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Effects of normobaric hypoxia on the activation of motor and visual cortex areas in functional magnetic resonance imaging (fMRI)

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1 English abstract

Aims: Hypoxia due to high altitude or otherwise altered fraction of inspired O_2 affects cerebral mechanisms. Human brain function can be assessed indirectly via examination of local changes in haemodynamics in fMRI. The aim of this study was to examine if adaptation to normobaric hypoxia determines divergent activation in the brain regions supplied by the main cerebral arterial vessels.

Methods: Visual and motor paradigms were used to shed light on the activation of different brain regions in fMRI under normobaric hypoxic conditions in 16 healthy male subjects. Hypoxia was produced by reducing the percentage of O_2 in an inhaled gas mixture resulting in normobaric hypoxia with an FiO_2 of 13 %. Participants had to complete a total of 3 MRI sessions to study different oxygen conditions: normoxia (FiO_2 = 0.21, normal pressure), short-time (7 ± 1 min, FiO_2 = 0.13, normal pressure), long-time hypoxia (8 h and 29 ± 24 min, FiO_2 = 0.13, normal pressure). Each session lasted approximately 30 min, consisting of two fMRI runs (1 visual task, 1 motor task) which were pseudo-randomized between participants, followed by the structural sequence. Cerebral symptoms of AMS were assessed by means of the LLS and it was examined if symptomatic AMS has consequences on brain activation patterns measured as ∆S values.

Results: Mean ∆S during normoxia was 2.43 ± 0.80 % due to motor stimulation, and 3.49 ± 1.41 % due to visual stimulation. During motor stimulation, the mean signal change due to short-time hypoxia was 0.55 ± 0.30 % and 0.82 ± 0,62 % due to long-time hypoxia. During visual stimulation, the mean signal change due to short-time hypoxia was 1.79 ± 0.69 %. Long-time hypoxia led to a mean signal change of 2.02 ± 1.18 %. Repeated ANOVA measures with factors task (motor, visual) and the hypoxic conditions (short-time hypoxia, long-time hypoxia) showed a main effect of task ($F(1, 15) = 52.10$, $p < 0.001$), but no main effect of the hypoxic condition ($F(1, 15) = 1.79$, p = ns).

Conclusions: Hypoxia led to diminished cerebral activation during motor and visual stimulation in spite of a preserved cerebral function. The oxygenation changes associated with brain activation seem more influential on the motor area, rather than the visual cortex. Therefore, the capability of the human brain to acclimatise to chronic hypoxic conditions may vary in the motor and the visual system.

2 Deutsche Zusammenfassung

Ziele: Hypoxie aufgrund großer Höhe oder eines anderweitig veränderten Anteils von eingeatmetem O_2-Gehalts beeinflusst zerebrale Mechanismen. Die menschliche Gehirnfunktion kann indirekt über den Nachweis lokaler hämodynamischer Veränderungen im fMRT bestimmt werden. Das Ziel dieser Studie war es, zu untersuchen, ob die Anpassung an normobare Hypoxie eine unterschiedliche Aktivierung in von den drei Hauptgefäßen versorgten Gehirnregionen erzeugt.

Methoden: Bei 16 gesunden, männlichen Probanden wurden visuelle und motorische Testparadigmen angewendet, um die Aktivierung verschiedener Hirnregionen im fMRT unter normobaren, hypoxischen Bedingungen aufzuklären. Hypoxie wurde mit Hilfe eines sauerstoffreduzierten Gasgemischs (O_2-Anteil 13%) erzeugt. Die Probanden mussten insgesamt 3 MRT-Sitzungen absolvieren, um verschiedene Sauerstoffzustände zu untersuchen: Normoxie (FiO_2 = 0,21), Kurzzeithypoxie (7 ± 1 min Hypoxie, FiO_2 = 0,13), Langzeithypoxie (8 h und 29 ± 24 min Hypoxie, FiO_2; = 0,13). Jede Sitzung dauerte ca. 30 min und bestand aus je zwei fMRI-Durchgängen (1 visuelle Aufgabe, 1 motorische Aufgabe). Die zerebralen Symptome einer Höhenkrankheit wurden mittels des LLS bewertet und der Einfluss einer Höhenkrankheit auf die Gehirnaktivierungsmuster im fMRT untersucht.

Resultate: Die mittlere BOLD-Signalveränderung während Normoxie betrug bei motorischer Stimulation 2,43 ± 0,80% und bei visueller Stimulation 3,49 ± 1,41%. Bei motorischer Stimulation betrug sie nach Kurzzeithypoxie 0,55 ± 0,30% und 0,82 ± 0,62% nach Langzeithypoxie. Bei visueller Stimulation betrug die mittlere Signaländerung aufgrund von Kurzzeithypoxie 1,79 ± 0,69 und aufgrund Langzeithypoxie 2,02 ± 1,18%. ANOVA-Messungen mit den Faktoren Aufgabe (motorisch, visuell) und hypoxische Bedingungen (Kurzzeithypoxie, Langzeithypoxie) zeigten einen Effekt der Aufgabe ($F(1, 15) = 52.10$, $p < 0.001$), aber keinen Effekt der hypoxischen Bedingung ($F(1, 15) = 1,79$, p = ns) auf die BOLD-Signalwertänderungen.

Schlussfolgerungen: Hypoxie führte zu einer verminderten Hirnaktivität im fMRT bei motorischer und visueller Stimulation trotz erhaltener Hirnfunktion. Die mit der Gehirnaktivierung verbundenen Veränderungen der Oxygenierung scheinen eher Einfluss auf den motorischen Bereich als den visuellen Kortex zu haben. Die Adaptationsfähigkeit an chronische hypoxische Zustände scheint sich demzufolge zwischen dem motorischen und dem visuellen System zu unterscheiden.

Content

3 Abbreviations

∆S	*BOLD signal intensity change*
2,3-BPG	*2,3-bisphosphoglycerate*
ACA	*anterior cerebral artery*
ADP	*adenosine diphosphate*
AMS	*acute mountain sickness*
ANOVA	*analyses of variance*
AP	*action potential*
ASL	*arterial spin labelling*
ATP	*adenosine triphosphate*
BA	*basilar artery*
BOLD	*blood-oxygen-level-dependent*
CBF	*cerebral blood flow*
CBVv	*venous cerebral blood volume*
CDO_2	*cerebral delivery of O_2*
$CMRO_2$	*cerebral metabolic rate of O_2*
CO_2	*carbon dioxide*
CVR	*Cerebrovascular reactivity*
deoxyHb	*deoxygenated haemoglobin*
EPO	*erythropoietin*
FiO_2	*fraction of inspired O_2*
fMRI	*functional Magnetic Resonance Imaging*
FTT	*finger tapping test*
FWE	*familywise error*
FWHM	*full-width at half-maximum*
Glc	*Glucose*
GLM	*general linear model*
GMN	*grey matter nulled*
HACE	*high altitude cerebral edema*
HAPE	*high altitude pulmonary edema*
Hb	*haemoglobin*
HE	*Hypoxic Encephalopathy*
HIF	*Hypoxia-inducible factor*

HRF ..... *hemodynamic response function*
LLS ..... *Lake Louise Score*
LMU ..... *Ludwig-Maximilians-Universität*
MCA ..... *middle cerebral artery*
MCAv ..... *middle cerebral artery blood velocity*
MNI ..... *Montreal Neurological Institute*
NMR ..... *Nuclear Magnetic Resonance*
NO ..... *nitric oxide*
O_2 ..... *oxygen*
OEF ..... *O_2 extraction fraction*
OXPHOS ..... *oxidative phosphorylation*
oxyHb ..... *oxygenated haemoglobin*
p.d.u. ..... *procedure defined unit*
$PaCO_2$ ..... *partial pressure of CO_2 in arterial blood*
PaO_2 ..... *partial pressure of O_2 in arterial blood*
PCA ..... *posterior cerebral artery*
PCO_2 ..... *partial pressure of CO_2*
PET ..... *positron emission tomography*
pH ..... *concentration of hydrogen ions*
PMC ..... *primary motor cortex*
PO_2 ..... *partial pressure of O_2*
POI ..... *point of interest*
PRES ..... *posterior reversible encephalopathy syndrome*
PVC ..... *primary visual cortex*
R_2* ..... *effective transverse relaxation rate*
RR ..... *blood pressure*
RT ..... *reaction time*
SaO_2 ..... *arterial oxygen saturation*
SD ..... *standard deviation*
SDB ..... *sleep-disordered breathing*
SNR ..... *signal to noise ratio*
TCD ..... *transcranial Doppler ultrasound*
TE ..... *echo time*
VASO ..... *vascular space occupancy*

Ve .....*expiratory Volume*

VEGF.....*Vascular endothelial growth factor*

$\bar{x}$ .....*arithmetic mean*

4 List of figures

5 Introduction

5.1 Hypoxia at altitude

Air is one of the foundations of life for humans, animals and plants. Humans can survive without food for about 40 days, without water for about five days, but can only survive without air for just a couple of minutes. Air contains 21 % oxygen (O_2), thus the fraction of inspired O_2 (FiO_2) is 0.21 accordingly. Humans need it to oxidize nutrients and keep the metabolism going. Every human cell is dependent on continuous influx of O_2. The influx of O_2 into the cell happens only by diffusion of O_2-molecules through the cell membrane. However, transportation of O_2 solely by diffusion is not possible in an organism as complex as the human body. According to Fick's second law, diffusion is only physiologically efficient over very short distances of ≤ 10 µm (Behrends, 2010), as diffusion velocity declines by the square of the distance (Erdélyi, 2013):

$$\frac{\partial \rho}{\partial t} = D \frac{\partial^2 \rho}{\partial x^2}$$

ρ is number of atoms per unit volume, x is distance in [m], t is time in [s], D is diffusion coefficient in [m^2/s]

Long distances can be covered with the help of transport mediums like haemoglobin (Hb) in erythrocytes. The main function of the lungs is intake of sufficient amounts of O_2 from the atmosphere and simultaneously the emission of metabolically generated carbon dioxide (CO_2), both via gas exchange in the alveoli. The alveolar membrane is the gas exchange surface. Lung capillaries carrying deoxygenated blood contact the alveoli and form the very thin membrane, so diffusion of gases between the inspired air and the blood can quickly happen. On average one capillary runs across three alveoli (Behrends, 2010) forming a short section in which the diffusion-driven gas exchange happens. While the blood flows through this contact section, the different partial pressures of capillary and alveolar gases gradually equalize. Diffusion equilibrium is normally reached after one third of the contact section (Behrends, 2010). During certain circumstances like physical exercise, excess CO_2 is produced, and cells require increased O_2. The body responds to this change by increasing the breathing and heart rate, maximizing the rate of possible gas exchange. This increased demand is not satisfied by increased diffusion, since it depends only on fixed variables. O_2 intake is therefore only increased by enhanced perfusion of lung capillaries. Lung

activity is regulated by chemoreceptors measuring the partial pressure of O_2 (PO_2), partial pressure of CO_2 (PCO_2) and concentration of hydrogen ions (pH) in blood and spinal fluid. In vertebrates, Hb can be oxygenated (oxyHb), or deoxygenated (deoxyHb), and it increases the O_2 carrying ability of a litre of blood from 2.7 ml physically dissolved to approximately 250 ml bound to haem (Behrends, 2010). O_2 is almost exclusively transported by Hb, so O_2 transport depends on Hb concentration. A Hb molecule consists of four subunits which are globular proteins with an embedded haem group. A haem group consists of an Fe^{2+} ion located in the centre of the porphyrin ring. The Fe^{2+} ion bound in fact is responsible for the reversible binding of O_2.

There are two conformational forms of Hb. If O_2 content in blood is low and none of the four haem groups has bound O_2, Hb is in a tense form and has low affinity to O_2. The Fe^{2+} ion sticks a little bit out of the plane of the whole porphyrin ring.

Binding of O_2 to the Fe^{2+} ion draws the Fe^{2+} more into the plane of the porphyrin ring. This causes a conformational shift to a more relaxed form. A relaxed state encourages O_2 to bind to the other haem groups within Hb. Hence, O_2 binding is cooperative. After O_2 has been bound to all four haem components, the Hb molecule is saturated. The affinity of Hb to O_2 is modulated by various factors, e. g. pH, CO_2 and 2,3-bisphosphoglycerate (2,3-BPG). Binding of O_2 itself influences the affinity, too. See chapter 5.1.1 for further details.

The driving force for the gas exchange in the peripheral tissue is the difference in local PO_2 and PCO_2 between incoming oxygenated arterial blood and surrounding tissue. In these peripheral tissues, local PO_2 and pH is low because cells consume O_2 and produce H^+ ions through oxidative phosphorylation (OXPHOS), while PCO_2 is high. OXPHOS is a pathway in human metabolism which cells use to oxidize nutrients and release energy to reform adenosine triphosphate (ATP). OXPHOS takes place at the inner membrane of the mitochondria.

At the semipermeable membrane of neurons, there is a potential difference. It is based on the different concentration of ions (Na^+, K^+, Cl^-) between the outside and the inside of the cell, resulting in ion gradients. The arrival of an action potential (AP) triggers a cascade that includes Ca^{2+} influx, neurotransmitter release, binding of neurotransmitter on the post-synaptic side, and opening of ion channels for Na^+ and K^+ (Buxton et al., 2004). Afterwards, Na^+, K^+ and Ca^{2+} must be transported against their gradients back into the cell to re-establish the original ion distributions and the synaptic cleft must be cleansed of Neurotransmitters before the arrival of the next AP (Buxton et al., 2004).

Thus, the energy cost of neural activity arises mainly from the regeneration from signalling processes. As in most biological systems, this energy is gained by the conversion of ATP to adenosine diphosphate (ADP).

Particularly sensitive to a lack of O_2 is the brain. Insufficient supply of O_2 to the brain tissue is only tolerated for a short period of time and brain cells start decaying less than 5 min after their O_2 supply terminates (Goldman and Schafer, 2016). Consequently, cerebral hypoxia can cause severe brain damage and eventually results into death. Such hypoxic situations can appear acutely by failure of circulation due to cardiac arrest or arrhythmias with delayed reanimation, burying or drowning accidents. In patients with chronic hindered lung function due to tumours or pulmonary diseases, decreased arterial O_2 saturation (SaO_2) might occur. In the last three days of life there are clinical phenomena which lead to dramatically declining SaO_2: apnoea periods, Cheyne-Stokes breathing, peripheral cyanosis and pulselessness of radial artery (Hui et al., 2015). Quite similar situations are well known in high altitude or mountain medicine since the PO_2 is lowered at high altitude. Barometric pressure is the hydrostatic pressure of air against surface of the earth. Since the air components at low elevations are compressed by the weight of the air components above them, the barometric pressure is great. At higher elevations, air components are more dispersed and barometric pressure is lower since there is less weight of air from above. The proportion of each gas component in the air is constant up to 12000 m although barometric pressure decreases, but gains of altitude result in a lower PO_2 in the inspired air. PO_2 in the atmosphere decreases with increasing altitude. At sea level PO_2 is ~ 160 mmHg. At 5500 m it is about half and at 8500 m it is only about one third of PO_2 at sea level.

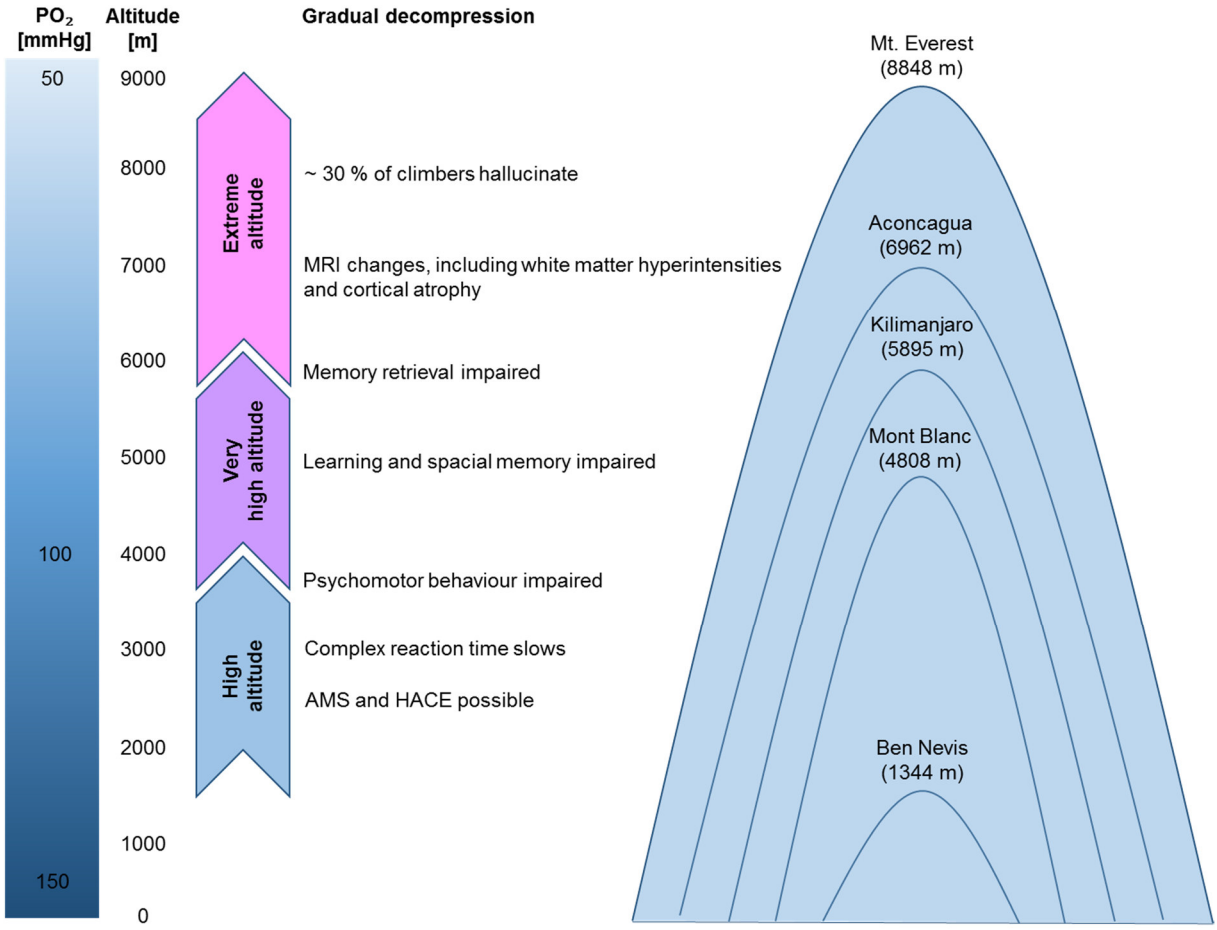

Figure 1: The gradual relation among atmospheric PO_2, altitude gain and the concomitant neurological effects. Gradual exposure, e. g. by walking, to lowered atmospheric PO_2 in high altitudes leads to neurological impairments. With increasing altitude, these impairments show increasing severity. On the right, some major mountains are displayed for reference (Wilson M. H. et al., 2009).

5.1.1 Adaption to hypoxia

Insufficient supply of the brain with O_2 poses an acute life-threatening situation. Exposure to high altitude and therefore hypobaric hypoxia can lead to a spectrum of pathophysiological effects on the brain. The first symptom usually is headache. Acute mountain sickness (AMS) may then develop. AMS rarely occurs at altitudes as low as 2000 m and symptoms typically develop within 6 to 10 hours after ascent, but sometimes as early as 1 hour (Hackett and Roach, 2001). The Lake Louise Consensus Group defined AMS as the presence of headache in an unacclimatised person who has recently arrived at an altitude above 2500 m plus the presence of one or more of the following symptoms: gastrointestinal symptoms (anorexia, nausea, vomiting, etc.),

insomnia, dizziness and lassitude or fatigue (Sutton et al., 1992). To diagnose AMS, the Lake Louise Score (LLS) is being used. AMS can develop in varying severity in individuals following rapid ascent to high altitudes (Sagoo et al., 2016, p. 1). Consequently, trekkers which climb up to high altitudes try to acclimatise themselves to hypoxia. Acclimatisation is the sum of physiological adaption processes in the human organism due to acute exposition to high altitude see. The main contributors are shown in figure 2. By acclimatization, a proper O_2 supply to the tissues can be maintained despite reduced PO_2. Acute exposition of the organism to a reduced PO_2 is compensated by an increased breathing frequency, pulmonary vasoconstriction, increased O_2 affinity of Hb, stimulated erythropoiesis, increased heart rate, increased blood pressure (RR) and increased urination (high altitude diuresis) (Feddersen and Ausserer, 2015). These mechanisms facilitate the sufficient supply of O_2 to the brain (Sagoo et al., 2016, pp. 1–2).

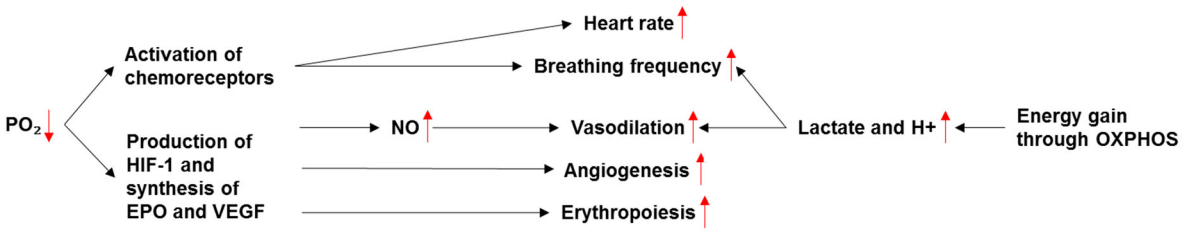

Figure 2: Simplified diagram showing the main processes of adaption to a decreased PO_2 at high altitude. Lowered atmospheric PO_2 activates chemoreceptors that increase heart rate and breathing frequency. Additionally, hypoxia-inducible factor-1 (HIF-1) through production of nitric oxide (NO), erythropoietin (EPO) and vascular endothelial growth factor (VEGF) cause vasodilation, angiogenesis and erythropoiesis. Metabolic processes of OXPHOS are also a substantial stimulus for vasodilation and breathing frequency. (Feddersen and Ausserer, 2015)

The increased breathing frequency is the foremost effect of hypoxia and results in an hypoxia-induced hyperventilation which reaches its highest level after two weeks (Berghold and Schaffert, 2009) of exposure to hypoxia. This hypoxic ventilatory response is based on a lowered PO_2 which is registered by the chemoreceptors in the glomus caroticum. Information from these receptors is conducted to the respiratory centre in the brain stem, breathing rate rises and the PO_2 in the blood increases. Hypocapnia due to this hyperventilation is an adverse, yet compelling effect. Hypoxic pulmonary vasoconstriction via the Euler-Liljestrand mechanism increases pressure in the arterial vessels and serves a homogenized ventilation/perfusion rate in the lungs. The increased pressure however rises the risk of outflow of fluid into the alveoli.

The affinity of Hb to bind O_2 rises at high altitude because of allosteric modulation of CO_2. It binds to the α-amino group of Hb and forms carbaminohaemoglobin (Lehninger et al., 2013). This decreases Hb's affinity for O_2 and is known as the Bohr effect (see figure 3). This mechanism shifts the SaO_2 curve to the right. On the other hand, when CO_2 levels in the blood decrease, CO_2 are released from Hb increasing the O_2 affinity. Up to altitudes of 2000 – 4000 m there is also an increase of 2,3-BPG in erythrocytes (Behrends, 2010). Allosteric modulation of Hb by 2,3-BPG counteracts the effect of hypocapnia and shifts the SaO_2 curve to the left and decreases O_2 affinity.

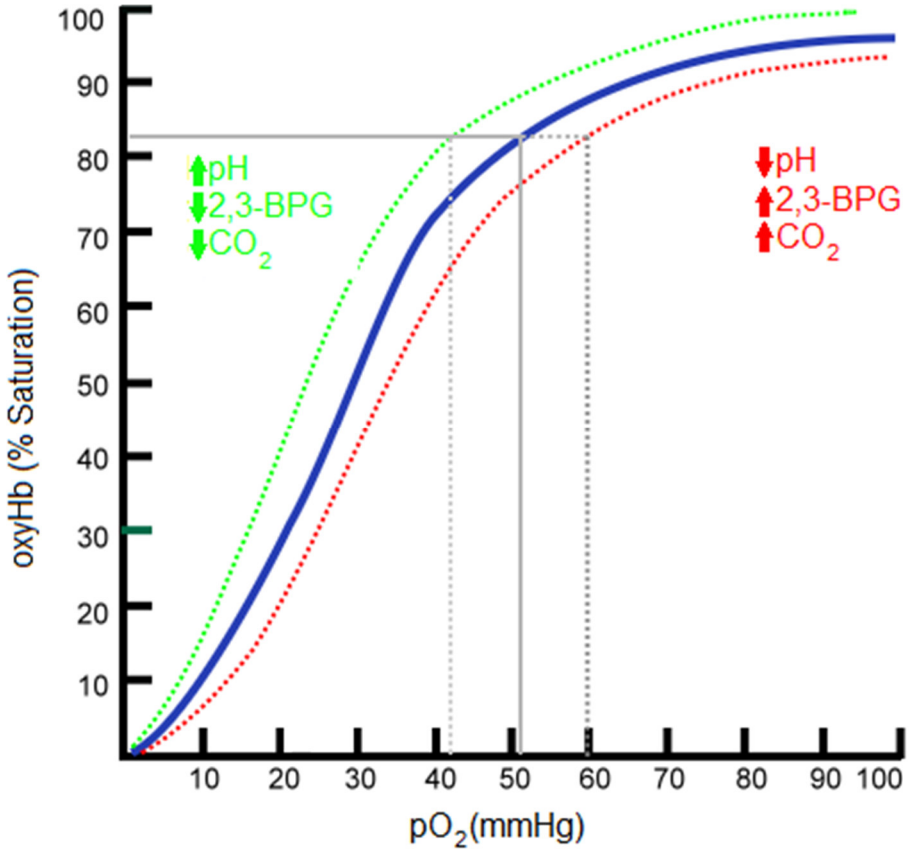

Figure 3: The oxyHb dissociation curve with SaO_2 on the vertical axis and PO_2 in the blood on the horizontal axis, supplemented by altering factors such as pH, 2,3-BPG and CO_2. The standard dissociation curve (blue) has a sigmoid shape due to the conformational change of the Hb molecule induced by the binding of O_2 to Fe^{2+}. Among other factors not displayed, pH, 2,3-BPG and CO_2 can shift the curve to the right or the left. A rightward shift (red dotted) indicates that Hb has a lowered affinity to O_2, making it easier for the Hb to release O_2. A leftward shift indicates the opposite of this condition (Behrends, 2010).

Besides hyperventilation, the most important acute temporal mechanism to maintain SaO_2 is a sympathetic increase of the cardiac output which is achieved mainly by an increase of the heart rate (Berghold and Schaffert, 2009). The heart rate decreases to previous levels after acclimatization took place (Feddersen and Ausserer, 2015)
During the first hours of altitude exposition, an elevated haematocrit can be observed. It originates of a loss of plasma through altitude diuresis and leads to a relative increase of blood cells per unit of blood and thus the O_2 transport capacity of the blood rises. HIFs are transcription factors that respond to decreases in O_2. Specifically, HIF-1 increases NO production through increased inducible NO synthase expression and

subunit 4-2 of cytochrome c oxidase expression (Poyton and Hendrickson, 2015). VEGF is a growth factor involved in angiogenesis that restores the O_2 supply to tissues when blood circulation is inadequate. VEGF binds tyrosine kinase receptors on the cell surface, causing them to dimerize and become activated through transphosphorylation (Ross et al., 2012; Shweiki et al., 1992) and leading to angiogenesis, thus ultimately increasing perfusion of the tissues (Palmer and Clegg, 2014). Additionally, an absolute increase in the number of erythrocytes manifests after about 2-3 weeks due to the kidney producing and secreting the cytokine EPO to increase the production of erythrocytes in the bone marrow.

5.1.2 Pathology of AMS, HAPE, HACE

Breathing hypoxic air reduces the driving gradient of O_2 and thus the attenuated O_2 cascade can compromise the adequate supply of O_2 to the tissues (Wilson et al., 2009). There have been promoted four stages of hypoxia to describe impairments in subjects exposed to acute hypoxia (Carrier, 2006):

1. Indifferent stage: People are not generally aware of the effects of hypoxia at this stage. The primary symptoms are a loss of night vision and a loss of colour vision. These changes can occur at relatively modest altitudes (as low as 1200 m). SaO_2 is typically 90 - 95 %.
2. Fully compensatory stage: In healthy people, this stage may occur at altitudes between 3000 – 4500m. The body generally has the ability to stave off further effects of hypoxia by increasing the rate and depth of ventilation and heart rate. SaO_2 during this phase is typically 80 - 90 %.
3. Partial compensatory stage: In this state, people are unable to compensate for the lack of O_2 and nervous system functioning begins to degrade. AMS occurs. Unfortunately, not everyone recognizes or experiences the signs and symptoms associated with this stage. SaO_2 during this phase typically is 70 - 80 %.
4. Critical stage: This is the terminal stage leading up to death. People are almost completely incapacitated physically and mentally. People in this stage will lose consciousness, convulsions may occur, breathing will be afflicted and finally death occurs. SaO_2 is less than 70 %.

The effects of hypoxia are being attributed to the reduced PO_2, regardless of how it is achieved. There are however, some physiological effects of hypobaria, apart from

those of hypoxia (West et al., 2012). Research data about the difference of hypobaric and normobaric hypoxia is sparse, but it seems that in normobaric hypoxia AMS occurs more seldom and expiratory Volume (Ve) is lower than in hypobaric hypoxia of the same PO_2 (West et al., 2012). However, other reports state that the cardiorespiratory parameters and the severity of AMS were similar between hypobaric and normobaric hypoxia (Richard et al., 2014).

A threshold altitude and barometric pressure for neurological symptoms, attributable to hypobaric hypoxia in resting individuals, can be assumed to be roughly between 2200 - 2500 m altitude and 560 – 585 mmHg barometric pressure (Muhm et al., 2007; Swenson and Bärtsch, 2014b). The severe hypoxia experienced by climbers at extreme altitudes is known to be associated with cerebral dysfunction (Bärtsch and Bailey, 2014; Virués-Ortega et al., 2004). This suggests that cerebral oxygenation might not be fully maintained through adaptive responses, which may include changes in cerebral blood flow (CBF) (Bärtsch and Bailey, 2014; Wilson et al., 2011, p. 2020). The reported prevalence of AMS varies widely (Wilson M. H. et al., 2009, p. 175), but the incidence of AMS was shown to be ~ 0 % at 2500 - 3000 m, ~ 10 % between 3000 - 4000 m, ~ 15 % between 4000 - 4500 m, ~ 50% between 4500 - 5000 m, and ~ 34 % over 5000 m (Bärtsch and Bailey, 2014; Vardy et al., 2006). Neurological consequences will vary greatly from person to person and with rate of ascent (Bärtsch and Bailey, 2014; Wilson M. H. et al., 2009).

AMS can be followed by high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE), both with potentially lethal outcome (Hackett and Roach, 2001). HACE is a severe form of AMS and a clinical diagnosis which is defined as the occurrence of encephalopathic signs of vertigo, ataxia, altered consciousness, or all of them in someone with AMS (Hackett and Roach, 2001). Thus, HACE represents the end-stage of AMS clinically and pathophysiologically (Hackett and Roach, 2001). Results of MRI studies of simulated ascents to very high altitudes in pressure chambers suggests that mild cytotoxic edema might be more prevalent in persons with symptoms of AMS (Feddersen et al., 2015; Wilson M. H. et al., 2009). Such cytotoxic edema may occur when O_2 delivery is lowered because of decreased perfusion in the posterior parts of the brain and this may result in dysfunction of Na^+/K^+-ATPase (Feddersen et al., 2015; Wilson M. H. et al., 2009). AMS evolving into HACE is characterized by dysfunction of the posterior parts of the brain (Feddersen et al., 2015, 2015). Although HACE is far more common at higher altitudes, there are case reports

of HACE at 2500 m (Wilson M. H. et al., 2009). HAPE is the pulmonary form of acute altitude illness and a type of noncardiogenic pulmonary edema (Zafren, 2014, p. 30). Cardinal symptom of HAPE is dry cough. Only late in the sickness does bloody sputum and respiratory distress develop (Hackett and Roach, 2001). As HAPE progresses, resting tachycardia, tachypnoea and cerebral symptoms (50 % of those with HAPE have AMS and 14 % have HACE) become more pronounced (Hackett and Roach, 2001). The estimated mortality among persons with untreated HAPE is 50 % (Bartsch and Swenson, 2013). The pathophysiology of HAPE is not completely understood, but it is characterized by high pulmonary artery pressures that lead to a protein-rich and mildly haemorrhagic edema and is a form of hydrostatic pulmonary edema with altered alveolar-capillary permeability (Swenson et al., 2002). The incidence of those severe forms of AMS however is much lower. It is 6 % - 15 % when people reach altitudes of 4500 m – 5500 m within 1 - 2 days (Bartsch and Swenson, 2013).

5.2 Physiological principles of fMRI

5.2.1 Nutrients and energy consumption of the brain

O_2 and Glucose (Glc) are essential for the metabolism of the brain to generate chemical energy in the form of the ATP molecule. The brain is a highly oxidative organ with only sparse endogenous reserve for energy metabolism, producing more than 90 % of its chemical energy through OXPHOS of Glc (Tuunanen and Kauppinen, 2006, p. 102). In the first stage of this metabolic pathway, glycolysis in the cytoplasm converts the Glc molecule to two molecules of pyruvate and generates two ATP molecules from ADP. Glycolysis uses no O_2 and ATP gain is low, but it is very fast (Behrends, 2010). Pyruvate and O_2 diffuse then into the mitochondria, enter the tricarboxylic acid cycle and result in six molecules of H_2O and CO_2, and the conversion of 36 ADP molecules to ATP (Behrends, 2010; Buxton et al., 2004). Much more ATP is generated in this second stage and the net Glc metabolism is as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O\ (+38ATP)$$

The cerebral O_2 consumption in normal, conscious, young humans is ~ 3.5 ml/100 g/min (Rowell, 1993). The brain therefore, which is only about 2 % of total body weight, accounts for about 20 % of the resting total body O_2 consumption, making it the most O_2 dependent organ in the body (Ainslie, Wilson et al., 2014).

5.2.2 Physiological cerebral delivery of O_2

O_2 reaches the brain via Hb molecules in the blood. The O_2 supply to the brain depends on SaO_2 and CBF (Ainslie, Wilson et al., 2014). The arteries contain almost only oxyHb until the Hb molecules in the blood reach the capillary bed where some of the O_2 is released to the cerebral neurons. Therefore, the capillary region and the draining venules contain blood with both oxyHb and deoxyHb.

The fraction of O_2 carried by an element of blood that is removed in passing through the capillary bed is called the O_2 extraction fraction (OEF) and is defined as follows (Xu et al., 2012):

$$OEF = \frac{[O_2]_{arterial} - [O_2]_{venous}}{[O_2]_{arterial}}$$

$[O_2]_{arterial}$ *and* $[O_2]_{venous}$ *(in mmol O_2/ml blood) are O_2 contents in arterial and venous blood.*

Under most circumstances, considerations of $[O_2]$ only need to focus on Hb bound O_2, as the amount dissolved in plasma is ~ 1.8 % of that bound to Hb and thus negligible (Xu et al., 2012). In the brain, OEF is typically ~ 40 % (Buxton, 2013, p. 3). Since the human brain has a limited capacity for substrate storage and a high cerebral metabolic rate e.g. of O_2 ($CMRO_2$), a precise regulation of CBF is critical for the maintenance of constant nutrient and O_2 supply (Brown and Ransom, 2007). One can assume that cerebral workload would increase $CMRO_2$ and CBF in the same way to maintain this supply. However, positron emission tomography (PET) and fMRI studies revealed that in humans large, stimulus-induced increases in CBF were accompanied by only small increases in $CMRO_2$ (Davis et al., 1998; Fujita et al., 1999). These data indicate that, during short-time functional activation, CBF and $CMRO_2$ are not directly coupled (Mintun et al., 2001).

5.3 Physiological basis of fMRI

OxyHb is diamagnetic and magnetically indistinguishable from brain tissue. Hb can also be desaturated of O_2 and, because of unpaired electrons, altered in its magnetic properties to become paramagnetic (Thulborn et al., 1982). Human neural activity is sampled indirectly with high spatial resolution indirectly, by detecting changes in blood oxygenation that are linked, but not equivalent, to changes in neuronal activity of regions of the brain that contain motor, sensory, language or memory functions (Frahm

et al., 1994; Menon et al., 1995; Mulert and Lemieux, 2010). Those are the so-called “functional areas”. In general, it has been observed that in brain images based on gradient echo techniques with a suitable echo time TE, signal amplitudes are temporarily enhanced in regions of neuronal activation (Mulert and Lemieux, 2010).

Functional magnetic resonance imaging (fMRI) is a non-invasive imaging technique based on the principles of nuclear magnetic resonance (NMR) to measure and localize those specific functions of the human brain (Mulert and Lemieux, 2010). In MRI, radiofrequency pulses are applied to induce precession of nuclear spin magnetic moments in the tissue or object of interest and electromagnetic induction produces a signal, which decays with a time constant called the effective transverse relaxation rate (R_2^*) (Rodgers et al., 2016). This relaxation rate is often expressed in terms of relaxation times T_2^* (Rodgers et al., 2016):

$$T_2^* = 1/R_2^*$$

The varying chemical and structural properties of tissues have characteristic effects on the time evolution of the MR signal, allowing the generation of images with widely varying contrast (Rodgers et al., 2016). Paramagnetic deoxyHb alters the magnetic susceptibility of blood (Thulborn et al., 1982), and the difference in susceptibility between blood in vessels and the surrounding tissue creates local magnetic field distortions that decrease the net MR signal (Buxton, 2013, p. 3).

The idea that changes in blood oxygenation could drive measurable signal changes in brain MRI was introduced by Ogawa and colleagues in 1990 (Ogawa et al., 1990). The blood-oxygen-level-dependent (BOLD) technique makes use of blood as an intrinsic factor (Ogawa et al., 1990), rendering intravenous application of paramagnetic contrast agents (Belliveau et al., 1991) or radioactive substances unnecessary (Raichle, 1983). In an animal experiment with this MRI technique that is sensitive to the local magnetic field distortions, it has been shown by Ogawa that the brain tissue surrounding these vessels had a low signal (Ogawa et al., 1992): When the rats breathed a gas mixture containing 10 % CO_2, there was much less signal loss near the venous vessels. It was proposed that breathing CO_2 increases CBF, decreases $CMRO_2$ and reduces OEF in the brain. The venous blood contained more oxyHb, and the total amount of deoxyHb was reduced (Buxton, 2013, p. 3). The MRI signal therefore was sensitive to OEF. This initial experiment still used CO_2 as an external agent to produce the change in blood oxygenation, but it was shown further that intrinsic changes in blood oxygenation

happen in normal physiology associated with changes in neural activity as well (Buxton, 2013, p. 3; Ogawa et al., 1992).

When a functional area of the brain is activated by a motor task, such as finger tapping or cognitive tasks, the additional neural signalling processes result in a locally increased requirement for energy. An increased $CMRO_2$ in the related brain area is the result (Buxton and Frank, 1997). As the local stores of O_2 in tissues adjacent to capillaries are consumed by glycolysis and waste products build up, various chemical vasodilatory signals cause a vasomotor reaction in arterial sphincters upstream of the capillary bed, causing vasodilation of these vessels (Glover, 2011, p. 2). By that hemodynamic response, the increased blood flow restores the local O_2 level to overcome the deficit. As mentioned, when an area of brain is activated, the blood flow increases much more than the $CMRO_2$ would demand (Fox and Raichle, 1986). Despite the increase in $CMRO_2$, the hemodynamic response leads to an additional reduction in the OEF, as there is more O_2 in the venous blood due to the overcompensating CBF. This means there are two primary consequences of neural activity: more local CBF and an increase in oxygenation concentration. The important physiological parameters that influence the BOLD effect are the $CMRO_2$, the CBF, and the venous cerebral blood volume (CBVv) (Mulert and Lemieux, 2010). Figure 4 shows these contributors linking neural activity to BOLD response.

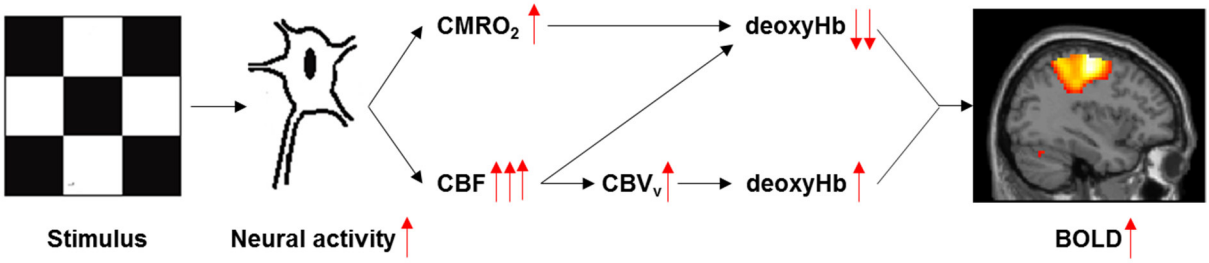

Figure 4: Schematic diagram showing the several physiologic contributions linking neural activity to BOLD response intensity. During neural activity, $CMRO_2$ and CBF increase. CBF far exceeds the additional $CMRO_2$ requirements due to activity. The result is a decreased OEF and thus decreased local deoxyHb concentration. Additionally, CBF independently increases CBVv, which acting on its own would increase deoxyHb concentration. Overall however, the excessive CBF effect dominates and causes a decrease in deoxyHb concentration as well as an associated increase in BOLD response intensity (Rodgers et al., 2016).

CBF can be quantified in terms of the rate of delivery of arterial blood volume $\Delta V_B/\Delta t$ to the capillaries of a particular volume V or mass m of brain tissue (Mulert and Lemieux, 2010). CBV is defined as volume of blood per volume brain tissue (Uh et al., 2009). $CMRO_2$, assuming both unidirectional O_2 transport from capillaries and close to zero tissue O_2 tension at mitochondrial sites, can be related to OEF and CBF (Ho et al., 2008):

$$CMRO2 = OEF * CBF * Yarterial$$

According to the O_2 limitation model, shown in figure 5, a large CBF/$CMRO_2$ ratio during brain activation is required to maintain a steep O_2 gradient between the capillary space and the site of brain tissue mitochondria, facilitating O_2 diffusion into the tissue due to limited diffusion of O_2 from capillaries to mitochondrial sites (Buxton and Frank, 1997; Ho et al., 2008).

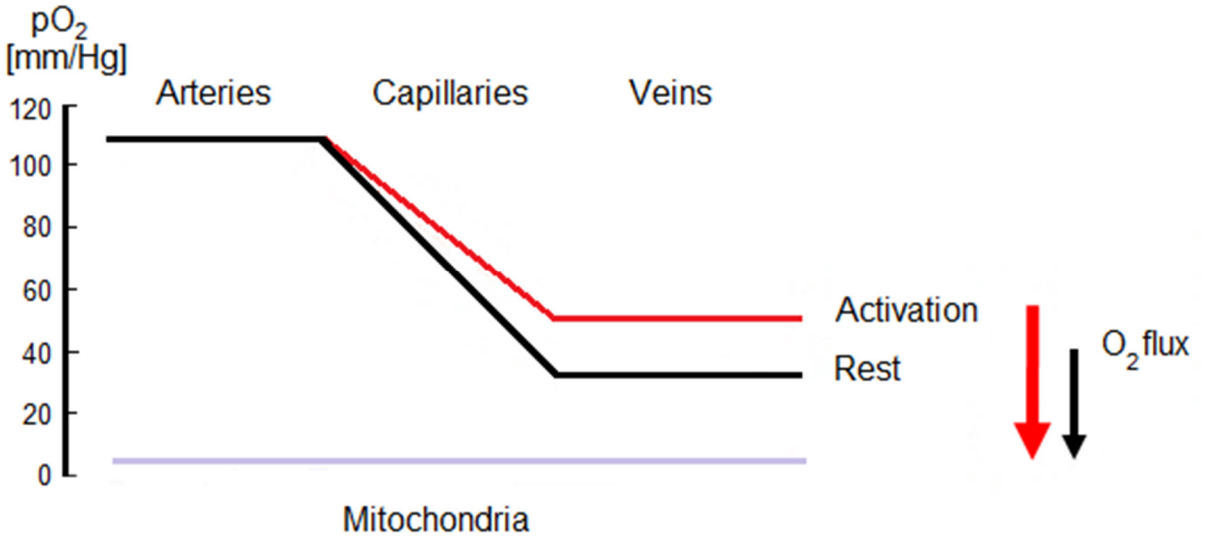

Figure 5: Illustration of the O_2 limitation model showing brain compartments, their corresponding PO_2 during rest and activation and the resulting O_2 flux from vessels to mitochondria (Gjedde, 2006). O_2 diffuses from the high concentration in the capillaries to a low concentration in the mitochondria. To match higher $CMRO_2$ due to activity, the gradient towards the mitochondria. Diffusion distance from capillaries to mitochondria is fixed and mitochondrial PO_2 is near 0, so mean capillary PO_2 must be increased must be increased to increase the O_2 flux (red arrow). This requires that the OEF must be reduced, so CBF must increase more than $CMRO_2$. (Uludag et al., 2005)

The results of Ho et al. showed a consistent CBF/$CMRO_2$ ratio, derived from additive BOLD responses to graded visual stimulation during elevated CBF baseline (Ho et al., 2008).

This O_2 limitation model implies that a drop in arterial O_2 tension should result in augmented CBF response and thus vasodilation in order to sustain a low OEF during brain activation (Ho et al., 2008).

If the brain is in resting state, neural activity is low and CBF is at base level. As mentioned before, 40% of O_2 is extracted from the blood in the capillary region. A constant OEF of the resting brain leads to a fixed deoxyHb/oxyHb ratio in the capillary region and venous vessels. When Hb loses some of its O_2 to become deoxyHb, the magnetic properties change and alter the magnetic susceptibility of blood. Venous blood contains a relatively high concentration of paramagnetic deoxyHb, but brain tissue is diamagnetic. The difference in magnetic conditions between blood vessels and the surrounding tissue creates local magnetic field distortions which lead to rapid

dephasing of excited spins that shorten T_2* and lead to a signal loss in T_2*-weighted images that decrease the net MR signal. In a 3 Tesla magnetic field, the level of deoxyHb in the venous vessels and capillaries is sufficient to reduce the MR signal in the brain by ~ 10% in the baseline state (Buxton, 2013, p. 3).

Directly after the onset of neuronal activation via an external stimulus (or even spontaneous brain activity) the $CMRO_2$ and the consumption of O_2 is increased. Thus, neuronal activity leads to an increased O_2 extraction and a higher concentration of deoxyHb. There is a slight signal decrease, resulting in an initial dip of the fMRI signal (Mulert and Lemieux, 2010). This initial dip is not always observed and has been reported for high field strengths (Buxton, 2001; Mulert and Lemieux, 2010). 2-4 s after stimulus onset haemodynamic response results in a strong increase in local CBF and CBVv, with opposing effects. More O_2 is transported to the site of activation, leading to a washout of deoxyHb and an oversupply of oxyHb in the vicinity of increased neuronal activity. Since oxyHb is diamagnetic, the magnetic properties of blood and brain tissue are more similar, field distortions are reduced, and the local image intensity increases (Deichmann, 2010). The increase in CBVv is associated with a higher concentration of deoxyHb and a lowering of the signal (Deichmann, 2010). However, the effect of the CBF increase outpaces the signal reduction caused by the higher $CMRO_2$ and CBVv values, resulting in a positive BOLD response for about 5 – 10 s (Deichmann, 2010). $CMRO_2$ and CBF return to their baseline levels after about 10 s, but the relaxation of CBVv is slower, so for a certain time there is an increased concentration of deoxyHb due to the higher blood volume, which reduces the signal, resulting in a signal undershoot (Deichmann, 2010; Mulert and Lemieux, 2010). Some researcher however state, that the origin of post-stimulus BOLD undershoot is still controversial (Kim and Ogawa, 2012, p. 1201). Figure 6 shows a generic haemodynamic response to a stimulus and the temporal relation of its contributing physiological processes (adapted from (Mulert and Lemieux, 2010)).

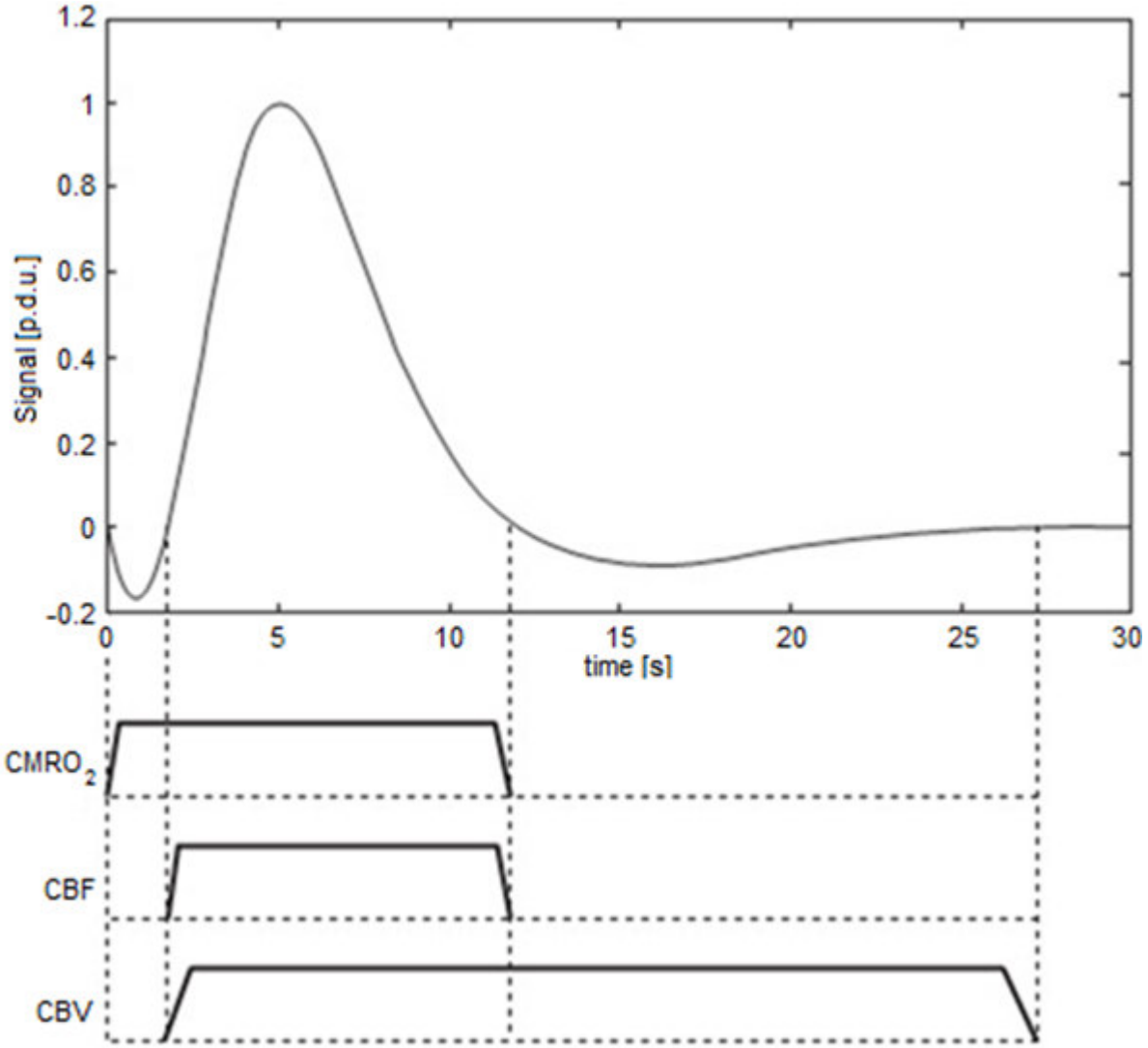

Figure 6: A typical haemodynamic response function following a stimulus, showing a negative initial dip, a strong positive BOLD response, and a subsequent negative undershoot (Mulert and Lemieux, 2010). Signal response is indicated in procedure defined units. These phenomena can be explained with the different time constants of the underlying physiological parameters: $CMRO_2$, CBF and CBVv. (Mulert and Lemieux, 2010)

5.4 CBF and altered FiO_2

Brain perfusion is highly sensitive to changes in the partial pressure of CO_2 in arterial blood ($PaCO_2$)(Davis et al., 1998; Fox and Raichle, 1986; Mandeville, Marota, Ayata, Moskowitz et al., 1999) and, to a lesser degree, the partial pressure of O_2 in arterial blood (PaO_2) (Kety and Schmidt, 1945). Early studies (Cohen et al., 1967; Kety and

Schmidt, 1948), demonstrated that resting CBF does change with hypoxia and hyperoxia, thereby suggesting that CBF regulates O_2 delivery, although it was noted that blood O_2 levels but not tissue O_2 levels likely triggered these CBF changes (Mintun et al., 2001). Complementary it was shown that, hypercapnia causes an increase in CBF and hypocapnia causes decreased CBF.

Given the occurrence of reduced SaO_2 in circumstances like exercise or ascent to high altitude, as well as during diseases such as chronic lung disfunction or heart failure, this might be a bit surprising. As mentioned, a fall in PaO_2 and thus hypoxia, produces cerebral vasodilatation. A number of fMRI studies have shown that during brain activation CBF can rise up to six fold higher values than those of $CMRO_2$ (Davis et al., 1998; Fox and Raichle, 1986; Kim et al., 1999). Although there is a crucial need for O_2, mild to moderate hypoxic hypoxia seems to be quite well tolerated and cerebral haemodynamic response, neural metabolism, and higher brain functions are well preserved within a relatively wide working range in SaO_2 (Ho et al., 2008; Mintun et al., 2001; Rostrup et al., 2005; Shimojyo et al., 1968).

Ascent to high altitude can result in an impairment in neuronal processing, e.g. arithmetic, memory, language, perception, and psychomotor skills (Ainslie, Wilson et al., 2014; Wilson et al., 2009). Such impairments in neural functioning may result in inadequate behaviour in dangerous circumstances during high altitude climbing, and are reflected in a number of deaths in high altitude above 8000 m (Firth et al., 2008). The underlying pathophysiological mechanisms may both relate to disturbed cerebrovascular function and apoptosis of neurons, i. e. by loss of grey and white matter tissue, even following return to sea level (Foster et al., 2015). Hemosiderin deposits as a result of microhaemorrhages, have also been shown in humans who have experienced HACE (Schommer et al., 2013). However, a comparison of studies is difficult due to numerous factors including varying altitude, length of stay, time of follow-up measurement or repeated altitude exposure between measurements. Cerebrovascular reactivity (CVR) to changes in CO_2 is an additional important marker for the functional capacity of cerebral vessels. Reduced magnitude of CVR to changes in CO_2 has been suggested to indicate impaired vascular function (Sobczyk et al., 2014). Although studies have shown that cortical and anterior to posterior CBF differences exist in the response to normobaric hypoxia (Binks et al., 2008; Willie et al., 2012), it remains unclear if regional (r) CVR differences assessed by BOLD MRI

remain upon return to sea level once acid-base balance has been restored and the lasting effects of high altitude exposure can be observed (Foster et al., 2015).
Hypoxia on its own is a cerebral vasodilator, reflected in a rise in CBF in proportion to the severity of isocapnic hypoxia (Cohen et al., 1967; Yang et al., 1994). Upon ascent to high altitude, the fall in PaO_2 tends to cause vasodilatation especially at levels below 40 - 45 mmHg (Ainslie and Ogoh, 2010; Swenson and Bärtsch, 2014a). However, the drop in PaO_2 stimulates the peripheral chemoreceptors and the hypoxia-induced activation of peripheral chemoreceptor activity leads to hyperventilation (Swenson and Bärtsch, 2014a). This mechanism of compensation lowers $PaCO_2$ which is a trigger for cerebral vasoconstriction (Ainslie and Ogoh, 2010). This way the cerebrovascular region gets contradictory signals during exposure to acute hypoxia. That means the balance between the degree of hypoxia and hypocapnia, controlled by changes in ventilation, are the critical determinants of CBF and O_2 supply to the brain respectively (Ainslie, Wilson et al., 2014; Swenson and Bärtsch, 2014a). In spite of the importance of these regulatory mechanisms, most studies have measured the velocity response of the middle cerebral artery (MCAv) to hypoxia, assuming blood gas reactivity is similar for different brain regions (Willie et al., 2012). Study of regional differences in human brain blood flow however, has been limited and even contradictory.

5.4.1 BOLD change in hypoxia

Using BOLD fMRI techniques, it is possible to study perfusion of the brain itself, instead of just measuring CBF in blood vessels supplying the brain. Several studies have examined different regions of the brain. During a short, 3 min lasting exposure to 12 % FiO_2, Bandettini et al found no change in BOLD response amplitude using finger tapping as a motor activation paradigm (Bandettini et al., 1997). Tuunanen et al. showed in their study, that BOLD activation volume decreased as a function of declining SaO_2 in the brain structures involved in execution of a motor task, but visual evoked potentials were not affected by hypoxia, which indicates that processing in the primary visual cortex (PVC) is sustained (Ho et al., 2008; Tuunanen and Kauppinen, 2006). Examining BOLD response during visual stimulation in the presence of hypoxic hypoxia (FiO_2 = 12 %), Ho et al. showed that relative to normoxia, hypoxic hypoxia caused a decrease in activation areas of T_2* BOLD responses, a decrease in the BOLD response size, a loss of the initial overshoot and a decrease in the size of the post-stimulus undershoot from BOLD response (Ho et al., 2008, p. 185).

5.4.1 Cerebral activation in hypoxia

The most commonly used test to detect motor speed dysfunction is the finger tapping test (FTT) from the Halstead-Retain Neuropsychological Battery. This test has proved its efficiency to discover slight motor dysfunctions (Peña-Casanova et al., 1997; Virués-Ortega et al., 2004). Other motor tests have obtained similar effects, for instance the Purdue Pegboard Test, sensitive to speed, motor coordination and precision (Bolmont et al., 2000; Virués-Ortega et al., 2004).

Effects of high altitude manifests as lower motor speed and precision, as compared to subjects' sea level performance (Berry et al., 1989; Virués-Ortega et al., 2004; West, 1984). The extent of environmental O_2 reduction required to demonstrate this effect is variable and in field studies motor deterioration may be confounded with fatigue, a variable associated with both motor delay and altitude gain (Bolmont et al., 2000). It is not clear if motor deficits are a direct consequence of altitude hypoxia, although the results of one study suggested that the effect of fatigue is probably spurious (Sharma et al., 1975).

Regard at al. noted that finger tapping was significantly impaired in 25 % of alpinists with a long history of high and extreme altitude exposures (Regard et al., 1989). Delayed reaction time (RT) in complex target-detection tasks is the most frequently reported effect of altitude (Virués-Ortega et al., 2006). Abnormal motor function has frequently been reported in the altitude literature, for example, evident in reduced speed and precision in finger tapping (Berry et al., 1989; Hornbein et al., 1989).

Roach at al. showed significant relationship of reaction time with acute altitude exposure: a marked increase in mistakes during reaction tests following cognitive testing emerges after ascent to altitude from sea level (Roach et al., 2014, pp. 816–817). However, following 16 days of acclimatization to high altitude, reaction test scores resemble those seen at sea level (Roach et al., 2014, pp. 816–817). This phenomenon has been observed in a variety of experimental settings, including high mountaineering expeditions and hypobaric chambers (Bolmont et al., 2001; Kramer et al., 1993) and significant impairment has been demonstrated at altitudes as low as 1500 m (Denison et al., 1966), although more consistent abnormality is found above 6000 m (Hornbein et al., 1989).

Petrassi et al. concluded in their review: some learning impairment has been robustly demonstrated on the manikin task at rest at ~ 2400 m, with increases in reaction time on orientation tasks from ~ 2100 m, and increased errors in arithmetic and decision

making by ~ 3700 m (Petrassi et al., 2012). Working memory has been affected as low as ~ 2800 m in some studies, but not until ~ 4300 m in others (Petrassi et al., 2012). In simple tests on the other hand, performance appears to be better preserved with increased altitude. This preservation of simple tasks suggests psychomotor performance is maintained and the insufficiencies in complex tasks are cognitive effects (Petrassi et al., 2012, p. 978).

Mild hypoxia at moderate altitudes of ~ 1200 m - 1500 m has been shown to cause visual degradation under scotopic conditions and under photopic conditions at ~ 3000 m (Petrassi et al., 2012).

Colour discrimination can also be altered (Bouquet et al., 2000; Leid and Campagne, 2001). Concentration of respiratory blood gases must also be considered, since hyperventilation (of room air) induced hypocapnia has been shown to improve visual sensitivity and contrast discrimination (Wald et al., 1942). Conversely, a rise in PCO_2 was associated with a decrease in rod sensitivity. It is unclear how hypocapnia affects visual sensitivity. Hypocapnia alone causes constriction of retinal blood vessels and hypoxia present at an altitude of ~ 4600 m, but not ~ 3800 m, is sufficient to overcome hypocapnic vasoconstriction (Brinchmann-Hansen and Myhre, 1990; Petrassi et al., 2012).

Other studies indicate that both PO_2 and PCO_2 affect dark adaptation and visual sensitivity, as these experiments show that early scotopic sensitivity is delayed by hypoxia and hastened by hypocapnia and hyperoxia (Connolly and Hosking, 2006). These results suggest that rod photoreceptor function is subpar when breathing air at sea level (Petrassi et al., 2012).

6 Aims

This work is a prospective observational study. The aim of the study was to examine the cerebral mechanisms of adaptation to normobaric hypoxia. Visual and motor paradigms were used to shed light on the activation of different brain regions in fMRI under normobaric hypoxic conditions. With an FiO_2 of 13 % (equivalent to an altitude of 4000 m) inhaled by healthy subjects, it was examined if adaptation to normobaric hypoxia determines divergent activation in the brain regions supplied by the different main cerebral arterial vessels: the anterior cerebral artery (ACA), the middle cerebral artery (MCA), the posterior cerebral artery (PCA), and the basilar artery (BA).

By using the FTT and visual stimulation through a checkerboard, it was examined if these motor and visual activations lead to a decreased activation in fMRI of the motor and visual brain area after 5 min exposition to 13 % O_2, and if it is being raised again after 8 h of adaption to hypoxia. Additionally, the question occurred if there is a different level of activation in the brain areas supplied by MCA and PCA during acute hypoxia and if 8 h lasting hypoxia influences the level of activation in the brain regions supplied by MCA and PCA. Finally, it was examined if symptomatic AMS, evident by a LLS ≥ 3, has consequences on brain activation patterns measured as BOLD ΔS values.

7 Subjects and Methods

7.1 Participants

A total of 16 healthy male subjects participated in the study. Arithmetic mean ($\bar{x}$) of age was 22.9 with a standard deviation (SD) of 2.2 years, ranging in age from 20 to 28 years. In the following, SD will be indicated by ±. Only male participants were included. Gender disposition to AMS seems unlikely, but cannot be completely ruled out (female cycle may influence development of AMS) (Berghold and Schaffert, 2009). None of the participants took any medication or had any disease. All participants were right-handed. Right-handedness was verified through the Edinburgh Handedness Inventory modified by Salmaso & Longoni ($\bar{x}$ laterality quotient 99.4 ± 2.4) (Salmaso and Maria Longoni, 1986) because they had to execute FTT with their right hand fingers. Furthermore, none of the participants had exceeded an altitude of more than 2500 m from six months before the study until the study took place (to neglect bias of existing altitude adaption) and none had a history of HAPE/HACE ever.

Since MRI examination had to be conducted, common exclusion criteria regarding MRI, e. g. claustrophobia, metal in the body, etc., were also applied. Inclusion and exclusion criteria were identified by a medical history questionnaire.

Recruiting of participants was done via an e-mail distributor by the student's council of the medical department of the Ludwig-Maximilians-Universität (LMU) in Munich (Breite Liste Gesundheit) and through multiple bulletin-board appeals at the medical faculty of the LMU, the Deutscher Alpenverein, the Deutsche Höhenmedizinische Gesellschaft (BExMed) and the Österreichische Höhenmedizinische Gesellschaft (ÖGAHM). A first preselection took place because recruitment could only be done on set times. Study conditions were presented at an informative meeting.

7.2 Study protocol

This study was part of a larger study on the influence of hypoxia on brain adaptation. Hypoxia can artificially be produced either by reducing the barometric pressure which results in hypobaric hypoxia (as in a decompression chamber) or by reducing the percentage of O_2 in an inhaled gas mixture resulting in normobaric hypoxia. The latter method was used in this study. The hypoxic gas mixture was composed of 13 % O_2 (FiO_2 = 0.13) balanced with N_2 (normal pressure). Hypoxia with FiO_2 = 0.13 was

maintained during the entire MRI sessions via a respiratory mask connected to an OxyMount hypoxia machine located outside of the scanner (Oxy Mount, Mountain Air 6001 /XA; OxyTherm GmbH, Coburg, Germany). The participants also wore this mask during the baseline condition without being connected to the hypoxia machine, to ensure identical conditions. SaO2 and heart rate were measured continuously using a finger-mounted pulse oximeter clip on the left index finger (9550 Onyx II; Nonin Medical, Plymouth, USA). MR imaging was performed on a 3 Tesla standard clinical MR scanner (Signa HDx, GE Healthcare, Milwaukee, USA) with 8 receiving channels. Structural imaging was performed using a T_1-weighted fast spoiled gradient-echo recalled (FSPGR) sequence (TR/TE = 6.9/3.2 ms, flip angle 15°, field of view 220 mm, matrix size 256 x 256, voxel size 0.9 x 0.9 x 0.6 mm). Functional imaging was made using a T_2*-weighted gradient-echo (GRE) multislice echo planar imaging (EPI) sequence (TR/TE = 2101.0/35 ms, flip angle 90°, field of view 240 mm, matrix size 64 x 64, voxel size 3.8 x 3.8 x 4.0 mm). Diffusion weighted data were acquired using an EPI sequence (TR/TE = 6200.0/88.7 ms, flip angle 90°, field of view 220 mm, matrix size 128 x 128, voxel size 0.9 x 0.9 x 5.5 mm). Each participant had to complete a total of three MRI sessions lasting approximately 30 min each, consisting of two fMRI runs (1 visual task, 1 motor task) which were pseudo-randomized between participants, followed by the structural sequence. Two sessions were executed on the first day. Thereby, the first session implied baseline conditions with room air (FiO_2 = 0.21, normal pressure) followed by hypoxia exposure to the hypoxic gas mixture (FiO_2 = 0.13, normal pressure) for an average of 7 ± 1 min before the second session started. In the following, the condition in this second run is referred to as short-time hypoxia. In the baseline condition, all five sequences were executed while in the short-time hypoxia the structural sequence was skipped since we did not expect any volume changes between baseline and short-time hypoxia. To measure effects under long-time hypoxia, on the subsequent day participants spent on average 8 h and 29 ± 24 min in a hypoxic chamber with FiO_2 = 0.13 balanced with N_2 (normal pressure) before the MRI trial started. During the long-time hypoxia exposition, the participants stayed in the rooms of a commercial centre for altitude training (Institut für Höhentraining – Höhenbalance München, Spiegelstraße 9, 81241 München) where hypoxia in the rooms was maintained by a hypoxia generator (VPSA-S330, Version V1.1, 2008, B-Cat High Altitude, 4004 MB Tiel, Holland). To ensure uninterrupted hypoxia between the hypoxic chamber and the MR scanner at Klinikum Großhadern Abt.

Neuroradiologie, gas respiratory masks connected to containers filled with a mixture of 13 % O_2 and 87 % N_2 were used.

Cerebral symptoms of AMS were assessed by means of the LLS in the hypoxic chamber every hour as well as before and after the MRI scan. Severe AMS was treated immediately according to the guidelines of the Wilderness Medical Society (Feddersen et al., 2015; Luks et al., 2010). LLS was used to investigate symptoms of AMS containing a self and foreign-rating scale ranging from 0 to 29 (Feddersen et al., 2015; Roach et al., 1992). Subjects with a score ≥ 3 were considered symptomatic, when headache and one other AMS symptom occurred.

During MRI examination, participants were lying in the MR scanner with their head carefully fixed. Both fMRI runs that were executed on each of the three sessions consisted of 8 active and 9 baseline blocks and were lasting 20 s each. This led to a total of approximately 6 min per run. In the visual paradigm, participants had to fixate the centre of a contrast-reversing (8 Hz) black and white checkerboard (Tuunanen, Vidyasagar et al., 2006) in the active condition and they had to fixate a stationary black square on white background in the baseline condition. In the motor task participants had to execute intermittent finger tapping with all five fingers of their right hand. Thereby participants' finger tapping was monitored by tapping on the two buttons of a response pad with the right index and middle finger (Lumina LP-400 response pads for fMRI, Cedrus Corporation, San Pedro, USA). They were told to start and stop the tapping following start and stop commands given through MRI compatible earphones.

7.3 Data analysis

7.3.1 Functional imaging

Functional imaging data of both fMRI runs of all three sessions of all 16 participants were processed the same way using statistical parametric mapping (SPM8) implemented in MATLAB 7.7 (MathWorks Inc., Sherborn, MA, USA) (Friston et al., 1994). Thereby, pre-processing consisted of motion correction, co-registration, segmentation, normalization, and smoothing. To correct possible head movement of participants during the fMRI run, motion correction was applied using the two pass procedure by registering all images of one fMRI scanning series to the mean of the images after the first realignment (Friston et al., 1996). Subsequently, the structural image volume was co-registered to the mean image of the corresponding functional

image series. The co-registered structural image volume was segmented and the estimated parameters to transform images into the standard space defined by the Montreal Neurological Institute (MNI) were used to normalize the functional and the structural images (Albrecht et al., 2010). Finally, images were smoothed using an 8 mm full-width at half-maximum (FWHM) isotropic Gaussian kernel to compensate for individual gyral variability and to attenuate high frequency noise to improve signal to noise ratio (SNR) (Albrecht et al., 2010).

After pre-processing was finished single subject analysis was executed by computing statistical parametric maps by means of the general linear model (GLM) (Friston et al., 1994). Thereby, regressors corresponding to the onsets of the tapping/visual blocks were convolved with the canonical hemodynamic response function (HRF). To suppress activation following head movement, realignment parameters were included as additional regressors.

To investigate activation in response to FTT/visual stimuli we used the primary contrast images and applied a random-effects group analysis while correcting by means of the familywise error (FWE) rate with $p < 0.05$ for the FTT/visual task. Thereby, FWE correction corresponds to the powerful Bonferroni-related procedure correcting for multiple comparisons across whole brain volume. Since the first session was executed under normal conditions independently from the other two sessions, that experiment was used as a pilot study applying the resulting activation as a mask for small volume correction to the other two hypoxia sessions.

To obtain physiological SNR, mean values for the tapping/visual condition and the corresponding constant (normoxia) in the activated regions were determined on single-subject level using a point of interest (POI) strategy. A POI was individually defined for each subject as the voxel in the functionally relevant area that displayed the highest activation amplitude during any of the runs (Table 1). These values of the individual subjects have been used to calculate the mean values of the subjects in the different MRI sessions (Table 1). T_2^*-weighted MR signal changes due to FTT and visual stimulation are referred to as BOLD signal intensity changes (ΔS) .

For statistical analysis of normoxia, short-time and long-time hypoxia, mean SNR values were submitted to repeated measures analyses of variance (ANOVA) where p values < 0.05 were considered significant.

7.3.2 Structural imaging

In order to identify possible tissue volume changes between the baseline and the long-time hypoxia condition we used the voxel-based morphometry (VBM8) plug-in for longitudinal data from SPM8 (Ashburner and Friston, 2000). One participant had to be excluded from this analysis due to missing structural images. To be able to compare tissue volumes of anatomical scans acquired on different time points, data had to be pre-processed using intra-subject realignment, bias correction, segmentation, and normalization. For statistical analysis of longitudinal data, flexible factorial analysis with the two factors subject (15 subjects) and time (2 time points) while correcting by means of the FWE with $p < 0.05$ were applied.

7.3.3 Statistical analysis

To compare the fractional stimulus-evoked BOLD responses of visual and motor activation during hypoxia, the normoxic baseline values were normalized to each other. Relative changes due to hypoxia were derived by adapting the corresponding values of the hypoxic conditions accordingly with respect to the values obtained in the 21% O_2 baseline condition. Thus, the results of the motor paradigm and the visual paradigm could be compared.

Subject-specific ΔS values were separated in groups of participants showing AMS (LLS ≥ 3) and participants not showing AMS (LLS < 3). ΔS of the two groups were examined on a significant impact of AMS on ΔS values.

As mentioned, all statistical tests were conducted using paired t-tests (two tails) with p values < 0.05 considered statistically significant. All values in the following are shown as mean ± SD.

7.4 Ethical Approvement

The local Medical Ethics Review Committee of the LMU in Munich approved *die medizinisch rechtliche Unbedenklichkeit* of the entire study (Projektnummer: 087-10), which was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained by all participants.

8 Results

8.1 Physiological data

There were no differences ($p > 0.05$) in mean heart rate between the baseline scans (70.4 ± 9.7 bpm), short-time hypoxia (71.3 ± 7.4 bpm) and long-time hypoxia scans (73.9 ± 11.3 bpm). The mean SaO_2 was significantly higher during the baseline scan (97.9% ± 1.2 %) compared to both hypoxia scans ($p < 0.001$), but no significant difference was found between short-time (84.1 ± 3.8 %) and long-time hypoxia (82.8 ± 4.4 %) scans ($p > 0.05$). Mean LLS increased from a baseline value of 0 ± 0 to 2 ± 1 after short-time hypoxia and to 3 ± 2 following long-time hypoxia. A LLS ≥ 3 was assessed in 5 subjects after short-time and in 8 subjects after long-time hypoxia.

8.2 BOLD data

The mean rate of finger tapping in the experiments was 0.65 ± 0.21 Hz during normoxia, 0.66 ± 0.31 Hz during short-time hypoxia and 0.66 ± 0.26 Hz. There was no significant difference.

The subjects' ΔS from subject-specific POIs during visual and motor stimulation are shown in Table 1. To distinct BOLD response due to activation from physiological noise, fMRI data were adjusted in SPM with motor stimulation height threshold: $T = 3.28$, $p < 0.001$ {unc.} and visual stimulation height threshold: 4.79, $p < 0.05$ {FEW}.

	Normoxia		Short-time Hypoxia		Long-time Hypoxia	
Subj	Visual	Motor	Visual	Motor	Visual	Motor
1	2,56	1,93	1,47	0,43	0,60	0,69
3	3,51	2,17	1,95	0,54	2,17	0,24
4	3,82	2,37	1,66	0,20	1,43	-0,51
6	7,87	2,76	3,05	0,74	6,00	1,27
7	1,92	2,42	1,58	0,47	1,01	0,77
8	4,13	2,49	2,11	0,36	1,72	0,22
10	1,94	4,29	1,17	0,80	2,34	2,30
11	4,30	3,40	1,11	0,07	2,11	1,21

12	3,15	2,30	2,10	0,95	1,77	0,74
13	3,24	1,55	1,67	-0,01	1,33	0,56
14	4,43	1,95	3,50	0,70	2,54	0,81
16	3,32	1,27	1,21	0,29	2,11	0,31
17	2,33	1,63	1,73	0,86	1,28	0,98
18	2,44	2,36	2,02	0,93	1,95	1,14
19	3,21	3,77	1,36	0,82	1,88	1,07
20	3,70	2,30	0,89	0,70	1,83	1,27
Mean	3,49	2,43	1,79	0,55	2,01	0,82
SD	1,41	0,80	0,69	0,30	1,18	0,62

Table 1: Individual and mean ∆S parameters of the 16 subjects. Columns show the BOLD results of the visual and motor paradigm during the 3 different conditions. ∆S were measured in the subject's POI and are indicated in p.d.u. Bottom rows show the mean ∆S and mean SD for each task and condition.

Repeated ANOVA measures with factors task (tapping, visual) and condition (normoxia, short-time hypoxia, long-time hypoxia) were done and ∆S are significantly different with regards to tasks and conditions (main effect of task: $F(1,15) = 19.43$, $p = 0.001$; main effect of condition: $F(1,15) = 67.09$, $p < 0.001$). Also, significantly different signal intensity change across conditions in motor area and visual cortex could be asserted (motor areas: $F(2,30) = 74.41$, $p < 0.001$; visual cortex: $F(2,30) = 28.18$, $p < 0.001$). Figure 7 shows the mean ∆S of motor and visual stimulation during normoxia (baseline) as well as short-time and long-time hypoxia.

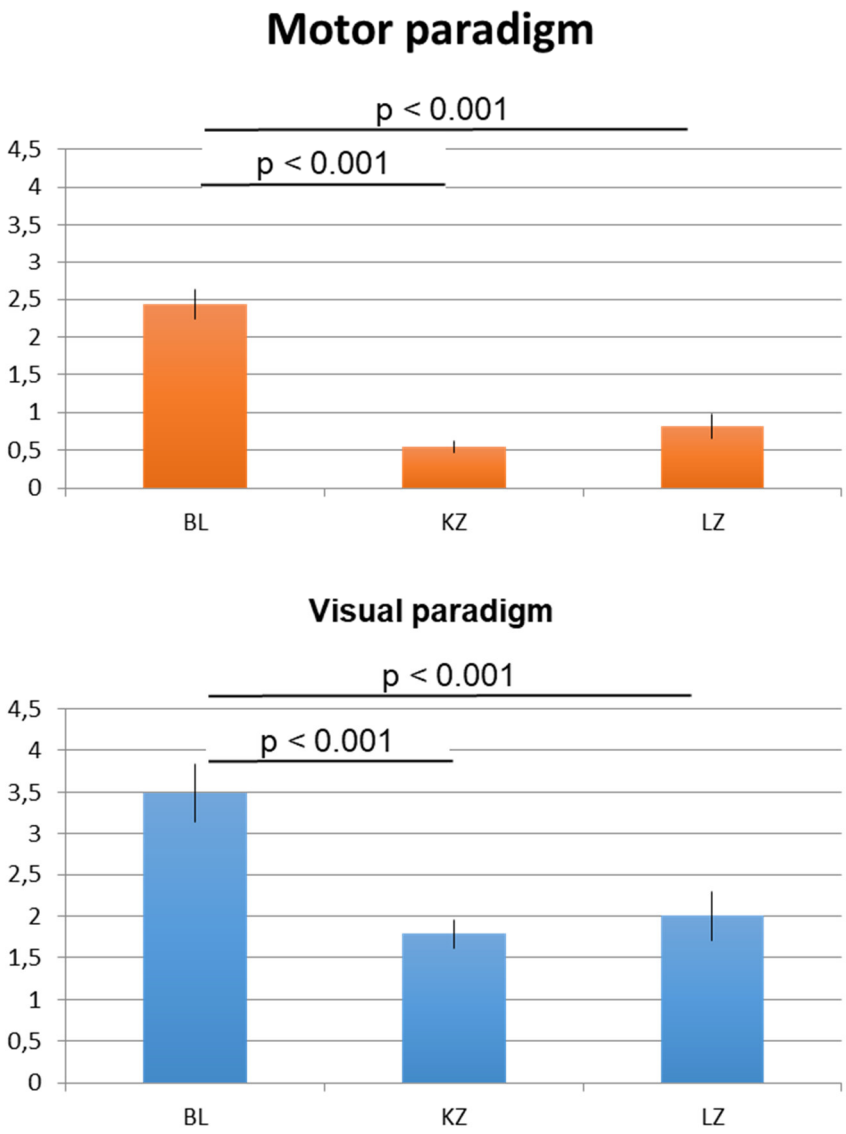

Figure 7: Mean ΔS under normoxic (baseline) and hypoxic (short-time hypoxia and long-time hypoxia) conditions during motor (Motor paradigm) and visual (Visual paradigm) stimulation. Data were obtained from the subject-specific POIs. SD is indicated by lines atop the bars. (*) indicates significant difference to baseline values (p < 0.05).

The mean ΔS during normoxia was 2.43 ± 0.80 % when the subjects were executing FTT, and 3.49 ± 1.41 % when they were looking at the checker board. The finger tapping paradigm was applied in all subjects and all the subjects had lower ΔS increase during hypoxia. During motor stimulation, the mean ΔS due to short-time hypoxia was 0.55 ± 0.30 % and 0.82 ± 0,62 % due to long-time hypoxia. During visual stimulation, the mean ΔS due to short-time hypoxia was 1.79 ± 0.69 %. Long-time hypoxia led to a

mean ΔS of 2.02 ± 1.18 %. Repeated ANOVA measures with factors task (FTT, visual) and the hypoxic conditions (short-time hypoxia, long-time hypoxia) showed a main effect of task ($F(1, 15) = 52.10$, $p < 0.001$), but no main effect of the hypoxic condition ($F(1, 15) = 1.79$, p = ns). So, on the one hand the two different paradigms yielded significantly different signal amplitudes in their corresponding activated brain areas relative to normoxia. On the other hand, the duration of hypoxia (7 ± 1 min versus 509 ± 24 min) had no significant influence on ΔS. To reveal the relative influence of hypoxia on mean ΔS values compared to ΔS values in normoxia, normoxic ΔS values were normalized using SPM8. Baseline ΔS values due to motor and visual stimulation were set to 5 (motor stimulation height threshold: $T = 3.28$, $p < 0.001$ {unc.}); visual stimulation height threshold: 4.79, $p < 0.05$ {FEW}) and the corresponding values of short-time and long-time hypoxia were aligned accordingly. Figure 8 shows the results after normalization of baseline values, and the calculated ΔS values indicate that task-related mean ΔS is higher due to visual stimulation (2.81 ± 1.01 %) than due to FTT (1.42 ± 0.90 %).

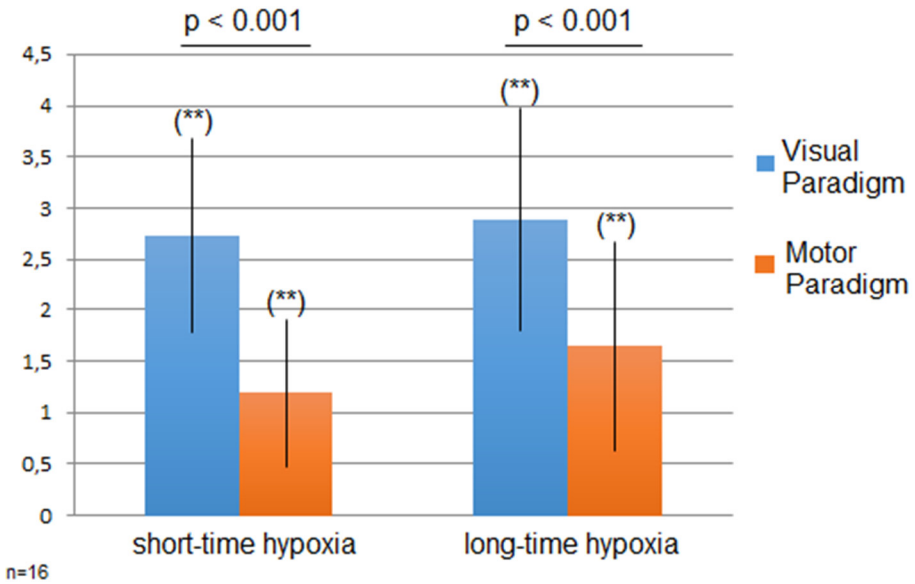

Figure 8: Normalized mean ΔS under short-time and long-time hypoxic conditions during visual (Visual paradigm) and motor (Motor paradigm) stimulation. Data were obtained from the subject-specific POIs. All ΔS were normalized with respect to normoxia (not shown) with corresponding stimulation. SD is indicated by lines atop the bars. (**) indicates no significant difference between short-time and long-time hypoxia values ($p > 0.05$).

To illustrate the extent of decline in a simple way, i. e. by means of percentage values, both normoxic short-time and long-time hypoxia baseline values were artificially set to 100%. The corresponding mean ΔS of motor and visual stimulation during short-time and long-time hypoxia were aligned to their baseline values as well (Figure 9). Hypoxia resulted in a signal that constituted approximately 28 % (motor) and 54 % (visual) of baseline values.

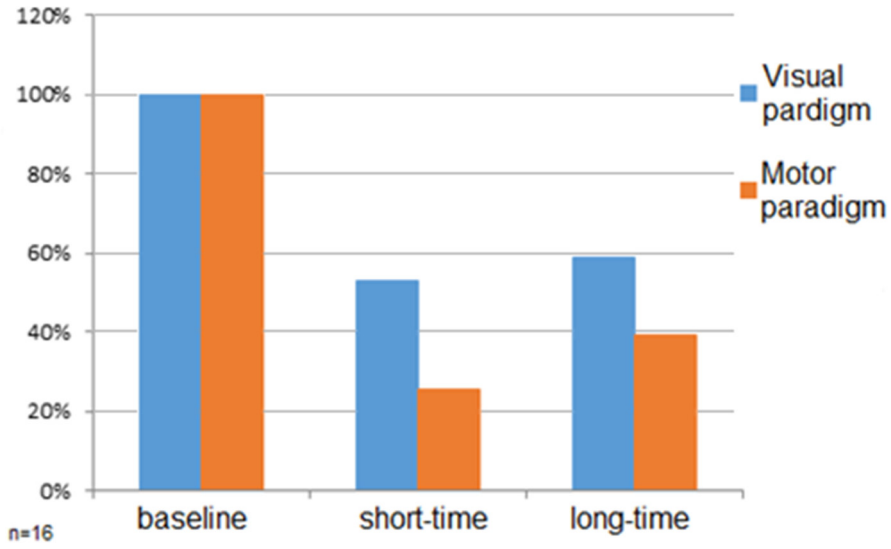

Figure 9: Normalized mean ΔS of motor and visual stimulation during baseline, short-time and long-time hypoxia. Values are indicated as proportion in % of normoxic ΔS values, which were given a value of 5 and set as 100 %.

Correlation of AMS (LLS ≥ 3) to subjects' ΔS values showed no main effect of symptomatic AMS on ΔS (p > 0. 05), probably due to the limited sample size.
To get a picture of the localisation of activated brain regions, a coloured map of BOLD image intensity changes showing the voxelwise average BOLD effect of visual and motor activation during normoxia and hypoxia has been created (Figure 10). The magnitude of the values evoked by the different hypoxic conditions were quite diverging in their amount. To create a presentable image of activation during all conditions, a certain threshold for activation values had to be set for each breathing condition to either distinguish signal based on activation from noise or irrelevant activation (e. g. eye movement, breathing, etc.) and to see activation of the brain at all. This means the image is only meant for ease of comprehension and does not allow a comparison of the values because different signal thresholds were used.

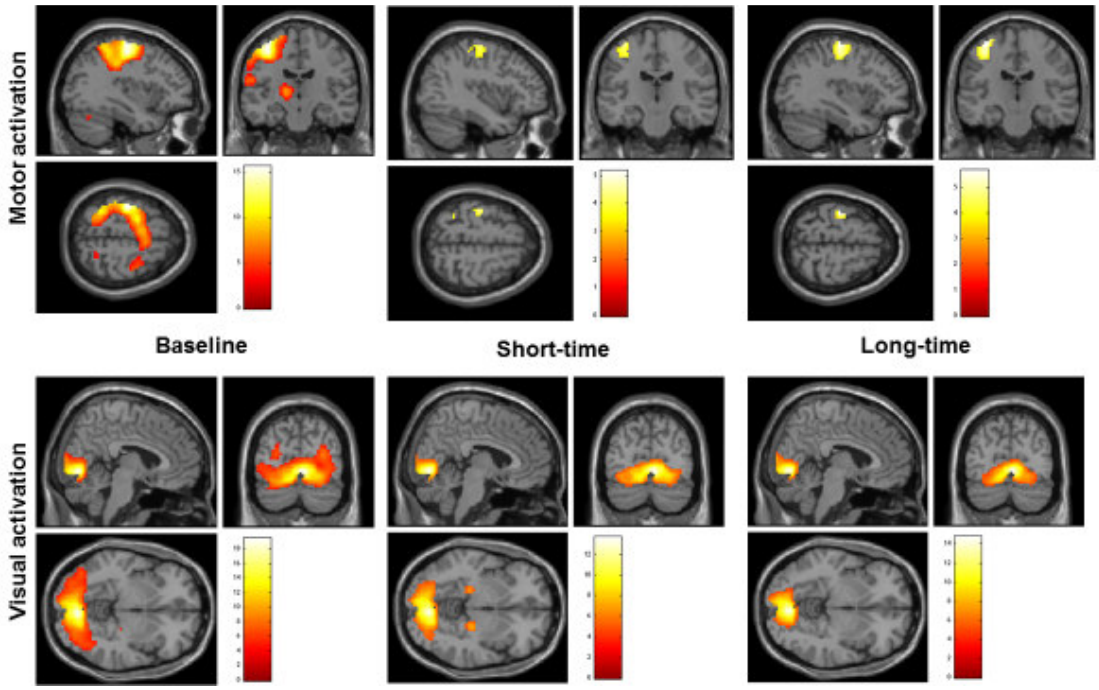

Figure 10: Pseudo-colour map of BOLD image intensity changes in response to visual and motor stimulation (Visual and Motor activation) during baseline condition (normoxia) as well as short-time and long-time hypoxia condition.

9 Discussion

ΔS in hypoxia were lower after visual stimulation than after motor stimulation compared to normoxic conditions ($p < 0.05$). However, the experiments didn't find significant ΔS in the visual or motor cortex, comparing both hypoxic conditions. But the results of the experiments show a pronounced effect of hypoxia itself on the BOLD response. The values revealed fewer activation areas of BOLD responses relative to normoxia, and a decrease in the BOLD response sizes. Visual stimulation in hypoxia led to a ΔS which on average was reduced by 46 % during low SaO_2. Motor stimulation led to a mean signal reduction by 72 %.

9.1 Changes of ΔS during hypoxia

In the past, some studies have reported that a decrease in SaO_2 reduces fMRI responses under an event-related stimulation paradigm. The general hypoxia effect on BOLD response found by the visual experiment is quite similar to the BOLD response described by other studies using a visual paradigm (Ho et al., 2008; Rostrup et al., 2005; Tuunanen, Vidyasagar et al., 2006). The study results demonstrate a reduction in the size of brain regions showing BOLD responses during visual stimulation in hypoxia (Ho et al., 2008; Tuunanen and Kauppinen, 2006). Examining BOLD response during visual stimulation in the presence of hypoxic hypoxia, Ho et al. showed that relative to normoxia, hypobaric hypoxia caused a decrease in activation areas of T_2^* ΔS and the thresholded areas in hypoxia were on average 55 % smaller for the BOLD scans (Ho et al., 2008, p. 185). Those groups also showed that the amplitude of BOLD response was reduced during acute hypoxia. There has been decreased amplitude, absence of initial sharp overshoot and a decrease in the size of the post-stimulus undershoot from BOLD response by decreasing SaO_2 (Ho et al., 2008).

That hypoxia decreases BOLD activation volume in the brain structures involved in execution of a motor task has been shown previously in animals (Sicard and Duong, 2005) and humans (Rostrup et al., 2005; Tuunanen and Kauppinen, 2006). Analysis for BOLD pixels activated due to normoxia and hypoxia showed that the number of pixels declines by ~ 50 % from the supplementary motor area, the supramarginal gyrus and the region around the central sulcus (Tuunanen and Kauppinen, 2006). The percentage decline in brain volumes showing BOLD in hypoxia was not different between the anatomical regions, however (Tuunanen and Kauppinen, 2006).

Attenuating effects on ΔS could also be reported by studies in animals (Sumiyoshi et al., 2012) and humans (Rostrup et al., 2005).

9.2 Reasons for BOLD changes in hypoxia: CBF, $CMRO_2$, CBVv

A detection of statistically significant BOLD response is regarded as a change in neural activity at a certain brain location, but interpreting the ΔS as a quantitative reflection of the magnitude of underlying change in neural activity or metabolism is problematic (Ances et al., 2008). The question arises whether a higher BOLD response in one particular region compared to another brain region indicates greater change in neural activity or O_2 metabolism as well (Ances et al., 2008). There are mainly two sources of physiological variability that could explain a discrepancy between the extent of the BOLD response and the extent of genuine physiological responses: the ceiling for the BOLD response magnitude is limited by the quantity of deoxyHb molecules present at baseline. Baseline conditions however can vary across the brain (Davis et al., 1998). In modelling the BOLD response, the effect of variable baseline conditions is described by the scaling factor M (Ances et al., 2008; Davis et al., 1998). As mentioned earlier, the important physiological parameters that influence the BOLD effect are the $CMRO_2$, CBF and the CBVv. ΔS in extravascular brain tissue can be approximated as follows (Kim and Ogawa, 2012):

$$\Delta S = M * \left(\frac{\Delta CBF/CBF - \Delta CMRO_2/CMRO_2}{\Delta CBF/CBF + 1} - \frac{1}{\beta} * \frac{\Delta CBVv}{CBVv} \right)$$

$$\Delta S = M * \left(\frac{\Delta SvO_2}{1 - SvO_2} - \frac{1}{\beta} * \frac{\Delta CBVv}{CBVv} \right)$$

M is the BOLD calibration constant. SvO_2 is the venous blood oxygenation level. β is 1.5 and is assumed accordingly to Davis and colleagues (Davis et al., 1998). SaO_2 is assumed to be 100 % at unaffected baseline condition. Haematocrit level in venous blood is assumed to be unchanged with stimulus.

M is a scaling parameter that represents the maximum possible ΔS that would be achieved by complete elimination of all deoxyHb (Whittaker et al., 2016). M comprises a number of factors determined by the baseline physiological state, as well the dependence of ΔR_2^* on TE, and thus is specific to a subject and region during a particular scanning session (Whittaker et al., 2016). The first term in the above equation relates to the mismatch between relative O_2 consumption and CBF change

and the second term relates to the relative CBVv change (Kim and Ogawa, 2012). This formula shows that an increase in SvO_2, and a decrease in OEF, will increase ΔS, while an increase in CBVv decreases ΔS (Kim and Ogawa, 2012). Another potential source of variability of the BOLD response is that the coupling of CBF and $CMRO_2$ could vary across the brain or potentially in the same area under different conditions (Ances et al., 2008).

9.3 CBF

Considering the importance of cerebral oxygenation, it's not surprising that physiological adjustments taking place during exposure to hypoxia usually can maintain global cerebral delivery of O_2 (CDO_2). The observation of an increased global CBF during hypoxia is well established (Ainslie and Subudhi, 2014). Consistent with the conservation of (O_2) mass principle, cerebral OEF is inversely proportional to CBF when metabolism is held constant, and directly proportional to metabolism when CBF is held constant (Ainslie et al., 2016). Hypoxia-induced vasodilatation occurs in all involved vessels, from the large extracranial and intracranial arteries (Willie et al., 2012; Wilson et al., 2011) to the arterioles in the pia mater (Wolff et al., 1930). The pial arterioles are the principal site for modulation of cerebrovascular resistance and thus the vasodilatation, both because their collective surface area is quite big and because their tone is coupled to the metabolic state of downstream neurovascular units (Iadecola, 2004). These findings of increased CBF persist upon ascent to high altitude despite marked hypocapnia, which typically provokes cerebral vasoconstriction (Ainslie et al., 2016). Although global CDO_2 is maintained at rest and even at maximal exercise in hypoxia (Smith et al., 2014), there are reported disparities in regional CBF and vascular reactivity (Binks et al., 2008) as well as an overall drop in tissue PO_2 (Ainslie, Shaw et al., 2014). Despite the decrease in tissue PO_2, cerebral OEF and $CMRO_2$ seem unaltered (Ainslie, Shaw et al., 2014; Kety and Schmidt, 1948). This suggests that the nutrient requirements of brain tissue are met when global CDO_2 is maintained, yet the mechanisms responsible for hypoxia-related neurological symptoms and deficits remain uncertain (Ainslie et al., 2016). There is also evidence that although global CDO_2 is sufficient to maintain overall metabolism, other extramitochondrial cellular processes are more sensitive to even small hypoxic insults (Ainslie et al., 2016). The synthesis of enzymes and related neurotransmitters (e.g. glutamate, serotonin, acetylcholine, dopamine) is very O_2 sensitive (Pulsinelli, 1985).

It's important to note that severe hypoxaemia has been associated with microhaemorrhages in the corpus callosum of patients with a history of HACE (Kallenberg et al., 2008). This could be considered as a situation when the increase in CBF is maladaptive. Erythrocyte extravasation was taken to reflect cerebral capillary stress failure subsequent to cerebral hyperperfusion and severe blood-brain barrier disruption, so these adverse outcomes suggest that the cerebrovascular changes that occur in response to severe hypoxaemic stress become maladaptive when they extend beyond a normal human physiological reserve (Ainslie et al., 2016).

The mechanisms that integrate the upstream sensing of SaO_2 and downstream transduction of the signal into vasodilatory reactivity are still not fully known, but receive much focus (Ainslie et al., 2016). The erythrocyte, in addition to its O_2 transport role, may also function as an O_2 sensor and a NO signal transducer capable of affecting vasodilatation in hypoxia, thus titrating O_2 supply against metabolic demand (Ainslie et al., 2016). Erythrocyte-mediated hypoxic vasodilatation mechanisms are dependent on the transition of Hb, i. e. from the oxyHb state to the deoxyHb state, which occurs at the site of arterioles through to the capillaries. The resultant vasodilatory signals can propagate in retrograde fashion to induce vasodilatation throughout all the involved cerebral vessels (Hoiland et al., 2016).

As mentioned, PET scan data reveal that the increase in CBF during isocapnic hypoxia is different between brain regions (Binks et al., 2008). Some brain regions receive a proportionally greater increase in regional CBF. The most prominent increases in regional CBF were seen in the nuclei of the basal ganglia as well as several other phylogenetically old brain regions, specifically the putamen, thalamus, nucleus accumbens and pallidum (Binks et al., 2008). These areas are mostly perfused by the lenticulostriate arteries which are branches of the MCA.

Investigations comparing the anterior and posterior circulation are sparse. Transcranial Doppler ultrasound (TCD) data at sea level indicate greater reactivity to hypoxia in the brainstem than cortex (Willie et al., 2012). Willie et al. measured a greater elevation in the vertebral artery's CBFV after a rapid ascent to 5260 m than in the ICA. It seems that there is a preferential maintenance of brainstem CBF upon acute exposure to hypoxia (Willie et al., 2012). On the other hand, Feddersen et al. report that a sojourn at high altitude led to a decrease of CBFV in the PCA and an increase in the MCA (Feddersen et al., 2015). It has been shown that there is an increase in sympathetic activity at high altitudes (Hainsworth et al., 2007), so this may be a relevant factor for

the CBFV changes as the posterior parts of the blood vessels are less innervated by the sympathetic nervous system (Beausang-Linder and Bill, 1981). Additionally, there are reports that sympathetic activity influences the reactivity of CBF to $PaCO_2$ (D'Alecy et al., 1979; Jordan et al., 2000). Hypoxia-induced elevations in sympathetic nerve activity seem to have the potential to affect CBF by both direct and indirect mechanisms, e.g. via systemically mediated changes in cardiac output being redistributed to the brain (Ainslie, Wilson et al., 2014). Whether that can explain the behaviour of CBFV in the PCA remains unclear (Feddersen et al., 2015). However, it is known that hypoperfusion in the PCA occurs in patients with hypoxic brain injuries. Cardiac malfunction or asphyxiation may cause brain injury in very hypoxia-sensitive areas such as the basal ganglia or the posterior brain areas (Feddersen et al., 2015). Hypoperfusion of cerebral tissue can lead to cytotoxic edema and accumulation of water in the stroma because of malfunction of the transmembrane pump (Moseley et al., 1990). An examination using diffusion-weighted MRI has shown that a lowered apparent diffusion coefficient directly preceded AMS symptoms (Hunt et al., 2013).

AMS may sometimes evolve directly into HACE, which is characterized by dysfunction of the posterior parts of the brain. A similar syndrome occurring during normoxia is the posterior reversible encephalopathy syndrome (PRES). It develops in patients with complex systemic conditions such as eclampsia, after transplantation, infection, autoimmune diseases and after cancer chemotherapy (Hunt et al., 2013). CT or MRI studies show that the edema is often widespread but predominantly in the parietal and occipital brain regions and these reports have detected reduced brain perfusion in regions of PRES (Bartynski, 2008). Brubaker et al. compared anterior with posterior hemispheric flow and demonstrated significant posterior brain hypoperfusion with increased mean transit time, reduced CBVv, and reduced CBF in PRES (Brubaker et al., 2005). Susceptibility-weighted MRI detected microhaemorrhages predominantly in the splenium of the corpus callosum (Schommer et al., 2013). These microhaemorrhages were a highly specific sign of HACE and correlated with the extent of the clinical presentation. It has been suggested that PRES develops because of the failure of autoregulation and hyperperfusion, but the theory of alternative endothelial dysfunction and hypoperfusion and vasoconstriction leading to altered integrity of the blood–brain barrier is favoured (Bartynski, 2008). Similar pathophysiological considerations might be valid for the development of HACE. Anatomical findings of the watershed area between the ACA and the PCA at the splenium might explain this

predilection for microhaemorrhages in HACE, in view of the continuous decrease of CBFV in the PCA. These findings indicate that the physiological response of CBFV to hypobaric hypoxia differs in the anterior and posterior supratentorial parts of cerebral circulation despite their similar physiological changes (Feddersen et al., 2015).
It has also been suggested that changes in global CBF can result from the intake of commonly used substances (e.g. caffeine, nicotine, and alcohol), changes in the concentration of endogenous substances (e.g. oestrogen and adrenaline), or experimental administration of various drugs (Kim and Ogawa, 2012). These global CBF changes can affect the dynamics and magnitude of BOLD (Cohen et al., 2002). Also, at higher baseline levels of SvO_2, the maximal allowable relative change in SvO_2 decreases, and as the relative change in SvO_2 diminishes, the BOLD response no longer linearly correlates with CBF changes, especially for extremely large CBF responses (Lee et al., 2002). However, within the range of normal physiological conditions, it is most likely that baseline-condition dependence of BOLD responses is due to baseline-condition dependence of CBF responses (Kim and Ogawa, 2012).

9.4 CBV

One of the major components contributing to BOLD contrast is the CBVv. Dynamic CBF and CBVv changes are intercorrelated, since CBF is dependent on CBVv and velocity changes (Kim and Ogawa, 2012). Total CBV change can be either estimated from CBF change (Grubb et al., 1974), measured with intravascular injection of a contrast agent (Mandeville et al., 1998) or measured by the vascular space occupancy (VASO) fMRI method. Total CBV should be subdivided into arterial and venous components. Approximately 60 % - 80 % of baseline total CBV is from CBVv (An and Lin, 2002). It therefore is assumed that stimulus-induced total CBV changes are dominated by CBVv changes (Mandeville, Marota, Ayata, Zaharchuk et al., 1999).
Delineated by VASO fMRI and grey matter nulled (GMN) fMRI, Shen et al. report that the effect of hypoxic hypoxia is a decline in the number of active voxels, meaning that the size of the active brain region is reduced during visual stimulation in hypoxic hypoxia (Shen et al., 2012). Previous studies using both BOLD and VASO techniques confirm these findings (Ho et al., 2008; Tuunanen, Murray et al., 2006; Tuunanen, Vidyasagar et al., 2006). Tuunanen et al. proposed a potential explanation to this observation (Tuunanen, Murray et al., 2006). Within the brain region surrounding the core brain activation region, there is a closer match between O_2 consumption and

delivery under decreased O_2 availability during hypoxic hypoxia, thereby reducing the BOLD effect (Shen et al., 2012). The CBVv responses to visual stimulation measured by GMN and VASO fMRI methods however, are similar under hypoxic and normoxic conditions (Ho et al., 2008; Tuunanen, Murray et al., 2006).This indicates that hypoxic hypoxia has little effect on HRF but a significant influence on ΔS (Shen et al., 2012). VASO data indicate that hypoxia does not further augment vascular response to brain activation and so the BOLD observations are likely to be due to differing OEF under the different oxygenation states (Ho et al., 2008). Furthermore, Tuunanen et al. determined OEF in the visual cortex during activation in hypoxic hypoxia with the VASO method and the visual cortex appeared to show heterogeneity in OEF, i. e. regionally varying $CMRO_2$/CBF ratio (Tuunanen, Murray et al., 2006).

9.5 $CMRO_2$

The CBF increase during hypoxia is known and has been discussed extensively (Ainslie and Subudhi, 2014), but uncertainty exists regarding changes in $CMRO_2$ (Vestergaard et al., 2016). MRI methods allow non-invasive measurement of mean $CMRO_2$, i. e. the brain's total O_2 consumption divided by the total brain volume, based on measurement of the concentration of deoxyHb in the sagittal sinus combined with measurement of total blood flow to the brain and Fick's principle (Vestergaard et al., 2016). Using such a technique, on the one hand there seems to be a significant increase in $CMRO_2$ during hypoxic exposure (Smith et al., 2013; Vestergaard et al., 2016; Xu et al., 2012). Using Kety-Schmidt techniques and blood samples on the other hand, found no change in $CMRO_2$ (Ainslie, Shaw et al., 2014; Kety and Schmidt, 1948). Low O_2 availability leads to maximized astrocyte glycolysis and lactate release, and the external lactate causes accumulation of prostaglandin E and subsequent vasodilation (Gordon et al., 2008). In rats, there has been shown that prolonged hypoxia increases the cerebral glycolytic rate (Harik et al., 1994). There is also an increased lactate release in the brain and it contributes up to 9 % of total energy turnover (Overgaard et al., 2012). Additionally, lactate may inhibit cAMP generation and cause a damping of glycolytic rate when lactate concentration rises (Lauritzen et al., 2014). Lactate might also act as a volume transmitter, regulating CBF and the brain energy turnover of groups of neurons (Bergersen and Gjedde, 2012).

It is also known that severe ischemia, e.g. in stroke, results in local release of the excitatory neurotransmitter glutamate and enhances neural activity, which is harmful

for the tissue (Vestergaard et al., 2016). Elevated ventilation rate and concomitant hypocapnia has also an effect on the cerebral metabolism, but the reported effect of hypocapnia is diverse (Vestergaard et al., 2016). A study on patients with cerebral vascular disease showed no conclusive result, as $CMRO_2$ did not change significantly during hypocapnia within the whole group of patients, because 10 out of 19 cases showed a decrease and other 9 showed an increase of $CMRO_2$ during hypocapnia (Tsuda et al., 1987). Another study on healthy humans reported a slight but not significant increase in $CMRO_2$ during normoxic hypocapnia (Alexander et al., 1968). So, for both hypoxia and hypocapnia, there are publications that imply increased metabolic rate and other publications indicating no change. Several reasons may contribute to these discrepancies, e.g. different species, experimental conditions and pathological effects The increased metabolic rate observed by MRI techniques could be a combination of the effects of increased excitability from hypocapnia and increased flow from hypoxia (Vestergaard et al., 2016).

9.6 Coupling of CBF and $CMRO_2$

The magnitude of the ΔS is dictated by neurovascular coupling, i. e. the relationship between CBF and $CMRO_2$, and the physiological phenomenon producing the BOLD response is a divergence in CBF and $CMRO_2$ changes (Whittaker et al., 2016). The hemodynamic origin of the BOLD response allows only a qualitative estimation of neural activity and the non-specific origin of these signal changes is particularly problematic for studies of subjects, since undetected pathologies or atypical brain physiology may confuse the interpretation of the BOLD response as a certain measurement of neural activity (Whittaker et al., 2016).

The ratio of fractional CBF and $CMRO_2$ responses is defined as n, and calibrated BOLD framework can be used to estimate the neurovascular coupling parameter n (Whittaker et al., 2016). This approach was introduced by Davis et al. It acquires BOLD responses and CBF responses simultaneously under distinct conditions of activation and mild hypercapnia (Ances et al., 2008; Davis et al., 1998). The CBF is measured with an arterial spin labelling (ASL) technique. This method uses the insight that BOLD responses depend on changes both in CBF and$CMRO_2$, whereas ASL signals depend solitary on changes in CBF (Blockley et al., 2013). There is evidence that n may be modulated by brain region (Ances et al., 2008; Chiarelli et al., 2007) as well as other

factors such as age (Schmithorst et al., 2015), attention (Moradi et al., 2012), adaptation (Moradi and Buxton, 2013), and stimulus intensity (Liang et al., 2013).

Previous studies using the calibrated BOLD framework have found values of n ranging from ~2 to 4.2 for cortical regions including the motor and visual areas (Ances et al., 2008). Specifically, Ances et al. report lower n within the subcortical lentiform nuclei of the basal ganglia, compared to parts of the visual cortex (Ances et al., 2008). So, if this is a general feature of differences between cortical and subcortical structures, then BOLD responses for similar changes in CBF may be substantially weaker in the subcortical regions (Ances et al., 2008). Chiarelli and colleagues performed an investigation of neurovascular coupling in three cortical brain regions, and found a larger proportional increase of n in the (PVC) region compared to the primary motor cortex (PMC) region (Chiarelli et al., 2007). An even larger coupling constant was observed in the supplementary motor cortex region. This further supports the notion that the disproportional rise in the inflow of fully oxygenated arterial blood and decreased levels of deoxyHb in the venous vasculature, is not uniform across brain regions.

The fMRI results of the present study suggest that the effect of altered blood oxygenation is more influential on the BOLD activation in the motor area supplied by the MCA, rather than the PVC region supplied by the PCA. A limitation of this study was the relatively small sample size, so the study results might not be representative. Previous research has shown the effect of factors like diet and physiological state on the cerebral hemodynamic response to stimulus. It may be possible that certain subjects showed such genuine physiological differences.

Quantitative Interpretation of the magnitude of a ΔS is problematic since it is a multifaceted occurrence, originating from changes in CBF, $CMRO_2$, and CBVv. The ΔS generated by a particular brain region can be characterized as mainly a function of CBF changes, but it is heavily adjusted by the physiological parameters M and n related to that given brain region. Experimental conditions also play a role. Thus, when comparing ΔS between varying cerebral regions, a larger magnitude does not mandatorily mirror a larger change in neural activation or cerebral metabolism. Brain regions with smaller values of n (assuming similar values of M) are characterized by weaker BOLD responses and smaller SNR for a certain change in CBF than regions with higher values of n (Ances et al., 2008). So, it is important to note that areas with equally substantial changes in neural activity could fail to show a detectable BOLD

response because of this. Significant BOLD response however can be interpreted as evidence for an underlying change in neural activity (Ances et al., 2008). Taking into account that n is larger in the PVC region compared to the PMC region (Chiarelli et al., 2007), this might apply to the results in this study. Motor stimulation led to weaker ΔS compared to visual stimulation. This could either be because of diverging influence of hypoxia on changes in BOLD in the separate brain regions or brain regions failing to demonstrate BOLD response, albeit significant underlying changes in neural activity, due to weaker n in the PMC region.

10 Conclusions

The brain in particular is very dependent on a continuous supply of blood. Perfusion of the brain is roughly 50 ml/100 g/min and it is coupled to cerebral metabolism (Goldman and Schafer, 2016). Local increase of brain function leads to increased metabolism and blood flow in these regions. Brain cells are very sensitive to a lack of O_2 and some brain cells start dying less than 5 minutes after their O_2 supply stops (Goldman and Schafer, 2016). Consequently, cerebral hypoxia can quickly lead to severe or even potentially deadly brain damage. Loss of consciousness already appears when blood supply of the brain drops to 35 – 40 ml/100 g/min or cerebral venous PO_2 decreases to 19 mmHg or less (McDowall, 1969). The various cell types of the central nervous system are different regarding their potential to withstand hypoxic stress. In descending order, especially susceptible to O_2 deficiency are Oligodendrocytes, astrocytes, endothelial cells, connective tissue cells and microglia (Hicks S. P., 1968). Many medical conditions can lead to hypoxic brain damage due to deficits of O_2 delivery (Garrido M. M. and Bayarri J. G., 2012):

- Heart failure followed by respiratory depression secondary to massive blood loss, septic or traumatic shock or heart disease (e.g. myocardial infarction or ventricular arrhythmia.
- Respiratory failure followed by cardiac arrest or malfunction as a result of low O_2 intake (e.g. tracheal obstruction, aspiration, or if the inspired air is poor in O_2).
- Reduced O_2 carriage by the blood in CO poisoning.
- Histotoxicity in cyanide poisoning.

The brain by itself can react to low O_2 supply by increasing the CBF and when this reaction is sufficient to preserve the amount of O_2 minimally required, the patient will stay asymptomatic. If the reaction does not suffice however, cerebral hypoxia will gradually lead to neurological consequences. Only mild hypoxia results in less severe neurological symptoms, including dizziness, difficulties with execution of complex tasks, diminished memory retrieval, impaired psychomotor behaviour and AMS. If the O_2 deficit is extended or severe, more serious consequences develop such as loss of consciousness, seizures, deep coma, cessation of brainstem reflexes, and brain death (Safar, 1986).

In the fMRI experiments of the present study, normobaric hypoxia led to decreased cerebral activation during motor and visual stimulation in spite of a preserved cerebral function. The effect of curtailed SaO_2 and its oxygenation changes associated with brain activation seems more influential on the activation in the motor area supplied by the MCA, rather than the PVC region supplied by the PCA. Therefore, the ability of the brain to adapt to chronic hypoxic conditions might differ between the PMC and the PVC. Caution should be used however, when interpreting a lower global BOLD response as reflecting lower O_2 delivery or consumption, since changes in CBF and perfusion are possible without changes in metabolism due to compensatory changes in OEF (Macey et al., 2014).

In infants affected by neonatal Hypoxic(-Ischemic) Encephalopathy (HE), the hypoxic injury causes damage to the sensorimotor cortex, basal ganglia, thalamus, and brain stem (Johnston et al., 2001; Martin et al., 1997). The susceptibility of these brain regions to hypoxic injury is likely to be a consequence of excessive activity of excitatory synapses (Johnston et al., 2002) and indeed, they have been confirmed to have high metabolic rate and are interconnected by functionally active excitatory glutamatergic neurons (Alexander and Crutcher, 1990; Johnston et al., 2002). Therefore the selective vulnerability of the different regions following neonatal hypoxic injury could be a consequence of their position within excitatory circuits (Rocha-Ferreira and Hristova, 2016). Damage to different neonatal brain regions depends on the severity, the duration of the insult, and the developmental stage of the brain (Schmidt-Kastner, 2015). However, comparison with adult brains is difficult. Actually, the immature brain is relatively resistant to hypoxia alone compared to the adult one due to its strong protective mechanisms such as the capability to increase CBF (Johnston et al., 2001). Advancing age is characterized by a decline in physiological functions and it is a complex state, characterized by an accumulation of many pathologies (Daulatzai, 2015). COPD and untreated sleep-disordered breathing (SDB), such as obstructive sleep apnoea and central sleep apnoea, with periodic breathing result in chronic hypoxia with intermittent exacerbations of acute hypoxia that are similar to the effects of high altitude (Daulatzai, 2015; Peppard et al., 2013). Decreases in SaO_2 levels will impose stress on the brain and SDB has been associated with substantial grey matter loss in several cortical regions (Macey et al., 2008). SDB subjects also show different fMRI signals in the sensory and supplementary motor cortex as well as in the thalamus,

cerebellar cortex and deep nuclei, cingulated cortex, medial temporal cortex, insula, right hippocampus, and midbrain (Macey et al., 2006).

In another study, a validated regional homogeneity method was employed in 16 adults who have immigrated to Qinghai-Tibet Plateau (2300 - 4400 m) for 2 years to investigate the local synchronization of resting-state fMRI signals (Chen et al., 2016). Compared with sea level controls, global mean regional homogeneity was significantly increased in high altitude immigrants as well as a regional increase in the right inferolateral sensorimotor cortex (Chen et al., 2016). Furthermore, they showed significant inverse correlation with memory search reaction time within the inferolateral sensorimotor area in high altitude immigrants (Chen et al., 2016).

Taking it all in all, the aforementioned observations support the notion that normobaric hypoxia seems more influential on the motor cortex and that the ability of the brain to adapt to chronic hypoxic conditions might differ between the motor and the visual system.

Hypoxia has been shown to decrease the magnitude of BOLD response to visual and motor stimulation. The effect on the BOLD response is tightly linked to CBF (or more precisely the Hb concentration) and underlying coupled $CMRO_2$ changes as well as to blood volume effects. These main confounders of ΔS seem different in brain regions, as shown in the preceding chapters. Changes in the O_2 carrying capacity may also influence BOLD mechanisms in a way, but such effects have not been investigated. In general, many different factors may contribute to a significant intra- and interindividual variation in BOLD magnitude (Rostrup, 2006). Future studies should address the issue how large precisely a fraction of this variation could be and by which measurable physiological variables it could be measured. It should be noted, that a study with relative few subjects may be prone to bias because of substantial interindividual variation in BOLD magnitude. Although physiological appropriate increases in CBF may occur in hypoxia, unnoticed neurotransmitter dysfunction may occur even during discreet hypoxic challenges that may be considered maladaptive despite the maintenance of CDO_2 and delineation of these mechanisms in humans remains a considerable challenge and focus of current research efforts (Ainslie et al., 2016).

11 References

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