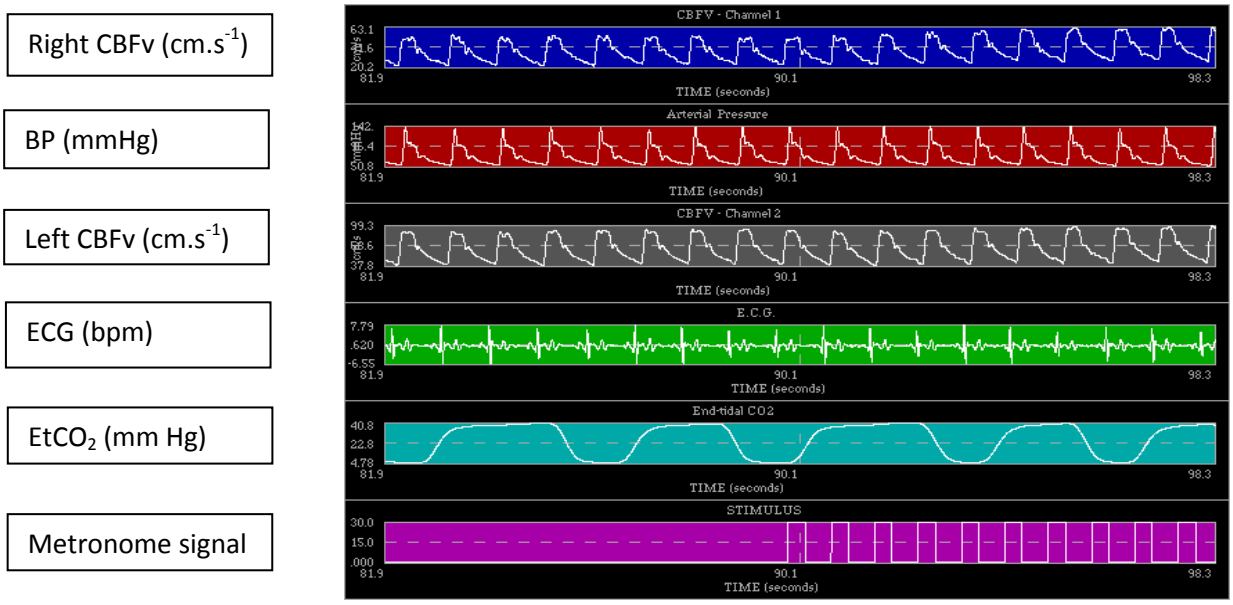

Fig 4.5 Signals displayed by editing software: CBFv, BP, heart rate and $EtCO_2$ traces

4.6.4 Data Analysis

Using the electrical output from the metronome, each variable was synchronized at the beginning of the paradigm responses and population coherent averages and standard deviation (SD) curves were obtained for each time sample value. Bilateral CBFv were normalized by their baseline values. From the temporal paradigm-synchronized population averages, mean values were extracted from the 30 seconds preceding the paradigm and from the 30s to 60s after the paradigm for baseline and recovery values, respectively.

Different data analysis (e.g. time-point averages selected to represent variables response) and statistical approaches were used to study cerebral haemodynamics in healthy and stroke group. The main methods are summarized below. A further description of the data analysis used is described in each chapter.

4.6.4.1 Baseline Blood Pressure Step Responses

The CBFv step response to the BP input was computed on the 5-minute baseline measurement, after a period of 100s of stabilization, as previously described (Katsogridakis *et al*., 2013). A fast Fourier transform was applied to the data, and the cross- and auto-spectra were estimated using the Welch method. The transfer function of the BP-CBFv dynamic relationship was then calculated with BP selected as the input and right then left CBFv as the output variables. An inverse fast Fourier transform was then applied to the complex transfer function, converting data back into the time domain, to calculate the CBFv step response (Katsogridakis *et al*., 2013). The autoregulatory index (ARI) was assigned to each recording by using the best least-squares fit between the CBFv step response and one of the 10 model ARI curves proposed by Tiecks *et al*.(1995) (as described in section 4.5.2.2.2).

4.6.4.2 Subcomponent Analysis

Decomposition of the CBFv change was performed to identify the relative contribution of its main determinants. The percentage change in CBFv (ΔCBF_v) was decomposed into standardized subcomponents describing the relative contributions of BP (V_{BP}), resistance area product (V_{RAP}) and critical closing pressure (V_{CrCP}) (Panerai *et al*., 2005b). At rest, CBF_V can be written as:

$$CBFv_0 = \frac{BP_0 - CrCP_0}{RAP_0} \qquad Eq.4.6$$

during activation, all variables in Eq. 4.6 change, leading to:

$$CBFv_0 + \Delta_v = \frac{(BP_0 + \Delta_p) - (CrCP_0 + \Delta_c)}{RAP_0 + \Delta_r} \qquad Eq.4.7$$

where Δ_v, Δ_p, Δ_c, and Δ_r represent small changes in CBFv, BP, CrCP, and RAP, respectively. Reference values of the quantities in *Eq. 4.6* were obtained by averaging data for the 10-s interval preceding the beginning of activation. The differences Δ_v, Δ_p, Δ_c, and $\Delta_r$were obtained by subtracting reference values from the total values during activation. For small changes $\Delta_r < RAP_0$,

$$\Delta_v = \frac{1}{RAP_o}\left(\Delta_p - \Delta_c - \Delta_r . CBFv_o\right) \qquad Eq.4.8$$

Defining

$$V_{BP} = \frac{\Delta_P}{RAP_O} \qquad Eq.4.9$$

$$V_{CrCP} = -\frac{\Delta_c}{RAP_O} \qquad Eq.4.10$$

$$V_{RAP} = -\frac{\Delta_r . CBFv_o}{RAP_O} \qquad Eq.4.11$$

Results in

$$\Delta_v = V_{BP} + V_{CrCP} + V_{RAP} \qquad Eq.4.12$$

Equation 4.12 expresses changes in CBFv around a reference value $CBFv_o$ as three different subcomponents representing the separate contribution of BP, CrCP and RAP. It has been suggested that V_{RAP} might reflect myogenic activity in response to BP changes, whereas V_{CrCP} is more indicative of metabolic control (Panerai *et al.*, 2012b, Panerai *et al.*, 2005b).

4.6.4.3 Estimation of parameters in multivariate ARMA modeling

A novel model was used to represent the influence of the inputs (BP, $EtCO_2$, and stimuli signal) on output (CBFv), as described in a previous study (Panerai *et al.*, 2012a). An ARMA model was adopted to express the dependence of CBFv, *v(t)* as a function of BP, $EtCO_2$ and the sensorimotor stimulation, represented by *p(t)*, *c(t)* and *s(t)*, respectively:

$$v(n)=\sum_{1}^{Nv} a_i v(n-i)+\sum_{0}^{Np} b_j p(n-j)+\sum_{0}^{Nc} d_k c(n-k)+\sum_{0}^{Ns} g_q s(n-q) \qquad Eq.4.13$$

where *n* is the discrete sample number and [N_v, N_p, N_c, N_s] are the model orders for each of the autoregressive (AR) and moving-average (MA) terms in $Eq.4.13$. a_i are the AR coefficients and b_j, d_r and g_q are the MA coefficients. To represent the stimulus signal *s(t)*, the electrical output of a metronome was continuously recorded generating a zero voltage signal when the metronome was *OFF* and a constant amplitude signal with arbitrary amplitude when the metronome and the paradigm were *ON* . The model parameters g_q will then reflect the amplitude of the contribution of *s(t)* to explain changes in *v(t)*.

Identification of suitable model orders [N_v, N_p, N_c, N_s] is a critical step in the use of ARMA models. From previous work (Panerai *et al.*, 2012a), initial model orders were [2,4,1,1]. Model coefficients were calculated by least squares and equation $Eq.4.13$ was used to calculate the model predicted time series of *v(t)* and the prediction error σ_e from the sum of squared differences in relation to real data. For each combination of model orders, it is possible to calculate the final prediction error (FPE) as

$$FPE = \sigma_e \frac{N+N_t}{N-N_t} \qquad Eq.4.14$$

where N is the number of samples in the record and N_t is the total number of model coefficients. The Student's t-value t_k can also be calculated for each estimated coefficient as:

$$t_k = \frac{c_k}{SD_k} \qquad k = 1, 2, \ldots N_t \qquad Eq.4.15$$

with c_k corresponding to the estimated coefficient value and SD_k its standard deviation.

Optimal model orders were identified by the compromise between minimum values of FPE and maximum number of coefficients with significant values of t_k. A multi-step, semi-automatic procedure was implemented to examine all combinations of model orders within a set range and select optimal values from inspections of 2 by 2 matrices of FPE and t_k. Finally, for the selected combination of model orders, the quality of model fitting was always confirmed by visual inspection of the predicted temporal pattern of *v(t)* ($Eq.4.13$) in comparison with real data.

The fraction of the total *v(n)* variance explained by the model, V_{MOD} was calculated from the squared Pearson correlation coefficient between the measured time-series of *v(n)* and model predicted values from $Eq.4.13$. A similar approach was adopted to calculate the relative contribution of each input variable, *p(n), c(n) or s(t),* as a percentage of V_{MOD}.

For any of the input functions, the corresponding CBFv step responses S_j (n) was calculated as:

$$S_j(n) = \sum_{1}^{Nv} a_i S_j(n\text{-}i) + \sum_{0}^{N} c_k \,, n = 1,2, \ldots Nd; j = 1,2,3 \qquad Eq.4.16$$

with the duration of the response set to Nd= 30s.

5 Effects of Active, Passive and Motor Imagery Paradigms on Cerebral and Peripheral Haemodynamics in Older Volunteers

5.1 Introduction

Previous studies using fMRI (Ogawa *et al.*, 1990), PET (Sitzer *et al.*, 1994), SPECT (Kuhl and Edwards, 1963) and TCD ultrasound (Aaslid, 1987, Hartje *et al.*, 1994) have shown a direct relation between specific cognitive and motor paradigms, and changes in cerebral haemodynamics in human subjects. Most of those methods of CBF quantification are expensive, complex and not widely available. TCD, on the other hand, is a non-invasive and easily operated method, which associated with its high temporal resolution, has become an important alternative technique for the quantification of dynamic adjustment in CBF accompanying cerebral activity in the human circulation (Aaslid *et al.*, 1982, Duschek *et al.*, 2008, Moody *et al.*, 2005).

Importantly, cerebral haemodynamic responses induced by active, passive and motor imagery paradigms have not been assessed in the same population using TCD methodology. More complex methods, such as PET and fMRI, have shown an activation of overlapping areas in the supplementary motor area (Guzzetta *et al.*, 2007, Jeannerod, 1994, Roland *et al.*, 1980, Weiller *et al.*, 1996), suggesting some degree of similarity in CBF responses evoked by these brain activation paradigms. Previous TCD studies showed that sensorimotor paradigms lead to a sudden CBFv rise in response to increased metabolic demand (Matteis *et al.*, 2001, Sato

et al., 2009), while the change in CBFv during mental paradigms appeared to be "task specific" (Duschek *et al.*, 2008, Tiecks *et al.*, 1998). Nevertheless, some of these studies only assessed the CBFv changes in the later period of each paradigm, and did not focus on beat-to-beat CBFv adjustments.

In addition, changes in the peripheral cardiovascular system, such as BP, $PaCO_2$ and heart rate have also been explored during motor and cognitive paradigms (Bragoni et al., 2000, Doering et al., 1998, Duschek et al., 2008, Groschel et al., 2007, Matteis et al., 2001, Silvestrini et al., 1993b). With few exceptions (Moody *et al.*, 2005, Sato *et al.*, 2009), the overall conclusion was that the extent of changes from baseline to activation was not significant. However, in keeping with CBFv studies, most of these investigations did not provide information about the temporal beat-to-beat variation of these variables in response to paradigms. Nevertheless, it is quite possible that fast changes in BP and $PaCO_2$ can trigger an autoregulatory mechanism responsible for adjusting CBF (or CBFv) and alter the cerebrovascular resistance, producing either vasoconstriction or vasodilatation (Aaslid *et al.*, 1989, Moody *et al.*, 2005, Panerai *et al.*, 1999).

Therefore, this chapter aimed to investigate the cerebral and peripheral haemodynamic beat-to-beat responses to active, passive and motor imagery paradigms, and to compare the responses between paradigms.

5.2 Methods

5.2.1 Research Participants

A total of twelve healthy participants, free from cerebrovascular disease and cardiovascular disease, were recruited from departmental staff and their relatives. Inclusion criteria were age ≥45 years and right-handedness according to the Edinburgh inventory (Oldfield, 1971).

Exclusion criteria comprised physical disease in the upper limb, poor insonation of both temporal bone windows, as well as any history of cardio- and/ or cerebrovascular disease.

5.2.2 Measurements

Recordings were performed as described in section 4.6.2.1. After a period of 15 minutes stabilization, participants performed three different paradigms each repeated twice in random order. Movement was performed only by the dominant side. All paradigms started with a 90-second baseline phase. Thereafter the active, passive and motor imagery paradigms were performed over 60 seconds, with a 90-second recovery phase.

5.2.3 Data Analysis

Data analysis was performed as described in section 4.6.3. In brief, after the signals were calculated with a uniform time-base, averages were performed for each variable synchronized from the beginning of each paradigm. Bilateral CBFvs were normalized by their baseline values. Mean values were extracted from the 30 seconds preceding the paradigm and from the 30s to 60s after the paradigm for baseline and recovery values, respectively. From the population synchronized averages, mean values of all variables were calculated at the beginning (between 5 and 10 seconds) and end (between 55 and 60 seconds) of the paradigms, which were expressed as time points 1 (*t1*) and 2 (*t2*), respectively.

5.2.4 Statistical Analysis

Paired t-tests were used to test changes from baseline to *t1*, from baseline to *t2*, and from *t2* to recovery for all variables. Bonferroni correction test was applied to multiple comparisons. Two-way repeated measures ANOVA was used to test for a) the effect of active, passive and motor imagery paradigm on CBFv, and b) the influence of lateralization. The influence of

active, passive and motor imagery paradigms on heart rate, $EtCO_2$ and BP were tested using one-way repeated measures ANOVA. For all comparisons $p<0.05$ was taken as statistical significance.

5.3 Results

All 12 subjects (8 male) with a mean age of 64 (SD 7) years completed the active, passive and motor imagery paradigms. The mean (SD) score of Edinburgh Inventory was 86.1 (6.8)%. Due to a technical fault and a poor left transtemporal insonation window, one male volunteer performed paradigms just once and one female participant had only the CBFv of the right MCA recorded, respectively. For these reasons only 11 subjects were considered for CBFv parameters. Table 5.1 provides mean (SD) values of recorded parameters for baseline and recovery phase during active, passive and motor imagery paradigms. Baseline and recovery values showed no significant differences for all recorded variables, except for ipsilateral CBFv estimates during the active paradigm where recovery values were significantly lower. No difference was found in baseline values preceding paradigms.

5.3.1 Cerebral Haemodynamic Changes

The pattern of CBFv response during active, passive and motor imagery paradigms revealed considerable similarity. Highly significant differences for the change from baseline to *t1* (Table 5.2), from baseline to *t2* (Table 5.3), and from *t2* to recovery (Table 5.3) were obtained for bilateral CBFv during the three paradigms. No significant effects due to type of paradigm or hemisphere were observed at either *t1* or *t2* (two-way ANOVA).

Paradigm-synchronized ipsilateral and contralateral CBFv averages for the whole population for the three paradigms are given in Fig. 5.1. Reviewing the ipsilateral and contralateral responses to the active motor paradigm, an increase in CBFv was found bilaterally as soon as

the metronome was turned on (grey bar), showing a peak after ~ 5s and decreasing gradually during the activity. Although the CBFv response to motor imagery paradigm was less pronounced, the sharp increase in CBFv bilaterally was again observed, as well as a gradual return to baseline. The pattern of cerebral haemodynamic response during the passive paradigm showed a steep rise in CBFv approximately 5s after the beginning of the task reaching a peak after 15s, and was maintained until just after the end of the exercise. In all three interventions, for both hemispheres, CBFv only started to return to baseline approximately 20 s after the paradigm was completed (Fig. 5.1).

Table 5.1 Mean (SD) values for cerebral and peripheral haemodynamic variables for the baseline and recovery phases.

Variable	Active			Passive			Motor Imagery		
	Baseline	**Recovery**	**p Value**	**Baseline**	**Recovery**	**p Value**	**Baseline**	**Recovery**	**p Value**
ipsi CBFv, $cm.s^{-1}$	49.1 (8.7)	47.7 (9.5)	0.03	50.2 (8.7)	49.6 (9.7)	0.4	49.1 (9.9)	49.5 (9.5)	0.1
cont CBFv, $cm.s^{-1}$	52.1 (9.4)	50.9 (10.2)	0.8	53.2 (9.7)	52.8 (10.2)	0.4	51.5 (10.7)	52.0 (8.0)	0.1
BP, mmHg	87.7 (11.7)	88.1(13.0)	0.6	88.3 (17.5)	87.4 (16.5)	0.4	89.0 (13.8)	88.2 (14.1)	0.1
Heart rate, bpm	62.0 (8.2)	62.5 (9.6)	0.5	61.7 (8.9)	62.4 (8.8)	0.08	62.4 (9.6)	62.6 (9.5)	0.6
$EtCO_2$, mmHg	38.0 (3.3)	38.1(3.3)	0.5	38.7 (3.2)	38.5 (3.0)	0.3	38.3 (3.3)	38.7 (3.1)	0.09

p value regarding comparisons between baseline and recovery values.

Ipsi CBFv, ipsilateral cerebral blood flow velocity; cont CBFv, contralateral cerebral blood flow velocity; BP, blood pressure, $EtCO_2$, end-tidal CO_2

Table 5.2 Mean (SD) values of cerebral and peripheral haemodynamic parameters changes from baseline to *t1* during active, passive and motor imagery paradigms.

Variable	Active	Passive	Motor Imagery
ipsi CBFv, %	8.3 (3.9)*	6.2 (4.7)*	6.8 (5.3)*
cont CBFv, %	9.9 (4.6)*	6.6 (4.3)*	7.5 (4.7)*
BP, mmHg	2.0 (1.8)*	0.5 (2.6)	1.4 (3.0)
Heart rate, bpm	3.1 (3.0)*	0.5 (2.2)	0.3 (1.5)
$EtCO_2$, mmHg	0.1 (1.0)	0.1 (0.9)	0.2 (0.9)

CBFv signals were normalized by their baseline values.

* $p<0.005$ for difference between baseline and *t1*. No significant differences between paradigms (two-way ANOVA). Ipsi CBFv, ipsilateral cerebral blood flow velocity; cont CBFv, contralateral cerebral blood flow velocity; BP, blood pressure.

5.3.2 Peripheral Haemodynamic Changes

Fig 5.2 represents the paradigm-synchronized averages for BP, heart rate and $EtCO_2$. The pattern of BP changes was similar for the three different paradigms (Fig. 5.2A) showing an initial rise peaking approximately 5 s after paradigm onset and a second higher peak around ~ 85s. A significant difference between baseline to *t1* was only found during the active paradigm (Table 5.2). All paradigms produced significant BP differences between baseline and *t2*, and also from *t2* to the recovery phase (Table 5.3). Heart rate also rose continuously during the task execution (Fig. 5.2B). With the exception of the active paradigm, no significant differences for the change from baseline to *t1* were obtained (Table 5.2). On the other hand, all paradigms led to significant heart rate changes from baseline to *t2*; and, with the exception of the passive paradigm, from *t2* to recovery (Table 5.3). Despite slight variation, no characteristic pattern was observed in the $EtCO_2$ response (Fig 5.2C). Contrary to CBFv (Fig. 5.1), both BP and heart rate started to return to baseline values as soon as the paradigm was completed (Fig. 5.2A&B).

When the peripheral haemodynamic responses were compared between the active, passive and motor imagery paradigms, no significant differences at either t1 or t2 were found.

Table 5.3 Mean (SD) values of cerebral and peripheral haemodynamic parameters changes from baseline (b) to *t2* and from *t2* to recovery (r) during active, passive and motor imagery paradigms.

Variable	Active		Passive		Motor Imagery	
	b Vs. *t2*	**r Vs. *t2***	**b Vs. *t2***	**r Vs. *t2***	**b Vs. *t2***	**r Vs. *t2***
ipsi CBFv, %	6.1 (5.4)*	9.0 (5.1)+	10.3 (11.3)+	12.3 (7.2)+	7.6 (9.1)*	5.3 (6.5)*
cont CBFv, %	7.9 (4.3)+	7.5 (3.3)+	12.1 (10.4)*	12.9 (8.1)+	7.5 (7.3)*	5.3 (6.0)*
BP, mmHg	4.0 (3.4)+	3.5 (4.2)*	3.3 (4.2)*	4.2 (4.4)*	2.0 (2.0)*	2.9 (2.0)+
Heart rate, bpm	4.2 (3.0)+	3.7 (2.6)*	1.8 (2.0)*	1.1 (2.0)	1.7 (1.8)*	1.5 (2.0)+
$EtCO_2$, mmHg	0.1 (0.5)	0.1 (1.2)	0.1(0.7)	0.1 (1.3)	0.3 (0.7)	0.4 (0.8)

CBFv signals were normalized by their baseline values. Ipsi CBFv, ipsilateral cerebral blood flow velocity; cont CBFv, contralateral cerebral blood flow velocity; BP, blood pressure

* $p<0.025$ for difference between baseline to *t2* and *t2* to recovery (after Bonferroni correction)

+ $p<0.0025$ for difference between baseline to *t2* and *t2* to recovery (after Bonferroni correction)

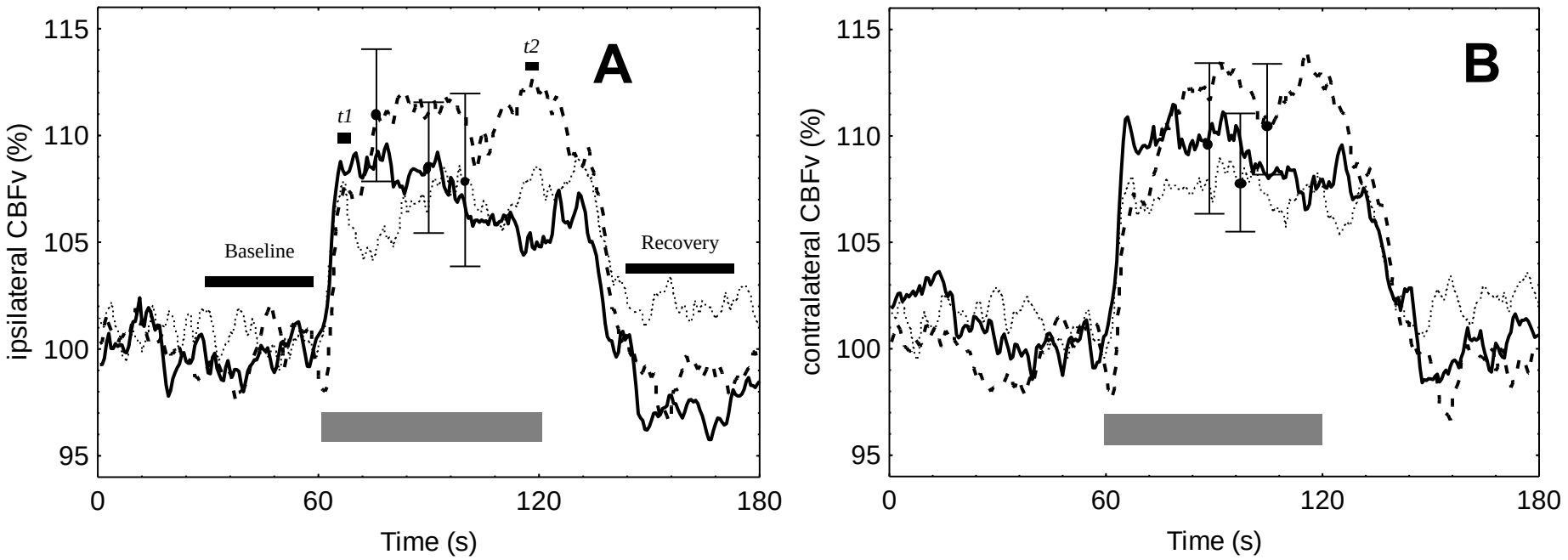

Fig 5.1 Population averages of changes in ipsilateral CBFv (A) and contralateral CBFv (B) before, during (grey bar) and after 60 s of active motor (continuous line), passive motor (dashed line) and motor imagery (dotted line) paradigms. For clarity, only the largest ± SE is represented at the point of occurrence.

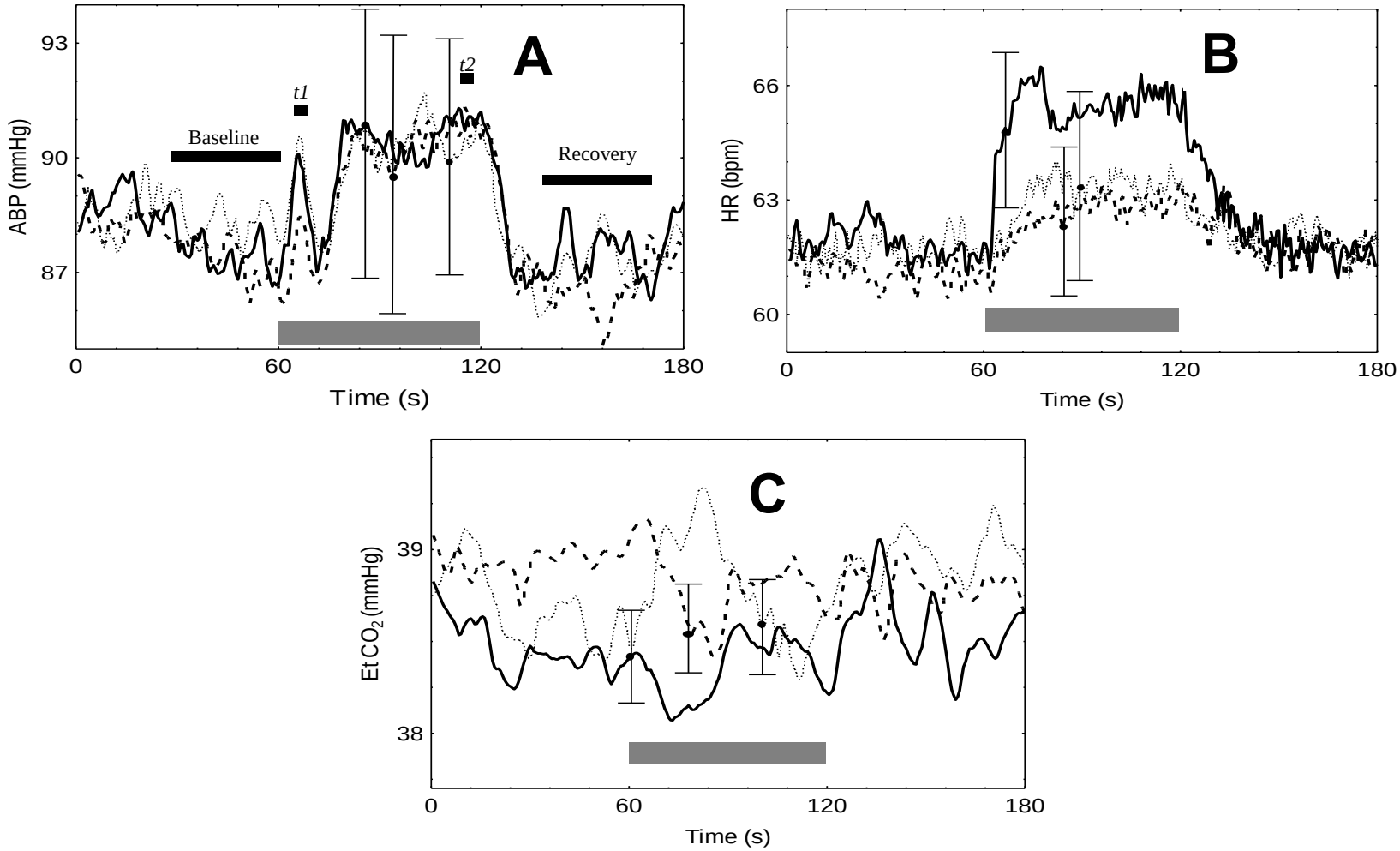

Fig 5.2 Population averages of changes in arterial BP (A), heart rate (B) and $EtCO_2$ (C) before, during (grey bar) and after 60 s of active motor (continuous line), passive motor (dashed line) and motor imagery (dotted line) paradigms. For clarity, only the largest ±SE is represented at the point of occurrence.

5.4 Discussion

5.4.1 Study Findings

The present study has demonstrated that active, passive and motor imagery paradigms, performed at a metronome-controlled rate, produce similar temporal patterns of cerebral and peripheral haemodynamic responses in healthy subjects aged ≥45 years. The main finding of our study is that the significant CBFv changes (at *t1* and *t2*) in response to neural activation onset were independent of the type of paradigm. Though the temporal pattern of activation was more noticeable on the contralateral side, there were no significant differences between contralateral and ipsilateral cerebral haemodynamic responses during active, passive and motor imagery paradigms at the beginning (between 5 and 10 seconds) and end (between 55 and 60 seconds) of the brain activation. This is likely to result from the fact that other variables, such as BP and heart rate, had a potential influence on the CBFv response (Aaslid *et al.*1989, Duschek *et al.*2008, Moody *et al.*2005), making the time points not sensitive to hemispherical lateralization. More work on this hypothesis is obviously needed to provide a better understanding of underlying mechanisms.

5.4.2 Active, Passive and Motor Imagery in the Literature

TCD as a non-invasive method has been widely applied to assess cerebral haemodynamic responses using cognitive tests, as well as, motor action tasks allowing continuous measurements of CBFv in real time. Despite several studies using a number of different cognitive stimuli to understand cerebral haemodynamic responses (as described in section 2.4.2.3), including arithmetic processing (Duschek *et al.*, 2008), constructional puzzle (Moody *et al.*, 2005) and reading (Droste *et al.*, 1989b) paradigms, the process of movement thinking (motor imagery) as a paradigm has not been well explored in the literature. Roland and Friberg (Roland and Friberg, 1985) have demonstrated that different types of thinking

can activate specific brain areas leading to different patterns of CBF response. Depending on the extent of mental stimulation, different cortical fields can be simultaneously activated, leading to global (multifield) CBF increases. Changes in cerebral haemodynamics either to active or passive stimuli have been previously documented, revealing a bilateral CBFv (or CBF) increase, with predominance of the contralateral side (Cuadrado *et al.*, 1999, Guzzetta *et al.*, 2007, Matteis *et al.*, 2001, Weiller *et al.*, 1996). However, in contrast to previous studies where values were either averaged over 30 to 60s (Bragoni *et al.*, 2000, Matteis *et al.*, 2001) or restricted to comparing values before and after the complete activation sequence (Silvestrini *et al*., 1998a, Tiecks *et al*., 1998), the continuous CBFv response in this study was synchronized to the metronome signals allowing more accurate detection of the beginning and end of each paradigm, as well as its precise temporal pattern. From our results, it is clear that prolonged averaging could attenuate the percentage of CBFv response.

In one subject, Roland and Larsen (1976) showed that the sensory component of the contralateral cerebral activation induced by hand movement could be eliminated by regional anaesthesia of the hand. Similarly, Friedman *et al.* (1992) measured CBF by PET during static handgrip before and after regional afferent blockade of the arm with lidocaine. They also concluded that brain activation through motor paradigms requires neural afferent feedback, because bilateral pre motor and motor sensory CBF increases during static handgrip before, but not after, blockade of afferent fibres from the contracting muscle. Based on these considerations, the proprioceptive aspects of the motor paradigm in healthy subjects and stroke patients should be taken into account.

5.4.3 Temporal Responses of Cerebral and Peripheral Haemodynamics

Turning now to the time course of cerebral and peripheral haemodynamic response to activation, we found corresponding time-matched changes from baseline between paradigms, as shown in Fig. 5.1 & 5.2, with no statistically significant differences between responses at time points 1 and 2. Similar patterns of CBFv increase have been described in the literature after active movement (Sitzer *et al.*, 1994), passive movement (Sato *et al.*, 2009) and cognitive paradigms (Droste *et al.*, 1989a, Moody *et al.*, 2005). Sato *et al.* (2009) have hypothesized that this fast adjustment in the vascular response was due to influences of the descendent central command of the brain rather than contractile force. The CBFv delay in return to baseline levels after the cessation of stimulation is in line with previous investigations (Droste *et al.*, 1989a, Moody *et al.*, 2005, Sitzer *et al.*, 1994), and may be related to remaining brain activity or replenishing of metabolite levels following increased brain activity (Donahue *et al.*, 2009, Droste *et al.*, 1989a).

5.4.4 Cerebral Dominance

Corresponding to cerebral dominance, several studies have addressed the issue of left-right differences in CBF responses (Guzzetta *et al.*, 2007, Hammond, 2002 , Matteis *et al.*, 2001, Solodkin *et al.*, 2001). There remains a lack of consensus regarding the relationship between handedness and brain activation, despite good evidence of anatomical asymmetries, especially regarding the lateral pre motor cortex (Hammond, 2002).

In some cases, brain activation in left- and right-handers has not been shown to be different (Matteis *et al.*, 2001), whereas in others, right-handers have larger ipsilateral activation in the primary motor cortex (Kim *et al.*, 1995) or left-handers (but not right-handers) have bilateral activation in the lateral premotor cortex during sequential finger movement (Solodkin *et al.*,

2001). Having said that, when analysing the correlation between handedness and volume, the number of areas and laterality during simple movement tasks (finger/thumb opposition, for example), no statistical significance difference in adult or children was found (Guzzetta *et al.*, 2007, Matteis *et al.*, 2001). On the other hand, during sequential movements Solodkin *et al.* (2001) found that left-handers activated larger volumes and number of brain areas than right-handers, and showed significantly less brain lateralization. The asymmetrical functions of the hands might reflect in an asymmetrical neural control (Hammond, 2002), as we use our hands asymmetrically. The dominant hand usually plays a manipulative role and the non-dominant usually acts as a stabilizing role. To avoid asymmetric responses, only right-handed healthy volunteers were recruited to facilitate interpretation of the CBFv response to neural activation.

5.4.5 Systemic Contributions on Cerebral Haemodynamics

Taking into account the systemic contributions on cerebral haemodynamics, we studied simultaneous changes in peripheral haemodynamic parameters showing increases of BP and heart rate during the interventions, in keeping with others (Duschek *et al.*, 2008, Moody *et al.*, 2005). Sato *et al.* (2009) have reported an increase in heart rate during the voluntary arm exercise and not during the passive. However, other groups have reported no change during activation paradigms (Bragoni et al., 2000, Matteis et al., 2001, Silvestrini et al., 1993b) compared to the rest phase. It is important to bear in mind that BP and heart rate changes during brain activation may influence the CBFv response, particularly where baroreceptor and cerebral autoregulatory mechanisms may be impaired as a consequence of age or disease. With regard to $EtCO_2$, Moody and colleagues (2005) showed a significant $EtCO_2$ decrease of the order of ~3 Torr during word generation and puzzle construction paradigms with TCD. Though systematic changes in $EtCO_2$ were not seen for the population as a whole in this

study, any variation in its levels would be likely attributed to hyperventilation induced by exercise and/or cognitive activity with the possibility of inducing changes in cerebral haemodynamic responses due to hypocapnic vasoconstriction (Ogoh *et al*., 2005a, Ogoh and Ainslie, 2009).

5.4.6 Clinical Implications

One important contribution of this study was the ability to examine beat-to-beat changes in both cerebral and peripheral haemodynamic responses in an older but healthy population. The population was older than the predominantly younger population of previous studies (Duschek and Schandry, 2007, Duschek *et al.*, 2008, Matteis *et al.*, 2001, Moody *et al.*, 2005, Tiecks *et al.*, 1998). This is important when considering disease-related changes, as age, per se, is known to influence changes in CBFv at rest and during simple motor tasks with a significant decrease of absolute CBFv and reduced lateralization response with increasing age (Groschel *et al.*, 2007, Orlandi and Murri, 1996, Stroobant and Vingerhoets, 2001).

Important insights into cerebral functional changes after focal ischaemic damage may be derived from motor and cognitive paradigms studies. The early stages post stroke often precludes the use of active motor tasks, therefore alternative paradigms for patients without sufficient voluntary motor control would be important. The use of passive movement to study stroke hemiplegic patients has been explored by preliminary PET investigations (Nelles *et al.*, 1999a, Nelles *et al.*, 1999b). Compared to healthy subjects, stroke patients showed a significantly larger increase in regional CBF bilaterally in the sensorimotor cortex during passive movement that was greater in the non-damaged hemisphere and in the parietal lobe bilaterally when the paretic hand was stimulated. Similar findings were also reported in PET and fMRI studies during active and cognitive paradigms in stroke patients (Carey *et al.*, 2005, Rehme *et al.*, 2011). Further study in a larger patient group using more readily available and

low-cost technology, such as TCD, is required, allowing a better understanding and interpretation of brain activation paradigms in a stroke population. Importantly, the natural history of CBFv response to such paradigms following stroke requires further assessment to determine prognostic significance, and to define more effective therapeutic strategies.

5.4.7 Limitations of the Study

This study has a number of limitations. First of all, TCD studies measure blood flow velocity data rather than absolute blood flow. Therefore, measurements of CBFv will be proportional to changes in blood flow only if the diameter of the insonated vessel remains constant. Previous investigations have failed to demonstrate a significant change in cross-sectional area of the MCA even when considerable changes in $PaCO_2$, BP and posture took place (Giller *et al.*, 1993, Newell *et al.*, 1994, Serrador *et al.*, 2000), but there is less evidence during cognitive and motor paradigms. Despite its advantages in temporal resolution, TCD findings of the MCA cannot explore more focal changes in cerebral haemodynamics in health as well as disease, including stroke. Another limitation of this method is occasional failure to locate an acoustic window, which led to the exclusion of one CBFv recording in our study. Finally, the lack of biceps and triceps electromyography recordings cannot exclude the absence of voluntary muscular activity during the 'passive' motor paradigm. However, similar to other brain activation studies (Guzzetta *et al.*, 2007, Nelles *et al.*, 1999a, Nelles *et al.*, 1999b, Sato *et al.*, 2009), subjects were closely monitored during the experiment for any active elbow movement.

5.5 Conclusion

In conclusion, it was demonstrated that active, passive and motor imagery paradigms with 60s duration in healthy participants (age ≥45) lead to similar beat-to-beat haemodynamic

responses in the ipsilateral and contralateral hemispheres. This was associated with significant changes in BP and heart rate, indicating a complex interaction between cerebral and peripheral haemodynamic responses to cognitive and sensorimotor paradigms. Further studies, benefitting from TCD as a commonly available and affordable technique, are required to establish the clinical value of interchangeable motor paradigms in patients with stroke and other cerebrovascular conditions.

6 Reproducibility of Cerebral and Peripheral Haemodynamic Responses to Active, Passive and Motor Imagery Paradigms in Older Healthy Volunteers

6.1 Introduction

Efforts have been made to understand and possibly detect potential indicators of brain recovery in stroke patients, since only up to 50% of the variance in recovery can be explained by the most commonly reported predictors, including lesion volume and initial stroke severity (Marshall *et al.*, 2009). However, it is vital to distinguish between changes specifically related to the stimulus or the effects of disease, and any genuine day-to-day variability (Carey *et al.*, 2000, Marshall *et al.*, 2004), since any change occurring as a result of an intervention must be greater than the intrinsic variability of the test (Brodie *et al*., 2009a).

It is therefore surprising that relatively little has been published on the reproducibility of cerebral activation responses derived from active, passive or motor imagery paradigms. Despite their widespread use, most studies have concentrated on methods such as fMRI (Loubinoux *et al.*, 2001, Marshall *et al.*, 2004, Yoo *et al.*, 2005) and PET (Carey *et al.*, 2000), rather than functional TCD. Although activation patterns appear to be qualitatively reproducible in some MRI studies, reported quantitative reproducibility measures were poor (Loubinoux *et al.*, 2001, Marshall *et al.*, 2004). TCD, on the other hand, has been shown to

produce reproducible CBFv measurements (Bishop *et al*., 1986, Brodie *et al*., 2009a, Totaro *et al*., 1992), and has been widely applied in humans to investigate the mechanism of neurovascular coupling in response to cognitive tests, as well as, motor action paradigms (Duschek et al., 2008, Matteis et al., 2001, Sato et al., 2009, Willie et al., 2011b). Nonetheless, the reproducibility of these results over time has not been investigated, either within groups or within individuals.

Therefore, the purpose of this chapter was to investigate the variability in CBFv response to active, passive and motor imagery paradigms using TCD over two visits. Owing to the contribution of other peripheral haemodynamic parameters, such as BP and heart rate to CBF (and CBFv) regulation, the reproducibility of these parameters was also examined.

6.2 Methods

6.2.1 Research Participants

A total of twelve participants were recruited from departmental staff and their relatives. Inclusion criteria were age ≥45 years and right-handedness according to the Edinburgh inventory (Oldfield, 1971). Exclusion criteria comprised physical disease in the upper limb, poor insonation of both temporal bone windows, as well as any history of cerebrovascular disease. The studied population comprises the same subjects who were analysed in chapter 5.

6.2.2 Measurements

Recordings were performed as described in section 4.6.2.1. After a period of 15 minutes stabilization, participants performed three different paradigms each repeated twice in random order. Movement was performed only by the dominant side. All paradigms started with a 90-second baseline phase. Thereafter the active, passive and motor imagery paradigms were performed over 60 seconds, with a 90-second recovery phase.

6.2.3 Data Analysis

Data analysis was performed as described in section 4.6.3. After the signals were calculated with a uniform time-base, averages were performed for each variable synchronized by the beginning of each paradigm. Temporal paradigm-synchronized population averages of the first and second execution of each paradigm per day were compared to evaluate qualitatively the CBFv response. The paradigm that achieved the highest amplitude of contralateral CBFv response was chosen to represent the participant`s response at each visit.

From the population synchronized averages, mean values of all variables were extracted from the 30 seconds preceding the paradigm and from the 30s to 60s after the paradigm for baseline (*b*) and recovery (*r*) values, respectively. Mean values were also extracted, at the beginning (between 5 and 10 seconds) and end (between 55 and 60 seconds) of each paradigm expressed as time points 1 (*t1*) and 2 (*t2*), respectively. Cerebral and peripheral haemodynamic responses were calculated as the percentage amplitude variation between the baseline and time point 1(*t1 - b*), the baseline and time point 2 (*t2 - b*), and the time point 2 and recovery (*t2 - r*) for each visit.

6.2.4 Statistical analysis

Two-way repeated measurements ANOVA was used to compare the amplitude response of selected time points between the visits and type of paradigms. The amplitude values (*t1-b*, *t2-b* and *t2-r*) obtained for each day were analysed to assess their reproducibility. Pearson correlations were performed to examine test-retest reproducibility of cerebral and peripheral responses. Standard error (SE) was used as an index of intra-subject reproducibility given as the square-root of the within-subject sum of mean squares (WSQ) of repeated measures ANOVA (Brodie *et al*., 2009a, Panerai *et al*., 2011). We report SE as an index of absolute

reproducibility, where high values of SE represent low absolute reproducibility, indicating large random variation within an individual.

The ICC (intraclass correlation coefficient) was then calculated from the ANOVA table using the expression proposed by Shrout and Fleiss (1979).

$$ICC = \frac{BMS - RMS}{BMS + (k-1)\,RMS} \qquad Eq.\ 6.1$$

where BMS is the ANOVA between-subject mean sum of squares, RMS is the residual error mean sum of repeated observations, *k* is the number of observations. 95% confidence intervals (CIs) were calculated. ICC is a measure of relative reproducibility as it expresses the amount of intra-subject variability in relation to corresponding values of inter-subject variability, thus reflecting the ability of a measurement or parameter to discriminate between different individuals (Brodie *et al*., 2009a). According to Shrout (1998), the ICC was classified into the following ranges: 0.00 to 0.10, virtually none; 0.11 to 0.40, slight; 0.41 to 0.60, fair; 0.61 to 0.80, moderate; 0.81 to 1.00, substantial reproducibility.

6.3 Results

Recordings were undertaken in thirteen subjects (7 men) on two occasions, a mean of 6 (SD 1) days apart. Due to a poor left transtemporal insonation window, one female participant had only the right MCA CBFv recorded on both visits; therefore only 12 subjects had contralateral CBFv parameters available for analysis. Subject characteristics were (mean (SD)): age 63.8 (4.2) years; Edinburgh Inventory 87.9 (3.2) %; systolic BP 124 (2.3) mmHg; diastolic BP 87 (0.9) mmHg; heart rate 64 (0.8) bpm; $EtCO_2$ 39 (1.4) mmHg. For baseline recordings on visit 1, CBFv was 55.3 (3.4) $cm.s^{-1}$ and 56.7 (2.1) $cm.s^{-1}$ for ipsilateral and contralateral MCA, respectively. On visit 2, ipsilateral CBFv was 55.9 (4.1) $cm.s^{-1}$ and

contralateral CBFv 57.7 (3.3) $cm.s^{-1}$. No significant difference was found in baseline values between the first and second visit.

Table 6.1 provides mean (SD) values for the amplitude variation between baseline and *t1* (*t1* - *b*), baseline and *t2* (*t2* - *b*), and *t2* and recovery (*t2* - *r*), for all recorded parameters, except $EtCO_2$. Two-way repeated measurements ANOVA did not show any significant amplitude differences at the selected time points between visits.

Table 6.1 Mean (SD) values for cerebral and peripheral haemodynamic variables for the difference change between t1 and baseline (t1-b), t2 and baseline (*t2-b*) and *t2* and recovery (*t2-r*) at day 1 and day 2.

Parameters		Active			Passive			Motor Imagery		
		***t1* - b**	***t2*– b**	***t2* - r**	***t1* - b**	***t2* – b**	***t2* - r**	***t1* - b**	***t2* - b**	***t2* - r**
CBFv ipsil (%)	day 1	10.3(4.4)	5.5 (6.3)	6.9(7.1)	9.9(4.7)	12.0(8.1)	14.1(6.7)	8.3(5.8)	4.8(7.7)	4.3(8.2)
	day 2	12.4(5.9)	5.8(6.9)	4.6(7.7)	10.2(6.7)	8.3(8.3)	10.7(5.6)	8.8(5.3)	4.6(4.6)	5.9(5.3)
CBFv cont (%)	day 1	11.7(4.7)	10.0(8.3)	9.2(8.0)	9.1(4.3)	13.4(8.6)	12.0(8.2)	8.4(5.9)	5.3(5.1)	5.1(6.5)
	day 2	12.0(5.1)	9.4(7.0)	7.4(7.9)	10.0(5.6)	10.9(8.2)	9.4(8.2)	9.3(4.3)	5.0(3.5)	6.5(4.7)
BP (%)	day 1	2.0(4.2)	3.0(3.4)	4.2(5.5)	3.0(6.4)	4.0(6.1)	4.1(4.7)	2.8(3.6)	2.7(2.7)	3.4(2.8)
	day 2	3.3(4.1)	4.7(1.9)	3.6(4.0)	4.4(5.5)	4.1(3.4)	2.4(2.6)	3.0(2.3)	4.4(3.5)	4.5(3.6)
Heart Rate (%)	day 1	7.2(4.2)	6.6(6.2)	7.5(3.7)	0.8(2.4)	3.0(3.2)	2.2(4.3)	1.2(5.2)	3.8(6.1)	3.3(5.5)
	day 2	9.1(5.9)	7.4(5.2)	7.7(4.7)	2.3(3.3)	4.3(4.4)	4.2(6.7)	4.5(5.9)	4.5(6.2)	4.9(5.1)

t1, time point 1; *t2*, time point 2; *b*, baseline; *r*, recovery; CBFv ipsi, ipsilateral cerebral blood flow velocity; CBFv cont, contralateral cerebral blood flow velocity; BP, mean arterial blood pressure.

None of the parameters showed significant differences due to the type of paradigm or day of measurement (two-way repeated measures ANOVA)

6.3.1 Reproducibility of the Cerebral Haemodynamic Responses

Paradigm-synchronized population averages on day 1 (continuous line) and day 2 (dashed line) for the contralateral and ipsilateral CBFv are given in Figs. 6.1&6.2, respectively. Although the amplitude of activation varies slightly for the passive (Figs. 6.1B&6.2B) and motor imagery (Figs. 6.1C&6.2C) paradigms, the time course of activation appears qualitatively reproducible. In keeping with this, the ICC values for contralateral CBFv indicate moderate to substantial relative reproducibility and for ipsilateral CBFv fair to moderate relative reproducibility (Table 6.2). The absolute reproducibility indices for all variables are also shown in Table 6.2. The SE of cerebral haemodynamic parameters ranged from 2.4 to 5.5%, as original parameters were normalised by baseline, and in this case the SE is the same as the coefficient of variation (CoV). The majority of test–retest correlations for CBFv were significant (Table 6.2). With the exception of one low value ($r = 0.25$), correlations ranged from 0.44 to 0.85.

6.3.2 Reproducibility of the Peripheral Haemodynamic Responses

Figs. 6.3, 6.4 & 6.5 represent the paradigm-synchronized averages for BP, heart rate and $EtCO_2$, respectively. No consistent pattern of response or statistically significant variations in $EtCO_2$ levels were seen during paradigms for the whole population (Fig. 6.5). For this reason, $EtCO_2$ data were not considered further. A similar temporal pattern of BP changes was observed at both visits for the three different paradigms (Fig. 6.3), showing an initial rise peaking approximately 5s after paradigm onset and a second higher peak around ~ 85s. The amplitude response of the initial BP peak demonstrated significant correlation in all paradigms, whereas, with *t2-b* and *t2-r* amplitude responses (with the exception of passive *t2-b*) showed poor correlation between paradigms (Table 6.2). Similarly, the temporal pattern of heart rate variation was matched during the paradigms at both visits, showing a significant

rise during task execution (Fig. 6.4). Moreover, active paradigms lead to a greater heart rate increase than passive and motor imagery paradigms. Although heart rate variation during active and passive tasks led to significant correlation, motor imagery demonstrated poor correlation between paradigms (Table 6.2). As shown in Table 6.3, the results indicate a moderate to substantial relative reproducibility for systemic haemodynamic parameters. However, in keeping with the measures of absolute reproducibility for cerebral haemodynamic parameters, the SE for peripheral haemodynamic parameters also varied substantially between 1.4 and 4.6% (Table 6.2).

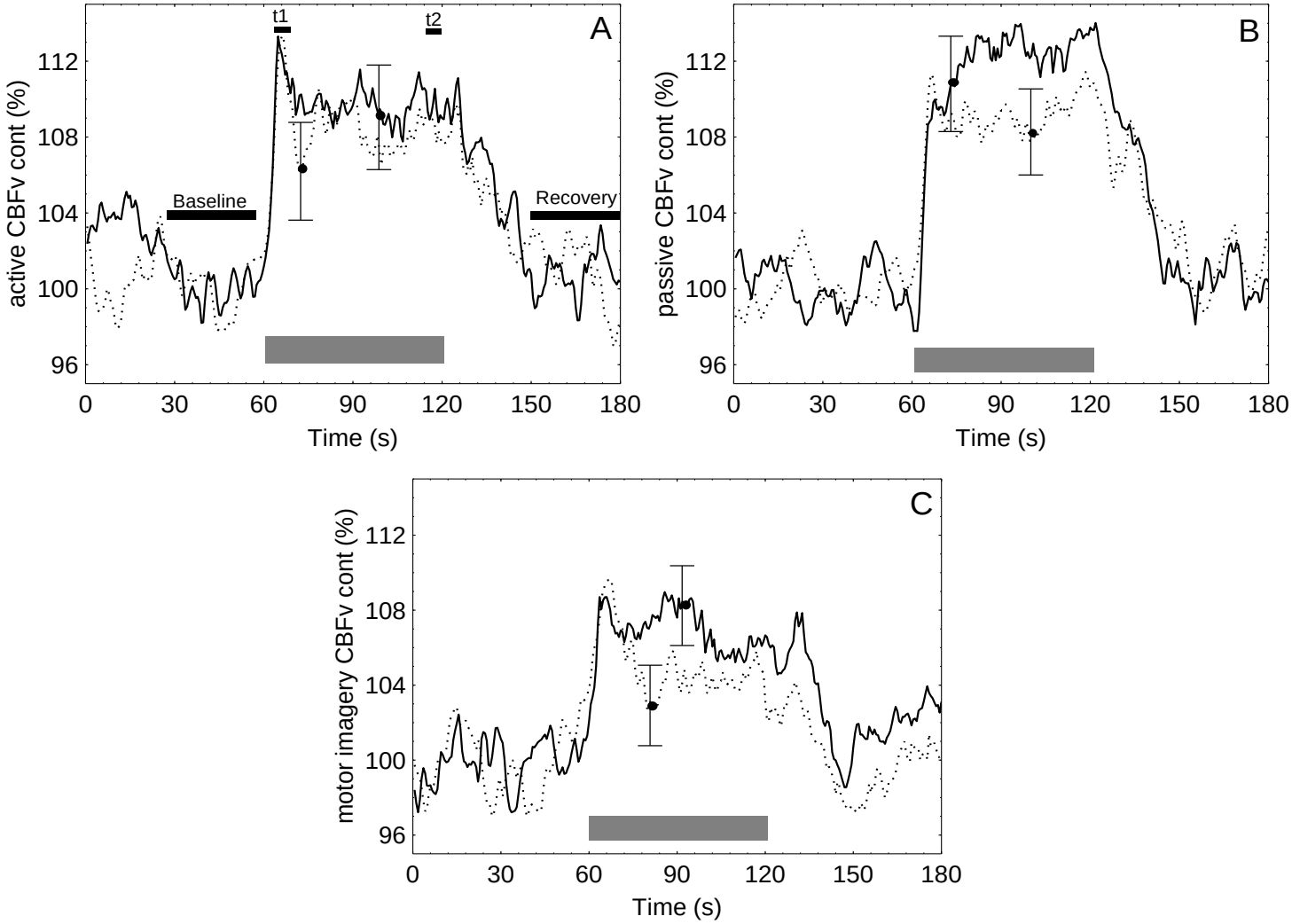

Fig 6.1 Population averages of changes in contralateral CBFv before, during (grey bar) and after 60 seconds of active (A), passive (B) and motor imagery (C) at day 1 (continuous line) and day 2 (dashed line). For clarity, only the largest ± 1SE is represented at the point of occurrence.

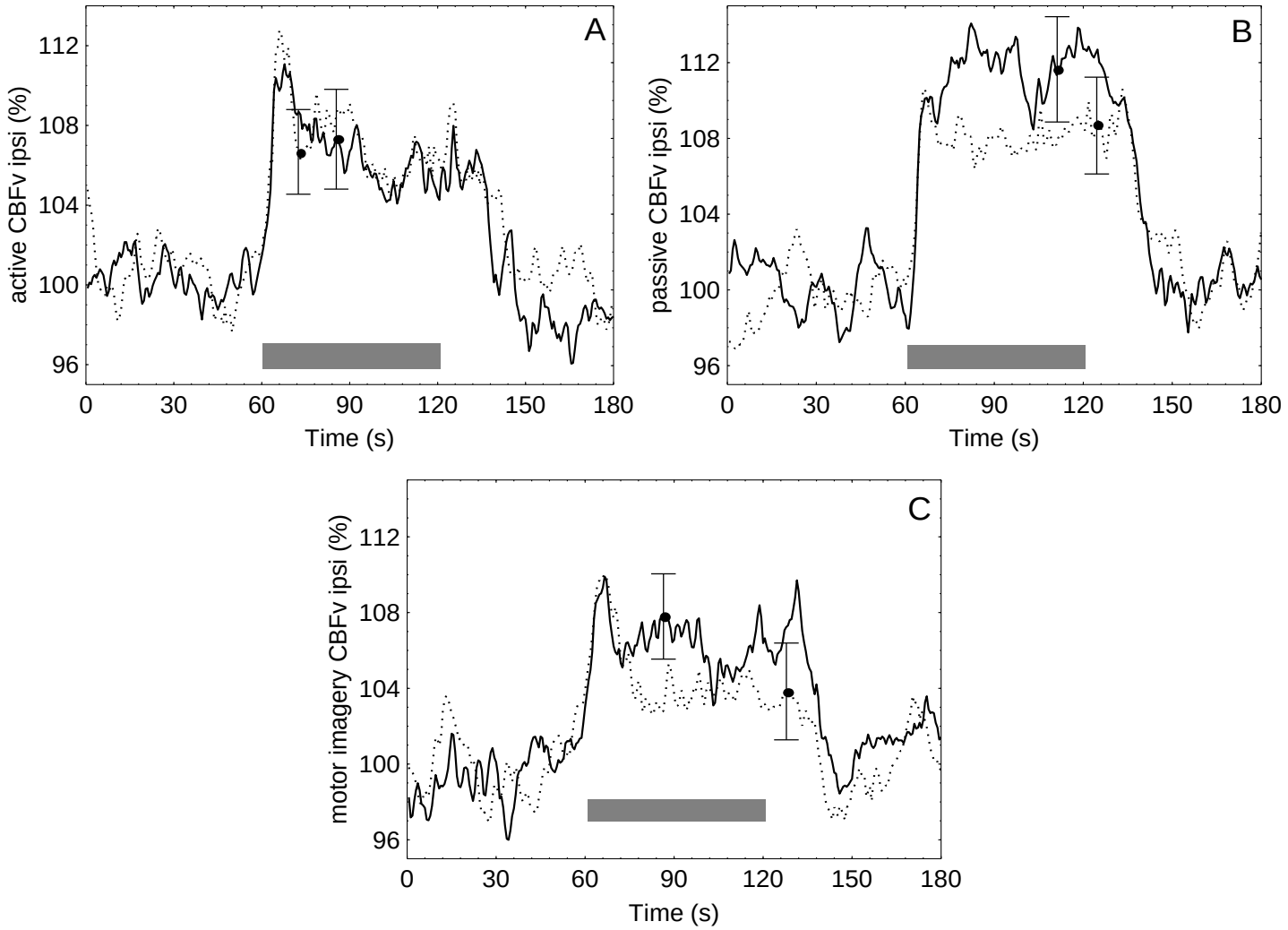

Fig 6.2 Population averages of changes in ipsilateral CBFv before, during (grey bar) and after 60 seconds of active (A), passive (B) and motor imagery (C) at day 1 (continuous line) and day 2 (dashed line). For clarity, only the largest ± 1SE is represented at the point of occurrence.

Table 6.2 Correlation coefficient, SEM and ICC for cerebral and peripheral haemodynamic parameters

Parameters	Active				Passive				Motor Imagery			
	Correlation Coefficient	SE (%)	ICC	95% CI	Correlation Coefficient	SE (%)	ICC	95% CI	Correlation Coefficient	SE (%)	ICC	95% CI
t1– b												
CBFv ipsil	0.49	3.7	0.66	0.15, 0.79	0.77*	3.0	0.72	0.31, 0.90	0.46	3.5	0.68	0.25, 0.89
CBFv cont	0.44	3.6	0.80	0.45, 0.93	0.70*	2.7	0.68	0.20, 0.89	0.55	3.5	0.62	0.55, 0.67
BP	0.87*	1.4	0.87	0.63, 0.95	0.72*	3.2	0.72	0.30, 0.96	0.75*	1.7	0.70	0.23, 0.89
Heart rate	0.58*	3.7	0.47	0.00, 0.80	0.69	1.9	0.61	0.06, 0.84	0.50	4.8	0.50	0.00, 0.81
t2– b												
CBFv ipsil	0.74*	3.3	0.74	0.34, 0.91	0.48	5.5	0.50	0.00, 0.80	0.79*	4.0	0.59	0.14, 0.65
CBFv cont	0.78*	3.7	0.70	0.21, 0.89	0.69*	4.5	0.68	0.24, 0.90	0.25	3.8	0.59	0.25, 0.81
BP	0.35	1.8	0.46	0.00, 0.79	0.60*	3.4	0.80	0.54, 0.91	0.19	2.7	0.68	0.19, 0.81
Heart rate	0.77*	2.9	0.76	0.38, 0.92	0.56*	2.9	0.50	0.00, 0.81	0.44	4.6	0.49	0.00, 0.78
t2– r												
CBFv ipsil	0.58*	4.8	0.61	0.09, 0.85	0.85*	2.5	0.69	0.40, 0.76	0.71*	4.1	0.64	0.16, 0.87
CBFv cont	0.72*	4.1	0.76	0.36, 0.92	0.70*	2.4	0.70	0.24, 0.90	0.55	3.9	0.66	0.20, 0.90
BP	0.53	3.4	0.50	0.00, 0.81	0.55	2.3	0.61	0.20, 0.83	0.40	2.5	0.40	0.00, 0.74
Heart rate	0.63*	2.6	0.61	0.11, 0.86	0.65*	3.6	0.60	0.81, 0.85	0.45	4.0	0.45	0.00, 0.77

t1, time point 1; *t2*, time point 2; *b*, baseline; *r*, recovery; CBFv ipsi, ipsilateral cerebral blood flow velocity; CBFv cont, contralateral cerebral blood flow velocity; BP, mean arterial blood pressure.

*$p<0.05$

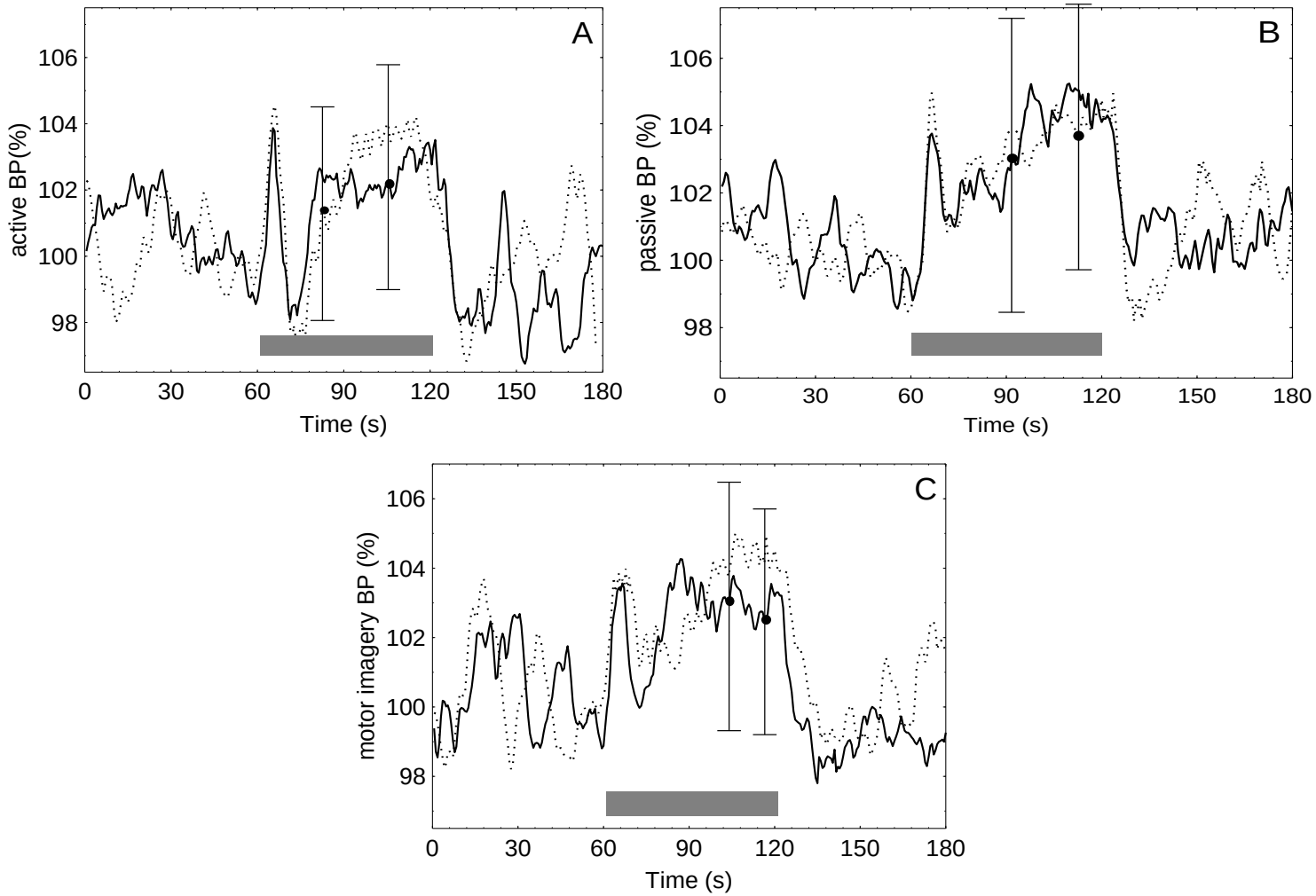

Fig 6.3 Population averages of changes in BP before, during (grey bar) and after 60 seconds of active (A), passive (B) and motor imagery (C) at day 1 (continuous line) and day 2 (dashed line). For clarity, only the largest ± 1SE is represented at the point of occurrence.

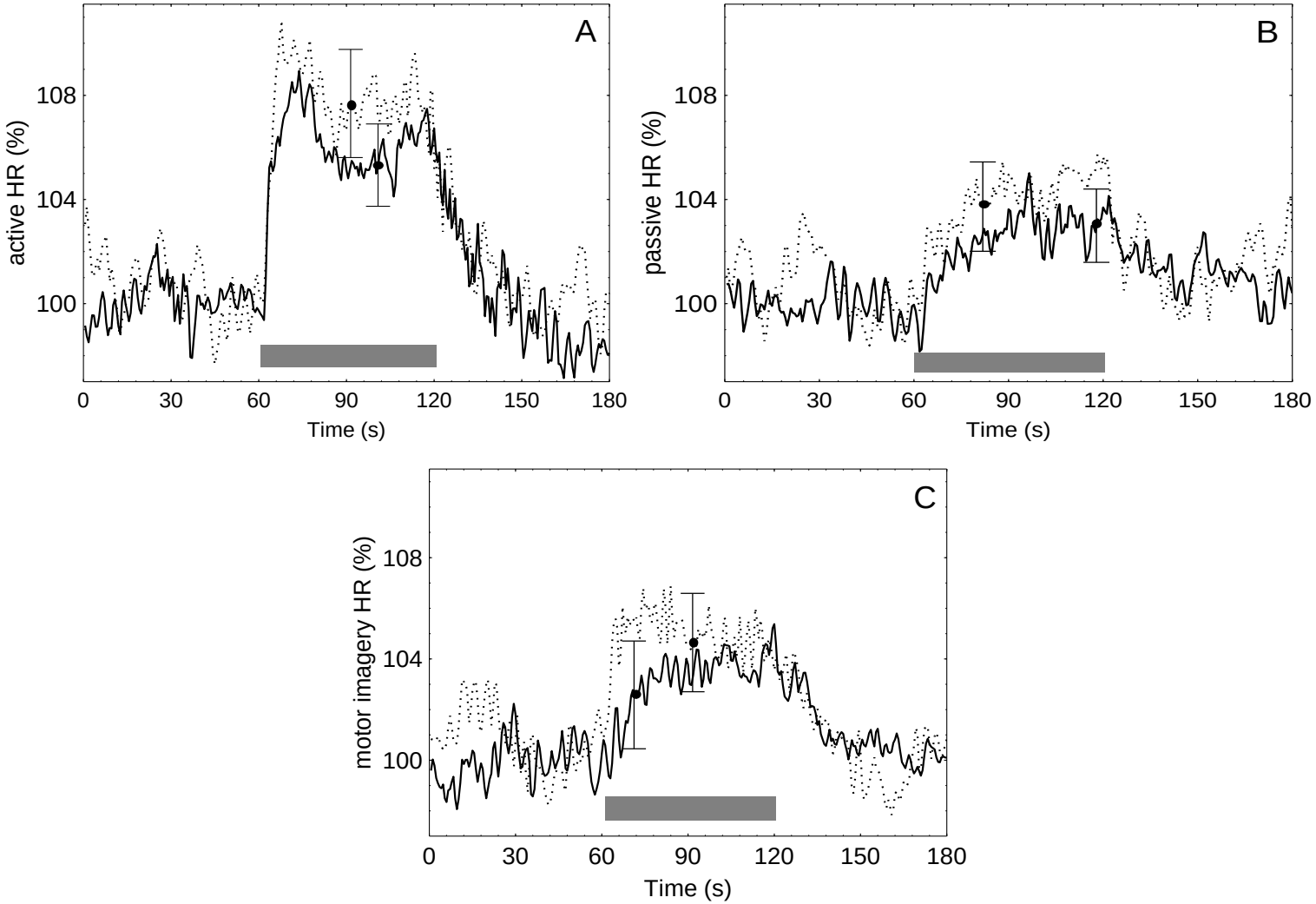

Fig 6.4 Population averages of changes in heart rate (HR) before, during (grey bar) and after 60 seconds of active (A), passive (B) and motor imagery (C) at day 1 (continuous line) and day 2 (dashed line). For clarity, only the largest ± 1SE is represented at the point of occurrence.

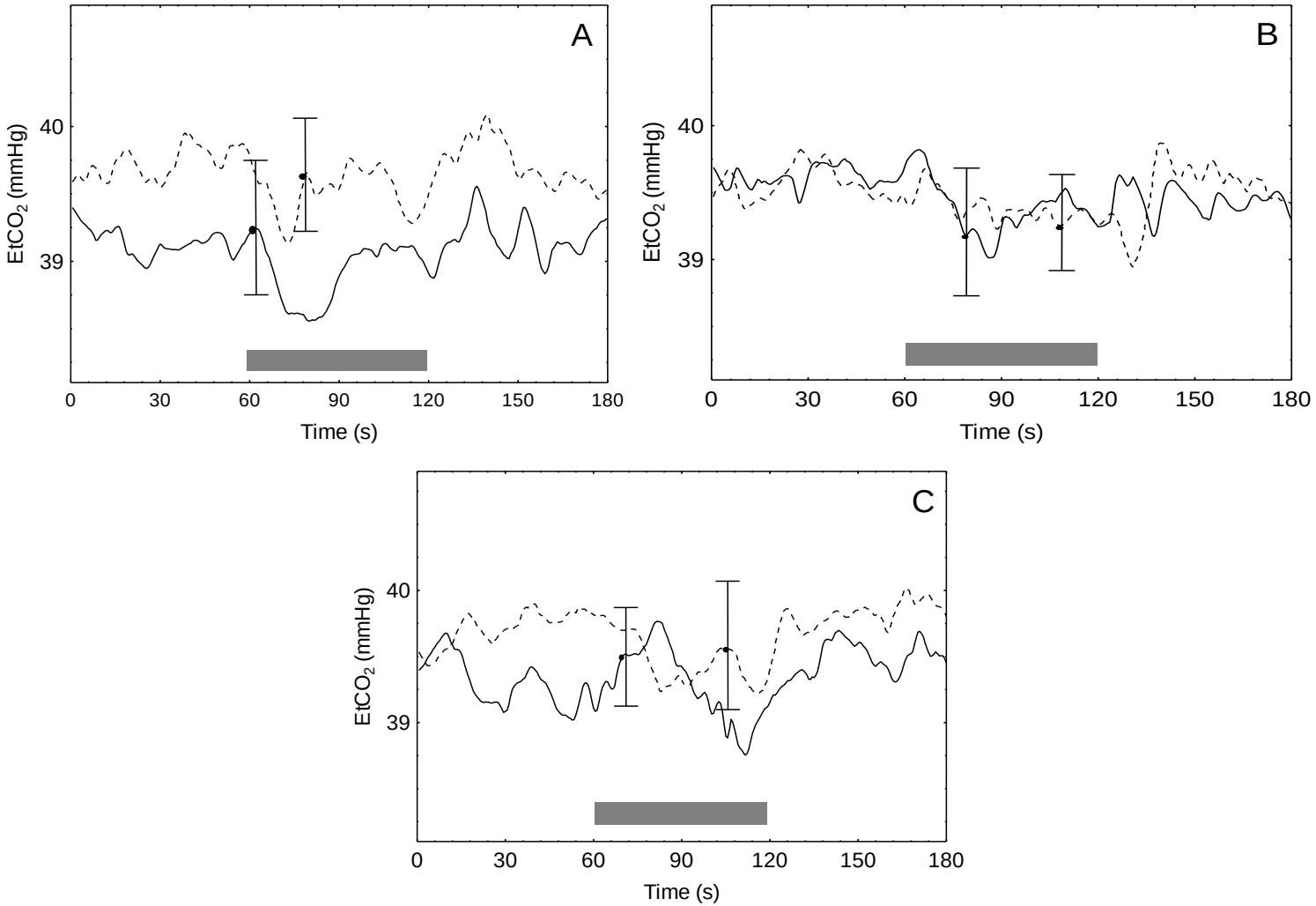

Fig 6.5 Population averages of changes in $EtCO_2$ before, during (grey bar) and after 60 seconds of active (A), passive (B) and motor imagery (C) at day 1 (continuous line) and day 2 (dashed line). For clarity, only the largest ± 1SE is represented at the point of occurrence.

6.4 Discussion

Many perfusion-sensitive techniques are available for the assessment of CBF during neuronal activity. Their advantages and limitations vary depending on the purpose of the study, for instance, fMRI allows quantitative measures of regional CBF, but does not allow laboratory and bedside assessment and is not tolerated by many patients. Despite its limited spatial resolution, TCD is easy to use and safe (with no exposure to ionizing radiation) in both healthy and disease states. Therefore, TCD has been widely used as a non-invasive method of assessing CBFv responses to neural stimulation tasks (Willie et al., 2011a). However, less attention has been given to the reproducibility of the cerebral response over time despite its clinical and physiological importance.

6.4.1 Reproducibility: Clinical Applications

Reproducibility is a key issue for the clinical application of task related CBFv responses, especially when longitudinal studies of age, disease, and therapeutic interventions on brain function are undertaken. Time-dependent changes in brain activity may be erroneously attributed to task-dependent effect rather than simply to random variation. This is relevant to studies of longitudinal changes in a stroke population, for instance, where cerebral haemodynamic responses may alter following brain recovery. It is important that the reproducibility of these responses is assessed to ensure that any reported changes are a consequence of disease, and not variability in the laboratory technique. Moreover, knowledge of the expected variation in matched healthy volunteers over time is essential to interpret change at an individual patient level. With this application in mind, simple paradigms were selected rather than more complex cognitive tasks or more demanding motor exercises to enable the future assessment of neurologically impaired patients. In addition, these paradigms are potentially feasible for measuring CBFv changes in the presence or absence of a motor

deficit as they have demonstrated consistent findings across studies (Matteis *et al.*, 2001, Roosink and Zijdewind, 2010, Weiller *et al.*, 1996).

6.4.2 Reproducibility of CBF Response to Neural Activation

Changes in cerebral haemodynamics either to active or passive stimuli have been previously documented, revealing a bilateral CBFv (or CBF) increase, though predominantly affecting contralateral CBF (Cuadrado *et al.*, 1999, Guzzetta *et al.*, 2007, Matteis *et al.*, 2001, Weiller *et al.*, 1996). In a previous study (Panerai *et al.*, 2012a), we have investigated the individual contributions of BP, $EtCO_2$ and an active motor stimulus per se to the beat-to-beat CBFv responses to an active motor paradigm. This demonstrated that the initial CBFv response was influenced by BP, with the active motor stimulus producing a slow increase plateauing at 15s, whilst the influence of $EtCO_2$ was erratic. However, that study did not address the reproducibility of cerebral and peripheral haemodynamic responses to the variety of paradigms addressed in the present study, nor are we aware of any other previous studies assessing the beat-to-beat reproducibility of the temporal cerebral and peripheral haemodynamic responses to sensorimotor stimulation. Previous work on CBF reproducibility has concentrated on identification of a significant difference between rest and activation, without considering the temporal pattern of response. Using the ^{133}Xe technique, Skolnick *et al.* (1993) assessed the reproducibly of regional CBF activation to spatial and visual paradigms in 16 elderly participants. In line with our results, despite methodological differences, they found correlation coefficients of r= 0.76 and 0.55 for the verbal and spatial tasks, respectively.

In this study, we have shown that active, passive and motor imagery paradigms led to a similar pattern of CBFv response, with reproducible and correlated amplitude of the

haemodynamic response at the majority of the selected time points. These responses were associated with reproducible variations in heart rate and BP. However, interpretation of the confidence intervals around the ICC values for cerebral haemodynamics leads to the conclusion that, even considering the best-case scenario (ICC 0.80 – contralateral CBFv response to an active motor paradigm), the boundaries of the 95% CI vary widely from slight (0.45) to substantial (0.93) reproducibility. This raises concerns about the ability of CBFv activation responses to discriminate between individuals. Although SE values (Table 6.2) are usually less than half the corresponding mean changes in CBFv, considerable intra-subject variability is also implied by the corresponding 95% confidence limits given by $\pm 1.96 \times$ SE. It is noteworthy that the smallest values were found for *t2-r* during the passive manoeuvre, which should be kept in mind when choosing physiological markers in future studies. The variability expressed by the SE may arise from the effect of biological factors. For example, increases in BP and heart rate may be the result of sympathetic nervous system activation as a consequence of stress caused by the research environment. Importantly, such variations in BP may have a major effect on the regulatory mechanisms of CBFv (Panerai *et al.*, 2001). More general psychological and situational factors can also play an important role, as reproducibility can be affected by mood, alertness and mental activity.

6.4.3 Study Power Calculation

This study can also provide estimates of sample sizes that could be of interest to other investigators. Starting with the simple case of a single point evaluation, assuming 80% power and confidence level of 5%, the number of subjects required to detect a difference of ΔCBFv between two groups of equal size is given by $\sigma^2 \times 15.8/\Delta CBFv^2$, where σ is the population standard deviation. This quantity can be estimated by the mean variance for the two separate measurements given in Table 6.2. Therefore, to detect a difference of 3% in the contralateral

CBFv response to motor stimulation, the number of subjects required would range from 33 for $t2-b$ during motor imagery to 124, also for $t2-b$, during passive movement. Furthermore, longitudinal studies would require additional repeated measurements per person and correlation between the repetitions. As a first step for the calculation of a sample size for longitudinal studies, the following formula proposed by Diggle *et al.* (2002) is suggested:

$$Sample\ size = \frac{[WSQ * 15.8] * [1 + (n-1)\rho]}{n.\Delta CBF^2} \qquad Eq.6.2$$

where ρ is the coefficient correlation and n number of repeated observations. Therefore, for n=4 and ΔCBF=3% in the contralateral side at *t2* again, 25 subjects would be required in each group for active paradigm, whereas 34 and 31 subjects be needed for passive and active paradigms, respectively. The above examples provide a more palpable measure of the typical variability shown by CBFv responses to motor stimulation and may be useful to other investigators designing cross-sectional and longitudinal studies of cerebral haemodynamics based on the responses to similar paradigms. This figure also helps determine the sample size for further studies presented in this thesis.

6.4.4 Limitations of the Study

In addition to the points described in section 5.4.7, this Chapter had also others limitations. Firstly, we did not investigate reproducibility within one visit. Instead, we opted for the selective acceptance of the best amplitude CBFv responses. Though we are not aware of any other study using the same approach, it was considered that this would have the greatest potential to improve between visit reproducibility, which would be of particular relevance to clinical studies monitoring brain recovery over time. Secondly, we selected four different time points to assess cerebral and peripheral haemodynamic responses during active, passive

and motor imagery paradigms. Therefore, the reported reproducibility values refer to these selected points.

6.5 Conclusion

In conclusion, CBFv evaluation during neural stimulation is a useful method to investigate the longitudinal effects of age and/or disease on brain function. The current study demonstrates the ability of active, passive and motor imagery paradigms to produce reproducible changes in the pattern of CBFv on two different visits. Though moderate to substantial relative reproducibility was found for most cerebral and peripheral haemodynamic parameters, high SE values reflecting poor absolute reproducibility were also identified. This highlights the importance of study power for the investigation of individual differences over time and their relation to ageing and disease.

7 Active, Passive and Motor Imagery Paradigms: Components Analysis to Assess Neurovascular Coupling

7.1 Introduction

Sensorimotor paradigms have become increasingly popular in the field of neuronal recovery studies, since impairment, preservation, and rehabilitation of sensorimotor function is a pivotal issue in many neurological disorders. Over the years, TCD has been widely used to detect CBFv modulation during neural activation, as it provides continuous information about dynamic CBFv adjustments and its facility in incorporating peripheral haemodynamics monitoring.

Neural activation studies have provided useful information regarding the adaptive mechanisms of cerebral haemodynamics after stroke (Bragoni *et al*., 2000, Matteis *et al*., 2003, Silvestrini *et al*., 1995a, Silvestrini *et al*., 1998b), as well as in Parkinson's (Rosengarten *et al.*, 2010) and Alzheimer's (Girouard and Iadecola, 2006) diseases. One important limitation of previous studies though is that co-variates of the CBF response, such as influences of changes in BP and $PaCO_2$ have not been taken into account. It is possible that the interplay of other cerebral mechanisms (CA and CVR) and the contribution of covariates (BP and $PaCO_2$) may affect the accuracy of the raw CBF response in representing underlying cerebral activity (Azevedo *et al.*, 2007). To derive more robust NVC measures, Panerai *et al.* (2012a) have recently proposed an innovative methodological approach to assess the

individual contribution of BP, $EtCO_2$ and the metabolic stimulus to the CBF responses. This new approach has considerable potential to improve the sensitivity and overall diagnostic accuracy of NVC studies in stroke. As a first step, in this chapter, it was analysed the dynamic CBFv response to active, passive and motor imagery paradigms in a healthy older population to test the hypotheses that a) these different paradigms stimulate the brain in a similar fashion and b) the contribution of BP and $PaCO_2$ is the same for different paradigms.

7.2 Methods

7.2.1 Research Participants

A total of nineteen participants (age ≥45 years), without vascular risk factors and without symptoms or history of cardiovascular or cerebrovascular disease, were recruited from University staff and their relatives. An additional exclusion criterion comprised physical disease in the upper limb. All subjects were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). Data from thirteen participants used in chapters 5 and 6 were re-analysed and grouped together with a new set of participants (n= 6).

7.2.2 Measurements

Recordings were performed as described in section 4.6.2.1. After a period of 15 minutes stabilization, participants performed three different paradigms each repeated twice in random order. Movement was performed only on the dominant side. All paradigms began with a 90-second baseline phase. Thereafter the active, passive and motor imagery paradigms were performed over 60 seconds, with a 90-second recovery phase.

7.2.3 Data Analysis

Data analysis was performed as described in section 4.6.3. After the signals were calculated with a uniform time-base, averages were performed for each variable synchronized by the beginning of each paradigm.

A multivariate autoregressive-moving average (ARMA) model was used to represent the influence of the inputs (BP, $EtCO_2$, and stimulus) on output (CBFv). As described in section 4.6.4.2 and in previous work (Panerai *et al.*, 2012a), the ARMA model allows quantification of the simultaneous influences of BP, $EtCO_2$ and stimulus to the CBFv response to stimulation. Briefly, the separate contributions of BP, $EtCO_2$ and stimulus to CBFv response were obtained as model predictions, with the use of ARMA coefficients. The order of these models, representing the number of past samples adopted for the autoregressive (AR) and moving average (MA) terms, was thoroughly considered as described in the Section 4.6.4.2. The beginning of stimulation was used as the point of synchronism to obtain population mean and SD curves for each separate contribution (BP, $EtCO_2$, stimulus) for the ipsilateral and contralateral hemispheres.

CBFv response patterns from two executions of each paradigm for each subject were qualitatively compared and the manoeuvre that achieved the highest amplitude of contralateral CBFv response was chosen to represent the participant`s response, as described in Chapter 6. Mean CBFv values were extracted from the 30 seconds preceding the paradigm for baseline. CBFv responses to stimulation were calculated at the first 10 seconds (for evaluating the initial impact of the paradigms) and last 30 seconds of each paradigm expressed as time points *t10* and *t30*, respectively. For the same time intervals, the mean of the predicted contributions of BP, $EtCO_2$ and stimulus to CBFv responses were also calculated. All parameters were expressed as percentages (%) of baseline values.

7.2.4 Statistical Analysis

Using two-way repeated measures ANOVA, baseline values for CBFv were compared between active, passive and motor imagery paradigms, as well as between the side of recording (ipsi- and contralateral hemispheres). At the selected time points (*t10* and *t30*), two-way repeated measures ANOVA with paradigms (active, passive and motor imagery) and side of recording (right, left hemispheres) as the within-factor for CBFv variations, and for the predicted contributions of BP, $EtCO_2$ and stimulus to CBFv responses was used. Tukey's honest significant difference test was adopted for post hoc analyses. A correlation analysis was performed in order to compare the similarity of the values of variables at the *t10* and *t30* time points. A value of $p<0.05$ was adopted to indicate statistical significance.

7.3 Results

Two participants were removed from the study due to poor insonation of the temporal acoustic window. No data were discarded following visual inspection. Therefore, data from 17 subjects (12 male) were included in this study. Included subjects had a mean (SD) age of 64.9 (4.9) years and Edinburgh Inventory of 91.0 (2.1)%. Baseline peripheral and cerebral haemodynamic parameters were: systolic BP 124 (2.9) mmHg, diastolic BP 82 (1.0) mmHg, heart rate 65 (0.7) bpm, right MCA CBFv 59.0 (1.9) $cm.s^{-1}$ and left MCA CBFv 61.9 (2.1) $cm.s^{-1}$. Baseline $EtCO_2$ was 39 (1.7) mmHg.

Ninety-seven of the 102 recordings analysed showed satisfactory model fitting as given by the comparison between model predicted CBFv responses and the real data (Panerai *et al.*, 2012a). Five recordings from two subjects resulted in poor fitting using the original model orders (orders [2,4,1,1]). However, adjusting model orders, mainly by increasing the order of the stimulus terms, solved this problem.

7.3.1 Temporal Pattern of CBFv Responses and Its Contributors

Two-way ANOVA did not show significant CBFv differences between the three paradigms, or between ipsi- and contralateral hemispheres at baseline. During paradigms, CBFv showed bilaterally a steep peaked rise, followed by a plateau phase, which outlasted the duration of stimulation (Fig. 7.1A&B). On the other hand, the contribution of paradigms (Fig. 7.1C&D) yielded a much simpler pattern, with a steady plateau of similar duration to the CBFv response. The contributions of $EtCO_2$ (Fig. 7.2A&B) and BP (Fig. 7.2C&D) showed both positive and negative values. $EtCO_2$ increased before motor imagery paradigm onset and contributed to the CBFv rise, especially in the contralateral response (Fig. 7.2B). $EtCO_2$ levels decreased gradually during motor imagery and active paradigms (reaching a minimum after the end of paradigms' performance – Fig. 7.2A&B). In all three paradigms, BP showed a clear peak at the beginning of stimulation coinciding with the initial peak in the CBFv response (Fig. 7.2C&D).

7.3.2 Cerebral Blood Flow Velocity Responses

The amplitude of CBFv and the input parameters variation at *t10* and *t30* are summarized in Table 7.1. At *t10*, no significant difference was found. At *t30*, two-way ANOVA showed significant differences (p= 0.04) in CBFv response between the three paradigms. Post-hoc comparisons revealed significant differences (Tukey's post-hoc p=0.04) between motor imagery and passive CBFv responses in the contralateral hemisphere, and showed a trend towards a significant difference during motor imagery when compared to the active paradigm (Tukey's post-hoc p= 0.05). A similar trend for a reduced CBFv response during motor imagery was also present in the ipsilateral responses (Table 7.1) showing a marginal significance when compared to the passive paradigm (p= 0.05). The correlation between *t10* and *t30* CBFv values ranged from 0.24 (motor imagery, p= 0.5) to 0.52 (active, p= 0.2) for

ipsilateral responses, and from -0.03 (motor imagery, p= 0.9) to 0.57 (active, p= 0.1) for contralateral responses, therefore not showing any strong relationships.

7.3.3 Contribution of Individual Inputs to CBFv Responses

Table 7.2 gives the distributions of explained variance for each input. Although Table 7.2 is presenting higher values for the stimulus contribution in all paradigms, Tukey's post hoc showed significant differences only between active contralateral BP and stimulus contribution (p= 0.03). A significant difference was also found between passive contralateral BP and $EtCO_2$ contributions (p= 0.03, Tukey's post-hoc p= 0.04). Note that in each paradigm, the total variance explained by the model is given for ipsilateral and contralateral hemispheres. Nevertheless, a better insight into the contribution of each input is gained by studying their temporal patterns (Fig. 7.1C&D and 7.2) and the corresponding values of *t10* and *t30* (Table 7.1).

No significant difference between the three paradigms was found in the BP and $EtCO_2$ contribution at *t10* and *t30* (Table 7.1). However, two-way ANOVA revealed differences of stimulus contribution on CBFv responses at both *t10* and *t30* (p= 0.03 and p= 0.04, respectively). At *t10,* stimulus contributions differed significantly between motor imagery and active (Tukey's post-hoc p=0.04) and passive paradigms (Tukey's post-hoc p= 0.03) in the ipsilateral hemisphere (Table 7.1). Moreover, contralateral stimulus increase during motor imagery was significantly lower when compared to active (Tukey's post-hoc p= 0.001) and passive (Tukey's post-hoc p= 0.007) paradigms (Table 7.1). At *t30,* the contralateral change in motor imagery stimulus was also reduced when compared to the other two paradigms using Tukey's post-hoc (active p= 0.02, passive p= 0.03) (Table 7.1).

The contribution of BP showed no or poor association between *t10* and *t30*. Correlations for the ipsilateral hemisphere ranged from 0.28 (motor imagery, p= 0.4) to 0.51 (passive, p= 0.1), whereas for the contralateral hemisphere ranged between -0.01 (motor imagery, p= 0.9) and 0.42 (passive, p= 0.2). On the other hand, with the exception of the $EtCO_2$ contribution during ipsilateral motor imagery (r= 0.3, p= 0.3), highly significant correlations were found in $EtCO_2$ and stimulus ranging from 0.76 (contralateral motor imagery $EtCO_2$ contribution) to 0.93 (ipsilateral passive stimulus contribution).

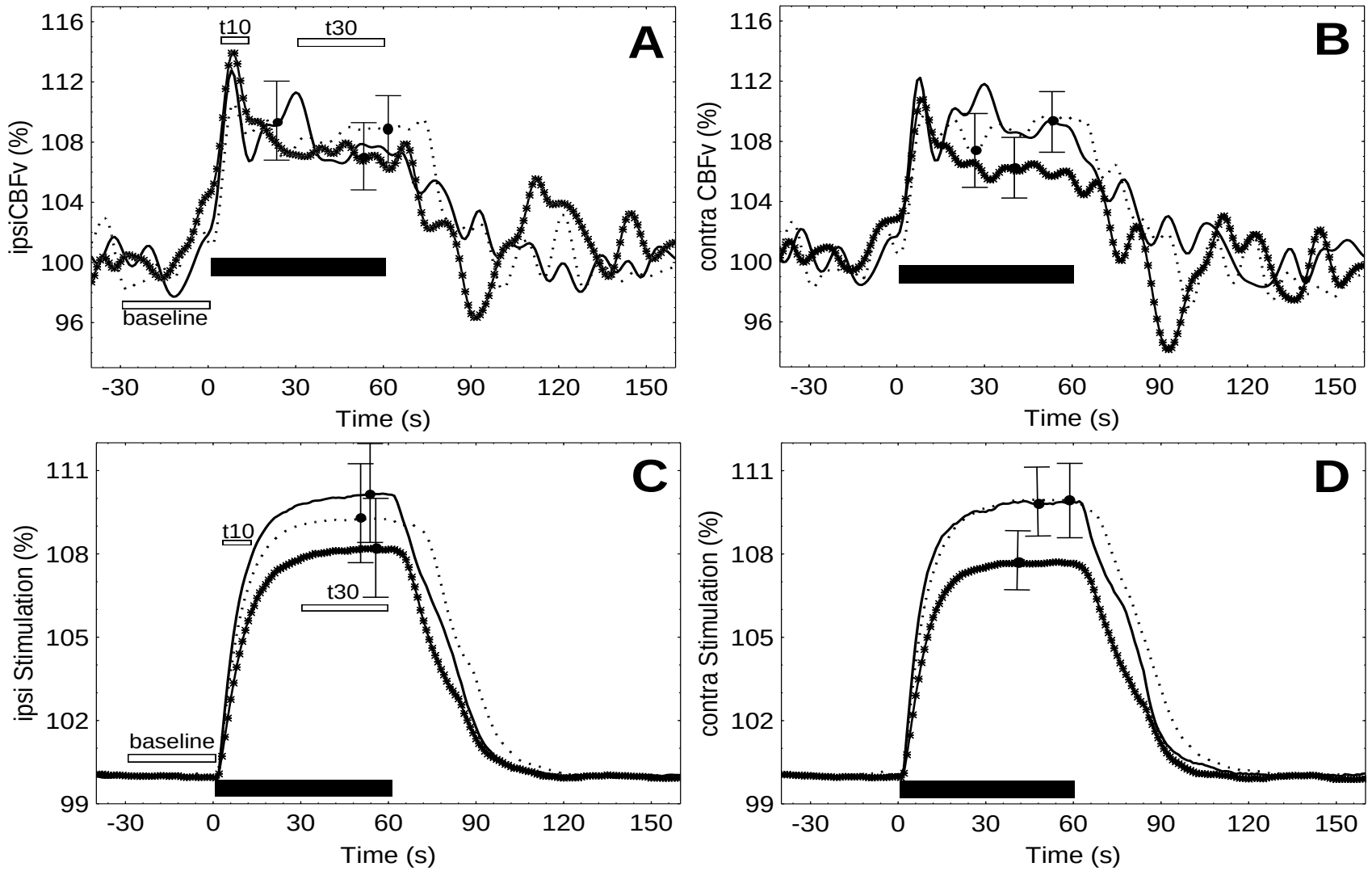

Fig 7.1 Population averages of changes in ipsilateral (A) and contralateral (B) CBFv responses and stimulus contribution (ipsilateral, C; contralateral, D) to active (continuous line + symbol), passive (continuous line) and motor imagery (dotted line). The black horizontal bar shows the duration of stimulation. Error bar represents the largest ±1 SE at the point of occurrence

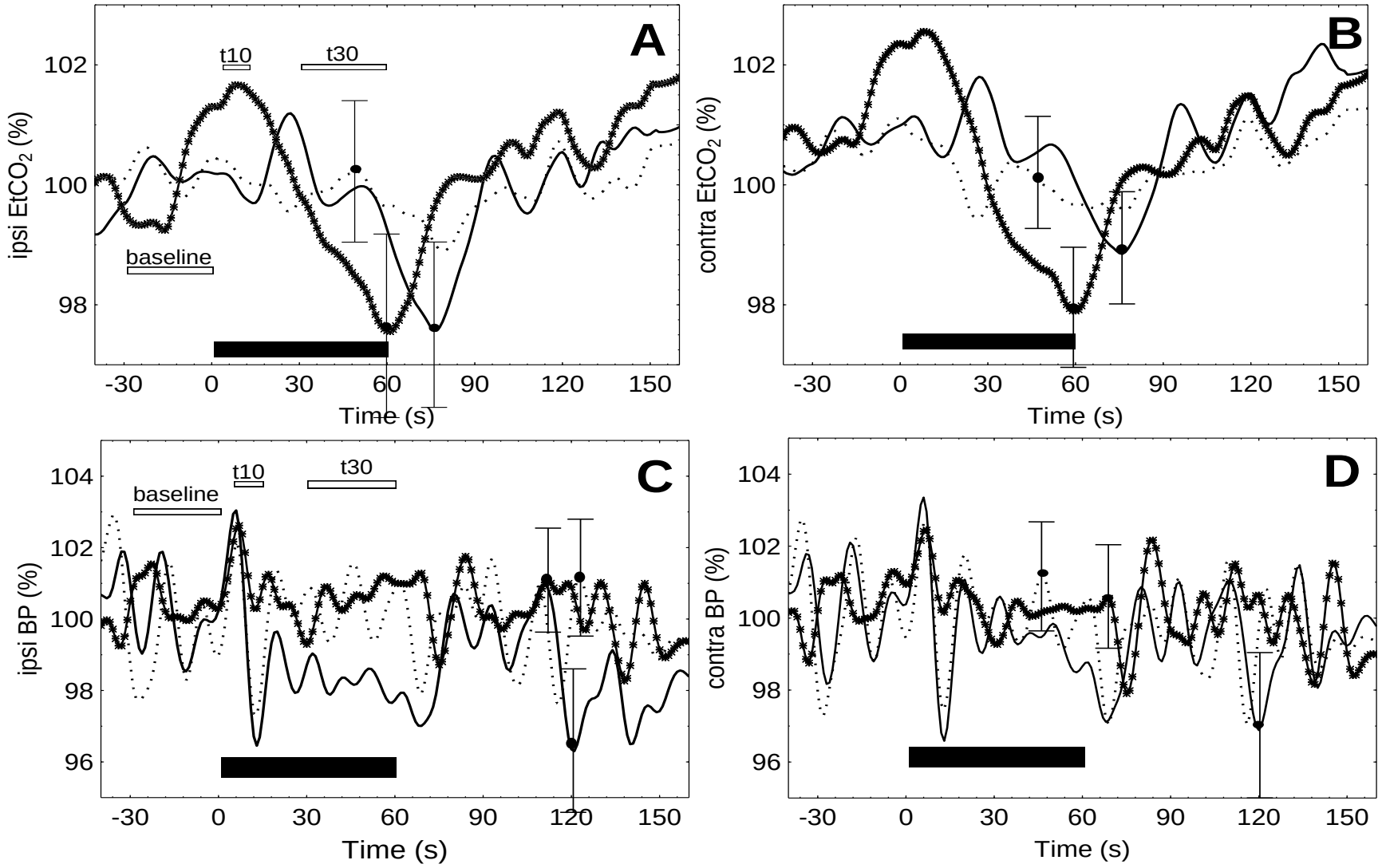

Fig 7.2 Population averages of the contributions of $EtCO_2$ (ipsilateral, A; contralateral, B) and BP (ipsilateral, C; contralateral, D) to CBFv response to active (continuous line + symbol), passive (continuous line) and motor imagery (dotted line). The black horizontal bar shows the duration of stimulation. Error bar represents the largest ±1 SE at the point of occurrence.

Table 7.1 Comparison of CBFv and separate contributions from BP, $EtCO_2$ and the stimulus during active, passive and motor imagery paradigms at the beginning (t10) and end (t30) of stimulation.

CBFv and contributors	IPSILATERAL			CONTRALATERAL		
	active	passive	MI	active	passive	MI
t10						
CBFv, %	108.3 (4.9)	108.0 (5.0)	109.9 (5.6)	108.9 (4.8)	107.7 (4.2)	107.6 (5.3)
BP, %	100.2 (1.7)	99.7 (3.5)	100.8 (2.5)	100.2 (1.9)	100.3 (2.4)	100.9 (3.3)
$EtCO_2$, %	100.5 (1.4)	101.8 (1.6)	102.0 (1.7)	100.7 (1.4)	101.0 (2.0)	101.9 (4.0)
Stimulus, %	104.1 (2.9)	105.0 (2.4)	103.0 (2.4)*	106.8 (4.3)	105.1 (2.1)	103.2 (1.8)*
t30						
CBFv, %	107.3 (8.0)	109.2 (8.1)	104.3 (7.0)#	108.3 (6.9)∞	110.1 (6.7)	105.9 (4.7)+
BP, %	100.0 (2.9)	100.1 (2.6)	101.0 (1.8)	99.2 (2.2)	100.1 (2.0)	99.9 (1.5)
$EtCO_2$, %	99.7 (5.0)	101.3 (9.3)	99.9 (5.2)	98.5 (7.1)	100.4 (4.7)	99.1 (5.0)
Stimulus, %	108.7 (5.9)	109.0 (4.6)	106.1 (5.4)	110.6 (5.2)	109.4 (3.6)	106.0 (4.0)*

CBFv, cerebral blood flow velocity; BP, mean arterial blood pressure; $EtCO_2$, end tidal CO_2; MI, motor imagery

* $p<0.05$, Tukey's post-hoc for differences between MI and active, MI and passive paradigms

$p=0.05$, Tukey's post-hoc for differences between passive and MI

+ $p<0.05$, Tukey's post-hoc for differences between passive and MI

∞ $p=0.05$, Tukey's post-hoc for differences between active and MI

Table 7.2 Percentage of total CBFv variance explained by model (V_{MOD}) and corresponding distribution of individual contribution of input variables as percentage of V_{MOD}.

	VARIANCE CONTRIBUTION (%)					
	IPSILATERAL			CONTRALATERAL		
	active	passive	MI	active	passive	MI
V_{MOD}	62.8 (21.3)	61.7 (19.4)	55.9 (20.8)	71.6 (15.8)	74.5 (14.8)	64.5 (16.3)
BP	25.5 (19.2)	25.8 (23.6)	30.8 (24.7)	23.2 (18.6)+	25.7 (22.6)*	35.3 (28.6)
$EtCO_2$	30.9 (25.3)	28.7 (26.7)	29.4 (29.6)	29.4 (24.1)	25.5 (19.5)	21.4 (22.8)
Stimulus	43.6 (21.4)	45.5 (26.6)	39.8 (20.6)	47.4 (21.7)	48.8 (25.4)	43.3 (25.3)

BP, mean arterial blood pressure; $EtCO_2$, end tidal CO_2; MI, motor imagery

+$p<0.05$, Tukey's post-hoc for differences between BP and stimulus contribution

* $p<0.05$, Tukey's post-hoc for differences between BP and $EtCO_2$ contribution

Table 7.3 Comparison of CBFv and separate contributions from BP, $EtCO_2$ and the stimulus during active, passive and motor imagery at t0-5, t5-10 and t0-15

CBFv and	IPSILATERAL			CONTRALATERAL		
contributors	active	passive	MI	active	passive	MI
t 0-5						
CBFv, *%*	106.2 (4.2)	105.3 (5.0)	105.0 (4.7)	105.5 (4.6)	103.3 (4.8)	104.7 (5.2)
BP, *%*	102.0 (1.9)	101.4 (2.9)	102.9 (2.7)	100.9 (1.5)	100.4 (2.1)	101.1 (2.9)
$EtCO_2$, *%*	100.7 (1.3)	101.9 (1.8)	102.1 (2.0)	101.1 (1.4)	101.6 (2.3)	101.9 (3.9)
Stimulus, *%*	103.7 (2.8)	104.1 (2.5)	101.5 (2.5)*	104.9 (4.3)	104.1 (2.7)	102.5 (1.8)*
t 5-10						
CBFv, *%*	109.3 (4.4)	108.9 (4.7)	109.9 (5.0)	108.2 (4.9)	108.0 (4.7)	108.0 (5.5)
BP, *%*	99.5 (1.8)	99.7 (2.5)	101.0 (2.2)	98.5 (1.8)	99.1 (1.1)	100.3 (3.0)
$EtCO_2$, *%*	100.1 (2.0)	101.2 (1.9)	101.9 (3.0)	99.8 (1.5)	101.8 (2.0)	101.2 (3.2)
Stimulus, *%*	107.4 (2.2)	107.5 (1.9)	104.3 (2.0)*	109.4 (2.9)	107.2 (4.1)	104.9 (0.9)*
t 0-15						
CBFv, *%*	106.9 (4.9)	107.1 (4.7))	107.3 (4.8)	106.6 (5.7)	106.9 (4.7)	106.9 (5.9)
BP, *%*	99.5 (1.9)	98.9 (2.1)	100.9 (2.0)	99.1 (3.0)	100.0 (0.9)	100.7 (3.2)
$EtCO_2$, *%*	99.1 (2.7)	100.9 (2.0)	102.9 (2.9)	99.9 (1.2)	101.6 (2.1)	101.5 (3.8)
Stimulus, *%*	105.2 (2.0)	105.7 (2.1)	104.1 (2.3)*	107.0 (3.9)	105.7 (3.9)	104.7 (1.3)*

CBFv, cerebral blood flow velocity; BP, mean arterial blood pressure; $EtCO_2$, end tidal CO_2; MI, motor imagery

* $p<0.05$, Tukey's post-hoc for differences between MI and active, MI and passive paradigms

7.4 Discussion

7.4.1 Main Findings

To our knowledge, this is the first time that the individual influences of the active, passive and motor imagery stimulus and other peripheral covariates on CBFv responses have been assessed and compared by multivariate modelling. Though the temporal course of beat-to-beat CBFv response across the three paradigms is consistent with previous chapters, CBFv response and its separate inputs were significantly different during motor imagery compared to active and passive motor responses. Stimulus was shown to be the major contributor of CBFv increases during the paradigm performance ranging from 103.0 (2.4) % to 110.6 (5.2) %, as detailed in the Table 7.1 and 7.2. These findings suggest that as well as differences in the metabolic components of the CBFv response caused by active, passive and motor imagery paradigms, rapid influences of peripheral haemodynamics (BP and $PaCO_2$) may also be involved in cerebral haemodynamic changes. The fluctuating BP and $EtCO_2$ modulation suggest that both parameters contributed to either increasing or decreasing the CBFv response during paradigms.

7.4.2 Cerebral Blood Flow Response to Neural Activation

The initial temporal pattern of CBFv response, involving a steep bilateral increase, is consistent with former fTCD studies using a broad variety of brain activation paradigms (Azevedo *et al*., 2007, Duschek *et al*., 2008, Moody *et al*., 2005, Panerai *et al*., 2012a, Rosengarten *et al*., 2003a, Sato *et al*., 2009, Sitzer *et al*., 1994). In keeping with our results, Duscheck *et al.*(2008) reported that the effects of BP on CBFv responses during arithmetic processing were also more pronounced during the first seconds of the response. From our analysis, it can be seen that this fast CBFv increase was mainly a response of a sharp BP rise rather than a neural metabolic

response during the three paradigms, indicating that the first 10s of CBFv response should not be used as a sole index of NVC; a concern also raised by Panerai *et al.* (2012a). In addition to the observed BP contribution, greater influences of $PaCO_2$ were also observed during motor imagery and active CBFv (Fig. 7.2A&B), contributing to decreasing CBFv during such paradigms. Most studies have ignored the influences of breath-by-breath $PaCO_2$, although Moody *et al.* (2005) and Ances *et al.* (2001) showed that $PaCO_2$ variations during brain activation may modulate cerebrovascular responses. The significant variation of systemic haemodynamics might reflect the fact that both paradigms impose greater demands on attention thus leading participants to higher levels of stress and associated changes to their breathing pattern.

Functional imaging studies have suggested the use of motor imagery and passive paradigms to induce neural activation in areas normally involved in the control of voluntary motor activity (Blatow *et al*., 2011, Roosink and Zijdewind, 2010, Stippich *et al*., 2002). In chapters 5 and 6, we have demonstrated that CBFv responses to active, passive and motor imagery have similar and reproducible patterns of CBFv response in healthy volunteers, with no significant differences between paradigms. However, when the contribution of each stimulus was extracted from the raw CBFv responses, differences between paradigms could be detected showing a greater sensitivity of model derived estimates to detect differences between paradigms (Fig 7.1C&D). On the other hand, the lack of significant differences at *t30* is possibly due to limitation in statistical power since a similar trend was observed in that case (Table 7.1). Whilst all paradigms led to CBFv increases, the motor imagery stimulus showed a lesser contribution to the CBF response than the motor paradigms, which is in agreement with previous reports (Roosink and Zijdewind, 2010, Stippich *et al.*, 2002). Though active and motor imagery share common functional circuits, motor imagery leads to smaller BOLD signals (Stippich *et al.*, 2002) and

motor evoked potentials (Roosink and Zijdewind, 2010) as cortical excitability increases with increases in task complexity.

In this study, and in agreement with the literature (Blatow *et al*., 2011, Doering *et al*., 1998, Matteis *et al*., 2001, Weiller *et al*., 1996), active and passive paradigms led to similar CBFv responses. The similarity persisted even when the contribution of other variables was excluded from the raw CBFv response. Whilst both active and passive paradigms during CBF (or CBFv) measurement may be used to obtain information about the mechanisms of neuronal adaptation after disease states (Caramia et al., 2000, Matteis et al., 2003, Seitz et al., 1998, Silvestrini et al., 1993b), our experience would recommend the use of passive paradigms to study the recovery of neuronal function as it allows the inclusion of patients with various degrees of motor impairment in all disease stages. Moreover, it was previously demonstrated (in chapter 6) that the passive paradigm leads to as reliable cerebral haemodynamic responses as voluntary movement.

7.4.3 Limitations of the Study

In addition to the point raised in previous chapters in regard to the absence of electromyography signal during the passive paradigm, a number of limitations of the present chapter should be noted. Firstly, TCD cannot be used to discriminate focal CBF during neural activation. It is possible that the paradigms we adopted did not provide just a focal sensorimotor stimulation, but also a non-specific mental stimulus involving attention, concentration and motivation. However, since TCD has a good temporal resolution in spite of a relatively poor spatial resolution, it may serve as a complementary tool for investigating cortical motor control in normal and disease-related stages. Indeed, the similarities shown in this study are in keeping with previous functional imaging data (Blatow *et al*., 2011, Guzzetta *et al*., 2007, Weiller *et al*., 1996). Although the

choice of parameters *t10* and *t30* was somewhat arbitrary, CBFv correlation analysis suggested that they have the potential to provide independent information. As hypothesized above, *t10* CBFv responses seem to be mostly driven by BP variations whereas *t30* may reflect more the metabolic response of the paradigms. A sensitivity analysis (Table 7.3) also indicated that *t10* did not change significantly in comparison with other alternatives in the range 0-15 s (0-5, 5-10, 0-15). Another limitation results from TCD measuring CBFv rather than absolute CBF (as described in previous chapters). Finally, the internal carotid artery was not examined in all participants. Future neurophysiological research in this area could certainly benefit from an extracranial vascular evaluation to exclude subjects with carotid artery stenosis.

7.5 Conclusion

In conclusion, the first hypothesis was rejected, since motor cognitive paradigms did not stimulate NVC in a similar fashion. On the other hand, the second hypothesis could not be rejected since the contribution of peripheral co-variates did not differ between paradigms. These findings have important implications for the interpretation of previous neurovascular coupling studies, and for the design of future studies assessing impairment of neurovascular coupling due to disease and its natural history. In particular, the use of motor imagery paradigms to assess patients' CBF and monitor their recovery is discouraged due to its poorer response to stimulation compared to active and passive paradigms. Instead, the passive paradigm is recommended for this purpose given its similar responses to active paradigms, its reproducibility of CBFv responses (as described in chapter 6) and lesser dependence on patient cooperation.

8 Neural Activation after Acute Ischaemic Stroke: a Failure of Myogenic Regulation?

8.1 Introduction

Stroke is one of the major causes of permanent disability worldwide with significant functional consequences. The potential for long-term rehabilitative response means that a more detailed understanding of the mechanisms underlying functional recovery and potential reorganization processes may help improve existing rehabilitation strategies, and even develop alternative neurorehabilitative interventions.

As described in previous chapters, triggered by several deleterious mechanisms during the ischaemic cascade (Brouns and De Deyn, 2009), stroke is typically associated with impaired neuronal activation, CBF regulation and/ or metabolism both local and distant to the ischaemic core (Dawson *et al*., 2000, del Zoppo, 2010, Gsell *et al*., 2000); such impairments leading to secondary brain injuries (Brouns and De Deyn, 2009, Lin *et al*., 2011). In the absence of brain ischaemia, CBF control and modulation during neural activation depends upon NVC (Carmignoto and Gomez-Gonzalo, 2010, Girouard and Iadecola, 2006, Koehler *et al*., 2009). Therefore, the CBF response to neural activation paradigms has been widely used as an index of neuronal activity and metabolism in healthy and pathological conditions (Gsell *et al*., 2000, Marshall *et al*., 2009, Mimura *et al*., 1998, Sharp *et al*., 2010).

However, changes in CBF following stimulation cannot be solely interpreted as the response to increased metabolic demand in the presence of parallel changes in confounding factors. Chapter 7 and recent studies have shown that significant variations of BP and $EtCO_2$ affect the CBF response in healthy participants (Moody *et al*., 2005, Panerai *et al*., 2005b, Panerai *et al*., 2012a, Panerai *et al*., 2012b), as well as in stroke patients (Silvestrini et al., 1993b, Silvestrini et al., 1995a, Silvestrini et al., 1998b). This suggests that neural activation paradigms may lead to a more complex CBF response, probably involving the interaction of many regulatory mechanisms. In a healthy young population, Panerai *et al*. (2005b) assessed contributions to the CBF velocity (CBFv) response during word finding and puzzle solving paradigms using a new approach described by a two-parameter model; CrCP and RAP. They demonstrated that together with the vasodilatation response to the metabolic stimulation, a myogenic vasoconstriction was also observed bilaterally.

The assessment of CBF changes following alterations in neural activity forms the basis of an increasing number of functional recovery studies after stroke. Better understanding of the different mechanisms contributing to the CBF response could have considerable potential to improve the clinical benefits of this approach. As a contribution in this direction, the present chapter addressed the following questions: firstly, does the beat-to-beat CBFv response to a passive motor paradigm differ between acute stroke patients and healthy control subjects? Secondly, does the subcomponents analysis of the CBFv response add to the interpretation of differences between the CBFv responses of stroke patients in comparison with control subjects?

8.2 Methods

8.2.1 Research Participants

Nineteen stroke participants fulfilling the criteria described in section 4.6.1. were recruited in this study. Seventeen control participants (same participants group of chapter 7) were also enrolled in the study.

8.2.2 Measurements

Recordings were performed as described in section 4.6.2.1. Briefly, after a period of 15 minutes stabilization, participants performed just the passive paradigm repeated twice in random order. Movement was performed only on the affected side in the stroke group and on the dominant side in control. The paradigm began with a 90-second baseline phase. Thereafter the passive paradigm was performed over 60 seconds, with a 90-second recovery phase. Prior to recordings, the paradigm was trialled twice to avoid the need for any verbal instructions during the recordings.

8.2.3 Data Analysis

Data analysis was performed as described in section 4.6.3. After the signals were calculated with a uniform time-base, averages were performed for each variable synchronized by the beginning of each paradigm. As described previously (section 4.6.4.1), the instantaneous relationship between BP and CBFv was used to estimate CrCP and RAP for each cardiac cycle using the first harmonic method (Panerai, 2003) and the CBFv responses decomposed into its main subcomponents. In summary, the percentage change in CBFv changes (ΔCBF_v) was decomposed into standardized subcomponents describing the relative contributions of BP (V_{BP}), resistance

area product (V_{RAP}) and critical closing pressure (V_{CrCP}). Therefore, the total CBFv changes during activation were represented as the sum of these three subcomponents. It has been suggested that V_{RAP} might reflect myogenic activity in response to BP changes, whereas V_{CrCP} is more indicative of metabolic control (Panerai *et al*., 2005b, Panerai *et al*., 2012b).

Using the electrical output from the metronome, each variable was synchronized at the beginning of the paradigm responses and population coherent averages and standard deviation curves were obtained for each time sample value. Mean CBFv, heart rate, BP and $EtCO_2$ values were extracted from the 30 seconds preceding the paradigm for baseline comparisons.

8.2.4 Statistical Analysis

T-tests were used to compare baseline values of CBFv, heart rate, BP and $EtCO_2$ between stroke patients and control subjects. To compare changes in CBFv and its subcomponents between strokes and controls, the area-under-the-curve (AUC) was calculated for their differences from the beginning of the manoeuvre, up to 20 s after the end of passive arm movement. Statistical analysis was performed using two-way ANOVA with group (control and patient) as the between factor, and side of recording (right, left, affected, unaffected hemispheres) as the within factor for ΔCBFv, V_{BP}, V_{RAP} and V_{CrCP}. Post-hoc comparisons (Tukey's test) were performed when appropriate. T-test for independent samples was used to compare $EtCO_2$ AUC values between the two groups. A value of $p<0.05$ indicated statistical significance.

8.3 Results

8.3.1 Participants' Characteristics

Nineteen stroke patients (11 male) were included in this study; two total anterior circulation, 11 partial anterior circulation and six lacunar strokes according to the Oxfordshire Community Stroke Project (OCSP) classification (Bamford *et al.*, 1991). Demographic and clinical characteristics are summarized in Table 8.1. There were no significant differences between the stroke and control groups in sex and age. With the exception of two stroke patients, all other participants were right-handed. A left hemisphere lesion was found in eight stroke participants. In the stroke group, carotid stenosis ranged from 10% to 80% with a population mean of 44.4 (13.1) %. Four out of nineteen stroke participants presented with severe stenosis (≥50%) in the ipsilateral internal carotid artery, as well as mild stenosis (10-20%) in the contralateral artery. The ultrasound scan also revealed a mild-to-moderate stenosis (10-40%) in the ipsilateral internal carotid artery in two patients. Four stroke patients had not recovered any voluntary control of elbow flexion and extension, and one had only partial recovery. No signs of severe proprioceptive dysfunction in the paretic upper limb (classified by score 2 in sections 8 and/or 11 of the NHISS scale) or neglect were found.

Table 8.1 Participant characteristics

Variables	Stroke n=19	Control n=17
Age, years (SD)	62.0 (11.2)	62.2 (7.5)
Male	12	11
Hypertension	6	-
Diabetes mellitus	2	-
Hypercholesterolaemia	1	-
Ipsilateral ICA stenosis (<30%)	1	-
Ipsilateral ICA stenosis (30-49%)	1	-
Ipsilateral ICA stenosis (≥50%)	4	-
Contralateral ICA stenosis (<30%)*	4	-
Onset to assessment, hours (SD)	28.1 (12.0)	-
NIHSS, (SD)	4.4 (3.6)	-
mRS, (SD)	1.4 (1.2)	-

ICA: internal carotid artery, NIHSS: National Institute of Health Stroke Scale, mRS: Modified Rankin score, SD: standard deviation.
*bilateral stenosis

8.3.2 Transcranial Doppler Recordings

Resting values of the recorded parameters were similar in controls and patients, with the exception of affected hemisphere vs. left CBFv, and $EtCO_2$, which were significantly lower in the stroke group compared to controls (Table 8.2).

Both patient and control groups were able to perform the sensorimotor paradigm correctly. Fig. 8.1 (A&B) shows the averaged CBFv change for both stroke and control groups during the passive paradigm performance. The TCD profile revealed a marked bilateral increase of ΔCBFv in stroke and control groups, with significant differences between groups (Table 8.3). A post-hoc comparison revealed significant differences between affected hemisphere (stroke) and left hemisphere (control) in ΔCBFv, and between unaffected hemisphere (stroke) and right hemisphere (control) in ΔCBFv (Table 8.3).

The breakdown of ΔCBFv into its subcomponents during the paradigm performance is presented in Fig. 8.1(C&D) and 8.2. At the beginning of stimulation, V_{BP} showed a higher peak value in the stroke group (Fig. 8.1C&D), but its overall amplitude, as expressed by the AUC, was not significantly different from controls (Table 8.3). A secondary peak in V_{BP}, was observed for both groups before the end of stimulation (Fig. 8.1C&D). Positive changes in V_{RAP} and V_{CrCP} are caused by reductions in RAP and CrCP, contributing to increases in CBFv. The contribution of CrCP (Fig. 8.2A&B) was more pronounced when compared to the other subcomponents, but its overall amplitude was not significantly different between groups (Table 8.3). On the other hand, V_{RAP} showed marked differences between patients and controls. Positive peaks in V_{RAP} were found in the control group during and immediately after the paradigm performance indicating maintained vasodilatation, whereas in the stroke group this response was missing or actually negative, indicating vasoconstriction, as shown in Fig. 8.2C&D. The AUC for V_{RAP} was significantly different between strokes and controls bilaterally (Table 8.3). Fig. 8.3 depicts the temporal pattern of variations in the population average of $EtCO_2$ during the passive paradigm. Mean baseline values were lower in stroke patients (Table 8.2), but the AUC after normalization showed no difference between groups.

Table 8.2 Mean baseline values (SD) of CBFv, heart rate, BP and $EtCO_2$.

Variables	Stroke	Control	p Value
CBFv (affected vs. left), $cm.s^{-1}$	39.4 (17.9)	50.6 (10.5)	0.04
CBFv (unaffected vs. right), $cm.s^{-1}$	47.2 (15.6)	45.8 (10.5)	0.77
BP, mmHg	91.1 (12.2)	86.5 (15.8)	0.32
Heart Rate, bpm	64.6 (8.7)	60.6 (6.6)	0.15
$EtCO_2$, mmHg	33.9 (3.9)	38.1 (4.6)	0.007

CBFv, cerebral blood flow velocity; BP, mean arterial blood pressure; $EtCO_2$, end-tidal CO_2. p-values for independent Student's t-test

Table 8.3 Mean values (SD) for area under the curve (AUC) for differences in CBFv variation, its subcomponents and $EtCO_2$ between patients and controls

Variable	Side	Stroke AUC (SD)	Control AUC (SD)	p value*	p value Tukey**
CBFv, %	AH vs. LH	4.5 (6.5)	9.5 (6.4)	0.01	0.01
	UH vs. RH	4.1 (3.6)	9.4 (6.9)		0.008
V_{BP}, %	AH vs. LH	2.5 (5.0)	2.4 (6.8)	0.7	
	UH vs. RH	2.4 (5.2)	2.4 (6.8)		
V_{CrCP}, %	AH vs. LH	2.4 (7.5)	3.9 (4.3)	0.4	
	UH vs. RH	1.9 (4.6)	4.1 (6.3)		
V_{RAP}, %	AH vs. LH	-0.9 (4.0)	2.9 (2.3)	0.03	0.03
	UH vs. RH	-0.4 (5.0)	2.5 (2.8)		0.01
$EtCO_2$, %	stroke vs. control	101.3 (10.6)	98.4 (6.3)	0.3	

CBFv, cerebral blood flow velocity; V_{BP}, relative contribution of mean arterial blood pressure; V_{CrCP}, relative contribution of critical closing pressure; V_{RAP}, relative contribution of resistance area product; $EtCO_2$, end-tidal CO_2; AH, affected hemisphere; UH, unaffected hemisphere; LH, left hemisphere, RH, right hemisphere.

*P value, p value of two-way ANOVA, with the exception of $EtCO_2$ (p value of t-test)

**P Tukey, p value of post hoc analysis when ANOVA showed significance.

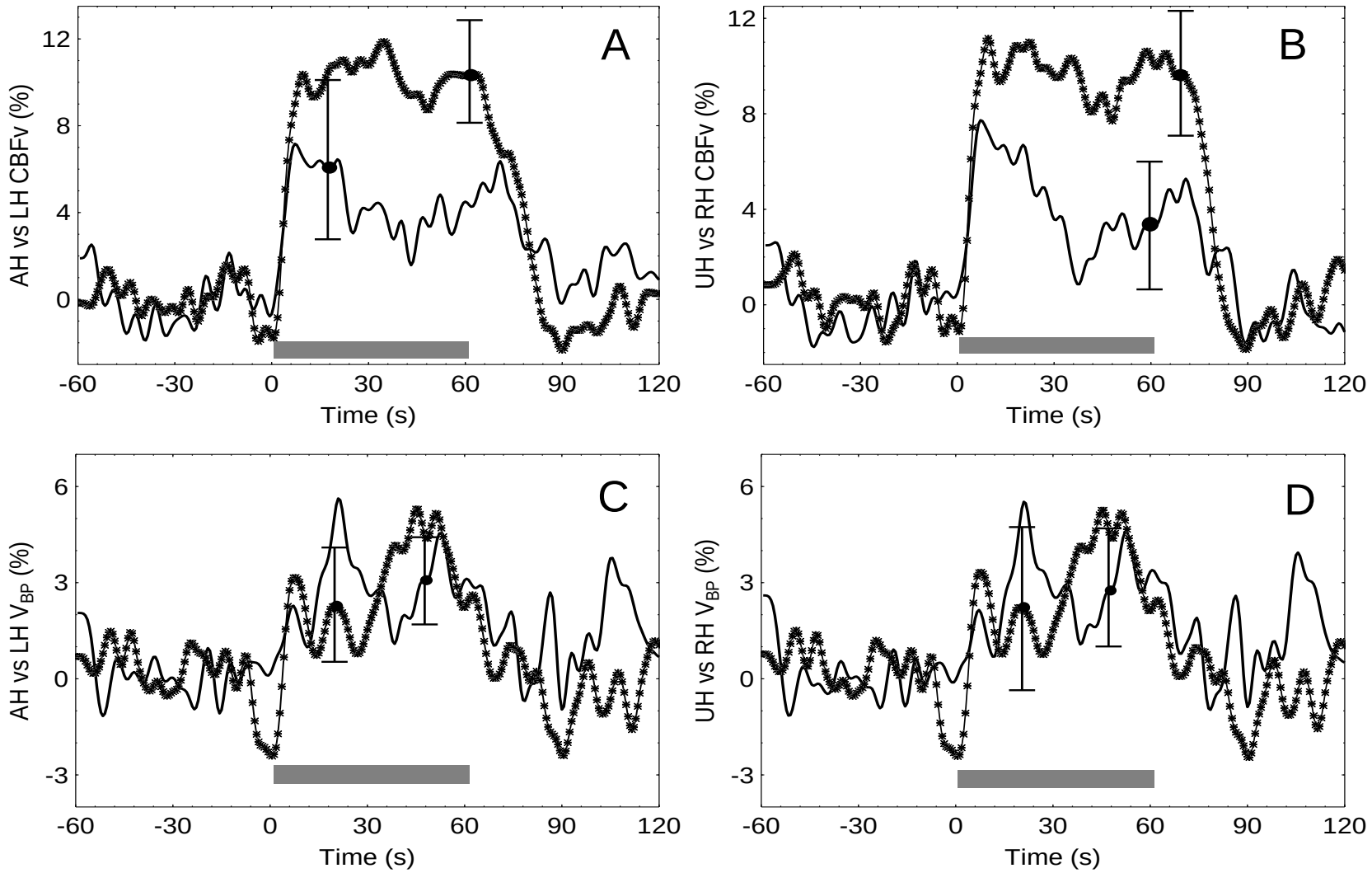

Fig 8.1 Changes in CBFv population averages (A&B) and blood pressure contribution (C&D) during the sensorimotor paradigm (grey bar). In each plot, averages for stroke patients (continuous line) are compared to corresponding averages for controls (continuous line+symbol). A&C correspond to the comparison of the affected hemisphere of stroke patients with the left hemisphere of controls. B&D compare the unaffected hemisphere of strokes with the right hemisphere of controls. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

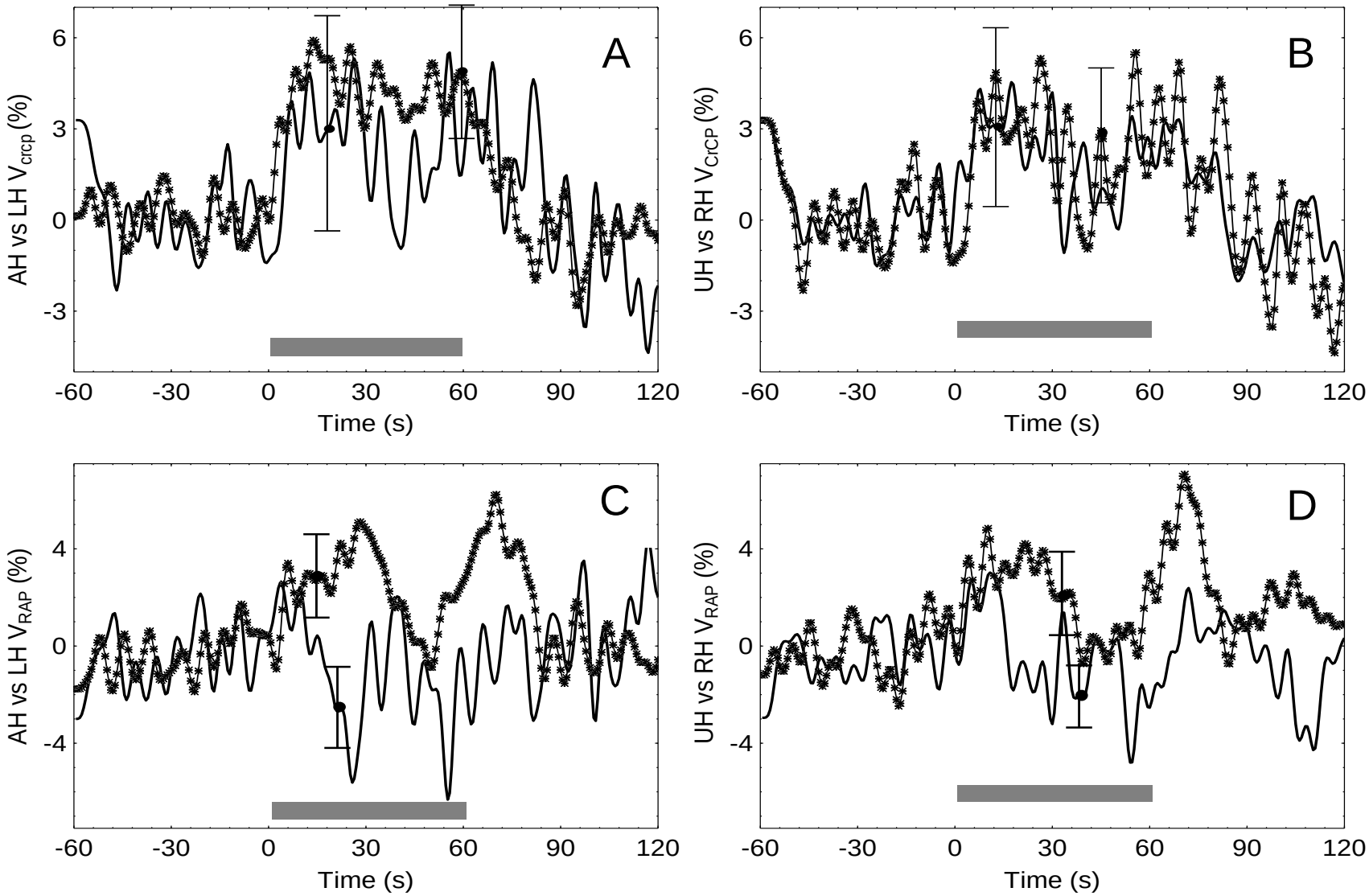

Fig 8.2 Population averages of CrCP (A&B) and RAP (C&D) during the sensorimotor paradigm (grey bar). In each plot, averages for stroke patients (continuous line) are compared to corresponding averages for controls (continuous line+symbol). A&C correspond to the comparison of the affected hemisphere of stroke patients with the left hemisphere of controls. B&D compare the unaffected hemisphere of strokes with the right hemisphere of controls. For clarity, only the largest ± 1 SE is represented at the point of occurrence

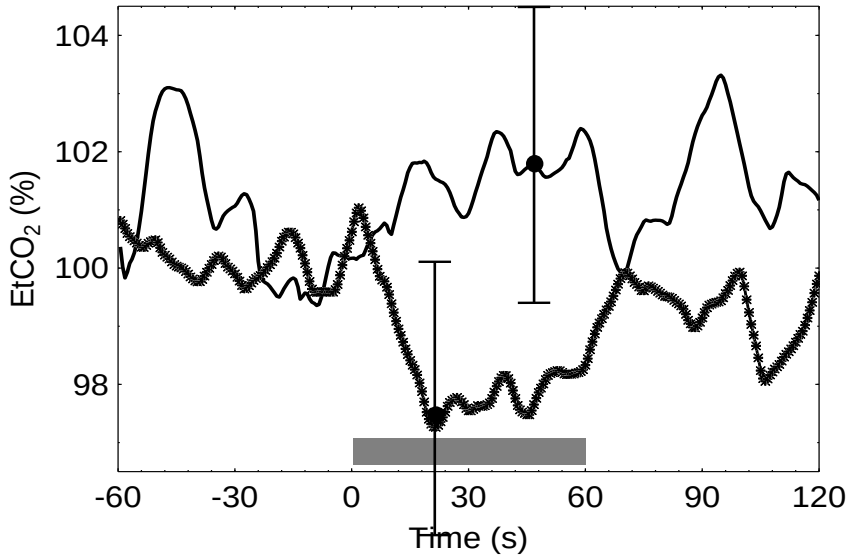

Fig 8.3 Population averages of $EtCO_2$ during the sensorimotor paradigm (grey bar). Normalised changes in $EtCO_2$ for stroke (continuous line) and control participants (continuous line + symbol). For clarity, only the largest ± 1 SE is represented at the point of occurrence.

8.4 Discussion

8.4.1 Cerebral Blood Flow Response

In the last 25 years, new non–invasive technologies, such as TCD and NIRS have enabled human cerebral haemodynamic responses to be studied, improving our understanding of the dynamic behaviour of CBF regulation mechanisms (Aaslid *et al.*, 1989, Markus and Cullinane, 2001, Panerai *et al*., 1999). Both technologies permit simultaneous monitoring of peripheral haemodynamic responses, which is limited in most other popular imaging techniques (i.e. fMRI and PET); allowing an assessment of the potential influences of peripheral haemodynamic variables on measures of NVC.

The relationship between CBFv responses to neural activation and stroke has been reported by several TCD studies in an attempt to find a functional pattern of cerebral recovery (Lin et al., 2011, Matteis et al., 2003, Silvestrini et al., 1993b, Silvestrini et al., 1995a, Silvestrini et

al., 1998b), as CBF increase has usually been interpreted as a reflection of additional metabolic demand due to neuronal stimulation (Gsell *et al*., 2000, Marshall *et al*., 2009). Results obtained thus far are conflicting (as described in the systematic review), though a lack of beat-to-beat CBFv measurements in these studies prevents further comparison. The above results showed CBFv increase in both affected and unaffected hemispheres, although the amplitude of such changes was significantly smaller in stroke than controls suggesting a bilateral NVC impairment. However, it is not clear if NVC impairment is related to impairment of myogenic or metabolic components, or both, which can be further explored by subcomponents analysis.

8.4.2 Subcomponents Analysis

In addition to confirming the importance of systemic haemodynamic parameters to CBFv responses, this study, for the first time, has also shown that RAP contributes differently to CBFv modulation following acute ischaemic stroke. The results showed that BP and CrCP contributed to CBFv increase during the 90 seconds of paradigm performance with no differences between groups. Previous studies have suggested that BP modulates CBFv only during the first seconds of activation (Moody *et al*., 2005, Salinet *et al*., 2012b). As a passive motor paradigm was the major stimulus of the measurement, I believe that the greater amplitude of V_{CrCP} contribution might represent the main manifestation of metabolic pathways. This hypothesis is supported by previous studies showing that changes in CrCP are influenced by $PaCO_2$, hyperaemia and cognitive paradigms (Panerai *et al*., 2005b, Panerai *et al*., 2012b).

On the other hand, the V_{RAP} component of the stroke group was significantly different from controls, possibly showing impairment of myogenic pathways. Interestingly, V_{RAP} negative troughs coincided with positive V_{BP} peaks in stroke patients. A study in neonates showed that

RAP reflected disturbances in dynamic cerebral autoregulation giving some evidence to support this possibility (Panerai *et al*., 1996). Previous studies have described impaired or even abolished CBF regulatory mechanisms after stroke in humans (Carusone *et al*., 2002, Dawson *et al*., 2000, Rossini *et al*., 2004b) and animals (Dijkhuizen *et al*., 2003) which could hamper the detection of brain activation induced by haemodynamic responses. Therefore, the present results suggest that the depressed NVC response observed in acute ischaemic stroke patients is due to a paradoxical response to the BP changes which accompany stimulation, rather than the result of damaged metabolic pathways, as could be represented by the CrCP contribution to CBFv. Further work will be needed to test this hypothesis under different protocols to allow better separation of the mechanisms involved. As an example, paradigms that can induce lower or greater influences on BP changes would be particularly relevant.

8.4.3 Influence of $PaCO_2$

At rest, acute ischaemic stroke patients had significantly lower values of $EtCO_2$ than controls. Previous studies have demonstrated that hypocapnia improves dynamic cerebral autoregulation, potentially enhancing any consequent CBFv response to neural activation after stroke (Panerai *et al*., 1999, Panerai *et al*., 2005a), but the possibility that the reduction in $EtCO_2$ levels could restrict the CBFv response due to vasoconstriction was also acknowledged (Jorgensen *et al*., 1993, Sitzer *et al*., 1994). Despite significant differences in baseline values between groups, $EtCO_2$ showed only minor fluctuations during the motor paradigm, which were not significantly different between groups. Therefore, these were not likely to have influenced the time course of mean CBFv responses or their separate subcomponents.

8.4.4 Limitations of the Study

A number of limitations of this chapter should be noted. Firstly, four stroke participants with moderate-to-severe carotid artery stenosis were included in the study. Aiming to test the effect of carotid stenosis on the subcomponents analysis, a total of four patients (ipsilateral carotid artery stenosis ≥50%) were excluded and the AUC values compared to controls. At baseline, no significant differences between the no-to-mild-stenosis stroke and control groups were found, with the exception of $EtCO_2$. Though mean CBFv increased in the affected hemisphere to 43.9 (12.8) $cm.s^{-1}$, when the moderate-to-severe carotid artery stenosis patients were excluded, this was not significantly different. Importantly, there were no changes to the results of the subcomponents analysis with the exclusion of these patients (Table 8.4). Secondly, the passive paradigm was performed only with the right side in controls, but either right or left (depending on the affected side) were stimulated in the stroke group. Despite the lack of consensus regarding the relationship between handedness and brain activation (Guzzetta *et al*., 2007, Hammond, 2002 , Matteis *et al*., 2001, Solodkin *et al*., 2001), for future research it would be better having two sides assessed and matched with the affected hemisphere in stroke. Another limitation to consider is the poor signal-to-noise ratio of RAP and CrCP estimates, which may lead to the exclusion of less than optimal recordings in clinical studies. However, previous studies have shown that robust estimates of RAP and CrCP can be obtained with the methods used in this study (Panerai *et al*., 2001, Panerai *et al*., 2012b). Finally, the sensorimotor paradigms led to significant CBFv responses in both MCAs rather than a lateralised response, suggesting that repetitive elbow flexion induced a more diffuse and non-specific mental stimulus regarding attention, concentration and motivation instead of a lateralised and focal response. From the perspective of more classical functional studies, such as BOLD fMRI, the lack of regional specificity would be a major disadvantage, but for clinical management of stroke patients, a global and non-specific response might

prove advantageous by providing greater sensitivity to detect abnormalities when faced with the diversity of ischaemic tissue anatomical localisation.

Table 8.4 Mean values (SD) for area under the curve (AUC) for differences in CBFv variation, its subcomponents between patients (no to mild stenosis, n=15) and controls (n=17).

Variable	Side	Stroke AUC (SD)	Control AUC (SD)	p value*	p value Tukey**
CBFv, %	AH vs. LH	4.5 (6.5)	9.5 (6.4)	0.01	0.01
	UH vs. RH	4.1 (3.6)	9.4 (6.9)		0.008
V_{BP}, %	AH vs. LH	3.2 (5.7)	2.4 (6.8)	0.7	
	UH vs. RH	3.5 (5.6)	2.4 (6.8)		
V_{CrCP}, %	AH vs. LH	2.3 (5.0)	3.9 (4.3)	0.5	
	UH vs. RH	2.1 (2.6)	4.1 (6.3)		
V_{RAP}, %	AH vs. LH	-1.4 (2.9)	-2.9 (2.3)	0.03	0.02
	UH vs. RH	-0.9 (4.1)	-2.5 (2.8)		0.009

AH, affected hemisphere; UH, unaffected hemisphere; LH, left hemisphere, RH, right hemisphere.
*P value, p value of two-way ANOVA,
**P Tukey, p value of post hoc analysis when ANOVA showed significance.

8.5 Conclusion

In summary, CBFv response to 60s passive flexion of the affected arm was bilaterally impaired after acute ischaemic stroke. Additionally, it was found that BP and CrCP represent the major contributors of CBFv responses in controls as well as in strokes. RAP contributed differently between groups suggesting impairment of the myogenic component of CBF regulation after stroke.

9 Neurovascular Coupling in Acute Stroke: Multivariate Analysis of its Peripheral and Central Contributors

9.1 Introduction

As stated in Chapter 1 and 2, cerebral haemodynamics is regulated by three main intrinsic mechanisms that rapidly adjust cerebral perfusion in a healthy brain. *Cerebral autoregulation* (CA) ensures that CBF is maintained relatively constant across a wide range of mean arterial BP, as long as this remains between 50-170 mmHg (Lassen, 1959). *Neurovascular coupling* (NVC) adapts regional CBF in response to neural activation (Girouard and Iadecola, 2006). These mechanisms are not fully understood, but there is evidence that the small arteriolar resistance vessels play an important role in both cases (Rosengarten *et al*., 2001). Importantly, small vessel resistance is also strongly influenced by $PaCO_2$ by what is known as *CO_2 cerebrovascular reactivity* (CVR) (Ringelstein *et al*., 1988).

CA is particularly challenged during acute ischaemic stroke. Efficient CA is important both during the acute vessel occlusion and during the reperfusion phase. With the impairment of CA acutely following infarction, it renders CBF totally reliant on systemic BP. Therefore, any reduction in the latter may have potential adverse consequences for the viability of the ischaemic penumbra. On the other hand, raised BP may result in increased cerebral oedema or haemorrhagic transformation of the infarct. CA and CVR have been reported to be

impaired after acute ischaemic stroke (Dawson *et al*., 2000, Immink *et al*., 2005, Aries *et al*., 2010).

Though CBF response to neural activation has been the target of many stroke recovery studies, the systematic review showed considerable diversity of findings. Despite consensus in sensorimotor and cognitive studies that the CBF responses after stroke indicate some degree of impairment, no agreement was found regarding the role of the unaffected hemisphere and the clinical significance of such impairment. Lack of a standard method for CBF assessment, diversity in brain activation paradigms, the number and type of parameters recorded, and different conceptual frameworks for the interpretation of results have limited further conclusions. In an attempt to better interpret and understand CBF responses to neural activation after stroke, a multivariate model assessing NVC, as well as CA and CVR mechanisms, was used in this study giving a global insight into neural and cerebral haemodynamic reorganization (Panerai *et al*., 2012a). Moreover, this model enables the effect of confounding factors (such as BP and $PaCO_2$) in the CBF response to be excluded, allowing a more robust assessment of NVC integrity, and therefore the quantification of cerebral dysfunction.

In summary, we sought to investigate the CBF response to neural activation in patients with a first-ever ischaemic stroke. By using TCD ultrasound, it was hypothesised that passive movement of the arm is associated with a different pattern of CBFv response to a control population, and that multivariate analysis can detect, in a single measurement, an impairment of NVC, simultaneously with altered function of CA and CVR.

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9.2 Methods

9.2.1 Research Participants

Twenty-seven stroke participants fulfilling the criteria described in section 4.6.1 and control participants were recruited in this study. The stroke group comprises in nineteen participants who were analysed in chapter 8 whereas data of ten new participants were included in the control group.

9.2.2 Measurements

Recordings were performed as described in section 4.6.2.1. Briefly, after a period of 15 minutes stabilization, participants performed just the passive paradigm repeated twice in random order. Movement was performed only on the affected side in the stroke group and on the dominant side in control. The paradigm began with a 90-second baseline phase. Thereafter the passive paradigm was performed over 60 seconds, with a 90-second recovery phase. Prior to recordings, the paradigm was trialled twice to avoid the need for any verbal instructions during the recordings.

9.2.3 Data Analysis

Data analysis was performed as described in section 4.6.3. After the signals were calculated with a uniform time-base, averages were performed for each variable synchronized by the beginning of each paradigm. The manoeuvre showing the largest CBFv response was chosen to represent the participant's response, as described in chapter 6.

Baseline Step Responses

The CBFv step response to the BP input was computed on the 5-min baseline measurement, after a period of 100s of stabilization, as previously described (Katsogridakis *et al*., 2013). A

fast Fourier transform was applied to the data, and the cross- and auto-spectra were estimated using the Welch method. The transfer function of the BP-CBFv dynamic relationship was then calculated with BP selected as the input and right then left CBFv as the output variables. An inverse fast Fourier transform was then applied to the complex transfer function, converting data back into the time domain, to calculate the CBFv step response (Katsogridakis *et al*., 2013). The autoregulatory index (ARI) was assigned to each recording by using the best least-squares fit between the CBFv step response and one of the 10 model ARI curves proposed by Tiecks *et al.* (1995). ARI was calculated for each subject for both hemispheres at baseline.

Neurovascular Coupling

As in Chapter 7, a multivariate autoregressive-moving average (ARMA) model was used to represent the influence of the inputs (BP, $EtCO_2$, and stimulus) on output (CBFv). As described in Section 4.6.4.2 and in previous work (Panerai *et al.*, 2012a), the ARMA model allows quantification of the simultaneous influences of BP (V_{BP}), $EtCO_2$ (V_{ETCO2}) and stimulus (V_{STIM}) to the CBFv response to stimulation (ΔCBFv). Briefly, the separate contributions of BP, $EtCO_2$ and stimulus to CBFv response were obtained as model predictions, with the use of ARMA coefficients. The order of these models, representing the number of past samples adopted for the autoregressive (AR) and moving average (MA) terms, was thoroughly considered as described in the section 4.6.4.2. The beginning of stimulation was used as the point of synchronism to obtain population mean and SD curves for each separate contribution (V_{BP}, V_{EtCO2} and V_{STIM}) for the ipsilateral and contralateral hemispheres. BP, CO_2 and stimulus step responses were calculated as described in the section 4.6.4.2.

Using a protocol used in the previous chapter, responses obtained from the affected hemisphere (stimulated side) in stroke were compared to the responses in the dominant (left) hemisphere in our right-handed control population, whereas the unaffected hemisphere results were compared to the right hemisphere in control.

9.2.4 Statistical Analysis

Mean CBFv, heart rate, BP, $EtCO_2$ values extracted from the 30s preceding the paradigm for baseline comparison. Paired T-tests for independent variables were used to compare baseline values of CBFv, heart rate, BP and $EtCO_2$ between stroke patients and control subjects. To compare changes in CBFv and the separate contributions of the three inputs between strokes and controls, the area-under-the-curve (AUC) was calculated for their differences from the beginning of the paradigm, up to 20s after the end of passive arm movement. Statistical analysis was performed using two-way ANOVA with group (control and patient) as the between factor, and side of recording (right, left, affected, unaffected hemispheres) as the within factor for ΔCBFv, V_{BP}, V_{EtCO2} and V_{STIM}. Similarly, two-way ANOVA was used to compare the differences in ARI (at baseline) and CBFv step responses to BP, $EtCO_2$ and stimulus, respectively. Mean values and standard deviation were extracted from the step responses between 20 and 25s for $EtCO_2$ and stimulus, and between 5s and 10s for the BP step responses during stimulation. Those time points were selected as they represent the most physiologically meaningful sections of the step responses. Post-hoc comparisons (Tukey's test) were performed when appropriate. A value of $p<0.05$ indicated statistical significance.

9.3 Results

9.3.1 Participants' Characteristics

Twenty-seven patients (16 male), of mean age 63 years (SD 11.7), were recruited after a mean of 32.6 (SD 14.0) hours from symptom onset. Fourteen had strokes in the right hemisphere, and neuroimaging (20 CT, 7 MRI brain scan) confirmed a single anterior circulation infarct. According to the OCSP classification (Bamford *et al*., 1991), the strokes were classified as three total anterior circulation, thirteen partial anterior circulation and eleven lacunar strokes. Mean NHISS and mRS scores at the time of scanning were 3.5 (SD 3.3) and 1.8 (SD 1.9), respectively. All stroke patients underwent carotid ultrasound, and measureable internal carotid artery stenosis was found in only six patients (mean 23.6% SD 13.6); with 80% stenosis in one patient. Five stroke patients had not recovered any voluntary control of elbow flexion and extension, and two had only partial recovery. No signs of severe sensory impairment or neglect were found. A past medical history of hypertension, diabetes mellitus and hypercholesterolaemia was found in ten, two and one patients, respectively.

Twenty-seven healthy control subjects (15 male), of mean age 61.4 years (SD 6.0) were recruited from University staff and their relatives. With the exception of two stroke subjects, all participants were right-handed (mean Edinburgh inventory score 90.2% (SD 16.8) and 91.7% (SD 7.4) for patients and controls, respectively).

9.3.1.1 Baseline Data

Resting values of the recorded parameters were similar in both groups, with the exception of affected hemisphere vs. left CBFv, and $EtCO_2$, which were significantly lower in the stroke group compared to controls (Table 9.1).

Table 9.1 Mean baseline values (SD) of CBFv, heart rate, BP and $EtCO_2$.

Variables	Stroke	Control
CBFv unaffected/right, *cm.s^{-1}*	41.1 (11.0)	46.9 (10.1)
CBFv affected/left, *cm.s^{-1}*	43.5 (19.2)	52.3 (11.2) ϕ
BP, *mmHg*	86.1 (20.1)	91.0 (19.3)
Heart rate, *bpm*	67.9 (10.8)	64.4 (11.0)
$EtCO_2$, *mmHg*	34.4 (3.4)	38.9 (4.5) ϕ

ϕ t-test, $p<0.05$ for the differences between stroke and controls.
CBFv, cerebral blood flow velocity; BP, mean arterial blood pressure;
$EtCO_2$, end-tidal CO_2.

9.3.1.2 Step Responses

CBFv step responses to the BP input obtained from baseline data (Figs. 9.1A & B) were similar to corresponding responses extracted during motor stimulation (Figs. 9.1C & D), based on spontaneous fluctuations in BP and CBFv. ARI calculated from the baseline BP step responses showed lower scores in the stroke group (ARI= 4.9 SD 1.7 for the unaffected hemisphere and ARI= 4.7 SD 2.4 for the affected hemisphere) compared to control (ARI= 5.6 SD 1.1 for the right hemisphere and ARI= 5.4 SD 1.1 for the left hemisphere). However, the two-way ANOVA did not reach statistical significance (p= 0.07). During stimulation, the 5s average selected from BP step responses also did not show a difference between groups (p= 0.09). On the other hand, corresponding step responses for the $EtCO_2$ (affected hemisphere 0.59 SD 0.7, unaffected 0.75 SD 0.8, right 1.27 SD 1.2, left 1.46 SD 0.7) and motor stimulus (affected hemisphere 0.20 SD 0.1, unaffected 0.22 SD 0.2, right 0.36 SD 0.2, left 0.40 SD 0.2) inputs were reduced in the stroke group compared to controls, as represented in Fig. 9.2. ANOVA revealed significant differences between groups (but no hemispheric interaction) for both step responses for $EtCO_2$ (p= 0.002) and for the stimulus (p= 0.003).

9.3.1.3 Neurovascular Coupling

No mirror or other extraneous movements were observed in either the practice movement or during the actual study recording for all participants. The CBFv response after stroke had similar temporal pattern as that of control subjects (Fig. 9.2). However, the increase in CBFv observed during the paradigm was lower in the stroke group than in controls for both affected and unaffected hemispheres (Fig. 9.2). Two-way ANOVA confirmed significant differences between groups ($p= 0.01$), but the post-hoc analysis only detected a difference in AUC between the affected (patient) and left (control) hemispheres CBFv responses (Table 9.2; Tukey $p<0.04$). It is of note that the difference in AUC between unaffected and right CBFv was of borderline significance ($p= 0.06$). No side interaction was found.

Table 9.2 Mean values (SD) for area under the curve (AUC) for CBFv responses and the contributions of BP, EtCO2 and stimulation.

Variables	Stroke		Control	
	Unaffected	Affected	Right	Left
CBFv, *%*	105.2 (4.8)	105.7 (5.4)	107.3 (6.2)	107.5 (4.3)$^{\phi}$
BP, *%*	101.7 (8.7)	100.7 (2.7)	100.7 (1.4)	100.5 (1.1)
$EtCO_2$, *%*	99.8 (2.3)	99.8 (2.7)	100.6 (3.4)	99.9 (4.0)
Stimulus, *%*	105.3 (6.2)	104.5 (4.5)	105.7 (6.0)	106.7 (3.3)$^{\phi}$

$^{\phi}$ $p<0.05$ Tukey's post-hoc for the differences between stroke (affected hemisphere) and controls (left hemisphere). CBFv, cerebral blood flow velocity; BP, mean arterial blood pressure; $EtCO_2$, end-tidal CO_2.

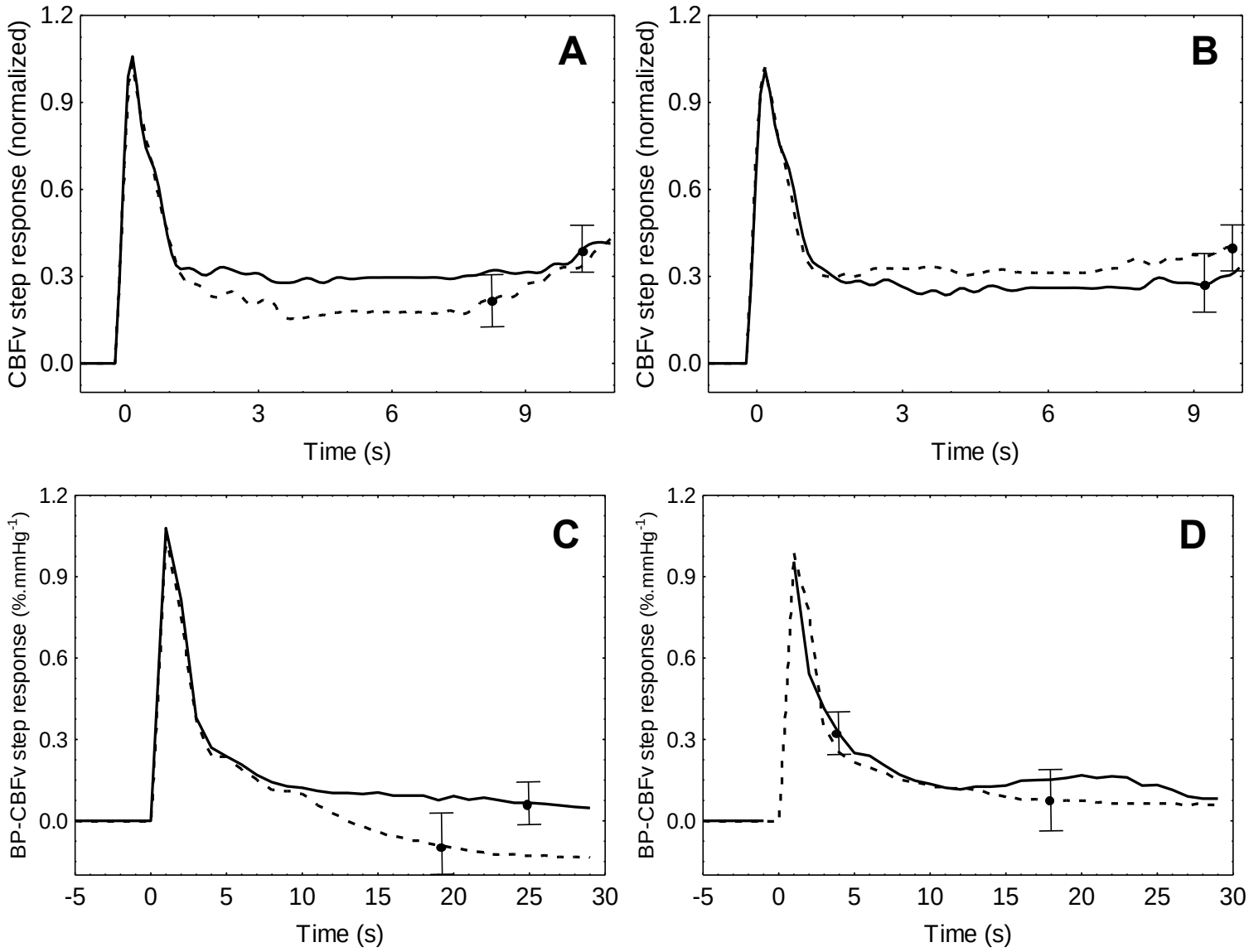

Fig 9.1 Step responses averages for stroke patients (dashed line) and controls (continuous line) at baseline (A, B) and during stimulus (C, D). A&C unaffected hemisphere of stroke patients and right hemisphere of controls. B&D affected hemisphere of strokes and left hemisphere of controls. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

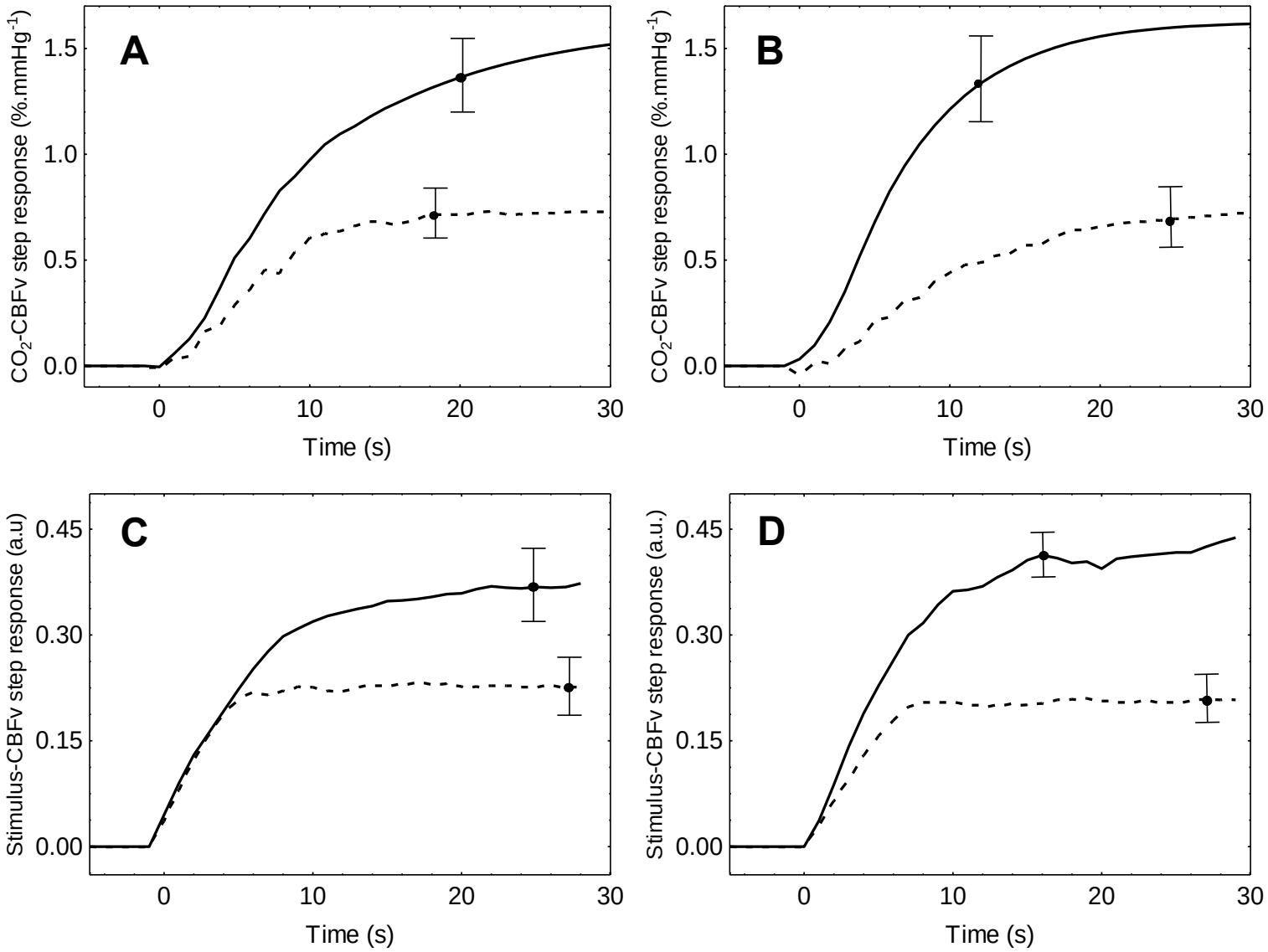

Fig 9.2 Population average CBFv step responses for stroke patients (dashed line) and controls (continuous line) due to the influences of $EtCO_2$ (A, B), and motor stimulation (C, D). A&C unaffected hemisphere of stroke patients and right hemisphere of controls. B&D affected hemisphere of strokes and left hemisphere of controls. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

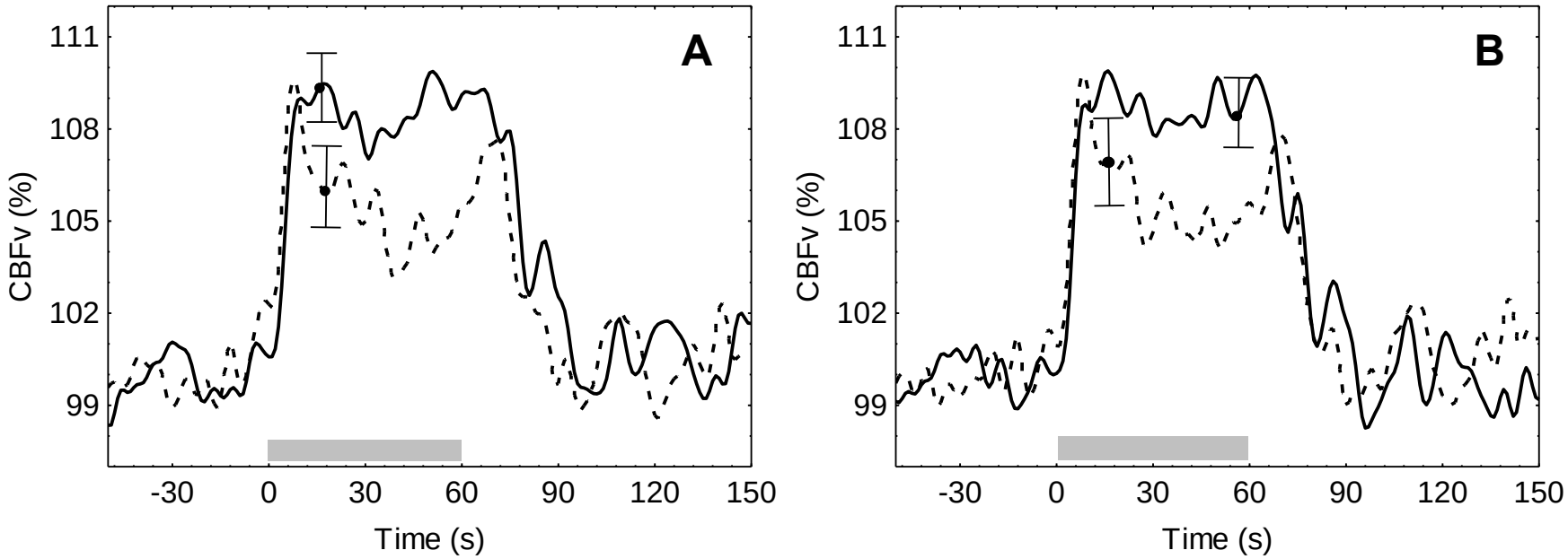

Fig 9.3 Population average changes in CBFv during passive motor paradigm (grey bar). Averages for stroke patients (dashed line) are compared to corresponding averages for controls (continuous line). A) comparison of the unaffected hemisphere of stroke patients with the right hemisphere of controls. B) comparison of the affected hemisphere of strokes with the left hemisphere of controls. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

9.3.1.4 Contribution of individual inputs to CBFv responses

Table 9.3 gives the distributions of explained variance for each input for both hemispheres in the stroke and control groups. Two-way ANOVA showed a significant interaction between the comparisons of the individual contributions (BP, $EtCO_2$ and stimulus) explained by the model (p= 0.0002), and not between groups. Of note, BP explained more of the variance in the stroke population and the stimulus explained more in the control population, though these differences did not reach statistical significance.

Fig. 9.4 presents the temporal pattern of the CBFv contributors during the passive paradigm. These curves suggest that BP is the major contributor of the initial peak in CBFv (due to a rapid rise in BP) and the transient reduction in CBFv (due to a drop in BP) after the end of the paradigm (Figs. 9.4 A&B). BP contribution (V_{BP}) at the beginning of the paradigm execution was higher in the stroke group than controls, but AUC values were not significantly different (Table 9.2). Despite $EtCO_2$ influencing CBFv, V_{EtCO2} showed no major contribution to CBFv responses modulation in both groups, neither in the temporal pattern (Figs. 9.2 C&D) nor AUC analysis (Table 9.2). However, the passive paradigm (V_{STIM}) itself made a significant contribution to the CBFv rise in both populations (Figs. 9.4 E&F). Furthermore, the paradigm contributed less to the CBFv increase in stroke patients when compared to controls (p= 0.008), especially in the affected side (Table 9.2; Tukey's post-hoc p= 0.02).

Table 9.3: Mean (SD) relative contributions of the model input variables to explain variance of CBFv response during passive stimulation.

Variables	Stroke		Controls	
	Unaffected	Affected	Right	Left
BP, *%*	47.3 (25.5)	46.4 (24.6)	36.4 (24.4)	34.9 (24.9)
$EtCO_2$, *%*	17.5 (17.1)	23.0 (20.0)*	22.7 (23.1)	19.6 (17.3) ϕ
Stimulation, *%*	35.2 (24.3)	30.6 (23.3)	40.9 (29.7)	45.5 (21.0)
Total variance explained by model, *%*	75.0 (10.0)	77.4 (11.5)	71.7 (14.4)	80.0 (8.5) ∞

ϕ $p<0.003$ Tukey's post-hoc for the differences between stimulus and $EtCO_2$ contributions

* $p=0.003$ Tukey's post-hoc for the differences between $EtCO_2$ and BP contribution

∞ $p=0.01$ Tukey's post-hoc for the differences of the total variance explained by model between left and right hemispheres in controls. BP, mean arterial blood pressure; $EtCO_2$, end-tidal CO_2.

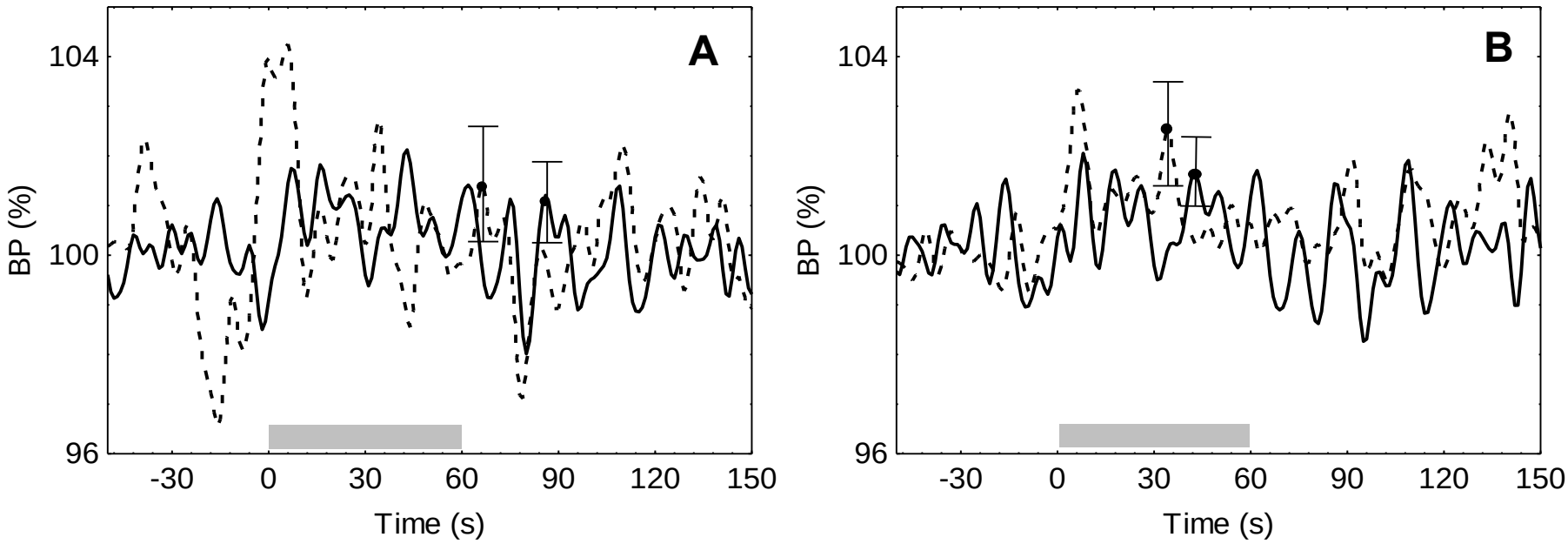

Fig 9.4 Population average of the contributions of BP during passive motor paradigm (grey bar). Averages for stroke patients (dashed line) are compared to corresponding averages for controls (continuous line). A) comparison of the unaffected hemisphere of stroke patients with the right hemisphere of controls. B) comparison of the affected hemisphere of strokes with the left hemisphere of controls. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

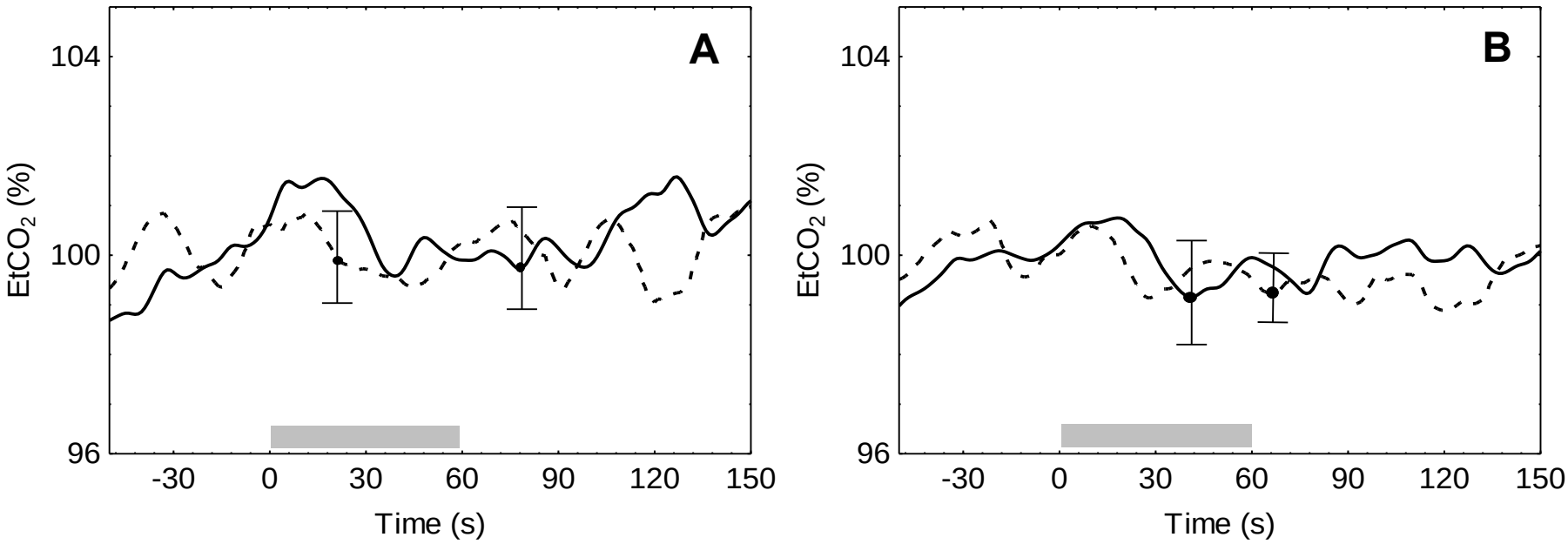

Fig 9.5 Population average of the contributions of $EtCO_2$ during passive motor paradigm (grey bar). Averages for stroke patients (dashed line) are compared to corresponding averages for controls (continuous line). A) comparison of the unaffected hemisphere of stroke patients with the right hemisphere of controls. B) comparison of the affected hemisphere of strokes with the left hemisphere of controls. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

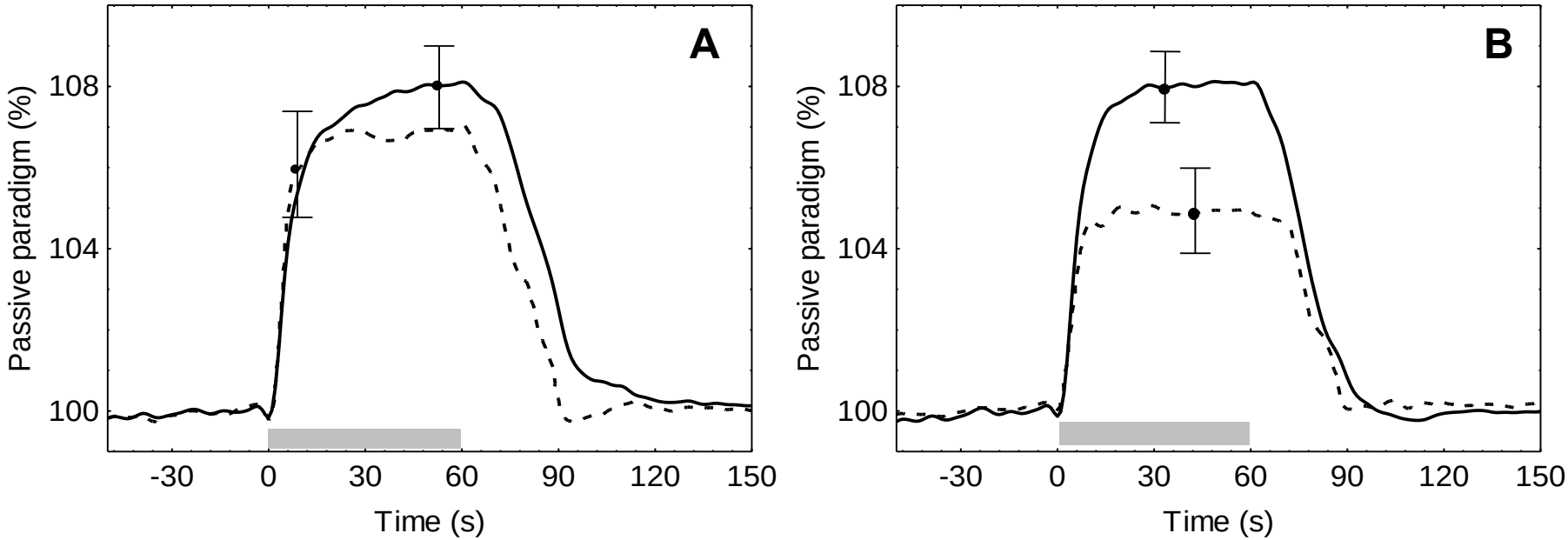

Fig 9.6 Population average of the contributions of the passive motor paradigm. Averages for stroke patients (dashed line) are compared to corresponding averages for controls (continuous line). A) comparison of the unaffected hemisphere of stroke patients with the right hemisphere of controls. B) comparison of the affected hemisphere of strokes with the left hemisphere of controls. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

9.4 Discussion

9.4.1 Main findings

Consistent with our original hypotheses, the present study has revealed two major findings. First, the CBFv step responses to $PaCO_2$ and stimulus showed differences between groups, being significantly reduced in acute stroke. In contrast, no differences in the CBFv step responses to the BP input were found. Secondly, the passive paradigm contributed less to CBFv increase upon neural activation in the stroke group when compared to controls. In combination, these findings suggest an impairment of both NVC and CVR mechanisms in the acute phase of stroke, but not in CA, which could be attributed to the relatively mild severity of the stroke population studied (mean NIHSS 3.5) and to the beneficial effect of the lower levels of $PaCO_2$ (Table 9.1). Nonetheless, overall our results give new insight to understanding the cerebral haemodynamic regulation after a vascular event providing simultaneous assessment of the major brain intrinsic vasoregulatory mechanisms (CA, CVR and NVC) in a single measurement, and emphasizing the importance of the co-variants (such as BP and $PaCO_2$) in influencing CBF modulation.

9.4.2 Clinical and physiological implications

Disturbances of cerebral haemodynamics, characterized by an increased oxygen extraction fraction, reduced vasodilatory reserve, and regional CBF redistribution, can lead to tissue hypoperfusion. In these circumstances, CBF levels might be adequate to sustain brain tissue viability, but not sufficient to maintain intact cerebral function (Ni *et al*., 1994). CBF, as well as other haemodynamic factors such as cerebral blood volume and cerebral haemodynamic reserve, have been related to the final infarct volume and long-term outcome after stroke (Kaps *et al*., 1992, Rubin *et al*., 2000). Moreover, perfusion abnormalities have been shown

to play a critical role in early deterioration (Alawneh *et al*., 2009). The acute physiological perturbations in cerebral haemodynamics may be further influenced by common therapeutic interventions (such as BP treatment and hyperacute physiotherapy interventions). Treatment aimed at the improvement of CBF regulation may play a role in optimizing the functional performance and future quality of life in an ischaemic stroke population. Therefore, the assessment and the potential effects of treatments on the fine-tuned blood flow adaptation and functional outcomes should be considered in the overall treatment strategy for patients with stroke (Aoi *et al*., 2012).

Whilst CA has been previously suggested to be impaired in stroke (Aries *et al*., 2010, Dawson *et al*., 2003, Eames *et al*., 2002), the lower values of ARI in the stroke group did not reach statistical significance in our study. Our seemingly contradictory observations may be explained by previous studies of CA in stroke patients. It is possible that CA was truly preserved in our cohort. Our patients had mild-to-moderate stroke severity, as evidenced by few total anterior circulation strokes, and low scores in NHISS and mRS. Indeed, there is some supporting evidence that CA impairment has been previously associated with poor clinical outcomes following brain injury and acute ischaemic stroke (Aoi *et al*., 2012, Reinhard *et al*., 2010, Reinhard *et al*., 2012). Moreover, our results revealed significantly lower $PaCO_2$ levels at baseline in the stroke participants that is well known to improve CA. In other words, if both groups were normalized to the same level of $PaCO_2$, it could be expected that CA would be significantly less efficient in the stroke group. Whether hypocapnia could be seen as a compensatory mechanism in these patients is an interesting teleological question that remains to be investigated. In line with our results, evidence has been accumulated over many years about the concept that cerebrovascular responsiveness to CO_2 operates independently from CA in healthy and disease states (Garnham *et al*., 1999,

Singhal and Markus, 2005). Although vasoactive dysfunction may partly underlie both cerebrovascular reactivity and autoregulation, it has been suggested that each reflects a different mechanism controlling cerebral blood flow, and interacting in a complex way (Carrera *et al*., 2009, Gommer *et al*., 2008).

Concerning the coupling between neural activity and cerebral haemodynamics, important insights into the specific mechanisms mediating the brain recovery after stroke were derived from neuroimaging studies. A decreased activation of the ischaemic hemisphere and recruitment of additional structures not normally involved in healthy cerebral function has been found (Calautti and Baron, 2003, Small *et al*., 2002, Ward *et al*., 2003). Triggered by the ischaemic cascade, brain oedema, inflammation, impaired neurotransmission and neuronal death are regarded (amongst others factors) as responsible for the neuronal activation dysfunction after stroke (Brouns and De Deyn, 2009).

The data in the stroke patients differed from those of the controls in showing a smaller CBFv response and neural activation contribution bilaterally, although only the affected hemisphere reached statistical significance. The passive movement of the affected arm resulted in CBFv responses somewhat similar to those established in previous studies (Cuadrado *et al*., 1999, Matteis *et al*., 2003). Also, the CBFv responses and their contributors that we observed in controls were similar to those described in previous chapters.

9.4.3 TCD as a Tool of Neurovascular Coupling Investigation

TCD ultrasound has been used extensively as a complementary tool for studying functional reorganization after stroke. Moreover, it has been the dominant technique to study CBF regulatory mechanisms (CA and CVR). Its non-invasiveness, as well as the possibility of obtaining instantaneous information on CBFv variability, makes TCD a reliable method for

monitoring a long term evolution of cerebral damage and CBF changes. Even if TCD is not as specific as other investigational techniques in regards to spatial resolution, it can provide, as suggested by our results and previous studies, rapid haemodynamic information during changes in neural activity.

fMRI is the most popular imaging technique for evaluating cerebral functional recovery (Buma *et al*., 2010, Calautti and Baron, 2003). Concerns about potential inaccuracies and difficulties in interpreting such results have been raised (Altamura *et al*., 2009, Kim *et al*., 2005, Sumiyoshi *et al*., 2012). Using BOLD contrast, the fMRI in a normal brain detects changes in the concentration of deoxyhaemoglobin dependent on a complex interplay among CBF, cerebral blood volume (CBV) and cerebral metabolic rate of oxygen consumption ($CMRO_2$), as stated in section 4.1.1. Triggered by a series of neurochemical processes unleashed after ischaemia, the resistance arterioles nearer to the ischaemic core may be transiently paralysed leading to a transient neurovascular decoupling (Bundo *et al*., 2002, Iadecola, 1998), and possibly disturbing the coupling between CBF and CBV to $CMRO_2$. Consequently, absent and reduced BOLD signals have been described in patients with cerebrovascular disease despite evidence of neural activation recovery (Altamura *et al*., 2009, Carusone *et al*., 2002, Pineiro *et al*., 2002, Rossini *et al*., 2004b). Moreover, the control of changes in BP and $PaCO_2$ and their possible influences on the CBF response have been largely ignored by the fMRI literature.

9.4.4 Limitations of the Study

Apart from those described previously, this study has a number of limitations. First of all, the passive paradigm was performed only with the right side in controls, but either right or left (depending on the affected side) were stimulated in the stroke group. Despite the lack of consensus regarding the relationship between handedness and brain activation, for future

research it would be better having both hemispheres assessed in the control population, and the appropriate hemisphere matched with the affected hemisphere in stroke. Another limitation to consider is the relatively mild stroke cases studied. Importantly, stroke is a heterogeneous condition and future studies should aim to recruit a wider range of stroke severity.

9.5 Conclusion

In conclusion, to my knowledge this is the first study to present an integrated model for assessing cerebral haemodynamics after ischaemic stroke. The results show an impairment of NVC and CVR after acute ischaemic stroke. No impairment of CA was found, but this could be due to the effect of hypocapnia at baseline and the relative mild stroke severity of the studied population. BP, $EtCO_2$ and the sensorimotor paradigm contributed to modulate CBF responses. Moreover, the paradigm contributed less to the CBFv increase in stroke patients when compared to controls, particularly in the affected hemisphere. The results highlight the importance of developing methods that remove the influence of covariates creating more robust and sensitive tools to assess CBF as an index of synaptic activity. Of equal importance, this original method allows the global assessment of the major CBF regulatory mechanisms in a single non-invasive assessment enabling accurate quantification of the CBF regulatory mechanisms during stroke recovery. Knowledge of cerebral haemodynamic regulatory mechanisms is crucial for the investigation of the potential risks and benefits of more effective hyperacute rehabilitative interventions and more aggressive and early management of physiological perturbations in BP to optimise stroke outcome. Future longitudinal studies on a larger population correlating the modelling response with the quality of recovery are needed to provide direction in the development of scientific-based treatment strategies targeting motor recovery.

10 The Natural History of the CBFv response to Active, Passive and Motor Imagery Paradigms after Acute Ischaemic Stroke

10.1 Introduction

Many lines of investigation have attempted to define the mechanisms for stroke recovery in the hope that understanding these mechanisms will improve our ability to enhance the recovery process. After the resolution of local oedema and reperfusion of the ischaemic penumbra, neuronal reorganization and restoration of the cerebral haemodynamic regulation have been proposed to explain further cerebral recovery (Alawneh *et al*., 2009, Dijkhuizen *et al*., 2001, Sumiyoshi *et al*., 2012).

Given the close temporal association between the sequential alterations of cerebral activity/haemodynamics and recovery, many of the dynamic changes observed in the brain may have a restorative function. Recent studies involving patients with variable motor recovery have reported the evolution of cerebral restoration after stroke (Aoi et al., 2012, Bragoni et al., 2000, Brumm et al., 2010, Cramer et al., 1997, Eames et al., 2002, Immink et al., 2005, Lin et al., 2011, Silvestrini et al., 1995a, Silvestrini et al., 1993b). However, most of these studies concerned patients investigated at a single time point, and usually after they reached full recovery.

This calls attention to the urgent need for investigating any changes in the pattern of cerebral haemodynamics over time. A longitudinal design could allow investigators to study, in the same patient, the evolution of the regulation of cerebral haemodynamics and the neural

activation pattern as recovery takes place, and with greater statistical power than possible with cross-sectional studies. Previous attempts to investigate the longitudinal assessment of cerebral activity and cerebral regulation have been limited to only two separate observations (Altamura *et al*., 2009, Askim *et al*., 2009, Calautti *et al*., 2001a, Carey *et al*., 2005, Cuadrado *et al*., 1999, Dawson *et al*., 2003, Matteis *et al*., 2003, Nelles *et al*., 2011, Reinhard *et al*., 2012). To my knowledge, only one of these studies examined patients on more than two occasions in different phases of stroke recovery (acute, subacute and chronic) (Cuadrado *et al*., 1999). Epidemiological studies have suggested that the greatest amount of recovery occurs during the first three months after stroke onset (Bonita and Beaglehole, 1988, Kuptniratsaikul *et al*., 2013), though the cerebral haemodynamic reorganizational processes during this period are largely unknown (Aries *et al*., 2010, Calautti and Baron, 2003, Salinet *et al*., 2013a).

This chapter aimed to investigate the temporal evolution of the changes in the CBF regulation after acute ischaemic stroke over a 3-month period. All stroke patients underwent four CBFv measurements during the acute, subacute and chronic phases. The first question addressed in this Chapter was whether CBFv regulation (assessing CA and NVC mechanisms) is impaired 72 hours after stroke onset. A second question evaluated the changes in CBFv regulation over time to determine the natural history during the recovery period, particularly the CBFv response to active, passive and motor imagery paradigms.

10.2 Methods

10.2.1 Research Participants

Stroke participants fulfilling the criteria described in section 4.6.1. were recruited to this study. Stroke participants were assessed in the acute phase (<72 hours) and at follow up (2

weeks, 1 month and 3 months after stroke onset), and compared with age- and sex-matched controls. For the stroke group, the neurological status was assessed at each session using the NIHSS and disability following stroke was assessed in the first and the last session using the mRS.

10.2.2 Measurements

Recordings were performed as described in section 4.6.2.1. After a period of 15 minutes of stabilization, a 5-minute baseline recording was taken. Then the participants performed three different neural activation paradigms each repeated twice in random order. Movement was performed only with the dominant side. All paradigms began with a 90-second baseline phase. Thereafter the active, passive and motor imagery paradigms were performed over 60 seconds, with a 90-second recovery phase. Recordings were performed as described in section 4.6.2.1. Movement was performed only by the affected side in the stroke group and by the dominant side (right) in controls. Prior to recordings, the paradigm was trialled twice to avoid the need for any verbal instructions during the recordings.

10.2.3 Data Analysis

Data analysis was performed as described in section 4.6.3. In brief, after the signals were calculated with a uniform time-base, averages were performed for each variable synchronized from the beginning of each paradigm. Bilateral CBFv were normalized by their baseline values and population coherent averages and standard deviation curves were obtained for each time sample value. Temporal paradigm-synchronized population averages of the first and second performance of each paradigm were compared to evaluate qualitatively the CBFv response. The paradigm that achieved the highest amplitude of contralateral CBFv response

was chosen to represent the participant`s response at each visit (as described in Chapter 6). If only one recording was available during each paradigm, this signal was used.

As described previously (Section 4.6.4.1), the instantaneous relationship between BP and CBFv was used to estimate CrCP and RAP for each cardiac cycle using the first harmonic method (Panerai, 2003) and the CBFv responses decomposed into its main subcomponents (as described in section 4.6.4.1). In summary, the percentage change in CBFv changes (ΔCBF_v) was decomposed into standardized subcomponents describing the relative contributions of BP (V_{BP}), resistance area product (V_{RAP}) and critical closing pressure (V_{CrCP}). Therefore, the total CBFv changes during activation were represented as the sum of these three subcomponents.

Mean CBFv, heart rate, BP and $EtCO_2$ values were extracted from the 30 seconds preceding each paradigm for baseline comparisons. Baseline BP-step response and ARI were calculated as described in Section 4.6.4.1 for both hemispheres.

10.2.4 Statistical Analysis

Baseline demographics were compared between the two study populations using a Student's t-test for independent samples and Fisher's exact test for nominal data. T-test for independent samples was used to compare baseline values of heart rate, BP and $EtCO_2$ between control and acute stroke participants (session 1). Baseline CBFv values were compared using two-way ANOVA with group (control and patient) as the between factor, and side of recording (right, left, affected, unaffected hemispheres) as the within factor. To compare the effect of the paradigms on CBFv and on peripheral haemodynamics, the area-under-the-curve (AUC) was calculated for their differences from the beginning of the manoeuvre, up to 20 s after the end of the paradigms. Again, a t-test for independent samples was used to compare heart rate,

BP and $EtCO_2$ AUC values between control and stroke (session 1), whereas two-way ANOVA was used to compare CBFv AUC values. Repeated measures ANOVA was used to assess the longitudinal data. Post-hoc comparisons (Tukey's test) were performed when appropriate. Correlation coefficient was calculated among the NIHSS (admission and first assessment scores) and CBFv response (affected and unaffected hemispheres), as well as among NHISS and ARI (affected and unaffected). A value of $p<0.05$ indicated statistical significance.

10.3 Results

10.3.1 Participants' Characteristics

A total of 51 eligible stroke subjects were approached in the hyperacute stroke unit at the Leicester Royal Infirmary and informed consent was obtained from 33 subjects. Poor insonation of the temporal windows lead to exclusion of 5 (female) participants. Eleven participants withdrew the consent after the first measurement, one after the second and one after the third. Therefore, a total 15 acute stroke subjects underwent all four sessions of data collection. Forty healthy controls were recruited in the study, but only twenty-two performed all three paradigms.

Of the fifteen stroke patients, there were two total anterior circulation, seven partial anterior circulation and six lacunar strokes according to the OCSP classification (Bamford *et al*., 1991). Demographic and clinical characteristics of the participants included in this chapter are summarized in Table 10.1. The acute assessment (session 1) was undertaken a mean (SD) of 36.6 hours (15.0) after the stroke onset, whereas the subacute (session 2) and chronic phases (sessions 3 and 4) were on average 14.0 (1.0), 30.7 (1.2) and 94.2 days (0.7) after stroke onset.

Table 10.1 Participant characteristics

Variables		**Stroke n= 15**	**Controls n=22**
Age, years (SD)		62.4 (9.0)	62.2 (7.5)
Male		12	16
Edinburgh Inventory, % (SD)		84.4 (6.2)	89.9 (3.6)
Hypertension		5	
MI		2	
Thrombolysis		2	
Diabetes mellitus		0	
Hypercholesterolaemia		1	
Ipsilateral ICA stenosis	>50	1*	
	30 to 49%	1*	
	<30%	1	
Contralateral ICA stenosis	30 to 49%	2	
Onset to assessment, hours (SD)		36.6 (15.5)	
NIHSS, (SD)	admission	7.8 (4.8)	
	session 1	3.4 (1.9)	
	session 2	1.9 (1.8)	
	session 3	1.2 (1.6)	
	session 4	0.7 (1.3)	
mRS,(SD)	session 1	2.1 (1.2)	
	session 2	0.4 (0.5)	

* ipsilateral and contralateral ICA stenosis

10.3.2 Baseline Recordings

Resting values of the recorded parameters during the 5-minute baseline period are illustrated in Table 10.2. Though bilateral CBFv in the stroke group during session 1 (< 72 hours) was lower than the control group (two-way ANOVA group interaction F= 4.1 p= 0.03), only the affected hemisphere CBFv was statistically lower than the left hemisphere in controls (Tukey's p= 0.02). A gradual increase in the affected hemisphere CBFv was seen on follow-up. On the other hand, the unaffected hemisphere CBFv dropped at the session 2 (decrease seen in 11/15 patients) which coincided with the drop in ARI values (Table 10.3). However, no statistical difference between assessments was found in the repeated measures ANOVA. Stroke participants were consistently more hypertensive (p= 0.03), tachycardic (p= 0.009) and hypocapnic (p= 0.003) than controls showing the same pattern over time. Though CrCP

and RAP values were higher in controls, no statistical difference was found between groups, as well as, between assessments (repeated measures ANOVA). Similar to the 5-minute baseline values, the resting values of the affected CBFv, BP, heart rate and $EtCO_2$ extracted from the 30s preceding the paradigms performance were significantly different between stroke (session 1) and controls (Table 10.4). No statistically significant correlation was found between ARI (affected and unaffected) and NIHSS (admission and first session) scores.

Table 10.2 Mean baseline values (SD) of CBFv, heart rate, BP and $EtCO_2$ during 5min resting period**.**

	CBFv, $cm.s^{-1}$		**BP, mmHg**	**HR, bpm**	**$EtCO_2$, mmHg**
	right/ unaffected	**left/ affected**			
control	48.9 (4.9)	50.7 (5.6)	87.2 (11.0)	58.8 (8.0)	37.7 (3.2)
session 1	49.5 (10.1)	45.4 (6.9)*	91.9 (8.7)*	65.3 (7.7)*	35.1 (2.6)*
session 2	43.9 (9.9)	48.3 (8.6)	93.7 (8.4)	68.5 (10.3)	35.6 (5.3)
session 3	46.0 (6.1)	48.8 (9.9)	93.8 (9.6)	64.3 (8.8)	34.9 (2.1)
session 4	47.9 (9.5)	49.0 (9.8)	94.8 (7.6)	67.0 (9.3)	35.4 (2.1)

* Tukey post-hoc $p < 0.02$ for the comparison between stroke (session 1) and control.

Table 10.3 Mean baseline values of ARI

	ARI	
	right/ unaffected	**left/ affected**
control	6.0 (0.9)	5.7 (0.9)
session 1	5.6 (1.3)	5.5 (1.3)
session 2	4.6 (1.3)*	4.7 (1.2)*
session 3	5.1 (1.3)	5.2 (1.8)
session 4	5.3 (1.0)	5.6 (1.0)

* $p<0.05$, Tukey's post-hoc for the differences between assessment 1 and 2

Table 10.4 Mean (SD) values for cerebral and peripheral haemodynamic variables for the baseline preceding the active, passive and motor imagery paradigms

	CBFv, $cm.s^{-1}$		BP, mmHg	HR, bpm	$EtCO_2$, mmHg	CrCP, mmHg		RAP, mmHg.s/cm	
	right/ unaffected	left/ affected				unaffected/right	left/ affected	unaffected/ right	left/ affected
Active									
control	49.1 (6.6)	50.3 (5.1)	85.3 (10.5)	59.3 (7.6)	37.8 (4.9)	18.6 (9.3)	19.8 (8.9)	1.4 (0.9)	1.6 (0.5)
session 1	49.5 (9.7)	45.9 (7.9)*	92.6 (9.0)*	64.9 (8.3)*	35.4 (2.9)*	11.7 (14.5)	13.6 (10.0)	2.0 (0.9)	1.6 (0.5)
session 2	44.1 (9.4)	48.1 (9.9)	93.3 (8.1)	68.7 (9.5)	36.1 (4.0)	13.5 (13.0)	12.5 (10.9)	1.9 (0.8)	1.5 (0.3)
session 3	46.8 (7.8)	47.8 (9.9)	93.3 (10.1)	66.8 (9.9)	35.6 (2.7)	13.6 (12.6)	14.1 (10.6)	1.9 (0.7)	1.7 (0.5)
session 4	47.7 (9.1)	48.2 (8.8)	94.0 (6.6)	66.4 (9.0)	35.6 (2.8)	14.3 (13.6)	14.8 (12.0)	1.8 (0.6)	1.8 (0.5)
Passive									
control	49.5 (5.5)	52.9 (5.0)	87.2 (11.0)	59.1 (7.6)	39.1 (3.1)	18.6 (10.2)	19.6 (8.4)	1.6 (0.5)	1.4 (0.5)
session 1	48.8 (8.0)	47.1 (9.2)*	93.6 (8.0)*	67.9 (9.6)*	34.8 (2.3)*	11.7 (15.1)	11.7 (13.1)	1.8 (0.9)	1.6 (0.5)
session 2	44.3 (9.6)	47.3 (8.8)	91.5 (7.6)	64.9 (9.7)	35.6 (3.4)	10.5 (13.3)	10.4 (12.0)	2.1 (0.9)	1.8 (0.6)
session 3	45.7 (8.0)	48.1 (9.6)	91.7 (7.5)	65.4 (10.0)	35.8 (3.2)	11.5 (17.2)	13.0 (9.9)	2.1 (0.9)	1.7 (0.7)
session 4	48.6 (8.9)	49.2 (8.9)	91.7 (7.6)	64.6 (10.3)	36.1 (2.3)	10.2 (14.5)	10.4 (12.0)	2.1 (0.9)	1.8 (0.6)
MI									
control	48.9 (6.2)	50.5 (4.9)	84.6 (11.0)	58.8 (8.0)	37.9 (5.5)	18.1 (8.8)	20.2 (9.4)	1.6 (0.6)	1.4 (0.5)
session 1	49.1 (9.5)	46.1 (8.6)*	92.0 (8.5)*	69.8 (8.4)*	35.3 (2.3)*	13.2 (11.8)	14.9 (9.5)	1.9 (0.7)	1.8 (0.6)
session 2	45.9 (9.9)	48.9 (9.1)	91.1 (7.3)	66.8 (8.9)	35.7 (3.6)	12.1 (12.3)	11.3 (10.6)	2.0 (0.7)	1.6 (0.6)
session 3	46.0 (8.5)	48.1 (9.4)	92.7 (8.0)	67.0 (9.0)	35.7 (4.3)	12.6 (9.9)	12.3 (8.8)	1.7 (0.5)	1.6 (0.5)
session 4	48.7 (9.3)	48.8 (9.6)	92.3 (9.0)	65.0 (8.4)	35.8 (2.4)	12.1 (10.1)	12.8 (13.0)	1.7 (0.4)	1.8 (0.7)

BP, blood pressure; HR, heart rate; $EtCO_2$, end-tidal CO_2; CrCP, critical closing pressure; RAP, resistance area product

* Tukey post-hoc $p < 0.01$ for the comparison between stroke (session 1) and control.

10.3.3 Sensorimotor Paradigms

All 15 patients performed the passive paradigm in the acute session as well as during the subsequent sessions. However, two participants were not able to voluntarily move their elbow and five were cognitively unable to mentally imagine the elbow movement in the acute phase. Therefore, only 13 and 10 stroke participants were included in the analysis of the active and motor imagery paradigms, respectively. Although five stroke participants performed the three paradigms just once in the acute phase, a complete set was recorded in sessions 2 to 4. The correlation between the admission NIHSS score was only significant with the affected hemisphere CBFv responses to the passive paradigm ($r= 0.74$ $p= 0.01$).

10.3.3.1 Active Paradigm

The active paradigm led to a bilateral increase in the CBFv in controls, as well as in all stroke phases (Figs. 10.1 & 10. 2). Fig. 10.1 illustrates the CBFv response in the right and unaffected (stroke) hemisphere at the acute/subacute (Fig. 10.1A) and chronic phases (Fig. 10.1B). With the exception of the 1-month assessment, the temporal pattern of the CBFv response was similar in both groups. CBFv increased sharply as soon as the active paradigm started followed by a gradual decrease up to 80 s into the response. In agreement with this, the left/affected CBFv responses showed a similar temporal pattern in the acute/subacute (Fig.10.2A) and chronic (Fig. 10.2B) phases, and the CBFv increase was significantly lower in the acute phase compared to control (Tukey's $p= 0.008$, Table 10.5). Though CBFv responses reached lower levels in all stroke phases, no statistical difference over time was found (Table 10.5).

Fig. 10.3 illustrates the paradigm-synchronized averages for BP in the acute/subacute (Fig. 10.3A) and chronic phase (Fig. 10.3B). A different temporal pattern of BP changes between

groups was seen. Though both groups showed an initial rise approximately 5 s after paradigm onset and extending to a few seconds after the end of the exercise, the rise was sustained throughout the paradigm performance in the stroke group. Heart rate also rose slightly during the active paradigm performance in both groups, decreasing gradually until the paradigm stopped (Fig. 10.4). Contrary to CBFv (Fig. 10.1), both BP and heart rate started to return to baseline values as soon as the paradigm was completed. Despite the tendency of $EtCO_2$ to decrease (due to hyperventilation) during the paradigm performance, no characteristic pattern was observed in the $EtCO_2$ response (Fig. 10.5).

Table 10.5 shows the mean values extracted from the beginning of the paradigm (second 0) to 20s after the paradigm (second 80). To avoid the interference of the differences in baseline values (described above – Section 10.2.2), the AUC analysis was performed with mean values normalised by baseline. A significant difference between controls and acute stroke (session 1) was only found in the percentage of BP increase of 102.9 (1.2) in controls and 106.0 (2.1) in acute stroke; p= 0.004.

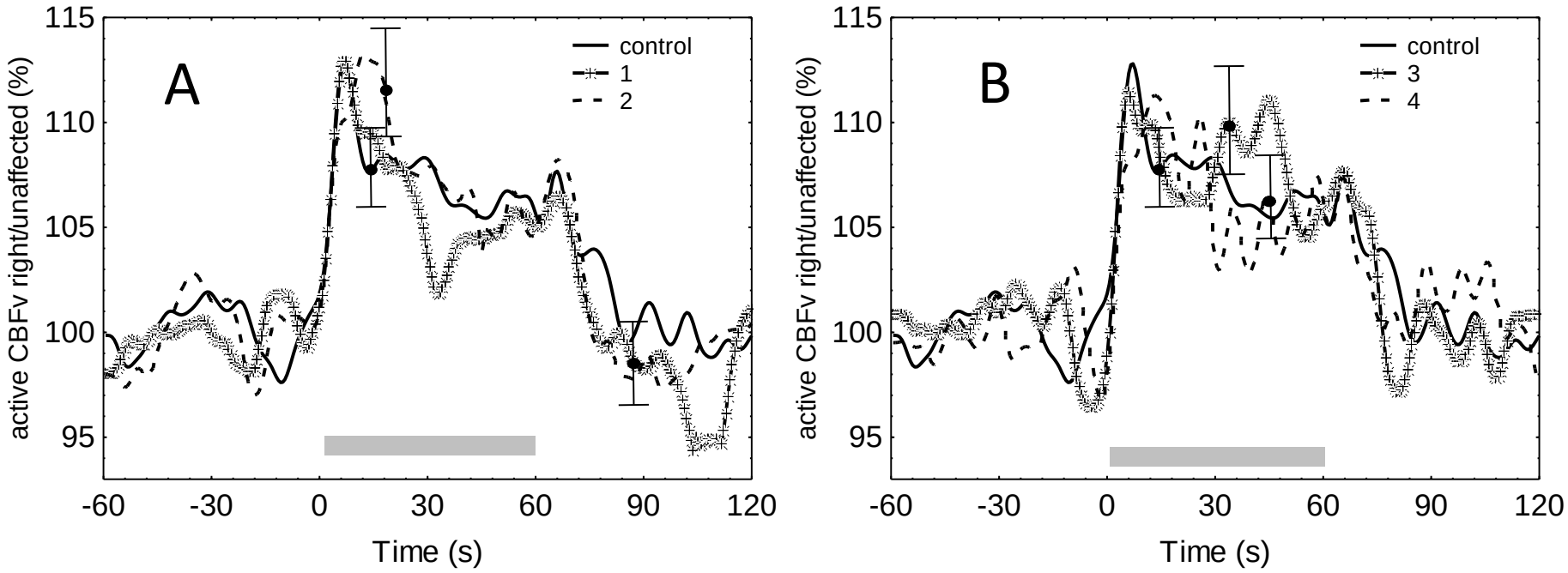

Fig 10.1 Population averages of CBFv in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the active paradigm. The grey bar shows the duration of the paradigm. The plots correspond to the comparison of the unaffected hemisphere of stroke with the right hemisphere of controls in acute (A) and chronic (B) phases. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

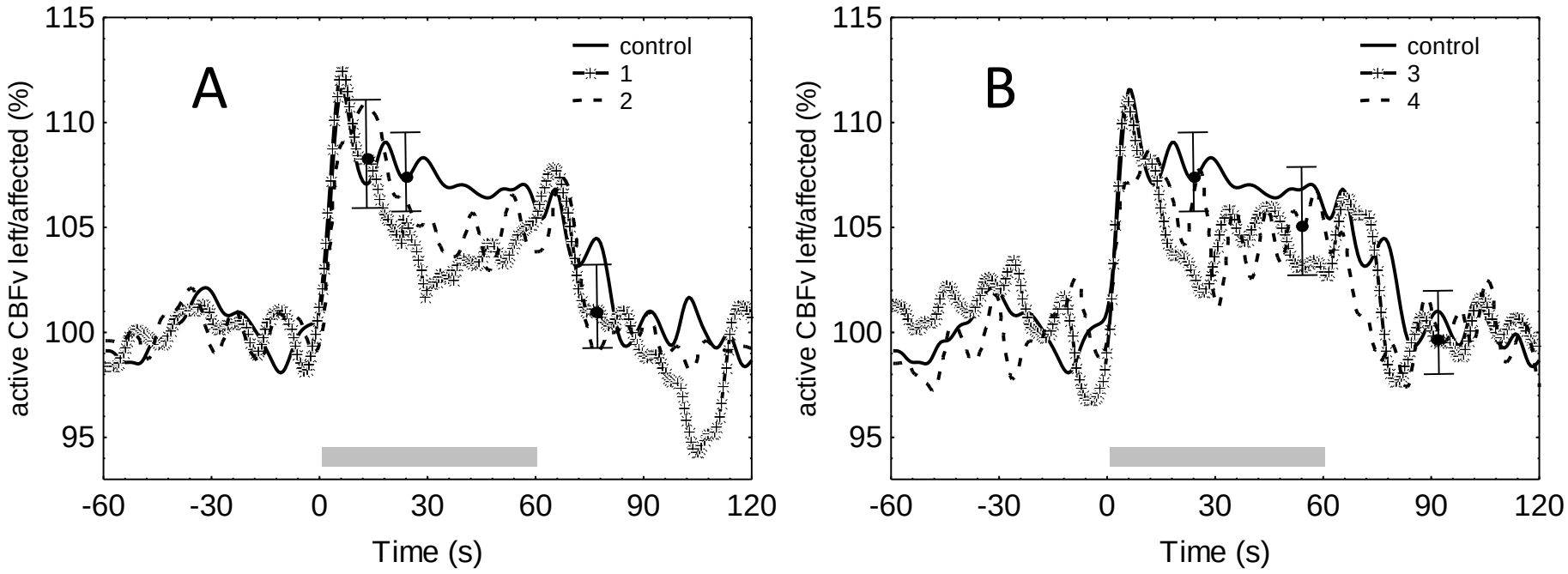

Fig 10.2 Population averages of CBFv in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the active paradigm. The grey bar shows the duration of the paradigm. The plots correspond to the comparison of the affected hemisphere of stroke with the left hemisphere of controls in acute (A) and chronic (B) phases. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

Table 10.5 Mean values (SD) for area under the curve (AUC) for differences in CBFv variation, BP, heart rate and $EtCO_2$ between patients (assessment 1 to 4) and controls.

	CBFv, %		BP, mmHg	HR, bpm	$EtCO_2$, mmHg
	right/ unaffected	left/ affected			
Active					
control	106.3 (6.0)	107.8 (5.6)	87.6 (10.7)	63.2 (8.7)	37.7 (4.6)
session 1	104.9 (4.9)	104.0 (5.1)*	97.6 (11.0)°	70.9 (10.4)	34.5 (2.6)
session 2	106.7 (6.6)	104.9 (4.9)	95.5 (9.3)	70.2 (9.7)	35.0 (3.1)
session 3	107.1 (6.9)	105.2 (5.6)	95.1 (8.1)	69.2 (8.9)	35.4 (3.9)
session 4	105.6 (5.1)	105.7 (6.1)	96.3 (6.8)	68.4 (11.0)	35.4 (2.4)
Passive					
control	107.8 (6.2)	108.3 (5.5)	90.5 (10.5)	60.7 (8.0)	38.8 (3.0)
session 1	105.4 (5.3)ϕ	104.1 (4.9)*	94.1 (9.3)	69.8 (10.9)	34.6 (2.4)
session 2	105.5 (4.3)	104.9 (2.9)	92.2 (10.1)	69.4 (11.7)	35.5 (2.7)
session 3	110.0 (6.9)¥	107.0 (4.7)	91.4 (8.9)	64.8 (10.4)	35.9 (3.2)
session 4	105.9 (5.8)	106.4 (4.8)	90.9 (8.1)	64.3 (12.8)	36.3 (2.3)
MI					
control	104.0 (4.5)	103.8 (4.1)	87.9 (10.2)	61.2 (8.7)	37.7 (4.9)
session 1	105.8 (5.1)	107.2 (5.1)	94.5 (10.0)	70.9 (11.3)	35.7 (3.5)
session 2	106.3 (6.4)	107.8 (5.5)	95.5 (9.2)	68.5 (11.0)	34.2 (4.2)
session 3	110.1 (7.8)	109.5 (6.7)	92.1 (11.0)	67.0 (9.3)	35.9 (4.3)
session 4	108.6 (6.3)	108.5 (5.1)	94.7 (10.1)	67.0 (11.0)	35.8 (1.9)

BP, blood pressure; HR, heart rate; $EtCO_2$, end-tidal CO_2

* Tukey's $p<0.01$ for the differences between control (left hemisphere) and stroke (affected hemisphere) session 1

° T-test $p=0.004$ for the differences of the normalised values of control and stroke assessment 1

ϕ Tukey's $p=0.06$ for the differences between control (right hemisphere) and stroke (unaffected hemisphere) session 1

¥ Tukey's $p<0.04$ between session 1 and 3, between session 2 and 3, between session 3 and 4

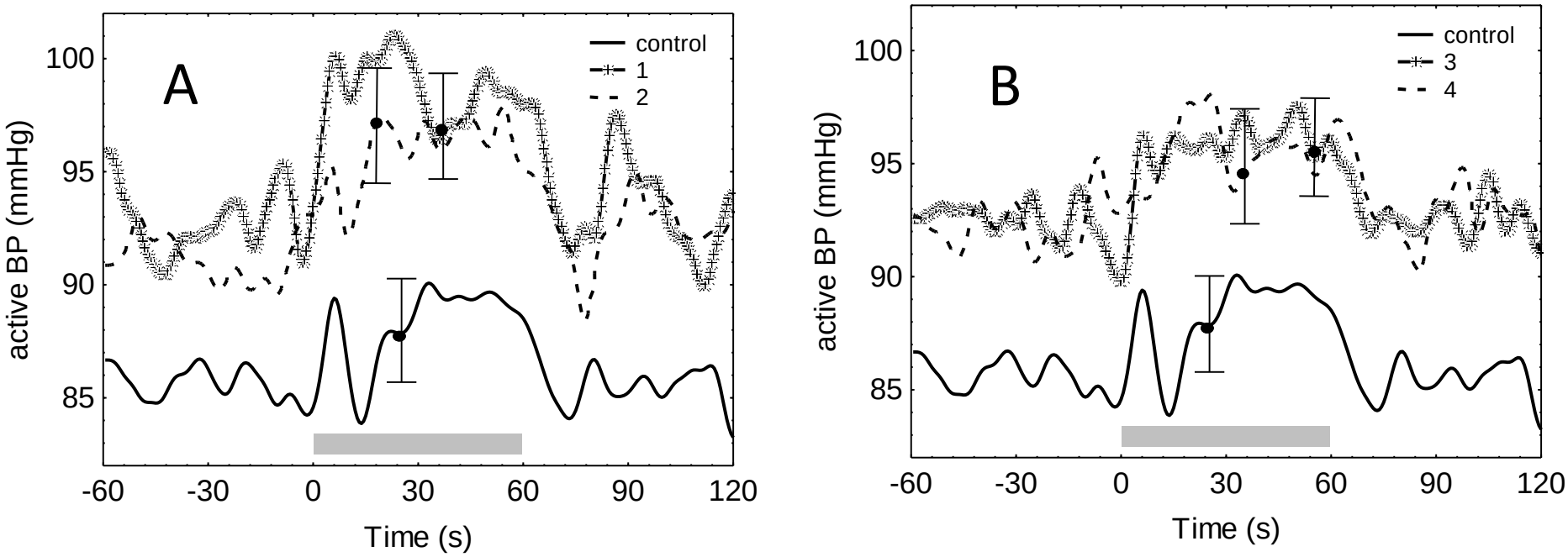

Fig 10.3 Population averages of changes in arterial BP in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the active paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

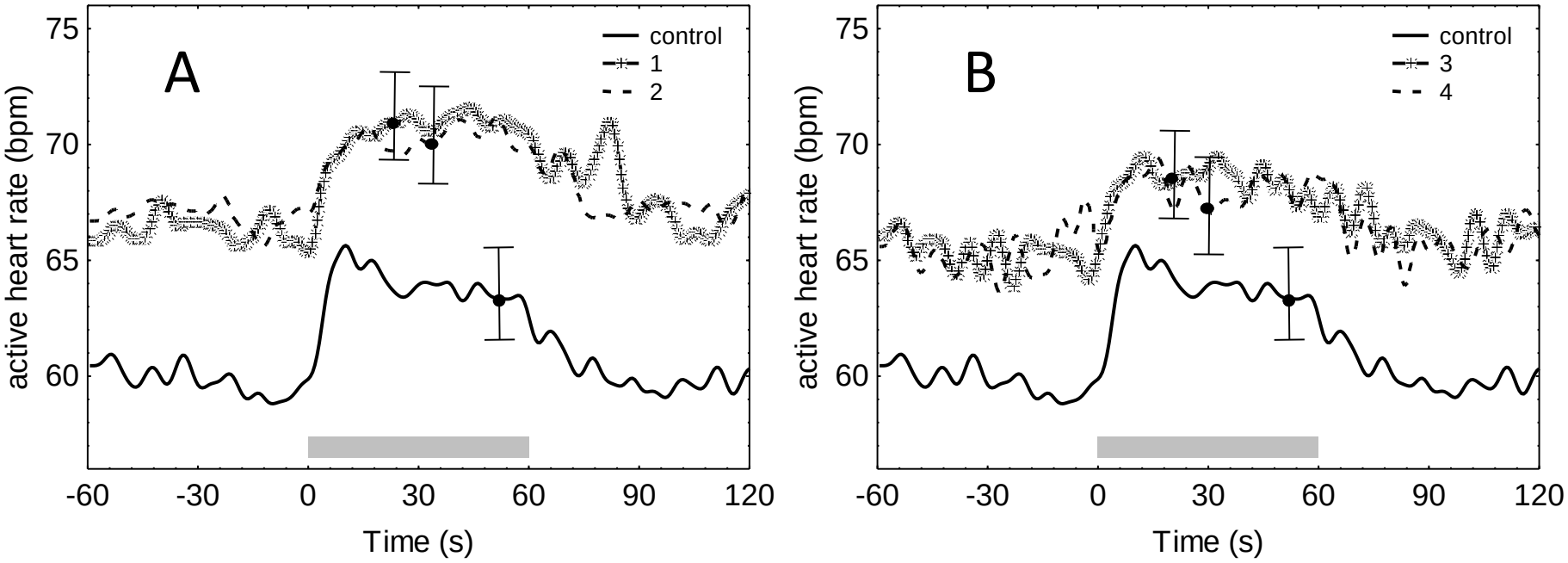

Fig 10.4 Population averages of changes in heart rate in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the active paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

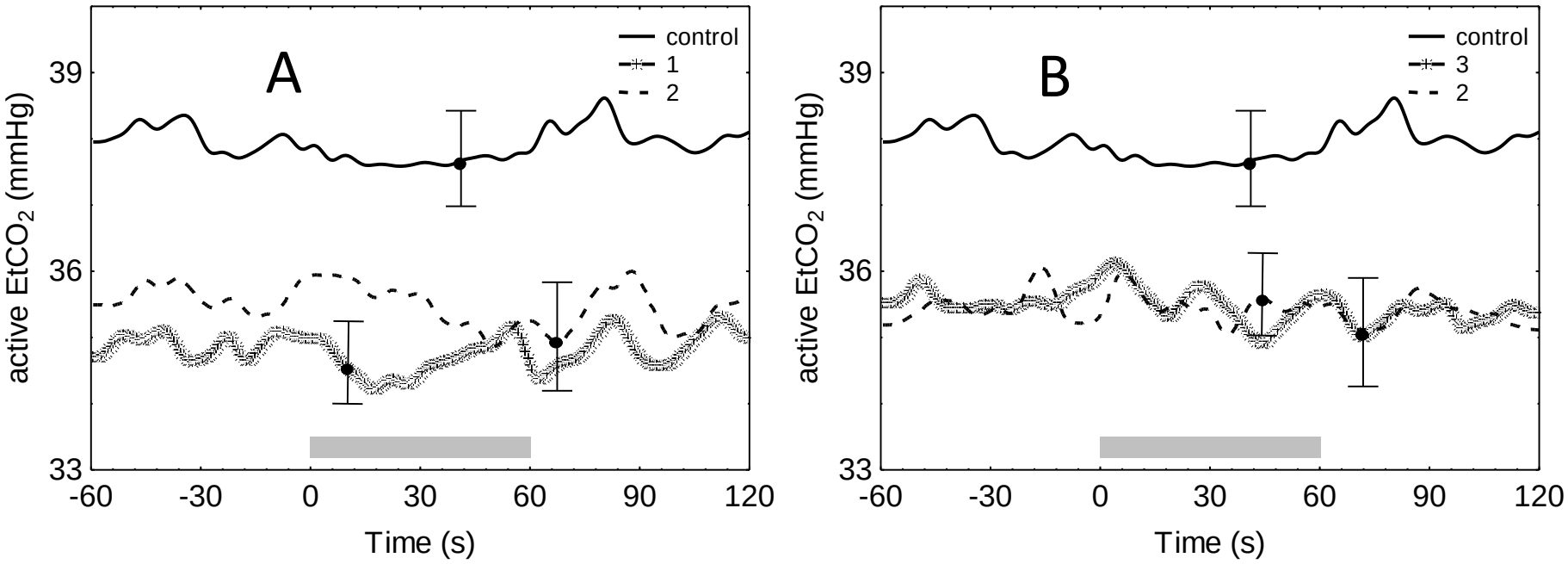

Fig 10.5 Population averages of changes in $EtCO_2$ in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the active paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

10.3.3.2 Passive Paradigm

The pattern of cerebral haemodynamic response in controls showed a steep bilateral rise in CBFv approximately 5s after the beginning of the task reaching a peak after 15s, and was maintained until approximately 20s after the end of the exercise (Figs. 10.6&10.7). Though the sharp increase in CBFv bilaterally, as well as a gradual return to baseline was also observed in stroke, the CBFv was less pronounced (with the exception of the unaffected response in session 3) and the return to baseline levels was faster in the acute/subacute phase. This was supported by the AUC analysis. Significant group interaction was found in ANOVA ($F= 5.4$ $p= 0.001$) between control and acute stroke (session 1). Tukey's post-hoc revealed differences between left and affected hemispheres ($p= 0.001$) and a marginal significance ($p= 0.05$) between right and unaffected hemispheres. As also observed in the active paradigm, the first assessment in the chronic phase (session 3) showed a slightly different pattern of response (Fig. 10.6B) confirmed by the repeated measures ANOVA (Table 10.5). CBFv values in the affected hemisphere increased over time, but no statistical differences were found.

The paradigm-synchronized averages for BP, heart rate and $EtCO_2$ during the passive paradigm are represented in Figs. 10.8, 10.9 & 10.10, respectively. Reviewing the BP and heart rate responses, the passive paradigm led to a slight increase in their values as soon as the metronome was turned on (grey bar). In keeping with the active paradigm, the BP rise in stroke was sustained throughout the performance of the passive paradigm whereas an initial peak approximately 5s after paradigm onset and a second higher peak around ~ 35s were seen in controls (Fig. 10.8). The AUC mean values are expressed in Table 10.5 and their normalised values did not show significant differences between groups or sessions. $EtCO_2$ varied slightly, but once again showing no specific temporal pattern of response (Fig. 10.10).

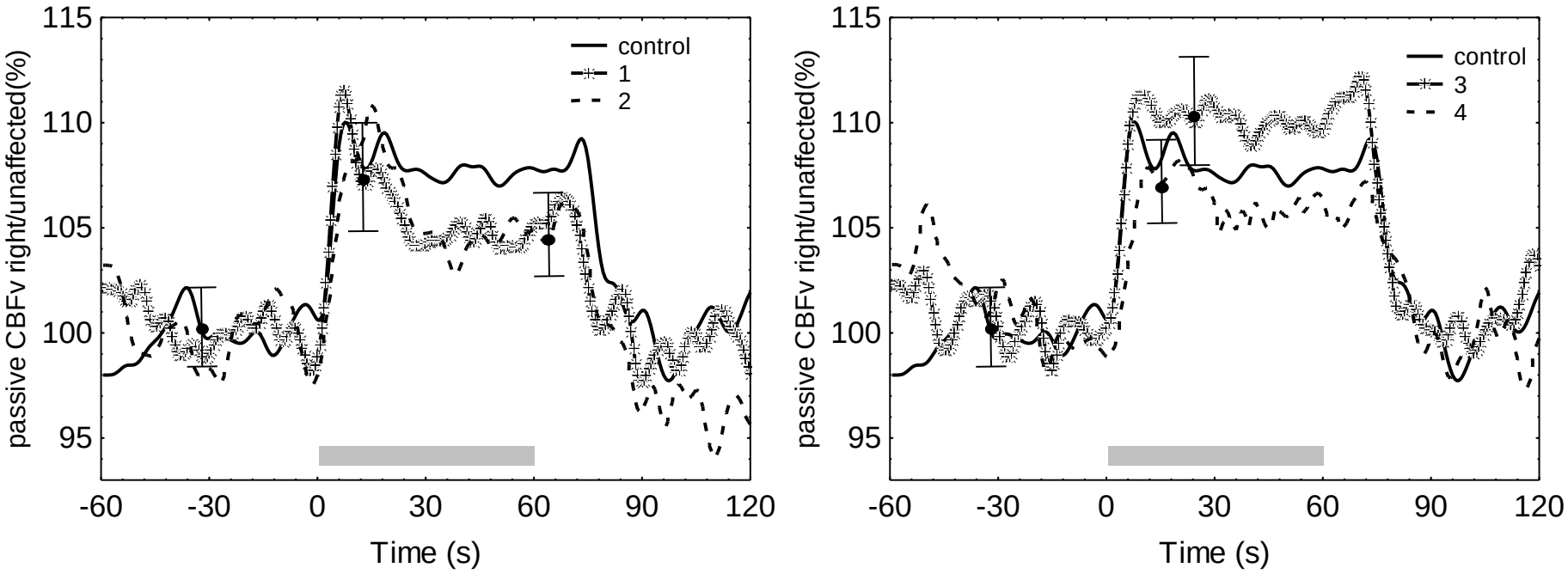

Fig 10.6 Population averages of CBFv in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The grey bar shows the duration of the paradigm. The plots correspond to the comparison of the unaffected hemisphere of stroke with the right hemisphere of controls in acute/subacute (A) and chronic (B) phases. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

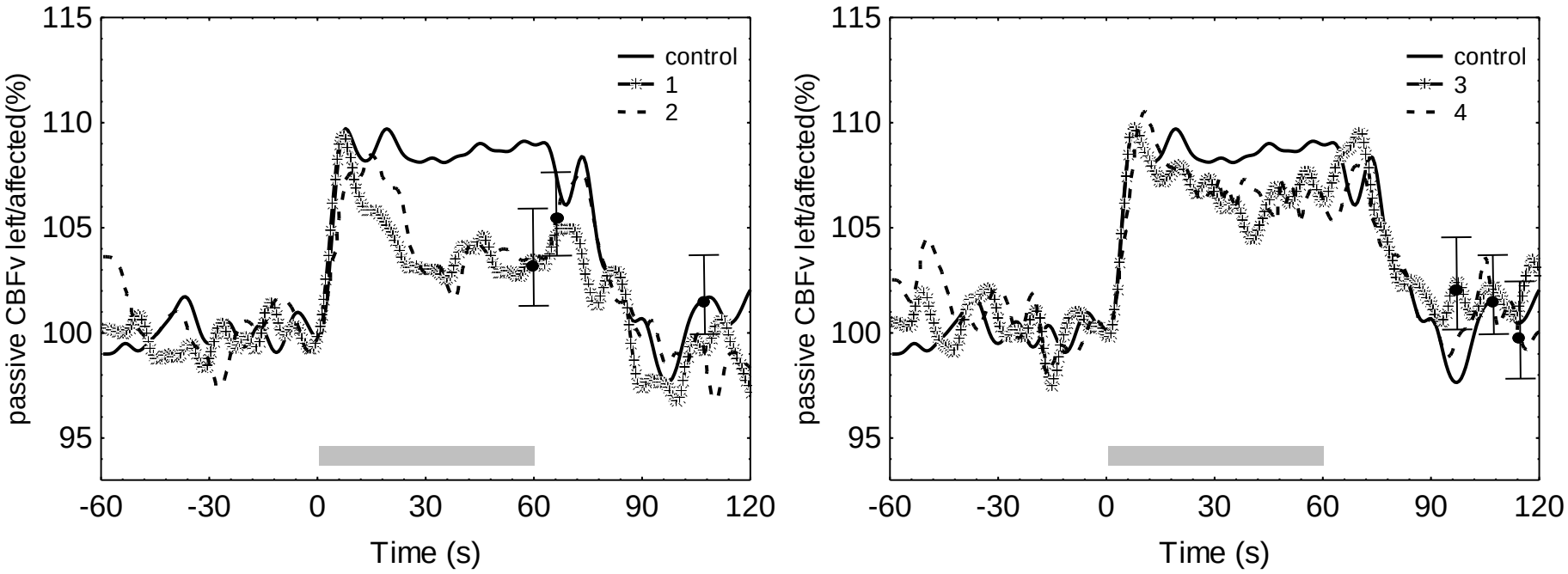

Fig 10.7 Population averages of CBFv in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The grey bar shows the duration of the paradigm. The plots correspond to the comparison of the affected hemisphere of stroke with the left hemisphere of controls in acute/subacute (A) and chronic (B) phases. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

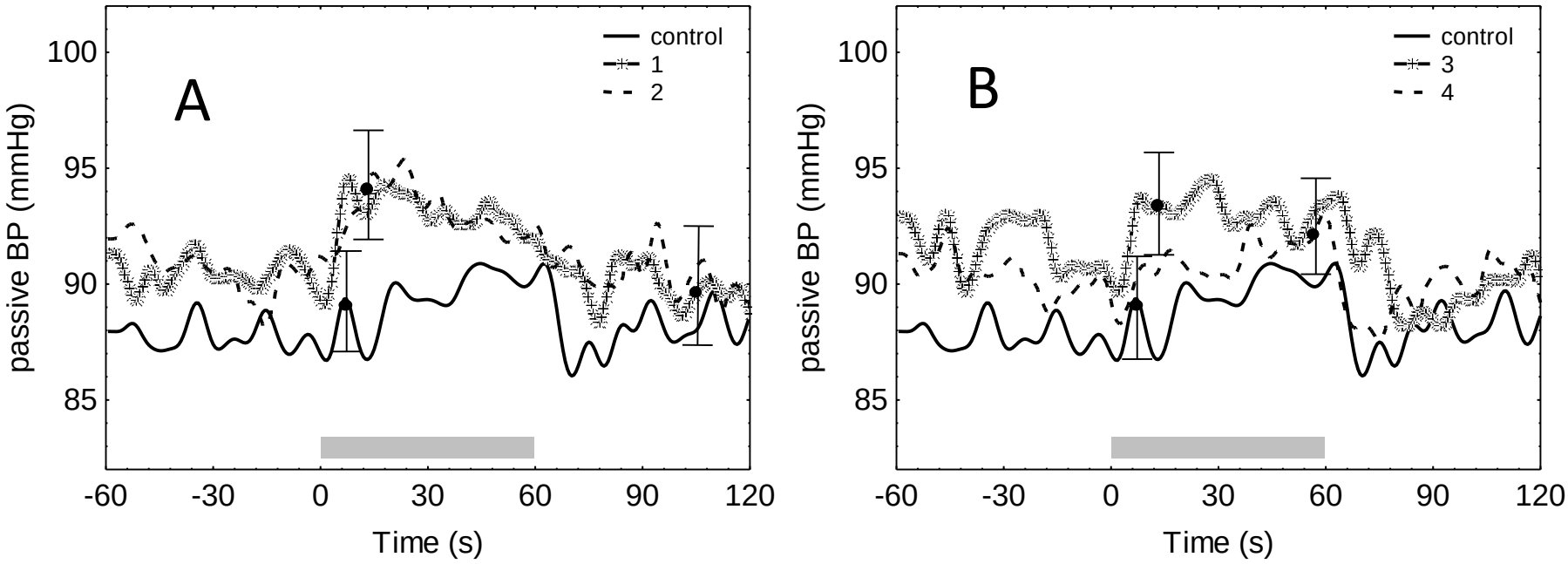

Fig 10.8 Population averages of changes in arterial BP in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

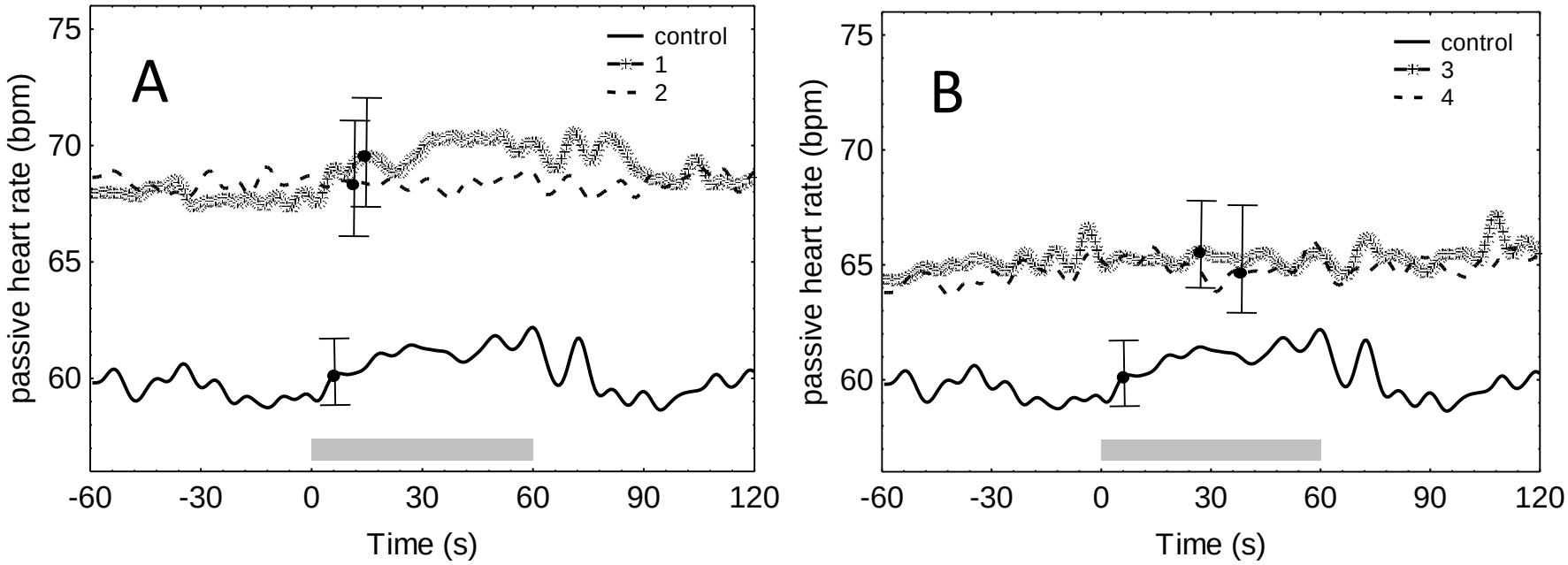

Fig 10.9 Population averages of changes in heart rate in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

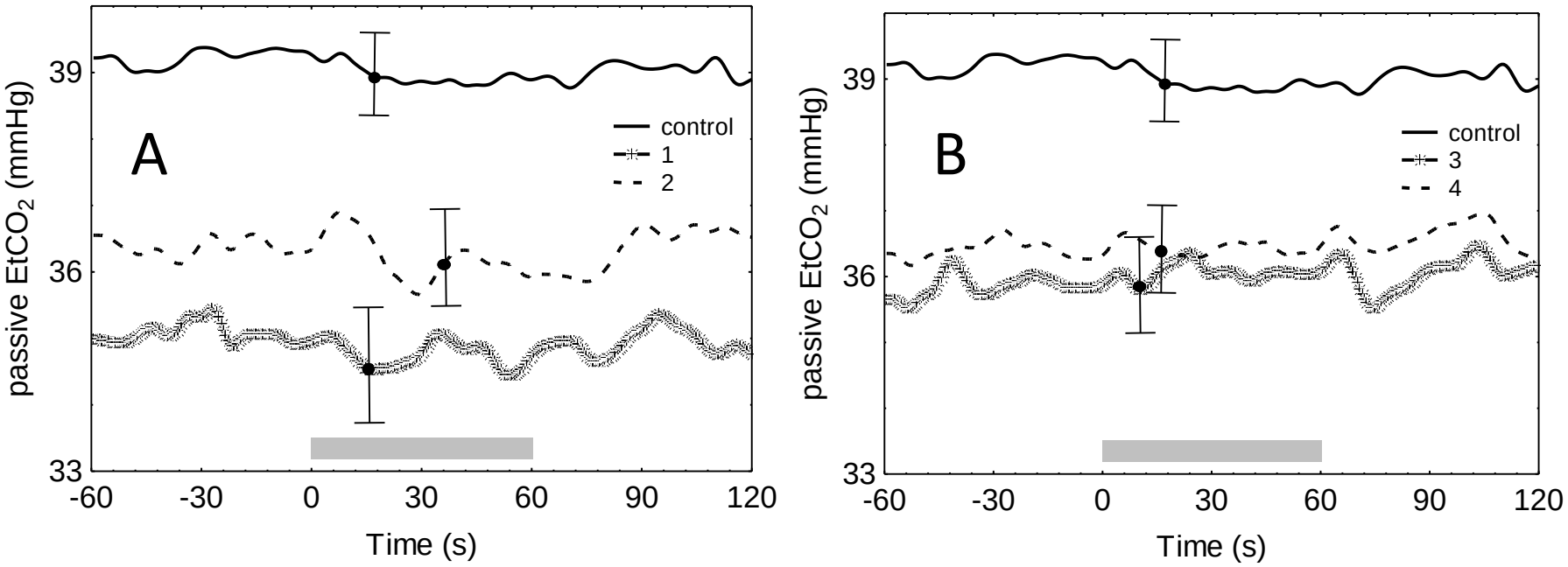

Fig 10.10 Population averages of changes in $EtCO_2$ in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

10.3.3.3 Motor Imagery Paradigm

Contrary to the other two paradigms, motor imagery led to a greater bilateral CBFv increase in stroke compared to controls, particularly in the chronic phases. However, no statistical significance was found either in the difference between controls and stroke, or among the four sessions as illustrated in Table 10.5. Session 1 (<72h) and control CBFv responses followed the same temporal pattern peaking straight after the beginning of the exercise and gradually decreasing to baseline values around 80s after paradigm onset (Figs. 10.11A & 10.12A). However, during sessions 2 to 4, CBFv showed a steep rise approximately 5s after the beginning of the task reaching a peak after 15s, and was maintained until approximately 30s (second 90) after the end of the paradigm (Figs. 10.11A&B and 10.12A&B).

The temporal pattern of BP variation during the motor imagery paradigm is represented in Fig. 10.13. An initial rise peaking approximately 5s after paradigm onset was seen in controls, as well as in acute and subacute phase of stroke (Fig. 10.13A). The heart rate did not change dramatically during the paradigm (Fig. 10.14 A&B). Despite slight variation (particularly during sessions 1 and 2), no characteristic pattern was again observed in the $EtCO_2$ response (Fig. 10.15A&B). The amplitude of such peripheral responses is represented in Table 10.5. ANOVA did not reveal statistical differences among groups or assessments when the AUC averages were normalised.

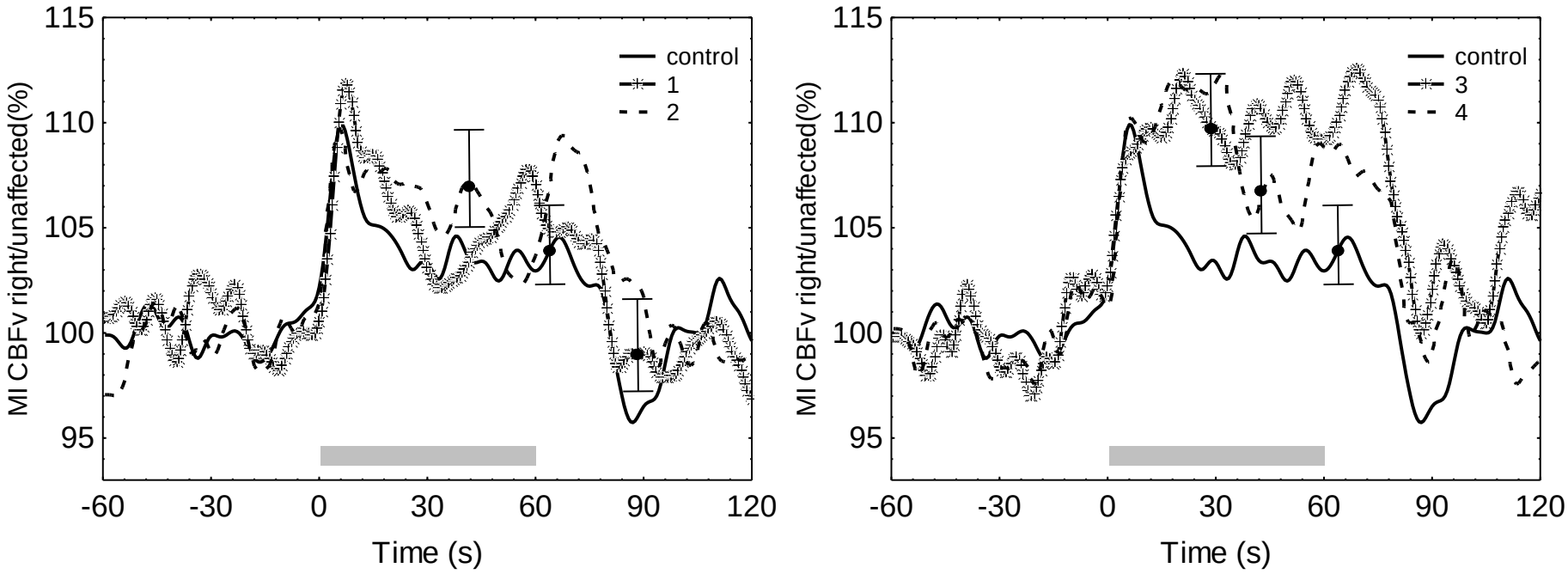

Fig 10.11 Population averages of CBFv in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the motor imagery (MI) paradigm. The grey bar shows the duration of the paradigm. The plots correspond to the comparison of the unaffected hemisphere of stroke with the right hemisphere of controls in acute/subacute (A) and chronic (B) phases. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

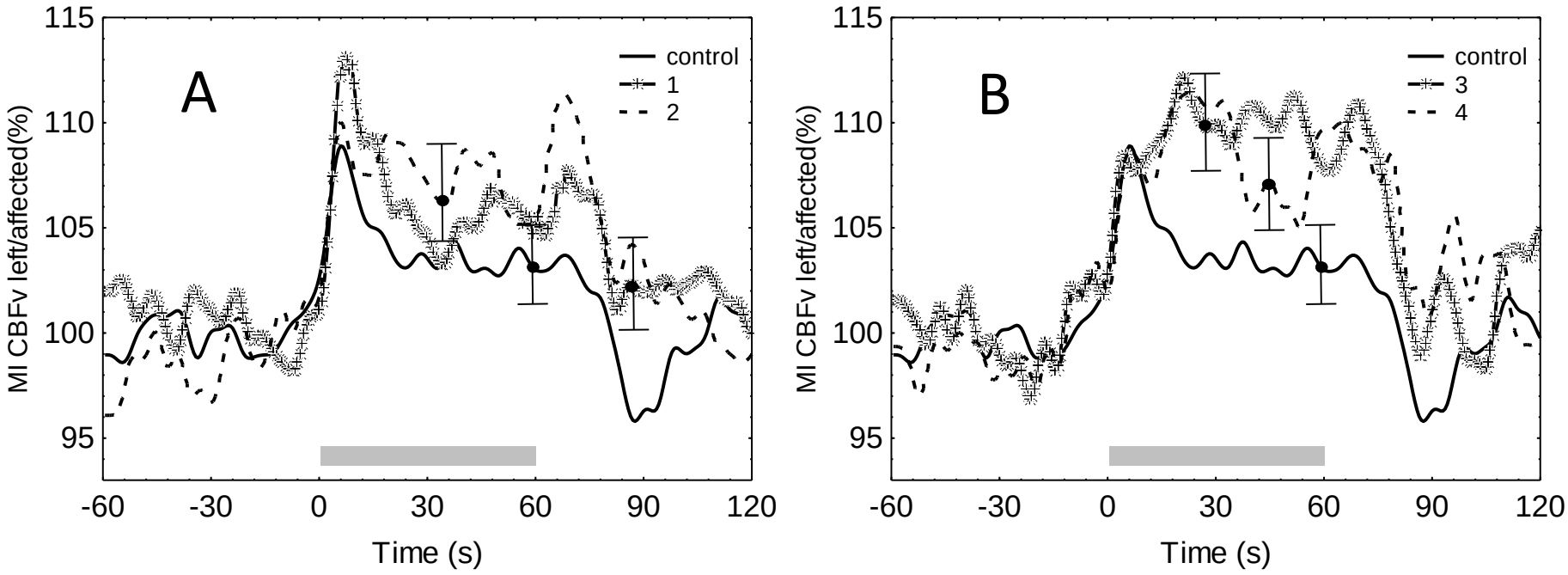

Fig 10.12 Population averages of CBFv in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the motor imagery (MI) paradigm. The grey bar shows the duration of the paradigm. The plots correspond to the comparison of the affected hemisphere of stroke with the left hemisphere of controls in acute (A) and chronic (B) phases. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

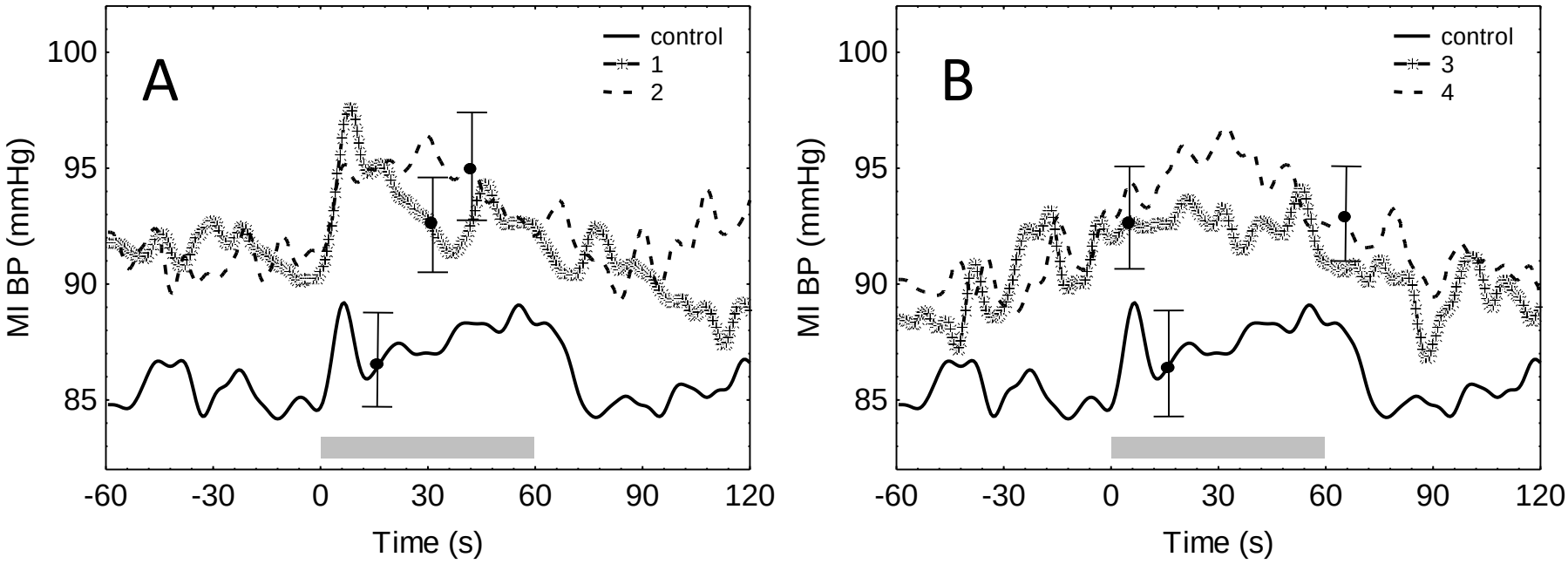

Fig 10.13 Population averages of changes in arterial BP in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the motor imagery (MI) paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

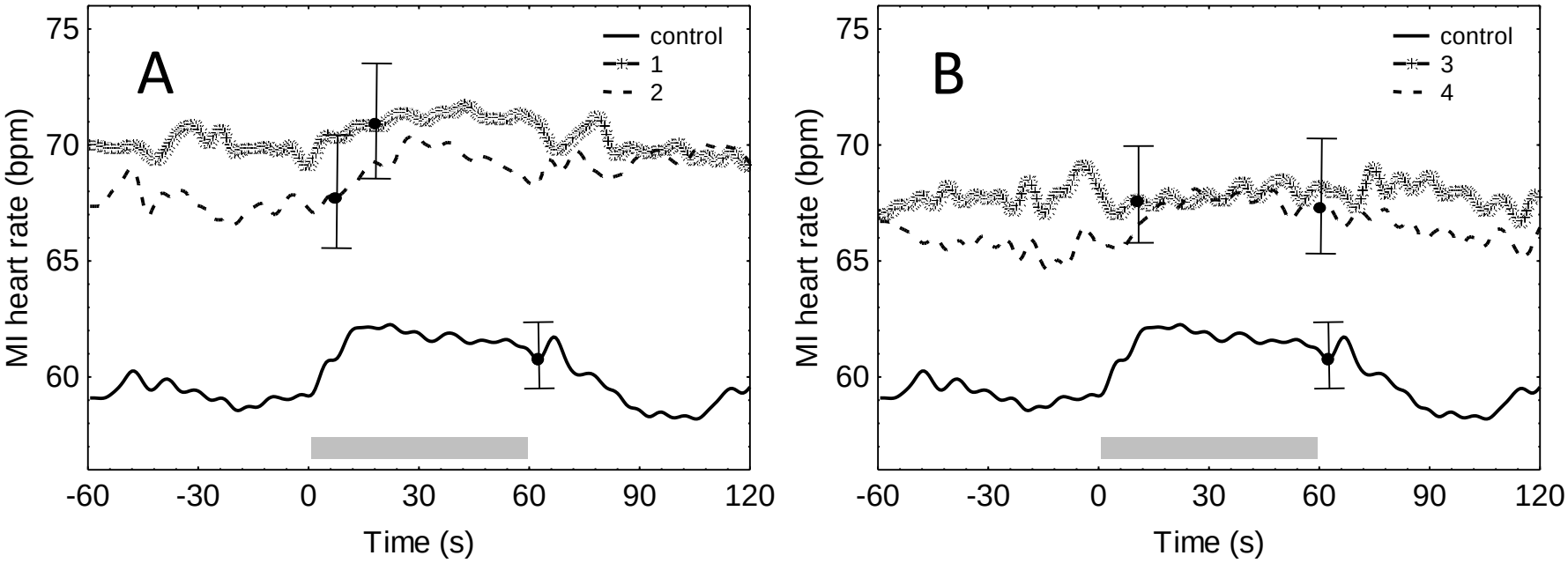

Fig 10.14 Population averages of changes in heart rate in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the motor imagery (MI) paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

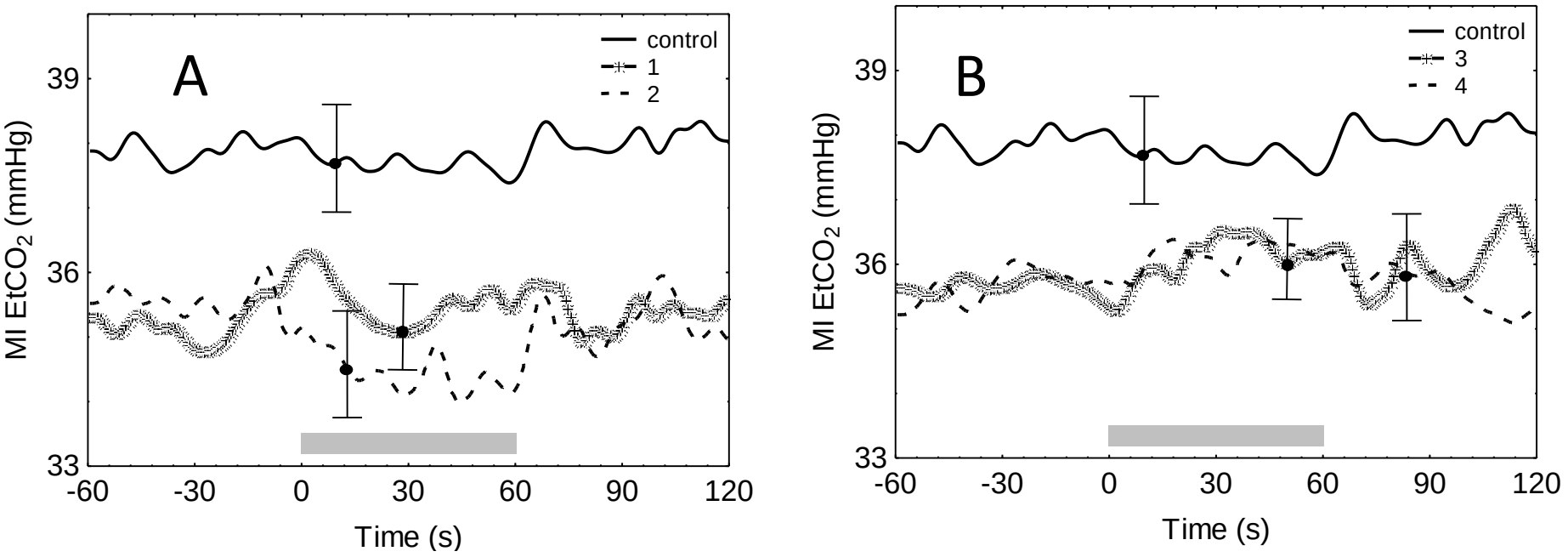

Fig 10.15 Population averages of changes in $EtCO_2$ in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the active paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

10.3.4 Subcomponents Analysis

The AUC analysis of the ΔCBFv broken down into its subcomponents is presented in Table 10.6. Though BP contributed more to the CBFv increase in the stroke group, ANOVA did not show a significant difference between groups or sessions. In agreement with the results in Chapter 8, the contribution of CrCP in controls was more pronounced when compared to the other subcomponents, but BP was the major contributor in all stroke phases during the three paradigms. The temporal pattern of the CrCP contribution is illustrated in Figs. 10.16 & 10.17 to the active paradigm, Figs 10.20 & 10.21 to the passive, and Figs. 10.24 & 10.25 to motor imagery. The overall amplitude of the V_{CrCP}, as expressed by the AUC (Table 10.6), was not significantly different either between groups or sessions. On the other hand, V_{RAP} showed marked differences between acute stroke patients and controls, as well as between acute/subacute and chronic phases. The AUC for V_{RAP} was significantly different between acute stroke patients and controls bilaterally (ANOVA p= 0.01 for active; p= 0.009 for passive) with the exception of the MI paradigm (ANOVA p= 0.8). No statistical differences between sessions were found (p=0.2 for active; p= 0.09 for passive, p= 0.7 for MI), although the contribution of RAP increased gradually over the two chronic phases. The temporal pattern of the RAP contribution is illustrated in Figs. 10.18 & 10.19 to the active paradigm, Figs. 10.22 & 10.23 to the passive, and Figs. 10.26 & 10.27 to motor imagery.

Table 10.6 Mean values (SD) for area under the curve (AUC) for differences in CBFv variation and its subcomponents between patients and controls during active, passive and motor imagery paradigms.

	CBFv (%)		**V_{BP} (%)**		**V_{CrCP} (%)**		**V_{RAP} (%)**	
	right/ unaffected	**left/ affected**	**right/ unaffected**	**left/ affected**	**right/ unaffected**	**left/ affected**	**right/ unaffected**	**left/ affected**
Active								
control	6.0 (4.9)	8.1 (4.5)	1.1 (6.0)	1.0 (6.3)	3.0 (4.7)	4.2 (6.1)	2.5 (3.3)	2.8 (3.9)
session 1	5.1 (3.4)	4.5 (3.7)*	4.2 (5.5) ϕ	4.0 (5.6) ϕ	2.0 (4.5)	2.8 (4.9)	-2.7 (3.3)*	-1.7 (3.4)*
session 2	6.7 (5.7)	5.7 (3.5)	4.0 (5.0)	4.7 (5.1)	3.5 (4.3)	2.4 (5.6)	-1.3 (3.4)	-2.1 (3.6)
session 3	7.3 (5.2)	5.8 (4.0)	3.7 (5.2)	2.1 (5.0)	2.0 (5.5)	2.7 (4.7)	0.7 (3.0)	1.4 (2.3)
session 4	5.6 (7.6)	5.5 (6.4)	2.3 (4.2)	2.0 (4.2)	2.1 (5.0)	2.5 (4.8)	0.1 (4.3)	0.9 (3.6)
Passive								
control	8.1 (5.4)	9.2 (4.4)	1.8 (4.2)	1.7 (4.1)	4.7 (4.7)	5.5 (3.7)	2.6 (3.0)	3.2 (3.1)
session 1	5.6 (5.5)	4.6 (4.7)*	3.5 (4.4)	3.0 (3.9)	2.9 (4.2)	2.0 (4.5)	-1.0 (3.8)*	-1.8 (2.4)*
session 2	6.4 (4.6)	5.3 (3.2)	2.6 (5.6)	3.0 (5.4)	3.9 (5.1)	3.4 (4.7)	-1.5 (3.2)	-2.0 (2.9)
session 3	9.2 (5.5)	7.9 (3.1)	3.6 (5.7)	3.3 (6.4)	3.0 (5.0)	3.9 (4.9)	0.8 (3.1)	0.1 (3.3)
session 4	6.2 (7.5)	7.3 (3.2)	3.5 (5.7)	3.1 (6.4)	2.0 (5.6)	3.0 (5.4)	1.5 (2.9)	1.7 (2.6)
MI								
control	4.8 (4.2)	3.4 (3.0)	1.6 (5.1)	1.5 (4.7)	2.6 (4.6)	2.7 (3.3)	1.0 (3.0)	0.6 (3.1)
session 1	5.4 (4.7)	7.3 (5.2)*	3.5 (4.8)	3.6 (4.9)	1.2 (4.6)	1.7 (4.0)	-0.9 (2.2)	-0.8 (2.9)
session 2	6.6 (6.6)	7.8 (5.0)	3.1 (5.1)	3.2 (5.2)	1.6 (5.0)	1.5 (4.2)	-1.0 (2.7)	0.8 (2.7)
session 3	9.5 (7.3)	8.9 (6.9)	3.2 (5.4)	3.1 (5.2)	4.0 (7.2)	2.8 (3.5)	1.6 (4.0)	2.1 (3.6)
session 4	9.2 (5.9)	8.4 (6.1)	3.1 (5.6)	3.0 (5.8)	2.4 (3.0)	2.3 (4.4)	2.0 (3.4)	2.3 (3.5)

CBFv, cerebral blood flow velocity; V_{BP}, relative contribution of mean arterial blood pressure; V_{CrCP}, relative contribution of critical closing pressure; V_{RAP}, relative contribution of resistance area product;

* P Tukey, p value (<0.03) of post hoc analysis when ANOVA showed significance

ϕ Tukey's p=0.06 for the differences between control (right hemisphere) and stroke (unaffected hemisphere) session 1

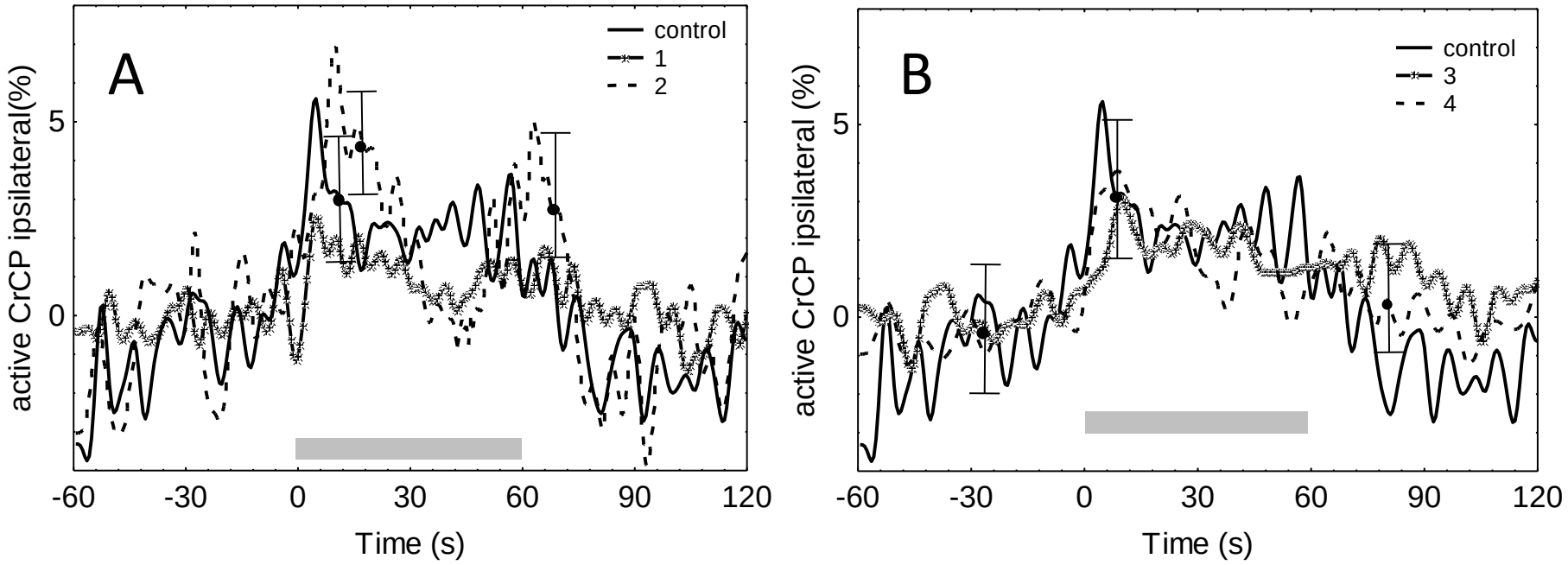

Figure 10.16 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

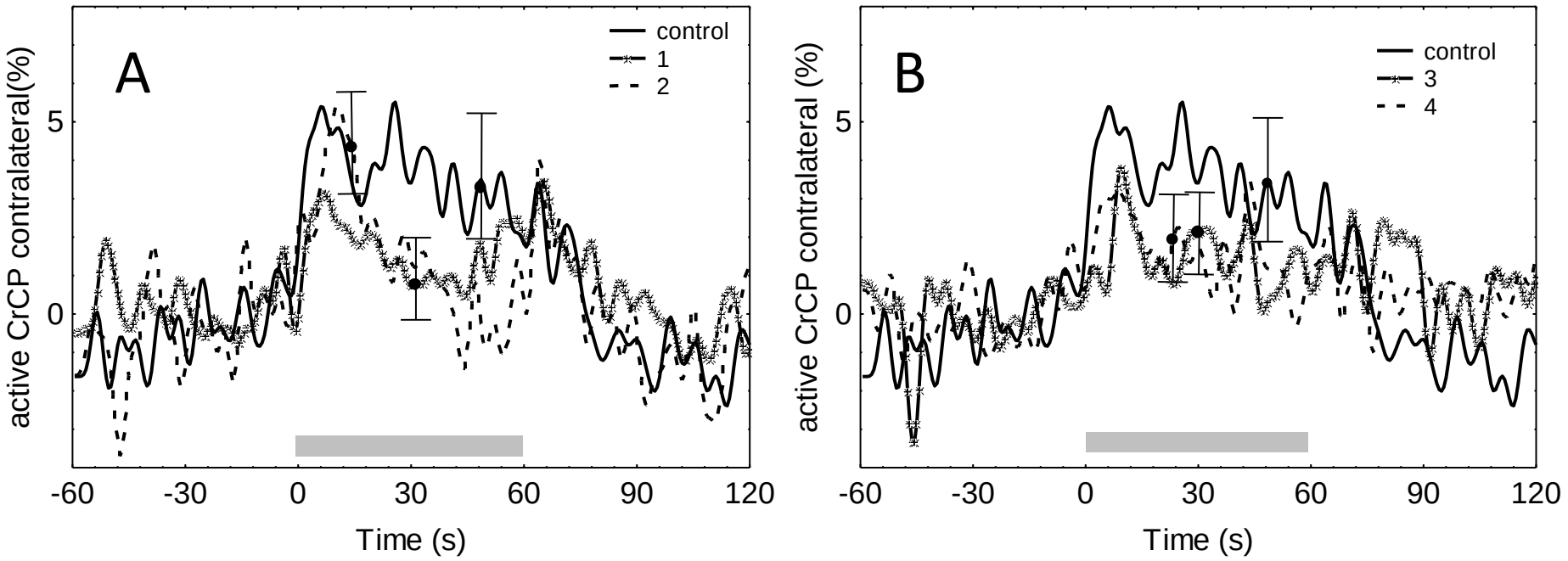

Fig 10.17 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

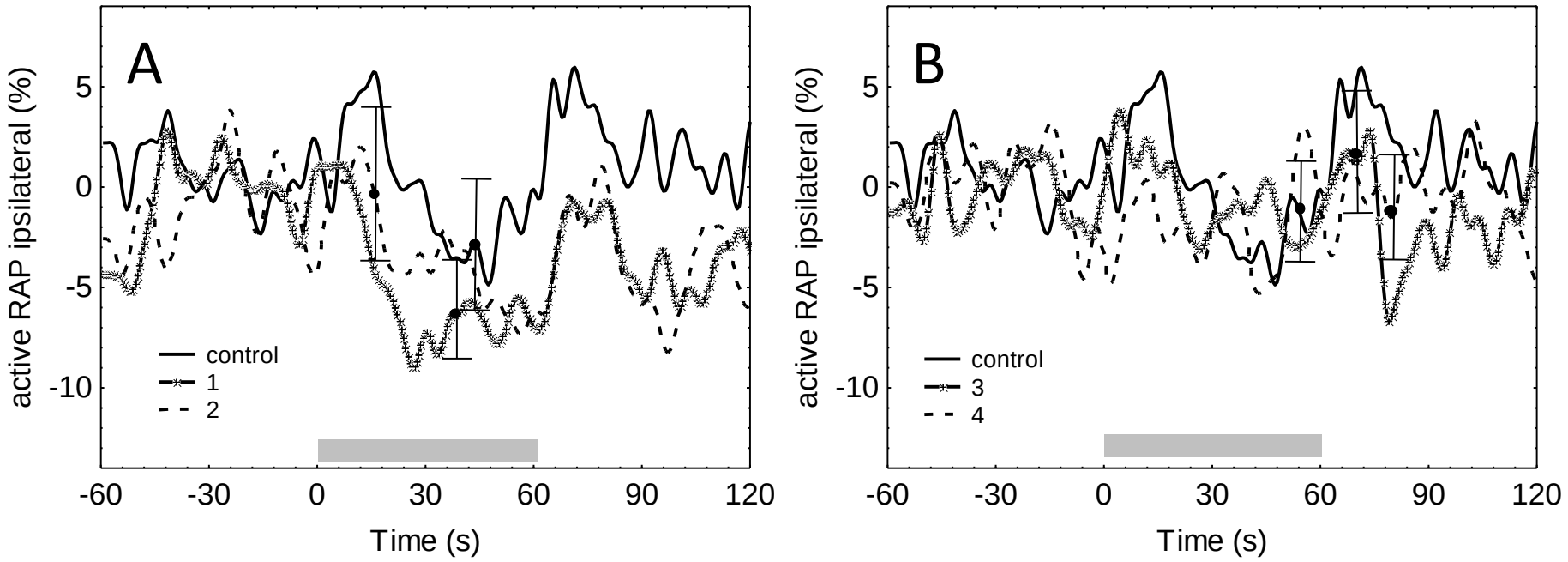

Fig 10.18 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

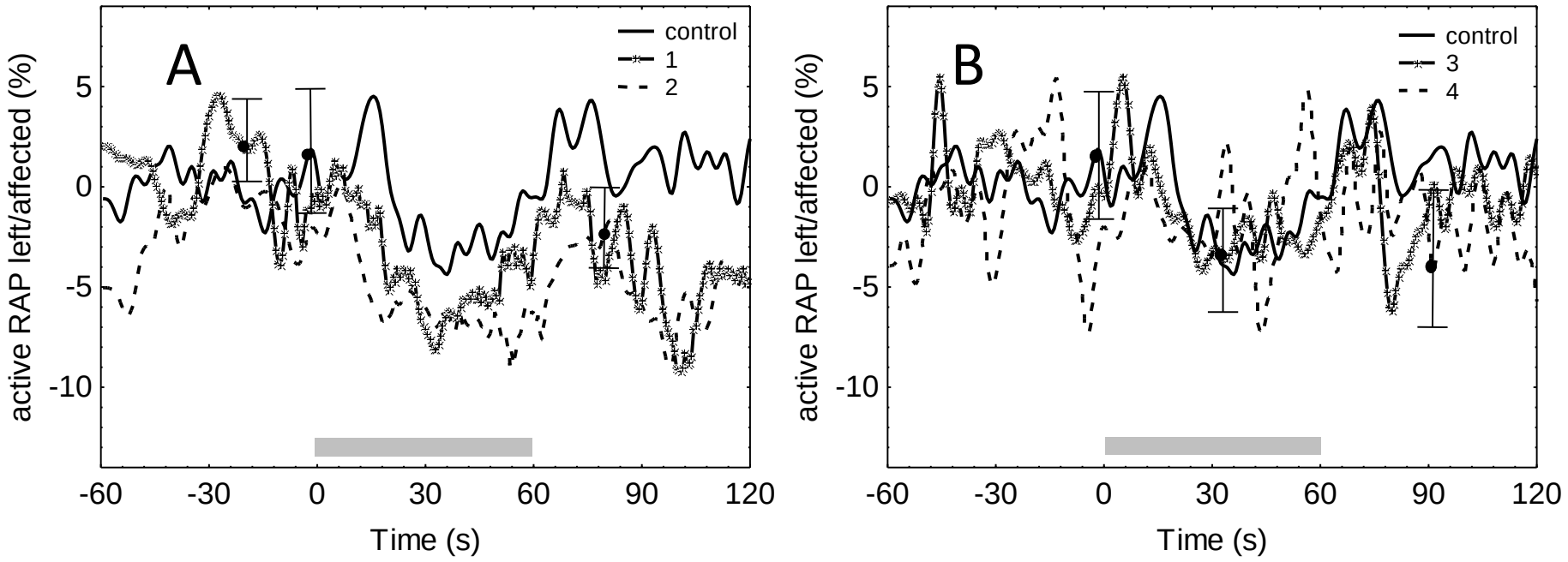

Fig 10.19 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

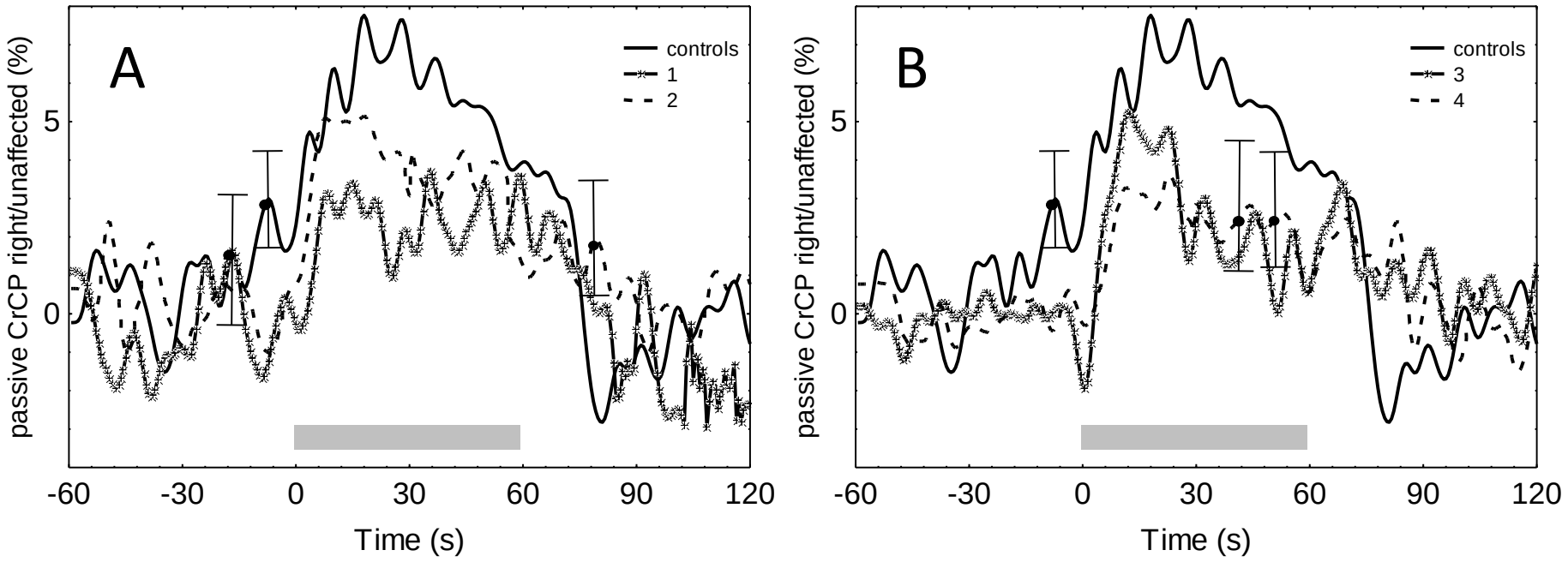

Fig 10.20 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

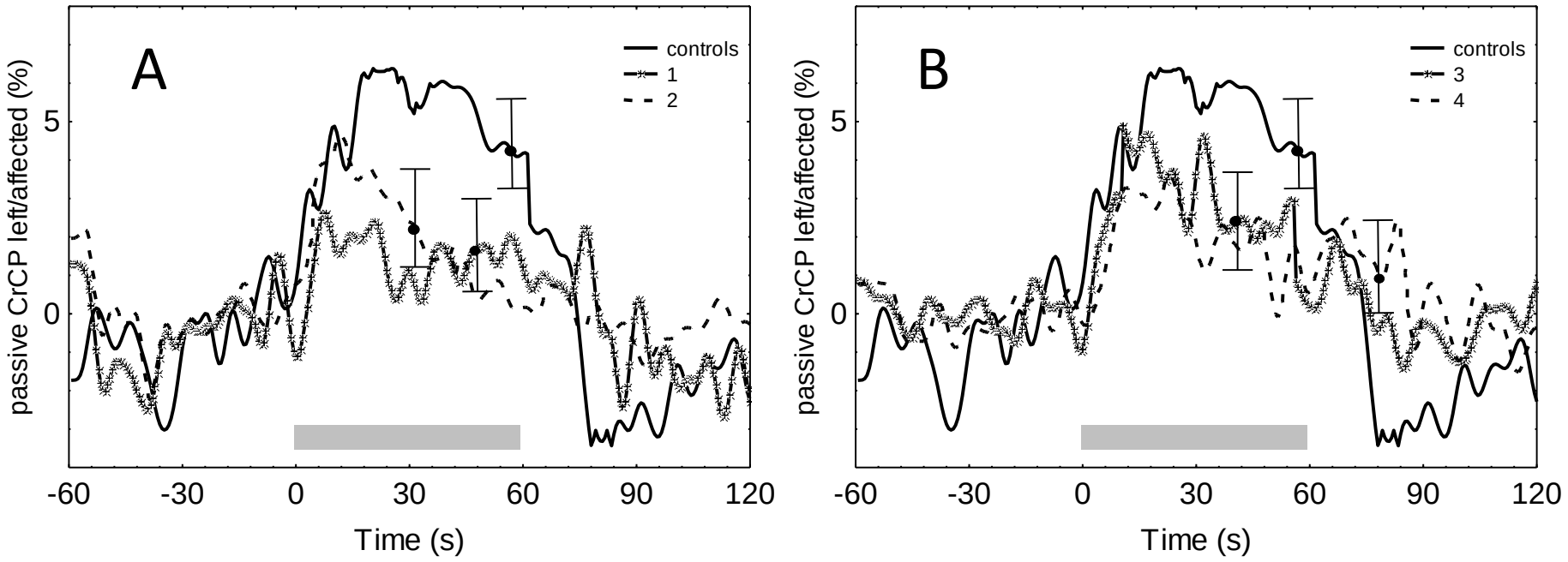

Fig 10.21 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

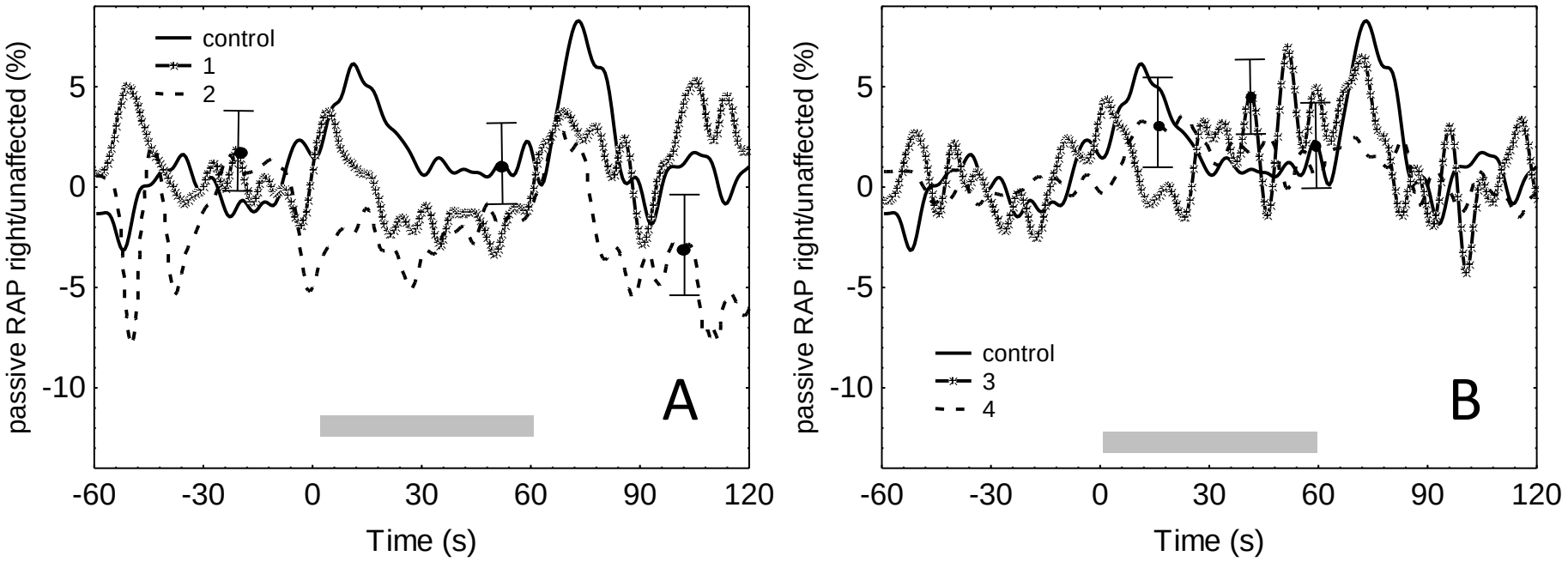

Fig 10.22 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

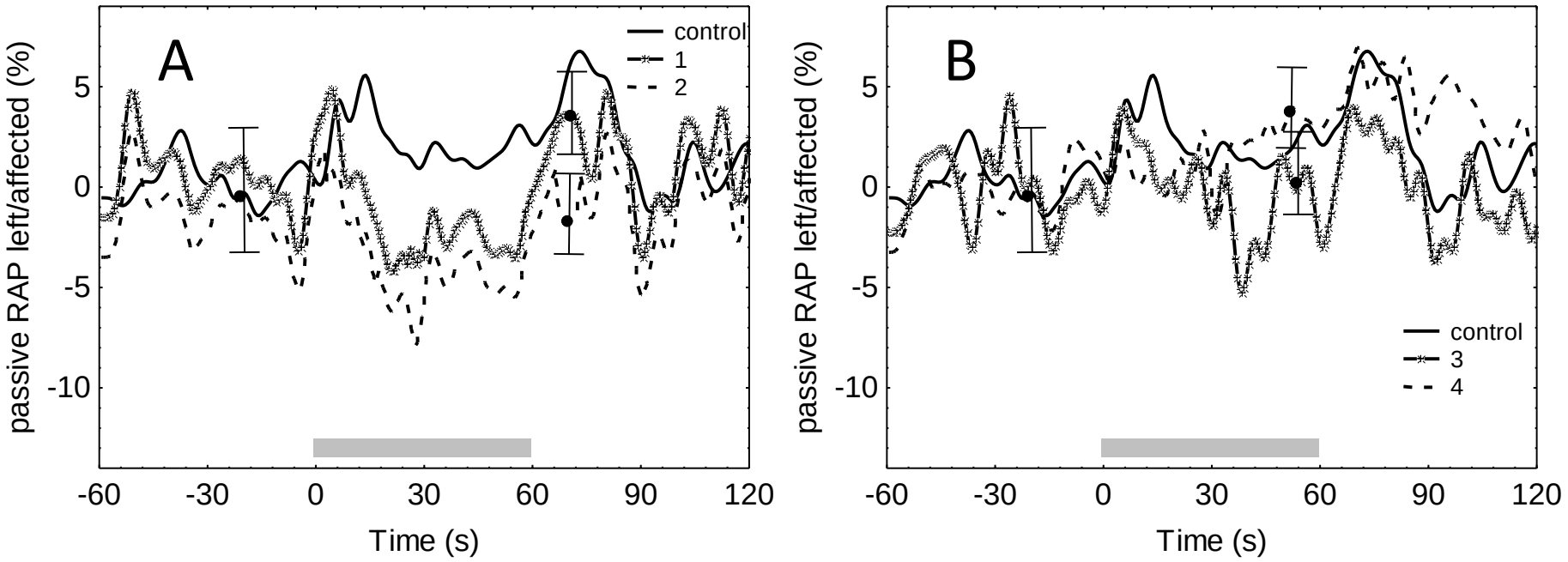

Fig 10.23 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

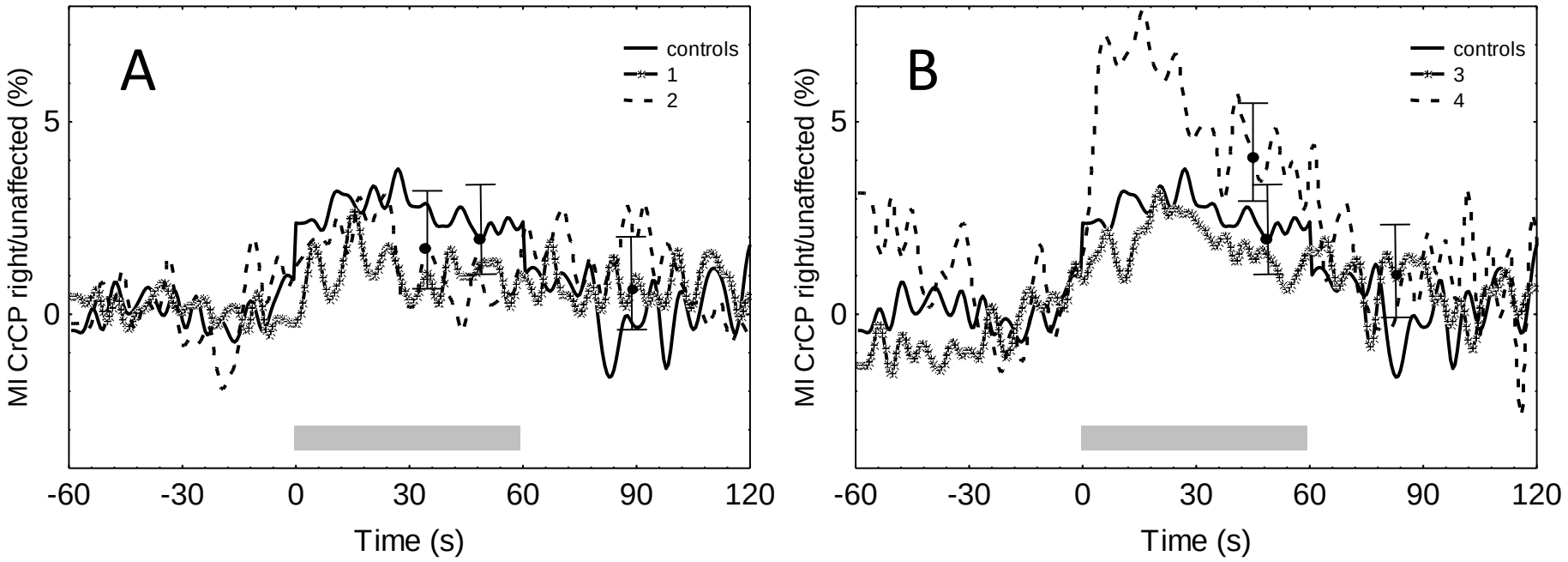

Fig 10.24 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

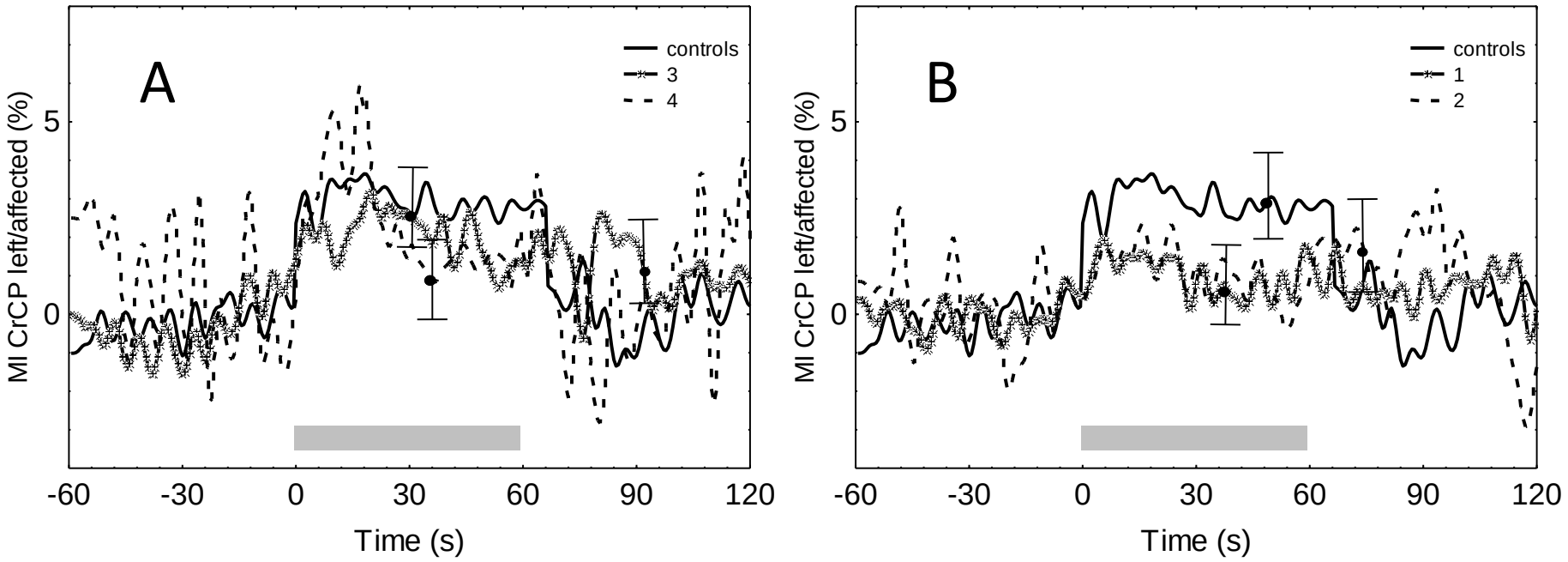

Fig 10.25 Population averages of CrCP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

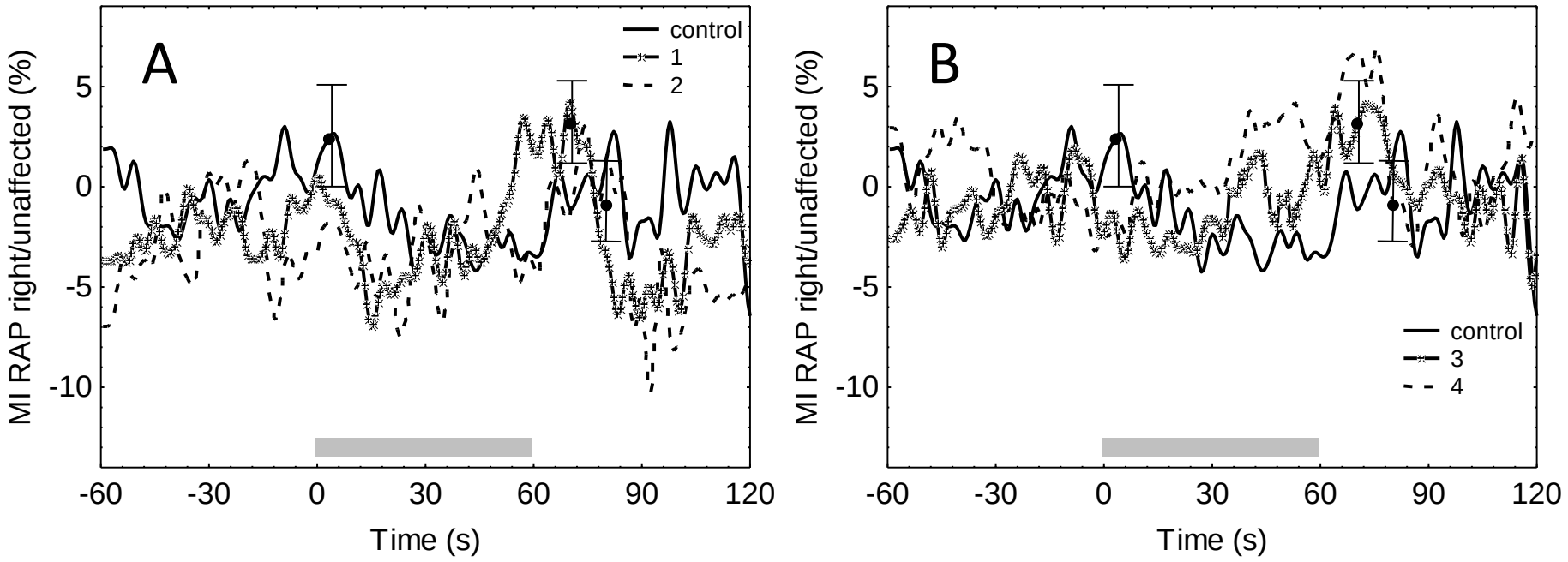

Fig 10.26 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

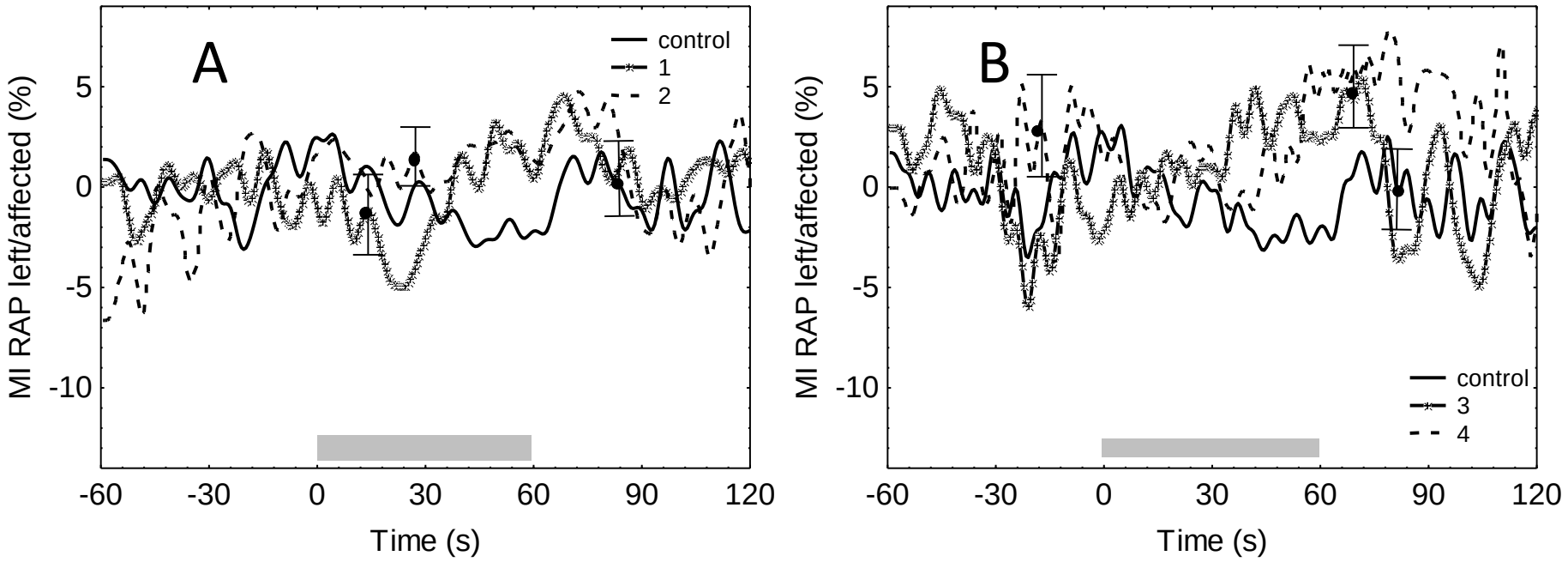

Fig 10.27 Population averages of RAP contribution in controls (continuous line) and stroke (continuous line+symbol and dashed line) during the passive paradigm. The plots correspond to the comparison between acute/subacute (A) and chronic (B) phases. The grey bar shows the duration of the paradigm. For clarity, only the largest ± 1 SE is represented at the point of occurrence.

10.4 Discussion

10.4.1 Main Findings

The study described in this chapter is the first to demonstrate the evolution of serial TCD assessments of CA and NVC mechanisms in the same stroke population over the first three months after stroke onset. The major novel finding of this study was that both regulatory mechanisms have showed some degree of impairment during the first weeks of stroke but improved over time.

In agreement with functional neural activation studies (Calautti *et al*., 2001a, Small *et al*., 2002), the natural history of the CBFv to neural activation (active and passive paradigm only) showed a tri-phasic trend from the acute to the chronic stage. The initially reduced bilateral CBFv response was followed by a CBFv increase in the unaffected hemisphere after a month, which was followed by a progressive approximation to the normal CBFv responses pattern by session 4. Other longitudinal studies have also shown signs of the progressive functional improvement of the CBF response or neural activation pattern in the stroke affected hemisphere (Altamura *et al*., 2009, Askim *et al*., 2009, Cuadrado *et al*., 1999). This observation was also consistent with findings from longitudinal studies in a rat stroke model in which functional improvement was accompanied by reactivation of the affected cortex over time (Dijkhuizen *et al*., 2001).

In recent years, several investigators have found some evidence for reorganization of the neural network after stroke, not only in the affected hemisphere but also in the unaffected hemisphere (Cuadrado *et al*., 1999, Marshall *et al*., 2000, Nelles *et al*., 1999b, Silvestrini *et al*., 1998a). Moreover, these studies found that this over-activation of certain areas in the unaffected hemisphere in the sub-acute phase was associated with clinical recovery (Carey *et al*., 2006, Nhan *et al*., 2004, Ward *et al*., 2006). Interestingly, Nelles *et al*. (2011) showed that

only patients with good recovery presented the over activation of the unaffected hemisphere. This is in line with our results, as the studied population all had a good recovery within weeks of stroke onset (see Table 10.1) and the higher CBFv response in the unaffected hemisphere was seen in almost all participants (for instance 13 out of 15 during the passive paradigm). The mechanisms of involvement of areas located in the unaffected hemispheres seems to be effective not only in recovery from motor deficits (Silvestrini et al., 1993b, Weiller et al., 1992a), but even in the favourable recovery of aphasia (Mimura *et al*., 1998, Silvestrini *et al*., 1998b).

Though impairment of CA was not found in the acute phase, a significant drop in the ARI was seen after two weeks coinciding with a drop in the baseline of the CBFv in the unaffected hemisphere. As stated in chapter 2, the intra-individual time course of dynamic autoregulatory disturbance has not been extensively studied in humans from the first days after cerebral ischaemia. Previous studies analysing the CA in acute stroke found a modest impairment of dCA (Dawson *et al*., 2000, Dawson *et al*., 2003, Eames *et al*., 2002) that lasted for at least 1 to 2 weeks (Dawson *et al*., 2003). Section 2.2.2 described that there is always the possibility of the pressure–velocity relationship being strongly affected by changes in physiological parameters like sympathetic activation, cerebral venous pressure, breathing frequency, and CO_2 levels. Because CO_2 has a marked influence on CBFv and also on autoregulation itself, it is possible that the hypocapnia in the studied stroke patients has masked any modest CA impairment in the acute phase, but not in the subacute phase. Further studies are needed to address this specific question.

In regions of poor perfusion pressure, arterioles are dilated and thus resistance is low; this results in impaired reactivity (CVR) in these territories (Zhao *et al*., 2009). CVR was not assessed directly in this study, but instead a two-parameter model has been used to express

the passive relationship between BP and CBFv comprised of CrCP and RAP (Panerai *et al*., 2005b). CrCP, which under certain conditions can represent the metabolic component of CA, was not affected at any stroke phase, whilst RAP, which possibly represents the myogenic response, was impaired in the acute and subacute phases of stroke (Panerai, 2003, Panerai *et al*., 2005b, Panerai *et al*., 2012b). It is important to note the temporal coincidence of CA and RAP both worsening in the subacute phase. Therefore, the smaller CBFv response to neural activation in the acute and subacute phases could be (partially) attributed to a "sluggish" vasodilatory response of the local vessels, as the vascular reserve was diminished but not entirely absent.

10.4.2 Neural Activation Paradigms

Control participants could perform the three paradigms without problem and use them interchangeably, but this was not the case in the stroke population. It was very difficult for acute stroke patients to concentrate during motor imagery as they were asleep and/or not mentally capable of undertaking this paradigm. Therefore, five participants (out of the fifteen patients that completed four assessments) could not perform it. Moreover, hyperventilation during the paradigm performance was quite common particularly in the subacute phase, as represented in Fig. 10.15. Motor imagery also led to a different pattern of CBFv responses compared to active and passive paradigms in the stroke group. The greater bilateral increase can be attributed to the decrease in $EtCO_2$ due to hyperventilation and/or to the sympathetic nervous system response to stress.

The active paradigm, as hypothesised in Chapter 5, was not suitable for patients with major deficits leading to the exclusion of two participants. Of note, twenty-seven patients were assessed in the acute phase and nineteen of them were not physically able to undertake voluntary movement of the elbow. On the other hand, the passive paradigm was well

tolerated by patients, as well as controls. No major increase in BP (Fig.10.8) and heart rate (Fig. 10.9) were seen indicating that the participants were not unduly stressed. Moreover, the contribution of BP (subcomponents analysis) was relatively lower during the passive paradigm in stroke patients than during the active and MI paradigms. As stated previously (chapters 5 and 7), the passive motor paradigm has been shown to be the most used in studying the evolution of cerebral haemodynamic regulation and functional recovery from acute ischaemic stroke independent of the patient's motor and/or cognitive skill.

10.4.3 Physiological and Clinical Considerations

As described in section 1.1.2, stroke has a multifactorial pathology that involves haemodynamic (involving reduction of CPP) as well as metabolic factors (such as inflammation, oedema and hypoxia). This pathophysiological heterogeneity probably in turn explains the difficulties faced so far in both identifying reliable predictors and understanding underlying mechanisms.

The reason for the bilateral impairment of cerebral haemodynamic regulation, which seems to be limited to the acute and subacute phases, is not clear. This phenomenon could be explained by arteriolar dysfunction that develops at the ischaemic site and spreads to remote areas later in the post-stroke interval. It was hypothesized that a vicious circle could start in the peri-infarct area by spreading local acidosis, and this is amplified by reperfusion (either spontaneously or induced by thrombolysis) with consequent dysautoregulation (Dohmen *et al*., 2007). In addition, bursts of oxidative stress induced by cerebral ischaemia lead to profound alterations in cerebrovascular regulation. In particular, reactive oxygen species can impair endothelial NO-mediated responses, vasodilation (mediated by K^+ channel activation), and vasoconstrictor mechanisms (Faraci, 2005). Moreover, pre-existing endothelial dysfunction (due to ageing, ICA stenosis, or hypertension), which may be exacerbated within

the acute phase shortly after cortical ischaemic stroke (Stevenson *et al*., 2010), when inflammatory or autonomic changes additionally affect the cerebral vasculature, needs to be taken into account.

Adding to the complexity of the interpretation of the cerebral haemodynamic regulation after stroke, the recruited patients showed a marked increase of BP in all sessions compared to control subjects. High BP was seen in 13, 11 and 10 patients during passive, active and motor imagery paradigms, respectively. Of note, only 5 out of 15 patients (3 out of 13, and 10 for active and MI paradigms, respectively) were hypertensive before the stroke onset. As described in section 2.3.3, biochemical and structural changes triggered by sustained hypertension can also induce dysfunction of cerebral haemodynamic regulation. Therefore, the results of this study need to be carefully interpreted, since it is possible that the impairment seen in the stroke population was just driven by the stroke per se.

The impairment of NVC and CA, and the evidence of impairment of the myogenic components of CBF regulation showed in this study might have implications for therapies directed at improvement of CBF regulation and perfusion. First, it was shown that impaired CVR can be improved using NO donors (Lavi *et al*., 2006). If effective, this approach may be useful to facilitate perfusion recovery, and consequently reduce the stroke progression and adverse events (such as early deterioration).

Secondly, a randomized study demonstrated that aerobic treadmill training improves CVR in chronic stroke patients (Ivey *et al*., 2011) which could consequently also improve CA and NVC. Very few studies investigated the evidence that exercise enhances CVR and to my knowledge, this evidence has not been tested in the earlier phase of stroke. Supporting this, previous studies in healthy controls showed that exercise (hand grip and cycling) do not impair CA (Ogoh *et al*., 2005b, Ogoh *et al*., 2010). However, CA was affected during heavy

(exhaustive) exercise (Ogoh *et al*., 2005a). Therefore, moderate and intense rehabilitative therapy should be undertaken with caution in the acute and subacute phases, since CBF regulation might be already impaired and be worsened during therapy leading to adverse events (increasing ischaemia areas and worsening functional recovery, for example).

The role of BP becomes particularly important whenever a large volume of hypoperfused but still viable issue, susceptible to changes in systemic BP, is present. The longitudinal findings revealed a deterioration of CA in the subacute phase being restored in the chronic phase. Indeed, in this case, any drop in BP cannot be compensated for by autoregulation mechanisms, which can in turn lead to tissue infarction. This adds to the debate over the use of antihypertensive therapy acutely following infarction, as described in section 1.4.1.

10.4.4 Limitation of the Study

There are several limitations of the current study. The small number of patients reduced statistical power. More than half of the recruited stroke participants withdrew from the study after the first session mainly for reasons unrelated to the research protocol, such as being discharged to rehabilitation (n= 7) or undergoing carotid endarterectomy (n= 2). A range of circumstances further reduced the number of participants, such as the absence of temporal windows. Strokes were located in a variety of cerebral areas presenting mild-to-moderate severity. As stated in previous chapters, stroke is a heterogeneous disease and the participants reflect this diversity. Moreover, it should be noted that this study required a number of follow-up assessments over a 3-month period and excluded patients with atrial fibrillation; both of these criteria excluded more severe stroke patients. It was not possible to assess the relevance of stroke sensorimotor deficits around 2 weeks, because most cases recovered relatively well. Therefore, the natural history of CBFv regulation for those who did not recover so well needs still to be addressed. The results of right and left strokes were

combined in this study, although the influence of hemisphere dominance on the CBF response is still not fully understood. Further studies should increase the sample size sufficiently to perform separate analyses of patients with right- and left-lesions. Most of the studied stroke participants were hypertensive whereas the control participants were all normotensive. Future studies will benefit from a second control group composed of non-stroke hypertensive subjects, in order to determine the separate contributions of stroke and hypertension to the findings of this chapter.

10.5 Conclusion

In conclusion, this chapter has reported longitudinal data on the evolution of cerebral autoregulation during baseline and neurovascular coupling during three different paradigms after acute ischaemic stroke. The findings indicate that the two major mechanisms of CBFv regulation are impaired at some point during the first weeks after stroke onset. A trend towards a higher CBFv increase in the unaffected hemisphere after the subacute phase and the bilateral return of the CBFv response to normal levels in the chronic phase was observed. Moreover, evidence of impairment of the myogenic component of CBFv regulation (represented by RAP) together with NVC and CA dysfunction was found. Further longitudinal studies of the regulation of cerebral haemodynamics in different deficits will improve our understanding of the evolution of the reorganizational processes paralleling (good and poor) clinical recovery. It would also be important to examine the efficacy of rehabilitative interventions targeting restoration of motor and/or language functions after stroke.

11 Main Findings, Future Work and Original Contributions

11.1 Introduction

The human brain has the capacity for recovery even in cases of severe damage. While clinical studies suggested that the final neurological outcome is strongly related to the reorganization processes in the first weeks of stroke, only little is known about the underlying improvement driving recovery of cerebral function. Nevertheless, experimental and clinical studies have suggested that neuronal and cerebral haemodynamic reorganization is one of the main mechanisms responsible for brain recovery, especially in the acute phase (Dijkhuizen *et al*., 2003, Alawneh *et al*., 2009, Sumiyoshi *et al*., 2012).

In order to assess the CBFv response to neural activation after acute ischaemic stroke, patients with a broad range of recovery were studied to better represent the stroke heterogeneity usually observed in a clinical setting. Aiming for the inclusion of as many patients as possible, three paradigms were included, active, and for whom voluntary movement was not possible, passive and motor imagery. After a systematic review, the overriding purpose of this thesis was to determine the pathophysiological changes and the natural history of the CBF regulation accompanying the recovery after acute ischaemic stroke. To accomplish those aims, it was necessary to understand firstly the CBF regulation in healthy control subjects. Therefore, this thesis has also sought to characterise the temporal

pattern and intensity of the CBFv increase during neural activation as well as its reproducibility.

Before highlighting the original contributions of the thesis, I will briefly summarise the main findings, and then go on to describe how the data presented here lead on to a variety of further research studies in cerebral haemodynamic regulation of stroke patients.

11.2 Main Findings of the Thesis

Do the proposed paradigms produce similar temporal pattern of cerebral and peripheral haemodynamic responses? (Chapter 5). The results suggest that active, passive and motor imagery paradigms can be used interchangeably to assess haemodynamic responses. This enables a more detailed non-invasive assessment of patients, where voluntary movement is not possible, but where abnormalities of cerebral hemodynamic control mechanisms can be anticipated.

Are the CBFv responses to the proposed sensorimotor paradigms reproducible? (Chapter 6). CBFv evaluation during neural stimulation is a useful method to investigate the longitudinal effects of age and/or disease on brain function. Time-dependent changes in brain activity may be erroneously attributed to task-dependent effect rather than simply to random variation. The temporal patterns of haemodynamic responses to active, passive and motor imagery revealed substantial reproducibility. For the CBFv response, the SEM ranged from 2.4 to 5.5% for the different paradigms, whilst the ICC ranged from 0.5 to 0.8 with better reproducibility occurring at the beginning of the paradigm. Though moderate to substantial relative reproducibility was found for most cerebral and peripheral haemodynamic parameters, high SE values reflecting poor absolute reproducibility were also identified. These findings have important implications for the design of studies and highlight the

importance of study power for the investigation of individual differences over time and their relation to ageing and disease.

Can multivariate dynamic modelling detect differences between the proposed paradigms by removing influences of covariates in the CBFv responses? (Chapter 7)**.** The association between neural activity and CBF has been used to assess NVC in health and disease states, but little attention has been given to the contribution of simultaneous changes in peripheral covariates. By removing the influences of other covariates using a multivariate autoregressive-moving average model, it was possible to detect differences between three proposed paradigms. Differences were found in the bilateral CBFv responses to motor imagery compared to active and passive motor paradigms, due to the contribution of stimulation. BP was the dominant contributor to the initial peaked CBFv response in all paradigms with no significant differences between paradigms, whilst the contribution of the stimulus explained the plateau phase and extended duration of the CBFv responses. Apparently similar CBFv responses to different motor-cognitive paradigms can be misleading due to the contributions from peripheral co-variates and could lead to less accurate assessment of NVC.

Is the neurovascular coupling mechanism impaired after acute stroke? Can subcomponents analysis help in the interpretation of CBFv responses? (Chapter 8) Bilateral CBFv responses to passive elbow flexion were significantly lower in the stroke group indicating an impairment of NVC in acute ischaemic stroke. Moreover, when CBFv changes to the passive paradigm were decomposed into standardized subcomponents, alterations in RAP suggested an impairment of the myogenic pathways of CBFv regulation thus giving a greater insight into the different mechanisms contributing to NVC.

Is NVC impaired after acute ischaemic stroke due to reduced neural activity and/or impaired cerebrovascular reactivity? Moreover, is NVC impairment accompanied by deterioration of CA? (Chapter 9) Using again a multivariate dynamic analysis model, it was possible to obtain information about CA, NVC and CVR from a single sensorimotor stimulation paradigm. The results suggest that the impairment of NVC is due to impairment in CVR and reduced neural activation. The absence of a statistically significant reduction in CA may reflect the mild stroke population studied and the 'protective' effect of low $PaCO_2$. Nonetheless, this novel approach has considerable potential in helping to understand better the complex changes in CBF following acute stroke, but needs to be confirmed in a larger, more heterogeneous stroke population.

How do the CBF regulatory mechanisms behave at different stages of stroke evolution? Can subcomponents analysis help to the interpretation of the CBFv responses to the three proposed paradigms? (Chapter 10) The findings indicate that the two major mechanisms of cerebral haemodynamics regulation are impaired at some point during the first weeks of stroke onset. A trend toward a higher CBFv increase in the unaffected hemisphere after the subacute phase and the bilateral return of the CBFv response to normal levels in the chronic phase was observed. Moreover, evidence of impairment of the myogenic component of CBFv regulation (represented by RAP) together with NVC and CA dysfunction was found.

11.3 Future Work

For all issues investigated in this thesis, specific aspects have been identified which would benefit from further work.

Corresponding to cerebral dominance, several studies have addressed the issue of left-right

differences in CBF responses (Guzzetta et al., 2007, Hammond, 2002 , Matteis et al., 2001, Solodkin et al., 2001). There remains a lack of consensus regarding the relationship between handedness and brain activation, despite good evidence of anatomical asymmetries, especially regarding the lateral pre motor cortex (Hammond, 2002). Unfortunately, in controls we only used one side in this study and cannot address this issue further, but for future research it would be better to have both hemispheres assessed and appropriately matched with the affected hemisphere in stroke.

Three different sensorimotor tasks were used as neural activation paradigms to induce changes in CBFv. As described in section 4.5.1.1, the active, passive and motor imagery paradigms share common functional circuits that include primary sensorimotor, premotor, supplementary motor and somatosensory areas (Guzzetta et al., 2007, Jeannerod, 1994, Roosink and Zijdewind, 2010, Sharma et al., 2009, Steuernagel et al., 2002, Stippich et al., 2002, Weiller et al., 1996). Stroke not only leads to sensorimotor deficits of the arm, but aphasia and hemianopia are also very common disabilities. Therefore, it would be interesting to assess the natural history of CBFv responses to other types of paradigms more specific for certain brain areas, e.g. language and visual stimulation.

The results of Chapter 8 and 9 revealed a significant higher BP in the acute stroke patients compared with controls and this was sustained until the last assessment, as revealed in Chapter 10. Hypertension modifies the structure and the function of cerebral blood vessels and therefore it can alter CA and NVC mechanisms (as described in section 2.3.4). Additional research is required to elucidate possible interactions between sustained hypertension, CBF and stroke recovery. Thus, future studies will benefit from a second control group composed of non-stroke hypertensive subjects, in order to determine the relative contributions of stroke and hypertension to the findings of this thesis. Moreover, the

findings highlight the need for refined treatment strategies to address cerebrovascular dysfunction in sustained hypertension. This will be of particular interest for therapeutic efforts aimed at inhibiting cerebral deterioration, preventing the development of secondary injury and developing a more effective rehabilitation plan.

Responses to rehabilitative interventions show large interindividual variation due to the heterogeneity of mechanisms underlying motor recovery (Takeuchi and Izumi, 2013). Therefore, an accurate prediction of motor recovery can help to determine the type, duration, and goals for individual stroke rehabilitation strategies. The early changes of CBFv regulation may be critical for the cerebral recovery and the regression of symptoms. Therefore, longitudinal studies relating CBFv regulation and rehabilitative interventions may be useful in examining the efficacy of such interventions and setting individualized rehabilitation goals. Moreover, the thesis highlighted the involvement (Chapter 5, 6, and 10) and individual contribution (Chapter 7, 8 and 9) of peripheral co-variates to measures of CBF regulation. Although the evidence base for (very) early stroke rehabilitation continues to grow (as described in section 1.4.3), the potential pros and cons, and the optimal time window for specific neurorehabilitation has yet to be elucidated. The relationship between the evolution of CBF regulation and/or neural activation, and time, intensity, and duration for specific rehabilitation techniques will benefit from future studies to facilitate the translation of basic scientific evidence into routine clinical application.

In this thesis, most of the included stroke subjects had mild-to-moderate stroke as explained in Chapter 10. Future studies should include patients with more severe stroke to address the clinical importance of CBF regulation in predicting patient recovery. One of the factors that led to the recruitment of more mild stroke was the exclusion of AF patients because the data analysis techniques rely on a measure of change in CBFv relative to each cardiac cycle, as

measured from the R-R interval, and this is inaccurate when the ECG trace is irregular and may give false estimates of dCA. Therefore, the use of different mathematical modelling techniques to permit the study of dCA in this group should be explored further.

The literature shows considerable diversity of indexes and parameters to gauge the NVC response to different stimulation paradigms, but none took into account the influences of covariates, such as BP and $PaCO_2$. The model used in Chapter 7 and 9 was the first step for the development of an index of NVC. Like CA and CVR, it would be of interest to have an index to investigate the effect of the neural activation on CBF without the influences of other covariates.

11.4 Original Contributions of the Thesis

- Only a few previous studies have presented the beat-to-beat CBFv responses to neural activation, as well as peripheral haemodynamic data (BP, heart rate and breath-by-breath $EtCO_2$) in a healthy older population and following stroke. No previous study, to my knowledge, has assessed such responses during active, passive and motor imagery paradigms in the same population. The beat-to-beat data generated characteristic patterns and also allowed assessment of the amplitude of the response. Some degenerative cerebral diseases (such as Alzheimer's and Parkinson's) have also been shown to reduce amplitude and/or alter temporal patterns (Girouard, 2006; Iadecola, 2004, Rosengarten et al. 2010).
- Though CBF responses to neural activation studies have provided a significant contribution towards understanding the mechanisms mediating stroke recovery, the reproducibility of such responses has been poorly investigated. I am not aware of any previous study assessing the reproducibility of CBFv responses using a TCD technique. As stated previously, any change occurring as a result of an intervention must be greater than the intrinsic variability of the test making the assessment of reproducibility of the test

response crucial. Moreover, the reproducibility data was also useful to provide estimates of sample sizes for cross-sectional as well as longitudinal studies that could be of interest to other investigators.

- The original approach proposed in Chapters 7 and 9 that separates the contributions of BP, $PaCO_2$ and stimulation from the raw CBF response shows considerable potential to increase the diagnostic accuracy of NVC assessment in healthy and disease states and hence warrants further investigation. Moreover, the multivariate analysis can also assess in a single measurement the main CBF regulatory mechanisms, NVC, CA and CVR. Only few studies have tried to extract the effect of neural activation from the CBFv responses and/or assess more than one regulatory mechanism in the same measurement: to my knowledge just two studies in healthy volunteers have attempted this (Rosengarten et al., 2001).
- Though CA and NVC impairment have been previously described in different stages of stroke, particularly in the first days from stroke onset, no previous study has assessed these mechanisms longitudinally in the same population.
- The deterioration of NVC and CA during the subacute interval of recovery highlights the potential to impact on cerebral networks not only immediately post injury but also during the subsequent rehabilitation phase. This raises concerns regarding the potential adverse effects of very early rehabilitation interventions, as well as aggressive pharmacological interventions to reduce BP in the hyperacute stroke period.

In summary, this thesis has advanced our understanding of cerebral blood flow regulation in healthy and diseases conditions and has demonstrated that new methods of assessment are needed to improve our knowledge of pathophysiological changes. The lack of understanding of crucial points of CBF regulation after ischaemic stroke limits the translation of the

knowledge into clinical practice. This thesis has contributed to the interpretation of the complex changes in cerebral haemodynamics following acute stroke, but needs to be confirmed in a larger, more heterogeneous stroke population. In addition, it will allow potential concerns about adverse effects of very early rehabilitation interventions, as well as pharmacological interventions to reduce blood pressure, to be explored further.

12 Appendix

Information leaflet and Consent Forms

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PATIENT INFORMATION LEAFLET

The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

You have been told that you have suffered a stroke by the doctors looking after you, and you are now being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

This is a small research study, which involve a 60-minute measurement of your blood pressure and blood vessels flow on four occasions over 3 months.

1. What is the purpose of the study?

Under normal conditions, your body carefully controls blood flow to the brain by a process called autoregulation. However, this process is impaired following stroke and it is not clear if it is restored over time. As a consequence blood flow becomes dependent on blood pressure (which itself is variable), and changes in blood pressure may be associated with further risk and damage. This research will use a small ultrasound probe applied to both sides of the head to see how blood flow changes during the activation of the brain in healthy and stroke conditions. In the future, this should help doctors and therapists to understand changes in the control of brain blood flow following stroke, to guide treatment; reducing risk of complications and improving chances of recovery.

2. Why have I been chosen?

You are being invited to participate in this study, because you have recently been admitted to hospital after a stroke.

3. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

4. What will happen to me if I take part?

If you agree to join this study, you will have 4 assessments; the first we will arrange for you to have whilst you are still in hospital, within 72 hours of your stroke. You will be asked to come back after 2 weeks, 1 month and 3 months for further studies.

For all the assessments, you will be asked to lie down quietly on a bed whilst a small cuff is attached to a finger on one hand to measure your blood pressure, 3 stickers to your chest to monitor your heart rate and a mask to measure the waste gas from your breathing. You will be asked to wear a head-frame, which will hold the small ultrasound probes that are used to measure blood flow against both sides of your head. After the readings have stabilised over a period of 20 minutes, a recording will then be made over 5minutes. Next, this will be followed by three shorter (4 minutes) recordings, in a random order, where will be asked to move your elbow, or to let the examiner move your elbow (without your help) or to think that you are moving you elbow (without actually moving the arm). These exercises will be performed two times in both arms. If you are not able to move your elbow, just the examiner moving your elbow and you imagining moving your elbow will be performed.

5. What treatment will be used?

No specific treatments are given as part of this small study.

6. What are the possible disadvantages and risks of taking part?

The blood pressure cuff applies only a gentle pressure to your finger to enable a blood pressure recording to be made every heart beat. This may cause a slight tingling in your fingers, but this should not be painful or cause any harm. Indeed, this type of blood pressure monitoring is often used routinely, e.g. in patients under general anaesthetic or in intensive care. The head-frame and ultrasound probes will exert a slight pressure against your head. However, this is not painful, and again is routinely used in many units to monitor blood flow to the brain.

The activity of moving or imagining you are moving your elbow should not be associated with any symptoms.

7. What are the possible benefits of taking part?

You should not expect to receive any personal benefit from taking part in this study. However, any travelling expenses that you incur will be reimbursed.

8. What if something goes wrong?

Medical research is covered for mishaps in the same way as for patients undergoing treatment in the National Health Service, i.e. compensation is only available if negligence occurs. Regardless of this or wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available for you.

9. Will my taking part in this study be kept confidential?

The blood pressure and blood flow data recorded during the study will be stored on computer for subsequent analysis. However, you will not be identified by name, and only your doctor will know that the information is related to you. Any information collected during the study will be treated with the usual degree of confidentiality under the data protection act. Your identity will not be revealed in any publication or presentation of the results from this study.

10.Who is organizing and funding the research?

This research is coordinated by Professor Thompson Robinson from the University of Leicester.

11.What if I have concerns?

If you have any concerns or other questions about this or the way it has been carried out, you should contact the investigator (Prof Thompson Robinson, telephone number 0116 252 3182), or you may contact the hospital complaints department.

Once again, thank you for taking time to read this information sheet and for considering taking part in this study.

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Patient Recruitment Number for this Study: …….

PATIENT CONSENT FORM

Title of the project: The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

Name of Principal Investigator:

	Please initial box
I confirm that I have read and understand the information sheet dated 01 May 2012 (version 3) for the above study and have had the opportunity to consider the information, ask questions and have these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw consent at any time, without giving any reason, without my medical care or legal rights being affected.	
I understand that sections of any of my medical notes may be looked at by responsible members of the research team or by representatives of the sponsor or regulator.	
I understand that my GP will be contacted about my participation in the study and by signing I agree to this	
I agree to take part in the above study.	

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

When completed 1 copy for the patient; 1 copy for the research file, 1 (original) to be kept in the hospital notes.

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GP INFORMATION LEAFLET (patient)

The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

Your patient was recently admitted to hospital with a stroke. He/she has been recruited to the above study which involves measurement of beat-to-beat blood pressure and heart rate, along with measurement of cerebral blood flow velocity using a transcranial Doppler. Participation in the above study is entirely voluntary. These assessments of cerebral haemodynamics will be undertaken in response to active and passive flexion and extension of the elbow, as well as cognitive paradigms. They will be undertaken at 4 time points: <72hours, 2 weeks, 1 month and 3 months of acute stroke onset. These assessments will not interfere with standard post stroke care, including secondary prevention therapy.

If you have any further questions regarding this research, then please contact:

Signed Prof. TG Robinson Telephone 0116 252 31 82

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PATIENT CONFIRMATION INFORMATION LEAFLET

(AFTER RECOVERY)

The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

While you were unwell, your relative agreed to your participation in a research study. Now that your condition has improved, you are being invited to decide for yourself whether you wish to stay in the study.

You will have been told that you have suffered a stroke by the doctors looking after you, and you are now being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

This is a small research study, which will involve a 60-minute measurement period of your blood pressure and blood vessels on four occasions over 3 months.

1. What is the purpose of the study?

Under normal conditions, your body carefully controls blood flow to the brain by a process called autoregulation. However, this process is impaired following stroke and it is not clear if it is restored over time. As a consequence blood flow becomes dependent on blood pressure (which itself is variable), and changes in blood pressure may be associated with further risk and damage. This research will use a small ultrasound probe applied to both sides of the head to see how blood flow changes during the activation of the brain in healthy and stroke conditions. In the future, this should

help doctors and therapists to understand changes in the control of brain blood flow following stroke, to guide treatment; reducing risk of complications and improving chances of recovery.

2. Why have I been chosen?

You are being invited to participate in this study, because you have recently been admitted to hospital after a stroke.

3. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

4. What will happen to me if I take part?

If you agree to join this study, you will have 4 assessments; the first we will arrange for you to have whilst you are still in hospital, within 72 hours of your stroke. You will be asked to come back after 2 weeks, 1 month and 3 months for further studies.

For all the assessments, you will be asked to lie down quietly on a bed whilst a small cuff is attached to a finger on one hand to measure your blood pressure, 3 stickers to your chest to monitor your heart rate and a mask to measure the waste gas from your breathing. You will be asked to wear a head-frame, which will hold the small ultrasound probes that are used to measure blood flow against both sides of your head. After the readings have stabilised over a period of 20 minutes, a recording will then be made over 10 minutes. Next, this will be followed by three shorter (4 minutes) recordings, in a random order, where will be asked to move your elbow, or to let the examiner move your elbow (without your help) or to think that you are moving you elbow (without actually moving the arm). These exercises will be performed two times in both arms. If you are not able to move your elbow, just the examiner moving your elbow and you imagining moving your elbow will be performed.

5. What treatment will be used?

No specific treatments are given as part of this small study.

6. What are the possible disadvantages and risks of taking part?

The blood pressure cuff applies only a gentle pressure to your finger to enable a blood pressure recording to be made every heart beat. This may cause a slight tingling in your fingers, but this should not be painful or cause any harm. Indeed, this type of blood pressure monitoring is often used routinely, e.g. in patients under general anaesthetic or in intensive care. The head-frame and ultrasound probes will exert a slight pressure against your head. However, this is not painful, and again is routinely used in many units to monitor blood flow to the brain.

The activity of moving or imagining you are moving your elbow should not be associated with any symptoms.

7. What are the possible benefits of taking part?

You should not expect to receive any personal benefit from taking part in this study. However, any travelling expenses that you incur will be reimbursed.

8. What if something goes wrong?

Medical research is covered for mishaps in the same way as for patients undergoing treatment in the National Health Service, i.e. compensation is only available if negligence occurs. Regardless of this or wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available for you.

9. Will my taking part in this study be kept confidential?

The blood pressure and blood flow data recorded during the study will be stored on computer for subsequent analysis. However, you will not be identified by name, and only your doctor will know that the information is related to you. Any information collected during the study will be treated with the usual degree of confidentiality under the data protection act. Your identity will not be revealed in any publication or presentation of the results from this study.

10.Who is organizing and funding the research?

This research is coordinated by Professor Thompson Robinson from the University of Leicester.

11.What if I have concerns?

If you have any concerns or other questions about this or the way it has been carried out, you should contact the investigator (Prof Thompson Robinson, telephone number 0116 252 3182), or you may contact the hospital complaints department.

Once again, thank you for taking time to read this information sheet and for considering taking part in this study.

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Patient Recruitment Number for this Study: …….

PATIENT CONSENT FORM

(AFTER RECOVERY)

Title of the project: The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

Name of Principal Investigator:

Please initial box

I confirm that I have read and understand the information sheet dated 01 May 2012 (version 2) for the above study and have had the opportunity to consider the information, ask questions and have these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw consent at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I understand that sections of any of my medical notes may be looked at by responsible members of the research team or by representatives of the sponsor or regulator.	☐
I understand that my GP will be contacted about my participation in the study and by signing I agree to this	☐
I agree to take part in the above study.	☐

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

When completed 1 copy for the patient; 1 copy for the research file, 1 (original) to be kept in the hospital notes.

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CONSULTEE INFORMATION LEAFLET

The Natural History of Cerebral Haemodynamics in Response to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

We feel your relative is unable to decide for himself/herself whether to participate in this research.

To help decide if he/she should join the study, we would like to ask your opinion whether or not he/she wants to be involved. We would ask you to consider what you know of his/her wishes and feelings, and to consider his/her interests. Please let us know of any advance decisions he/she may have made about participating in research. These should take precedence.

If you decide your relative would have no objection to taking part we will ask you to read and sign the consultee declaration form. We will then give you a copy to keep. We will keep you fully informed during the study so you can let us know if you have any concerns or you think your relative should be withdrawn.

If you decide that your relative would not wish to take part it will not affect the standard of care they receive in any way.

Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to your relative takes part.

Thank you for reading this.

This is a small research study, which will involve a 60-minute measurement period of his/her blood pressure and blood vessels on four occasions over 3 months.

1. What is the purpose of the study?

Under normal conditions, the body carefully controls blood flow to the brain by a process called autoregulation. However, this process is impaired following stroke and it is not clear if it is restored over time. As a consequence blood flow becomes dependent on blood pressure (which itself is variable), and changes in blood pressure may be associated with further risk and damage. This research will use a small ultrasound probe applied to both sides of the head to see how blood flow changes during the activation of the brain in healthy and stroke conditions. In the future, this should help doctors and therapists to understand changes in the control of brain blood flow following stroke, to guide treatment; reducing risk of complications and improving chances of recovery.

2. Why has your relative been chosen?

Your relative is being invited to participate in this study, because he/she has recently been admitted to hospital after a stroke.

3. Does your relative have to take part?

It is up to you to decide whether or not your relative takes part. If you do decide that he/she can take part, you will be given this information sheet to keep and be asked to sign a relative carer or independent legal representative consent form. If you decide that he/she can take part, you are still free to withdraw him/her at any time and without giving reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your relative receives.

4. What will happen to your relative if he/she takes part?

If you agree for your relative to join this study, he/she will have 4 assessments; the first we will arrange to have whilst he/she is still in hospital, within 72 hours of his/her stroke. He/she will be asked to come back after 2 weeks, 1 month and 3 months for further studies.

For all the assessments, he/she will be asked to lie down quietly on a bed whilst a small cuff is attached to a finger on one hand to measure his/her blood pressure, 3 stickers to his/her chest to monitor heart rate and a mask to measure the waste gas from his/her breathing. He/she will be asked to wear a head-frame, which will hold the small ultrasound probes that are used to measure blood flow against both sides of his/her head. After the readings have stabilised over a period of 20 minutes, a recording will then be made over 10 minutes. Next, this will be followed by three shorter (4 minutes) recordings, in a random order, where he/she will be asked to move his/her elbow, or to let the examiner move his/her elbow (without their help) or to think that he/she is moving his/her elbow (without actually moving the arm). These exercises will be performed two times in both arms. If he/she is not able to move his/her elbow, just the examiner moving his/her elbow and he/she imagining moving your elbow will be performed.

5. What treatment will be used?

No specific treatments are given as part of this small study.

6. What are the possible disadvantages and risks of taking part?

The blood pressure cuff applies only a gentle pressure to his/her finger to enable a blood pressure recording to be made every heart beat. This may cause a slight tingling in his/her fingers, but this should not be painful or cause any harm. Indeed, this type of blood pressure monitoring is often used routinely, e.g. in patients under general anaesthetic or in intensive care. The head-frame and ultrasound probes will exert a slight pressure against his/her head. However, this is not painful, and again is routinely used in many units to monitor blood flow to the brain.

The actual activity of moving or imagining he/she is moving his/her elbow should not be associated with any symptoms.

7. What are the possible benefits of taking part?

Your relative should not expect to receive any personal benefit from taking part in this study. However, any travelling expenses that are incurred will be reimbursed.

8. What if something goes wrong?

Medical research is covered for mishaps in the same way as for patients undergoing treatment in the National Health Service, i.e. compensation is only available if negligence occurs. Regardless of this or if you wish to complain, or have any concerns about any aspect of the way your relative has been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available for you.

9. Will your relative taking part in this study be kept confidential?

The blood pressure and blood flow data recorded during the study will be stored on computer for subsequent analysis. However, he/she will not be identified by name, and only his/her doctor will know that the information is related to him/her. Any information collected during the study will be treated with the usual degree of confidentiality under the data protection act. His/her identity will not be revealed in any publication or presentation of the results from this study.

10.Who is organizing and funding the research?

This research is coordinated by Professor Thompson Robinson from the University of Leicester and Mrs Angela Salinet is funded by CAPES (scholarship – Ministry of Education of Brazil).

11.What if I have concerns?

If you have any concerns or other questions about this or the way it has been carried out, you should contact the investigator (Prof Thompson Robinson, telephone number 0116 258 3182), or you may contact the hospital complaints department.

Once again, thank you for taking time to read this information sheet and for considering your relative taking part in this study.

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Patient Recruitment Number for this Study: ……

CONSULTEE DECLARATION FORM

Title of the project: The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

Name of Principal Investigator:

Please initial box

I _____ confirm that I have read and understand the information sheet dated 01 May 2012 (version 2) for the above study and have had the opportunity to consider the information, ask questions and have these answered satisfactorily. ☐

I understand that his/her participation is voluntary and that he/she and I are free to withdraw consent at any time, without giving any reason, without his/her medical care or legal rights being affected. ☐

I understand that sections of any of his/her medical notes may be looked at by responsible members of the research team or by representatives of the sponsor or regulator. ☐

I understand that his/her GP will be contacted about his/her participation in the study and by signing I agree to this. ☐

I agree to _____ taking part in the above study. ☐

_____	_____	_____
Name of consultee	Date	Signature
_____	_____	_____
Name of person taking consultation (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

When completed 1 copy for the consultee; 1 copy for the researcher site file, 1 (original) to be kept in the hospital notes.

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VOLUNTEER INFORMATION LEAFLET

The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

This is a small research study, which will involve a 60-minute measurement period of your blood pressure and blood vessels.

1. What is the purpose of the study?

Under normal conditions, your body carefully controls blood flow to the brain by a process called autoregulation. However, this process is impaired following stroke and it is not clear if it is restored over time. As a consequence blood flow becomes dependent on blood pressure (which itself is variable), and changes in blood pressure may be associated with further risk and damage. This research will use a small ultrasound probe applied to both sides of the head to see how blood flow changes during the activation of the brain in healthy and stroke conditions. In the future, this should help doctors and therapists to understand changes in the control of brain blood flow following stroke, to guide treatment; reducing risk of complications and improving chances of recovery.

2. Why have I been chosen?

You are being invited to participate in this study as a volunteer (or control subject), in parallel to a study which is currently ongoing in the patients who have had a recent stroke, where disorders of control of blood flow to the brain are common and important.

3. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving reason.

4. What will happen to me if I take part?

If you agree to join this study, you will have a single study test, for which we will arrange for you to come into the hospital for an hour. Travelling expenses will be reimbursed for this visit. You will be asked to lie quietly on your bed whist a small cuff is attached to a finger of one hand to measure your blood pressure, 3 stickers to your chest to monitor your heart rate and a mask to measure the waste gas from your breathing. You will be asked to wear a head-frame, which will hold the small ultrasound probes that are used to measure blood flow against both sides of your head. After the readings have stabilised over a period of 20 minutes, a recording will then be made over 10 minutes. Next, this will be followed by three shorter (4 minutes) recordings, in a random order, where you will be asked to move your elbow, or to let the examiner move your elbow (without your help) or to think that you are moving your elbow (without move it).

5. What treatment will be used?

No specific treatments are given as part of this small study.

6. What are the possible disadvantages and risks of taking part?

The blood pressure cuff applies only a gentle pressure to your finger to enable a blood pressure recording to be made every heartbeat. This may cause a slight tingling in your fingers, but this should not be painful or cause any harm. Indeed, this type of blood pressure monitoring is often used routinely, e.g. in patients under general anaesthetic or in intensive care. The head-frame and ultrasound probes will exert a slight pressure against your head. However, this is not painful, and again is routinely used in many units to monitor blood flow to the brain.

The actual activity of moving or imagining you are moving your elbow should not be associated with any symptoms.

7. What are the possible benefits of taking part?

You should not expect to receive any personal benefit from taking part in this study. However, any travelling expenses that you incur will be reimbursed.

8. What if something goes wrong?

Medical research is covered for mishaps in the same way as for patients undergoing treatment in the National Health Service, i.e. compensation is only available if negligence occurs. Regardless of this or wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available for you.

9. Will my taking part in this study be kept confidential?

The blood pressure and blood flow data recorded during the study will be stored on computer for subsequent analysis. However, you will not be identified by name, and only your doctor will know that the information is related to you. Any information collected during the study will be treated

with the usual degree of confidentiality under the data protection act. Your identity will not be revealed in any publication or presentation of the results from this study.

10.Who is organizing and funding the research?

This research is coordinated by Professor Thompson Robinson from the University of Leicester.

11.What if I have concerns?

If you have any concerns or other questions about this or the way it has been carried out, you should contact the investigator (Prof. Thompson Robinson, telephone number 0116 252 3182), or you may contact the hospital complaints department.

Once again, thank you for taking time to read this information sheet and for considering taking part in this study.

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Patient Recruitment Number for this Study: ……

VOLUNTEER CONSENT FORM

Title of the project: The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

Name of Principal Investigator:

Please initial box

I confirm that I have read and understand the information sheet dated 23 March 2011 (version 2) for the above study and have had the opportunity to consider the information, ask questions and have these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw consent at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I understand that sections of any of my medical notes may be looked at by responsible members of the research team or by representatives of the sponsor or regulator.	☐
I understand that my GP will be contacted about my participation in the study and by signing I agree to this	☐
I agree to take part in the above study.	☐

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

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GP INFORMATION LEAFLET (volunteer)

The Natural History of Cerebral Haemodynamic Responses to Active and Passive Motor, and Cognitive Paradigms Following Acute Ischaemic Stroke

Your patient agreed to participate in the above study as a volunteer. The study involves the measurement of beat-to-beat blood pressure and heart rate, along with measurement of cerebral blood flow velocity using a transcranial Doppler. These assessments of cerebral haemodynamics will be undertaken in response to active and passive flexion and extension of the elbow, as well as a cognitive paradigm, and will be undertaken on one occasion.

Participation in the above study is entirely voluntary.

If you have any further questions regarding this research, then please contact:

Signed Prof. TG Robinson Telephone 0116 258 3187

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