FUNCTIONAL IMAGING WITH NEAR-INFRARED SPECTROSCOPY (NIRS): CORRELATION BETWEEN BRAIN RESPONSE, APOE GENOTYPE, AND NEUROPSYCHOLOGICAL TEST PERFORMANCE

DISSERTATION

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"A prudent question is one-half of wisdom."

Sir Francis Bacon (1561-1626), statesman and philosopher

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ABBREVIATIONS

AD	Alzheimer's Disease
ApoE, ApoE-ε4	Apolipoprotein E, Apolipoprotein E ε4 allele
BA	Brodmann Area
BNT	Boston Naming Test
BOLD	Blood-Oxygenation-Level-Dependent
CBF, rCBF	Cerebral Blood Flow, regional Cerebral Blood Flow
CBV	Cerebral Blood Volume
CERAD-NAB	Consortium to Establish a Registry for Alzheimer's Disease-Neuropsychological Assessment Battery
CPT	Continuous Performance Test
CVLT	California Verbal Learning Test
Cyt-Ox	Cytochrome-C Oxidase
DPF	Differential Path Length Factor
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4^{th} edition
EEG	Electroencephalography
ERP	Event-Related Potential
HbO/oxy-Hb	Oxygenated Hemoglobin
HbR/deoxy-Hb	Deoxygenated/Reduced Hemoglobin
HbT	Total Hemoglobin
lf/LF	left frontal
rf/RF	right frontal
lp/LP	left parietal
rp/RP	right parietal
lt/LT	left temporal
rt/RT	right temporal
MEG	Magnetencephalography
Min, Max	Minimum, Maximum
MMSE	Mini-Mental State Evaluation
MRI, fMRI	Magnet Resonance Imaging, functional Magnetic Resonance Imaging
MWT-B	Mehrfachwahl-Wortschatz-Intelligenztest
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association

NIRS	Near-infrared Spectroscopy
NP	Neuropsychology
PET	Positron Emission Tomography
SD	Standard Deviation
S.E.M	Standard Error of the Mean
SPECT	Single-Positron Emission Computed Tomography
TMT/TMT A, B	Trail Making Test. Form A, B
WAIS-R	Wechsler Adult Intelligence Test – Revised
WCST	Wisconsin Card Sorting Test

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ABSTRACT

Near infrared spectroscopy (NIRS) is a non-invasive optical technique which measures concentration changes of oxygenated (HbO), deoxygenated (HbR), and total hemoglobin (HbT) in brain tissue. In conjunction with the ApoE genotype and neuropsychological measures it might reveal new insights into brain-behaviour relationships.

We studied 240 non-demented elderly individuals selected from the project BASEL cohort (69 females, 171 males; mean age = 72.3 ± 7.03 years) and 21 patients with probable, mild Alzheimer's Disease (12 females, 9 males; mean age = 76.2 ± 6.37 years; mean MMSE = 25.3 ± 2.08) with multi-channel (left and right frontal, left and right parietal, left and right temporal) NIRS during performance of either a verbal (letter) fluency task or a computerized labyrinth test. On the same day, non-demented elderly subjects obtained a comprehensive neuropsychological assessment - the patients with AD had their neuropsychological examination first, followed by the NIRS examination maximally 10 days later. In all study participants the ApoE genotype was determined. The proportion of ApoE-ε4-positives and –negatives was evenly distributed in both study groups (non-demented elderly/patients with AD: 45% ApoE-ε4 positives, 55% ApoE-ε4 negatives).

Mean concentration changes of HbO and HbR during cognitive activation with either task revealed a typical hemoglobin oxygenation response pattern in normal elderly subjects: an increase in HbO and a decrease of HbR over brain areas activated by the task. The NIRS parameter HbR turned out to be a specific marker of brain activation, as significant decreases in HbR were only seen with the verbal fluency task and over the frontal optode positions. In contrast, the parameter HbO exhibited a pattern of general activation across the whole brain and for both activation tasks and thus seems to reflect a state of arousal with a very high sensitivity but no specificity.

Using multiple regression analysis we found that neither the sociodemographic factors age, years of formal education, and gender, nor the ApoE genotype act as predictors of the NIRS response in normal elderly subjects. Specifically, ApoE-ε4 carriers did not differ from ApoE-ε4 non-carriers with respect to concentration changes of HbT at the left parietal position.

Investigating the relationship between covert and overt behaviour, we found a statistically significant correlation between the NIRS response during performance of the verbal fluency task and the NIRS task performance: Subjects able to bring the brain areas critical for successful performance in a cognitive task on-line, show better task performance.

Further, subjects with a typical NIRS response pattern at the left-frontal position (Left-frontal NIRS responders) performed significantly better in the NIRS verbal fluency task than subjects who did not show a typical NIRS response at the left-frontal position (Left-frontal NIRS non-responders). In conjunction with the neuropsychological data collected on the same day, we investigated the relationship between the NIRS response during performance of the verbal fluency task and neuropsychological test performance. We found that the NIRS parameters HbO and HbR at the left and right frontal and left temporal position were able to predict 42% of the performance in a test of executive function (Trail Making Test, quotient of Form B/A). On another route, we compared the neuropsychological test performance of left frontal NIRS responders with the performance of left-frontal NIRS non-responders. The results of these analyses were largely in line with our hypothesis: With the exception of the Wisconsin Card Sorting Test, left-frontal NIRS responders scored significantly better in tests of executive function. Further, the subjects with a typical NIRS response at the left frontal position performed markedly better in the WAIS Block Design and slightly better in the Boston Naming Test and the WAIS Similarities.

In comparison to our sample of non-demented elderly subjects, the patients with mild-stage AD did not show a reduced NIRS response during cognitive activation as compared to rest. However, in comparison to the cognitively normal subjects, they showed a clearly distinguishable profile in the relationship between covert and overt behaviour and between NIRS response and neuropsychological test performance: The NIRS task performance of the patient group was negatively correlated with the brain response. Further, left-frontal NIRS responders in the patient group showed a statistical trend towards lower scores in tests of executive function. In other words, the patients with AD – yet at a mild stage of the disease - could not make use of the cortical network required for an efficient task performance as did the group of cognitively normal elderly subjects.

In conclusion for future functional imaging studies, the analysis of brain-behaviour relationships might add valuable benefit to the investigation of the brain response alone.

1 Introduction

The data reported in this doctoral thesis were recorded in the context of the "Basel Study on the Elderly (BASEL)" project, an extensive multidisciplinary research project. The primary objective of the BASEL project is to identify preclinical markers of Alzheimer's Disease. Alzheimer's Disease (AD) is a progressive neurodegenerative disorder and affects more than 5% of individuals over the age of 65 and increases up to 50% in those over 85. As of today, there is no means to prevent or cure AD once the disease has been diagnosed. As a consequence, treatments aimed at preventing the disease will intervene too late at this stage. Therefore, the identification of preclinical markers of AD would be of tremendous benefit not only in the search for the etiopathogenesis of AD, but, even more importantly, would lead to the development of therapeutical measures at a very early stage of the disease. In order to address this complex question, the BASEL project applies a research strategy which combines multidisciplinary cross-sectional with longitudinal approaches in subgroups of individuals at lower and higher risks to develop AD. Once some of these subjects develop overt AD, baseline comparisons would identify the preclinical markers of this sample (Monsch, 2001).

In this thesis, the focus is on the cross-sectional aspect of the BASEL project, incorporating functional Near-Infrared Spectroscopy (NIRS) of the brain, neuropsychology and ApoE genotype data. The main objective is to correlate cerebral hemoglobin oxygenation data obtained during functional NIRS with NIRS task performance and with selected neuropsychological tests, thus providing a better understanding of the brain-behaviour relationships in healthy elderly subjects and in patients with AD. By incorporating sociodemographic factors such as age, gender, and years of formal education, and including a well-described risk factor for AD, the Apolipoprotein E (ApoE) genotype, we will investigate if and how these factors correlate with the NIRS data, and if they have a modifying effect on the brain response. For this purpose, the author could revert her analyses to a unique sample of 240 non-demented elderly individuals selected from the project BASEL cohort and 21 patients with mild probable Alzheimer Disease (DSM-IV criteria). In all individuals functional multi-channel NIRS was performed and cerebral hemoglobin oxygenation measured during cognitive activation and during rest. On the same day a comprehensive neuropsychological assessment was performed, of which selected tests were chosen for our analyses. In all study participants the ApoE genotype was determined and the presence or absence of at least one ApoE-ε4-allele used for combined analyses.

In the next chapter, the basic principles of NIRS, a non-invasive optical technique to monitor brain function, will be described and the cortical origin of the NIRS signal evaluated. By reviewing past functional NIRS studies conducted predominantly in young healthy volunteers and using different activation paradigms it will be examined whether a typical NIRS response pattern can be described, and how this pattern is altered in normal elderly subjects and in various patient groups.

Chapter 3 provides an overview of the current neuroimaging research investigating brain-behaviour relationships and using Verbal Fluency tasks as functional activation paradigm. The ApoE genotype will be introduced as a major genetic susceptibility factor for AD, and its importance in modulating the brain response evaluated.

Chapter 4 describes the methods, including an overview of the BASEL project and the relevant cross-sectional main examinations (neuropsychological assessment and selected neuropsychological tests, NIRS, ApoE genotype), subject disposition and key subject demographics, and overview of statistical methods applied. The study objectives and hypotheses are outlined in chapter 5 together with the corresponding statistical analysis plan. This order corresponds to the approach applied, e.g. the fact that all data had been collected and thereafter handed over to us for combined analyses.

Chapter 6 contains the result section.

A summary of the objectives and methods as well as a discussion of our major findings are provided in chapter 7. The doctoral thesis ends with conclusions for future research.

2 Near-infrared spectroscopy

Introduction

To date several techniques to examine functional brain activity are available. Historically, electroencephalography (EEG) was the first technology, discovered by the neurologist Hans Berger in the twenties of the past century (Borbély, 1991), followed by other technologies including positron emission tomography (PET) and single-positron emission computed tomography (SPECT), magnetencephalography (MEG), and most recently functional magnetic resonance imaging (fMRI).

A less known technology for monitoring of brain function, near-infrared spectroscopy (NIRS), uses the difference of absorption spectra of oxyhemoglobin and reduced hemoglobin as well as oxygenated cytochrome oxidase in the near infrared.

Optical spectroscopy is a long-established technique for the observation of oxygenation and hemodynamic effects in tissue. The specific interest in NIRS followed the first description by Jöbsis (1977), in which he demonstrated that near-infrared spectroscopy can be used as a new tool to non-invasively monitor cerebral blood oxygenation. Since that time, many hundreds of papers have been published demonstrating its potential not only in the monitoring of the brain but also in other organs (e.g. muscle, breast, liver, and kidney; see Cooper and Delpy, 1997). In clinical research NIRS has been widely used in neonates for early detection of cerebral hypoxemia, and recently also in adult patients with closed-head injury (Kirkpatrick, 1997).

In the first part of this chapter the basic principles of optical imaging and NIRS will be described in detail, and the physiological processes related to brain activity will be outlined. By reviewing studies which have combined NIRS with other, more popular brain-imaging techniques such as PET and fMRI, the cortical origin of the NIRS signal will be evaluated. At the end of the first part, the usefulness of NIRS as a new method to examine functional brain activity will be discussed by contrasting it against other techniques. In the next part, an overview of studies using NIRS as a new tool to non-invasively assess functional brain activation will be given. The question will be addressed whether a typical response pattern can be described in young healthy volunteers and how, if at all, this pattern is altered in normal elderly subjects and in various patient groups.

2.1 Optical imaging: interactions of light with tissue

> It is well known and widely used in medicine that the functional state of tissue influences its optical properties: Cyanosis indicates poor tissue oxygenation, paleness may be related to anaemia and yellow colouring may be due to increased bilirubin concentration indicating liver failure (Villringer and Chance, 1997, p. 435).

It was reported as early as 1949 that the activity of nerve cells is associated with changes in their optical properties (Hill & Keynes, 1949). Changes in optical properties of intact cortical tissue were first described by Jöbsis (1997), while later reports of brain cells' optical properties were described in cell cultures (Stepnoski et al., 1991) and in bloodless brain slices (Grinvald, Lieke, Frostig, Gilbert & Wiesel, 1986; MacVicar & Hochman, 1991).

Villringer and Chance (1997, p.435) define functional optical imaging as follows: "Functional optical imaging is the assessment of physiological changes associated with brain activity by optical methods, i.e. by investigating the interactions of photons with tissue". Photons that interact with tissue may undergo:

- **Absorption** which may lead to
  - Radiationless loss of energy to the medium,
  - Fluorescence or delayed fluorescence,
  - Phosphorescence,
- **Scattering**,
- **Doppler shifts** due to moving particles in the tissue (e.g. blood cells; Villringer, 1997; Villringer & Chance, 1997).

2.1.1 Measurement of Light Absorption

An optical apparatus typically consists of a light source by which the tissue is irradiated, and a light detector that receives light after it has been reflected from or transmitted through the tissue (Villringer & Chance, 1997). Light that is emitted by the light source will be multiply scattered and partly absorbed. To quantify changes in concentrations of absorbing molecules, a model of light diffusing through tissue is required (Strangman, Boas & Sutton, 2002), the traditional approximation to the full photon migration theory being the so called modified Lambert–Beer law (Figure 1).

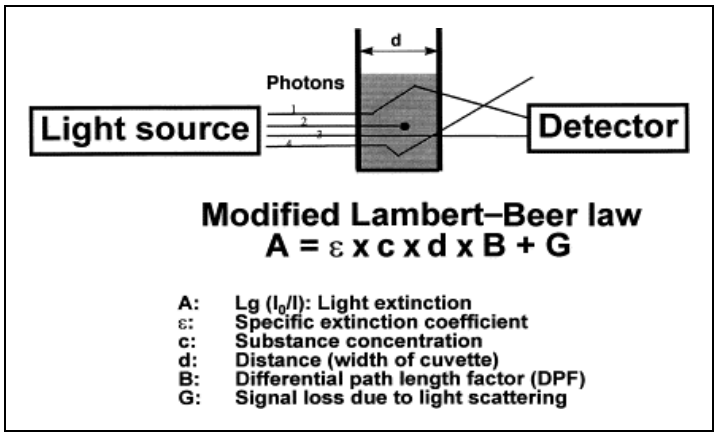


Figure 1: Modified Lambert–Beer law (taken from Villringer & Chance, 1997, p.436).

According to Figure 1, the concentration of a light-absorbing molecule in tissue can be determined similarly to the determination of a substance concentration in a photometer. Assuming infinitesimal substance concentrations, and no scattering in the medium, a concentration can be determined according to the original Lambert–Beer law, $A = \varepsilon \epsilon \times c \times d$, according to which the extinction of light [the logarithm of the ratio of incident versus measured light , $\lg(I_0 / I)$] is proportional to the concentration (c) of the absorber multiplied by the constant extinction coefficient ($\varepsilon\epsilon$) for the particular absorber and the distance (d) corresponding to the width of the cuvette. This law holds as long as photons are either absorbed (photon 2) or transmitted in a straight line directly to the detector (photon 3). With higher substance concentrations and significant light scattering, the formula must be modified to take into account the longer pathlength of light (see photon 1) and the loss of light (photon 4) due to light scattering. In the modified Lambert–Beer law as given in the Figure, therefore a term B which accounts for the longer pathlength and a term G, which is a measure of the signal loss due to light scattering and which depends mainly on geometrical factors are introduced. In certain situations only the difference ($\Delta\Delta c$) between two situations is of interest and under the assumption of a constant light scattering loss the term G cancels out in the subtraction. $\Delta\Delta A = \varepsilon \epsilon \times \Delta\Delta c \times d \times B$.

Ideally, B, the differential path length factor (DPF) should be determined in each experiment, however, technology to do this has not been available in commercial optical devices so far (Villringer, 1997). Therefore, until recently, DPF values from the literature were taken as a reasonable estimate (see Duncan et al., 1996 for measurement of DPF as a function of age).

2.2 The basic principle of near-infrared spectroscopy (NIRS): Measuring brain function with a light bulb

The method of NIRS relies on a simple basic principle: Biological tissue is relatively transparent to light in the near-infrared range between 700–900 nm. This is due to the fact that water absorption and hemoglobin absorption are relatively small within this wavelength region (Villringer & Chance, 1997). Therefore, this wavelength range represents an "optical window" for the non-invasive assessment of brain tissue, and allows light of these wavelengths to penetrate several centimetres through tissue and still be detected (Strangman et al., 2002). As described in section 2.1, (near-infrared) light administered into the head will be multiply scattered and partly absorbed, whereas the scattered light, which is not absorbed on its path, can in part be detected by a second optical probe, the light detector. The light source and the detector are coupled to the subject's head via fibre-optical bundles (optodes, see Figure 2a). The light-receiving optode is connected to a light-detecting system such as a photomultiplier or a charge-coupled device (CCD) camera (Obrig et al., 2000).

Since biological tissue is rather transparent to light between 700 and 900 nm enough light can be detected to allow for a spectroscopic analysis. The most important endogenous absorbers in brain tissue are the chromophores oxygenated hemoglobin (HbO), deoxygenated hemoglobin (HbR) and cytochrome-C-oxidase (Cyt-Ox). These substances have characteristic light absorption patterns in the near-infrared range (see Figure 2b) and thus can be used as biologically relevant markers of brain activity (Villringer, 1997). By adding the changes in HbO and HbR a fourth parameter, total hemoglobin (HbT), can be obtained corresponding to the corpuscular blood volume (Obrig et al., 2000).

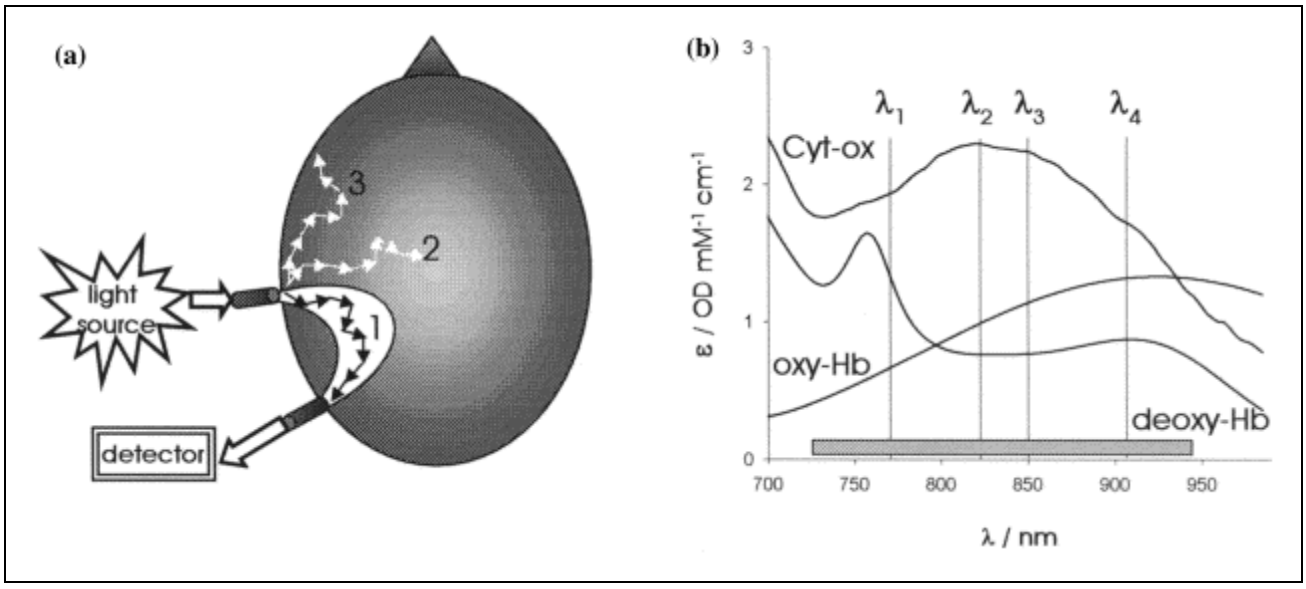


Figure 2: (a) Sketch of NIRS measurement of the adult head. Three possible photon paths are illustrated. Photon 1 undergoes a number of scattering events to reach the detector, photon 2 is absorbed after a number of scattering events, photon 3 leaves the head without being detected by the system. Changes in scatter and absorption in the banana like shaped sampling volume (white) will alter the amount of photons reaching the detector. (b) The spectra of HbO, HbR and Cyt-ox in the near-infrared. The lines denote the four wavelengths of the NIRO-500 monitor, the grey bar signifies the spectral region typically analysed in the CCD-approach (taken from Obrig et al., 2000, p. 126).

Based on different assumptions and modelling algorithms several models of the sample volume have been proposed (Villringer & Chance, 1997). Assuming a homogeneous tissue beneath the optodes, the sample volume is assumed to correspond to a banana-shaped volume beneath the optodes (see Figure 2a). This assumption is in agreement with the findings of Hock et al. (1997a) who found a correlation between the total hemoglobin measured with NIRS and the regional cerebral blood flow (rCBF) measured with positron emission tomography (PET) which was restricted to a semisphere volume of brain tissue with a limited depth of penetration of 1-3 cm. In recent more sophisticated approaches, a multi-layer model imitating skin, skull, CSF and brain tissue has been assumed (‘Monte-Carlo Method’) in which the role of the CSF layer with its potential `light tunnelling' properties is emphasized (Villringer & Chance, 1997).

2.2.1 Relationship between brain activity and optical parameters

As outlined above, the concentration of a light-absorbing molecule in tissue can be determined by measuring the extinction of light passing through the tissue (Villringer, 1997). By measuring light absorbance concentrations of endogenous tissue components or exogenous substances can be determined.

Optical measurements are classified as either extrinsic (using exogenous contrast agents)

or intrinsic (without exogenous contrast agents). One great advantage of the NIRS technology is that no exogenous contrast agent is required, thus with NIRS we are investigating endogenous optical parameters (Villringer, 1997; Villringer and Chance, 1997).

Figure 3 illustrates the relationship between brain activity and optical measurements, on the left part for intrinsic optical contrast agents, and for exogenous contrast agents on the right part respectively. As explained by Villringer and Chance (1997, p. 437) “Brain activity is accompanied by certain physiological events, e.g. an increase in cerebral blood oxygenation. These events influence the value of certain intrinsic physiological parameters, for example, the concentration of oxy-Hb, that, in turn, can be measured through their influence on optical parameters, for example, light absorption”.

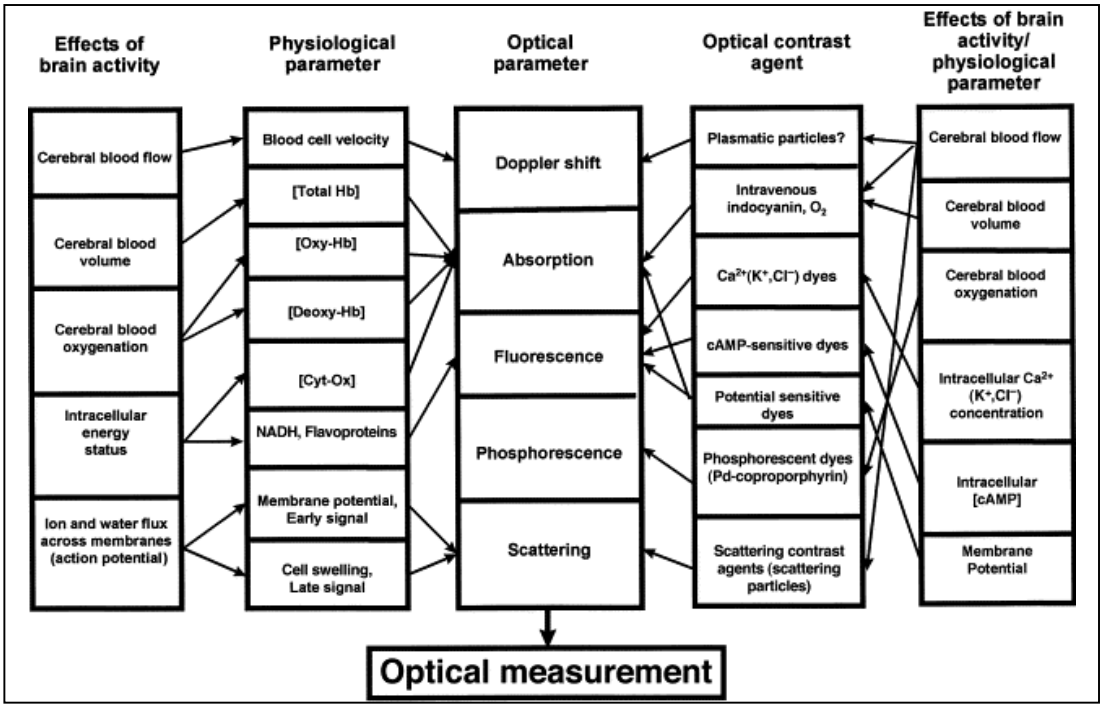


Figure 3: Assessment of brain activity by measuring optical parameters (taken from Villringer & Chance, 1997, p. 437).

2.3 Physiological processes associated with brain activity

Investigating brain activity using an indirect technology like NIRS has one great drawback compared to direct measures: "It is, however, a big step from the activation of a neuronal population, most directly measured by intracellular recordings to the vascular response as demonstrated by the different imaging techniques" (Obrig et al, 2000, p. 131). Thus, we need to understand the different steps from the activated neuron to the vascular response, or as in the example of NIRS, to the cerebral hemoglobin oxygenation.

Physiological events associated with brain activity can be subdivided into intracellular events or events occurring at cell membranes and those that are mediated by neurovascular coupling and occur within the vascular space (Villringer and Chance, 1997).

2.3.1 Cellular physiological events

The activity of neurons is characterized by ion and water fluxes across the neuron's membrane inducing a change in membrane potential, as well as electrical and magnetic field changes. The main ions involved are Na^+, K^+, Cl^- and Ca^{2+} with the ion shifts inducing changes in their intracellular and extracellular concentrations. Furthermore, second messenger systems, such as cAMP (Ca^{2+} itself also serves as a second messenger) are activated (Villringer & Chance, 1997).

Increased brain activity is correlated not only with oxygen consumption but also with glucose consumption. The brain has only negligible stores of glucose and therefore relies on circulating glucose and an active transport system to move glucose across the blood-brain barrier (Schmidt, 2003). Increased activity of brain cells is associated with an increase in glucose consumption and thus the intracellular glucose concentration might fall in the early activation period (Villringer & Dirnagl, 1995). This transient drop in glucose is accompanied by a transient rise in local lactate concentration (Villringer & Dirnagl, 1995). Recently, Magistretti and Pellerin (1999 a, b) have provided new insights on the role of astrocytes in coupling neuronal activity with energy metabolism: They propose an initial glycolytic processing of glucose occurring in astrocytes during activation, resulting in a transient lactate overproduction, followed by a recoupling phase during which lactate is oxidized by neurons.

Although NIRS is most commonly used to measure concentration changes in HbO and HbR, it can also be used to detect an oxygen-dependent signal from the mitochondrial enzyme cytochrome oxidase (Cooper & Springett, 1997; Cooper et al., 1997; Obrig et al., 2000). Cytochrome oxidase (Cyt-Ox) is the terminal electron acceptor of the mitochondrial electron transport chain and responsible for over 90% of cellular oxygen consumption (Cooper et al., 1997), and essential for the generation of cellular adenosine triphosphate (ATP; Cooper & Springett, 1997). Due to the potential interference with the much greater HbO signal and the lack of a gold standard for Cyt-Ox measurements, changes in Cyt-Ox are however a controversial issue (Villringer & Chance, 1997; Obrig et al, 2000).

Several investigations in blood-perfluoro-carbon-exchanged animals with NIRS have revealed small changes in Cyt-Ox redox state (e.g. Ferrari, Williams, Wilson, Thakor, Traystman & Hanley, 1995), and large reductions in NIRS measurements of Cyt-Ox were only found when oxygen delivery dropped significantly (Cooper & Springett, 1997; Cooper et al, 1997). In other words, there is at least preclinical evidence that under normoxic conditions, Cyt-Ox is highly oxidised, whereas reductions in Cyt-Ox can only be observed when the cerebral rates of oxygen delivery are very compromised (Cooper et al., 1997). Recent data in human subjects using visual stimulation revealed an increase in Cyt-Ox, which was smaller in amplitude than the increase in HbO and the decrease in HbR (Obrig et al., 2000; Wobst, Wenzel, Kohl, Obrig & Villringer, 2001), and in the study of Wobst et al. (2001) showed a roughly linear behavior of responses to stimulation periods of varying duration.

2.3.2 Intravascular events: Relationship between Blood Oxygenation, Blood Flow and Blood Volume

In addition to the events taking place intracellularly, local brain activity induces a local arteriolar vasodilation (Villringer and Dirnagl, 1995; Villringer & Chance, 1997). Although small arteries and arterioles probably contain less than 5% of the blood volume in the brain parenchyma, they control most of the resistance and therefore blood flow at a local level (Villringer & Dirnagl, 1995). As a consequence of local vasodilation the local cerebral blood volume (CBV) as well as the blood flow (CBF) increase. This relationship between neuronal activity and vascular response is termed "neurovascular coupling". At the capillary level, the increase in CBF is achieved mainly by higher blood flow per capillary, associated with higher blood flow velocity rather than with "capillary recruitment", e.g. the opening and closing of previously unperfused capillaries (Villringer & Dirnagl, 1995). The increase in CBF and oxygen delivery exceeds the increase in local oxygen consumption (Fox, Raichle, Mintun & Dence, 1988), therefore, cerebral blood oxygenation rises locally (Villringer and Dirnagl, 1995). In other words, the changes in HbR most probably reflect the match between oxygen supply and oxygen demand, whereas changes in HbO reflect the alterations in CBF, an overshoot in cerebral oxygenation during brain activation.

Malonek and Grinvald (1996, p.554) detected a large mismatch between oxygen consumption and oxygen supply after a brief sensory stimulation, which they expressed with the following metaphor: „Watering the entire garden for the sake of one thirsty flower". Using optical imaging spectroscopy from the exposed visual cortex their spectroscopic data suggest a sequence of three different physiological events:

1. an initial increase in HbR, indicating that „a localized increase of neuronal activity was accompanied by aerobic metabolism" (p. 553),
2. no complementary reduction in HbO, which suggests a second physiological event which immediately compensates for the initial HbO decrease,
3. and a "delayed increase of the global oxyhemoglobin and the delayed undershoot of the global deoxyhemoglobin signals presumably caused by the well-known, large activity-dependent increase of blood volume and flow to the tissue" (p. 553).

A spectroscopic analysis of the hemodynamic and metabolic responses to vibrissal stimulation in rat somatosensory cortex performed by Mayhew et al. (1999) is in partial agreement with the findings of Malonek and Grinvald, in that they also found an initial increase of HbR following increased neural activity. In contrast to Malonek and Grinvald, Mayhew and

colleagues did also find an early rapid decrease in HbO (followed by a larger HbO increase). However, they could not provide an explanation for this difference.

In a study of Jones, Berwick, Johnston and Mayhew (2001) the hemodynamic response in rodent barrel cortex was examined using concurrent optical imaging spectroscopy and laser Doppler flowmetry. Electrical stimulation of the whisker pad at varying intensities (0.8 –1.6 mA) resulted in a fast early increase in HbR followed by a decrease below baseline (reaching minima at approx 3.7 s), whereas HbO simply increased after stimulation (reaching maximum at approx 3.2 s). The time courses of changes in blood volume and blood flow were very similar: „Both increased within a second of stimulation onset and peaked at ~2.7 s, after which CBV returned to baseline at a slower rate than CBF“ (Jones et al., 2001, p. 1002). Using the CBF, HbT and HbR data, time series were generated to estimate changes in oxygen consumption. As a result, „Evidence for increased oxygen consumption was obtained even at the lowest stimulation intensity“ (Jones et al, 2001, p. 1014).

In conclusion of their studies reviewed on activation-dependent coupling, Villringer and Dirnagl (1995) put forward the following hypothesis:

> Coupling serves to increase the delivery of glucose to fuel glycolysis and to remove the lactate that is produced as a result of glycolysis. Lactate, which is increased in the early phase of the response, is washed out by the increased blood flow. Tissue and blood oxygenation might be decreased in the early phase of the response, but overshoots during sustained activation due to the rise in blood flow and only minor increase in oxidative metabolism (p. 256).

2.3.3 Studies combining NIRS and fMRI

Besides using a contrast agent as tracer to detect blood volume changes due to functional brain activation, a major development in brain activity mapping is the development of Blood-Oxygenation-Level-Dependent (BOLD) fMRI. BOLD fMRI follows changes in regional brain blood flow by deoxyhemoglobin serving as an endogenous paramagnetic contrast agent (Schmidt, 2003). It is known for some time that deoxygenated hemoglobin (HbR) is paramagnetic while oxygenated hemoglobin (HbO) is diamagnetic, and hence an increase in the concentration of HbR increases the volume susceptibility of blood. In BOLD fMRI deoxyhemoglobin serves as the contrast agent and determines signal intensity, with other words, „the increase in signal intensity during functional activation is therefore explained by a drop in the concentration of HbR“ (Villringer and Dirnagl, 1995, p. 247).

In a combined and simultaneous NIRS-fMRI study, Kleinschmidt et al. (1996) have

confirmed that this drop of HbR does occur in areas that emerge to be activated in fMR images. Simultaneous NIRS and fMRI measurements were conducted in nine healthy subjects performing a finger opposition task for 18 s followed by 36 s rest. The NIRS optodes were located over the precentral region of the left hemisphere according to a modified 10-20 system (1.5 – 2.0 cm medial and lateral position C3; thus the interoptode axis was superimposed onto the central sulcus, thought to correspond to the location of the sensorimotor hand area). The exact positioning of the optodes was assessed in the MR images by vitamin E capsules attached to the optodes‘ head. The results showed that a decrease in HbR was most pronounced when located over the area with the maximal BOLD signal increase, with the responses being significantly stronger for contralateral than ipsilateral finger movements. In summary, Obrig et al. (2000, p. 129) conclude that “the decrease in [deoxy-Hb] can be judged as a robust marker of cerebral oxygenation changes in response to cortical activation”.

Applying a similar methodology, Hirth et al. (1996) inquired the spatial precision of NIRS by determining the position of the optodes with MRI. NIRS measurements were obtained over an array of 8-10 different locations over the left hemisphere and the cerebral oxygenation was measured in five right-handed healthy subjects sequentially during performance of a sequential finger opposition task of the contralateral hand at each location. In all five subjects, a typical response pattern of a task-related increase of HbO and a decrease of HbR was observed with a regional maximum in measurement positions located at C3 or in a position adjacent to C3. Very interestingly, changes in HbR appeared to be more localized than changes in HbO and therefore according to the authors seem to reflect a closer relationship to activated brain regions.

Simultaneous measurements of cerebral oxygenation changes during a motor tapping task were performed in a study of Mehagnoul-Schipper et al. (2002) using NIRS and BOLD fMRI in healthy young and elderly subjects. As a result, NIRS and BOLD fMRI measurements of motor-task related changes in HbR over the left motor cortex showed strong correlation in young (r = -.70; $p < .001$) and elderly subjects (r = -.82; $p < .001$). They also found significant relations between the individual HbT responses measured by NIRS and the changes in maximum BOLD fMRI signal for the young subjects (r = -.55; $p < .001$), however not for the group of elderly (r = -.20; $p = .07$).

In a recent study published by Kennan, Kim, Maki, Koizumi and Constable (2002) hemoglobin changes were measured from an array of optical fibers on the scalp (so called “near infrared optical topography”) in addition to fMRI measurement in order to determine language lateralization of prefrontal areas to a widely used language task. The language task consisted of

visually presented sentences containing either syntactic or semantic errors (e.g. babies can fly, baby can crying) or syntactically and semantically correct sentences. Subjects were required to respond by pressing a button if sentences were fully correct or either semantically-syntactically incorrect. The control task consisted of a line decision task, e.g. a test designed to tap a subject's attention to physical characteristics of nonlinguistic stimuli. The findings of the study revealed a strong correlation between the two methods in terms of laterality indices ($r^2 = 0.6$). Thus, the authors conclude, that near infrared optical imaging could be used to make predictions of hemispheric dominance consistent with fMRI. (The areas of strongest hemoglobin responses were recorded in optodes which are thought to be associated with Broca area and pre motor cortex of the left hemisphere).

2.3.4 Studies combining NIRS and PET

Positron Emission Tomography (PET) is based upon the coincidence detection of paired 511 keV annihilation photons arising from the collision between an electron and a positron emitted from a radionucleotide. The collision results in the release of two high-energy photons that travel in opposite directions (180°) and can be measured at detectors spaced around the head (Kutas and Federmeier, 1998). The detectors are arrayed in pairs around the bore of the camera. When a pair of detectors senses photons simultaneously, this is recorded as a ‚true' event, whereas many millions of such events are recorded during a PET scan necessary to create an image of isotope distribution (Schmidt, 2003). In a PET experiment, subjects are injected with a radiolabeled substance, of which nanomolar concentrations can be detected, provided it has been taken up by the brain.

Whereas [^{18}F]deoxyglucose is most commonly used to investigate cerebral metabolism, [^{15}O]-labeled water is used to follow blood flow changes associated with transient physiological increases in neuronal activity (Kutas and Federmeier, 1998). Using the [^{15}O]-labeled water approach, the distribution of a freely diffusible tracer in response to changes in functional neuronal activity can be mapped. Cortical areas are termed ‚activated' if the tracer concentration is higher under test as compared to under rest (Obrig and Villringer, 1997). The underlying assumption that neuronal activation is coupled to an increase in regional cerebral blood flow (rCBF) is broadly accepted and has been demonstrated by many techniques (Villringer and Dirnagl, 1995).

In a simultaneous PET and NIRS study Villringer et al. (1997) compared changes in HbO, HbR and HbT as measured by NIRS over the left forehead, with cerebral blood flow measurements followed with PET using [^{15}O]-labeled water. The light-emitting diode and the

detector placed on the head for NIRS had an interoptode distance of 4 cm and were radioactively labeled in order to localize them on the PET image. Two PET measurements were performed at rest, two measurements during performance of a Stroop task, and two measurements during performance of a calculation task in five healthy subjects. The results of the study showed that changes in HbT correlated best with changes in CBF at a penetration depth of near-infrared light of 0.9 cm ($r = .88$; $p = .048$). There was a trend for positive correlation between HbO and CBF and a negative correlation between HbR and CBF, however not statistically significant. According to the authors these data confirm the intracerebral origin of the NIRS signal, which, using an interoptode distance of 4 cm, "most probably stems from the outer 1 cm of the brain, i.e. mainly from the brain cortex" (Villringer et al, 1997; p. 151).

In a more ambitious study conducted by members of the same group (Hock et al., 1997a), simultaneous NIRS and PET measurements were performed in ten patients with Alzheimer's disease (65 ± 13 years; diagnosis according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association). The activation paradigm used was a modified Stroop test, in which subjects have to name the colour of congruent and incongruent colour words. The NIRS optodes were placed on the right parietal region to cover a portion of the right superior parietal cortex, where changes in rCBF were expected based on previous findings (Pardo. Fox & Raichle, 1991). To localize the position of the optodes on the PET image, they were labeled radioactively. PET images were obtained under rest as well as during performance of the Stroop task. For comparison of the hemodynamic changes measured by NIRS with changes in rCBF an analysis of regions of interest (ROI) was performed (activation – rest), for which the individual optode coordinates were transformed into the stereotactic space of Talairach (Talairach & Tournoux, 1988) and projected to the cortex surface by an automated algorithm. By using these cortex coordinates a hemispheric sample volume with the radius corresponding to the assumed penetration depth of NIRS was defined. To address the question of overlap between changes in rCBF (PET) and changes in cerebral hemoglobin oxygenation parameters HbO, HbR, and HbT (NIRS) correlations were calculated for different assumed penetration depths of near-infrared light (i.e. from 0.45 cm to 3.38 cm). As a result, the correlations were significant for all three NIRS parameters up to a semisphere radius of 1.35 cm, and decreased with increasing assumed penetration depth of near-infrared light into the brain tissue. The highest correlation was found between changes in HbT and changes in rCBF at a semisphere radius of 0.68 ($r = 0.93$; $p < .001$). Based on these data the authors conclude that the NIRS field of view is restricted to the outer 1-3 cm of the brain cortex, which is in agreement with reports demonstrating that the

NIRS signal is predominantly derived from the cerebral grey matter (Okada, Firbank & Delpy, 1995).

2.4 Classification of brain imaging techniques: making the case for NIRS

The usefulness of a specific method as a functional neuroimaging method is determined by its non-invasiveness, practicability to be performed in humans, and spatial and temporal resolution (Villringer and Dirnagl, 1995). In the following section, the different methodologies are described according to these criteria, and a conclusion for the use of NIRS is made.

One way to classify brain imaging techniques is whether they provide direct or indirect information about brain function (Gratton, Goodman-Wood & Fabiani, 2001). Direct methods include EEG (including event-related potentials; ERPs) and MEG, from which EEG and ERP record the electrical fields generated by neuronal activity, while MEG records the magnetic fields induced by such activity (Strangman et al., 2002). PET, SPECT, fMRI and NIRS, in contrast to EEG/ERP and MEG, are indirect measures as they monitor hemodynamic and metabolic changes consequent to brain electrical activity, i.e. as a function of the so called neurovascular coupling.

Another way of categorizing brain imaging techniques is whether the use of an exogenous contrast agent is required. PET, SPECT, fMRI and NIRS, all measure changes in the concentration of some intrinsic or extrinsic tracer with specific radioactive, magnetic or optical properties. PET and SPECT are both based on the introduction of extrinsic radioactive tracers into the blood stream (Cherry and Phelps, 1996): The blood stream carries the tracer to the brain, where its local concentration is measured. As this concentration is higher in areas with an increased blood flow and/or metabolism, areas of the brain that are active during a particular task can be mapped in this fashion (Gratton et al., 2001). If a tracer with pronounced magnetic properties is used, changes in blood flow can be measured by using magnetic resonance imaging (MRI). For fMRI however the administration of an external contrast agent becomes obsolete by the fact that the intrinsic chromophore deoxyhemoglobin is itself a substance with pronounced paramagnetic properties (Kwong et al., 1992; Ogawa et al., 1992). This type of fMRI is labeled blood-oxygen level dependent (BOLD) MRI. In addition to possessing distinct magnetic properties, oxy- and deoxyhemoglobin also differ in their absorption spectra of both visible and near-infrared light. These properties can be used for investigating functional changes in the concentration of oxy- and deoxyhemoglobin in the brain non-invasively, which

is the basis of NIRS. Thus, in the case of NIRS, no additional external tracer is needed.

Another approach of categorizing different brain imaging methods is in terms of their spatial and temporal resolution as shown in Figure 4. The direct measures MEG and EEG/ERP are strong in temporal sensitivity (i.e. in the range of milliseconds), but on the other hand relatively weak in terms of spatial resolution. In contrast, fMRI, PET and SPECT have an advantage when it comes to spatial resolution, however are weak in terms of temporal sensitivity. Diffuse optical methods, such as NIRS, in comparison to the other techniques, can provide excellent temporal sensitivity and, depending on the type of optical measurement technique, also reasonable spatial sensitivity (Strangman et al., 2002).

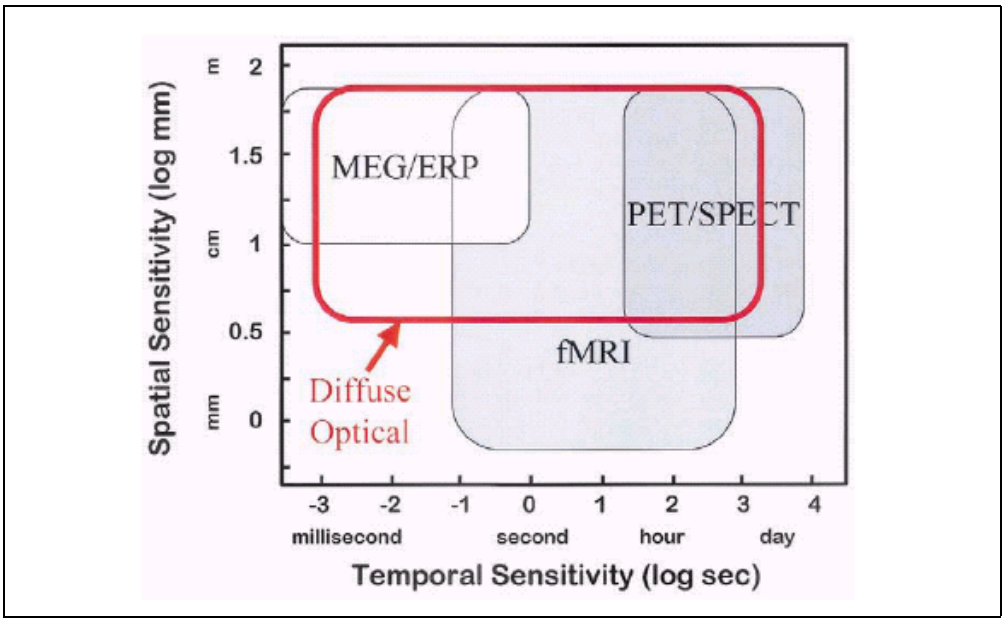


Figure 4: Comparison of the spatial and temporal sensitivities of commonly-used neuroimaging methods (taken from Strangman et al, 2002).

In NIRS functional activation studies performed so far, the spatial resolution however was a major shortcoming (Obrig et al., 2000). According to Hock et al. (1997a) "it is obvious that the spatial resolution (in the order of several cm^3) is the most critical issue for the further development of the method" (p. 301), and, "the NIRS method ... may be used as a research tool with rather high temporal but low spatial resolution in clinical studies focussing on brain activation, aging, or brain diseases" (p. 301-302).

The strength of NIRS lies beyond the categories of temporal and spatial resolution. In contrast to PET and fMRI the NIRS instrumentation can be made portable (applicability at the bedside) and compared to other techniques has low expense (Obrig et al., 2000). Another advantage of NIRS is its noninvasiveness as no radioactive compound is needed, thus

measurements may be repeated as many times as one likes (Hock et al., 1997a; Strangman et al., 2002). With the current methodology in use, the poor spatial resolution can be partly overcome by multiple site recordings (Obrig et al., 2000). The excellent temporal resolution and the relative simple relationship between signal and chromophore concentration make NIRS a suitable method to better understand the transformation of neuronal activity to the vascular or oxygenation response (Wobst, Wenzel, Kohl, Obrig & Villringer, 2001).

Altogether, although the spatial resolution of the current NIRS methodology is clearly behind fMRI, NIRS does have a role in functional brain activation research as it is has a high biochemical specificity, a high temporal resolution, and the potential of measuring metabolic (concentration changes in Cyt-Ox) as well as vascular events (concentration changes in HbO, HbR, and HbT) simultaneously and thus may be used to better understand the basis of neurovascular coupling. For the clinician, NIRS provides a simple, relatively inexpensive and non-invasive tool to monitor cerebral hemodynamics even in difficult populations such as infants, small children, or patients with claustrophobia.

2.5 NIRS in functional activation studies

Several NIRS studies conducted in the past ten years have demonstrated that activation-induced changes in brain activity can be assessed non-invasively during the performance of visual activation (Gratton, Corballis, Cho, Fabiani & Hood, 1995; Kato, Kamei, Takashima & Ozaki, 1993; Villringer, Planck, Hock, Schleinkofer & Dirnagl, 1993;), motor activity (Maki et al, 1995; Hirth et al., 1996; Obrig et al., 1996), and cognitive tasks (Hoshi and Tamura, 1993a, b; Villringer et al., 1993).

In this section, by reviewing NIRS functional activation studies, it will be explored whether a typical, activation-induced response pattern can be described for the NIRS parameters HbO, HbR, HbT, and Cyt-Ox. In the first part, studies in young healthy volunteers will be presented. In the second part, the question of how this typical response pattern may be altered in normal aging and in different pathological conditions, such as Alzheimer's disease and major depression, will be addressed.

2.5.1 Typical response in healthy volunteers

An overview of functional activation studies in healthy volunteers is provided in table 2-1. The most consistent finding is an increase in HbO in response to the stimulus applied, i.e. either visual, motor or cognitive performance. HbT, which equals the sum of concentrations of

HbO and HbR, is generally reported to have the same response direction as HbO, which is due to the fact that changes in HbR are smaller in response amplitude than changes in HbO (Obrig & Villringer, 1997). As can be seen in Table 2-1, there is only sparse data for Cyt-Ox, which may be partly related to the problems of detecting the Cyt-Ox signal in the presence of the much greater concentrations of HbO and HbR (Cooper et al, 1997, see also section 2.3.1).

Table 2-1: Overview of NIRS functional activation studies in healthy volunteers

Study	Sample size	Type of activation	Single- vs multi-site	Parameters	Results (mean changes)	Comments
Meek et al. (1995)	10 HVs	visual	2 sites: right occipital, right frontal	HbO, HbR, HbT	HbO↑ HbR→ HbT↑	The right frontal position was used as a control to the right occipital position. There was no significant rise in HbO over the frontal area during stimulation. Therefore, the changes observed over the occipital area are unlikely to be the result of increased blood flow in extracerebral tissue of skull and skin, neither are they due to a movement artefact.
Kato et al. (1993)	5 HVs	visual	2 sites: frontal and occipital, n.s.	HbO, HbR, HbT, Cyt-Ox	HbO↑ HbR→ HbT↑ Cyt-Ox→	There was no change of any of the NIRS parameters over the frontal region.
Wenzel et al. (1996)	16 HVs	visual	right occipital (at the calcarine sulcus level)	HbO, HbR, HbT	HbO↑ HbR↓ HbT↑	Localization problems were minimized by localizing the optodes individually according to previously acquired 3-D-MRI.
Heekeren et al. (1997)	12 HVs	visual	right occipital (at the calcarine sulcus level)	HbO, HbR	HbO↑ HbR↓	In contrast to the previous study of Wenzel et al. (1996) a prolonged visual stimulation over 5 min was applied.
Hirth et al. (1996)	5 HVs	motor	multi-site: 8-10 sites over central sulcus and surrounding cortical structures	HbO, HbR	C3 and adjacent to C3: HbO↑ HbR↓	Positions of the optodes were determined by 3-D-MRI. Changes in HbR were more localized than changes in HbO.
Obrig et al. (1996)	44 HVs (Experiment 1)	motor	single, C3'	HbO, HbR, HbT	HbO↑ HbR↓ HbT↑	All 44 subjects showed a Hb response to the stimulation. 33/44 subjects showed a a decrease in HbR in parallel with an increase in HbO.
Kleinschmidt et al. (1996)	9 HVs	motor	single, C3'	HbO, HbR, HbT	HbO↑ HbR↓ HbT→	NIRS measurements were combined with simultaneous fMRI. HbR responses were smaller for ipsi- than for contralateral finger movements, whereas increases in HbO were comparable for both conditions. HbR thus seems to be more specific.
Hoshi and Tamura (1993a)	14 HVs	cognitive (solving mathematical problems, calculating)	single left frontal, n.s.	HbO, HbR, HbT	Subjects who had difficulty in solving mathematical problem: HbO↑ HbR↓ HbT↑ Subjects who solved problem without difficulty: HbO→ HbR→ HbT→	Cerebral oxygenation varied with degree of difficulty of a problem and was individual. Subjects who found difficulty in solving a problem showed an increase in HbO, a slight decrease in HbR and an increase in HbT. Subjects who solved the problem without difficulty showed no significant changes in Hb oxygenation.

Table 2-1: Overview of NIRS functional activation studies in healthy volunteers

Study	Sample size	Type of activation	Single- vs multi-site	Parameters	Results (mean changes)	Comments
Villringer et al. (1993)	16 HVs	cognitive (calculating; n=10), visual (n=6)	cognitive task: left frontal, n.s.; visual: right occipital, n.s.	HbO, HbR, HbT	cognitive task: HbO↑ HbR↓ HbT↑ visual task: HbO↑ HbR↓ HbT⇡	Simultaneous measurement of skin blood flow using Laser Doppler flowmetry showed that the NIRS signal is not influenced by alterations in skin blood flow.
Hoshi & Tamura (1993b)	7 HVs	visual, auditory, cognitive (solving mathematical problems, calculating)	multi-site: left/right frontal (BA 10), left/right occipital (BA 17) left/right temporal (BA 41)	HbO, HbR, HbT	visual, left occipital: HbO↑ HbR→ HbT↑; auditory, left temporal: HbO↑ HbR ↑HbT↑; mental task, frontal: HbO↑ HbR↓ HbT↑; temporal: HbO↑, HbR↑, HbT ↑	It is not clear whether the reported results reflect individual responses or the group's mean response. Regional variations in Hb oxygenation state were shown.
Hoshi et al. (1994)	33 HVs	cognitive (solving mathematical problems, calculating)	single left frontal, n.s.	HbO, HbR, HbT	Unexpected results in 9/33 HVs (27%): HbO↓ HbR ↓ HbT ↓ whereas 6/9 also showed HbO↑ HbR ↓ HbT →	Subjects performed calculations of the type 6696x93, root of 4096 and 8!. In 2 subjects with these unexpected results simultaneous PET measurments of rCBF confirmed the reliability of NIRS. Results for typical responders are not reported
Fallgatter and Strik (1997)	10 HVs	cognitive (Continuous Performance Test)	2 sites: FP1/F3 and FP2/F4	HbO, HbR	Right frontal: HbO⇡ HbR⇣ Left frontal: HbO ⇡HbR ⇡	
Fallgatter and Strik (1998)	10 HVs	cognitive (Wisconsin Card Sorting Test)	2 sites: FP1/F3 and FP2/F4	HbO, HbR	HbO↑ HbR⇣	The increase of HbO during the performance of the WCST was significant in both hemispheres.
Fallgatter et al. (1998)	10 HVs	cognitive (reading aloud)	2 sites: FP1/F3 and FP2/F4	HbO, HbR	HbO⇣ HbR ↑	There were no hemispheric differences for both parameters during the reading task.
Schroeter et al. (2002)	14 HVs	cognitive (Stroop Test)	multi-site: F7/8, F3/4, FC3/4, C3/4, P3/4, O1/2	HbO, HbR, HbT, Cyt-Ox	F3/4; F7/8; FC3/4 HbO↑ HbR↓ HbT↑ Cyt-Ox →	The vascular response was higher at F3/4, F7/8 and FC3/4 in the incongruent than in the neutral and congruent condition, and was greater at C3 when subjects responded with their right, i.e. contralateral hand.
Herrmann et al. (2003)	14 HVs	cognitive (letter fluency)	2 sites: FP1/F7-F3 and FP2/F4-F8	HbO, HbR	Right frontal: HbO↑ HbR↓ Left frontal: HbO ↑HbR ↓	There was no hemispheric difference between left and right prefrontal activation. No significant correlation was found between task performance and changes in HbO

Abbreviations

HV= healthy volunteer; n.s.=not specified; HbO = oxygenated hemoglobin; HbR = reduced hemoglobin; HbT = total hemoglobin; Cyt-Ox = Cytochrome oxidase

BA = Brodmann Area; C3', FP1/F3, FP2/F4, F7/8, F3/4, FC3/4, C3/4, P3/4, O1/2 positions according to international 10/20 system

↑ = significant increase; ↓= significant decrease; →no change

⇡ = trend for increase; ⇣= trend for decrease

In three experiments Obrig et al. (1996) systematically investigated a total of 56 young, healthy subjects to determine whether the response measured by NIRS reflects a localized hemodynamic response. Using a motor stimulation (sequential opposition task, i.e. opposing the thumb to each of the other four fingers of the right hand); NIRS data were acquired over the left hemisphere corresponding to the contralateral hand region of the sensorimotor cortex (C3‘ position) in 44 subjects. 18 subjects also performed the motor task with their left hands, i.e. ipsilateral to the optode position. In the third experiment, the frequency of finger tapping was controlled in 12 subjects, such that subjects were asked to oppose their fingers at frequencies of 1, 2, and 3 Hz, both ipsilateral and contralateral to the NIRS optode. The study revealed the existence of a typical stimulus-associated NIRS response during motor performance, which consists of an increase in HbO and a decrease in HbR. The contralateral response was significantly greater than the ipsilateral response for all parameters, and there was a trend for greater response amplitudes with higher performance rates. Summarizing the homogeneity of their findings in this first systematic approach, the authors conclude that the observed NIRS response indeed reflects cortical hemodynamic changes in response to functional stimulation.

In NIRS studies applying *cognitive* activation tasks such as a calculation task (Hoshi and Tamura, 1993a; Hoshi et al., 1994; Hock et al., 1995; Villringer et al, 1993), the Stroop task (Villringer et al., 1997; Schroeter, Zysset, Kupka, Kruggel & von Cramon, 2002), the Wisconsin Card Sorting Test (WCST; Fallgatter and Strik, 1998), a letter fluency test (Herrmann, Ehlis & Fallgatter, 2003) or the Continuous Performance Test (CPT; Fallgatter & Strik, 1997) an activation of the frontal region could be detected as reflected by an increase of HbO and a decrease of HbR during these tasks.

In their NIRS study in healthy subjects Fallgatter and Strik (1997) found indications of right frontal activation during the performance of a CPT. Subjects showed an initial increase of HbR and a parallel initial decrease of HbO in the right frontal region, followed by an inverse trend after some seconds, which lasted until the end of the task. This prevalently right-hemispheric frontal activation is in line with Buchsbaum et al. (1990) who showed an increase in glucose metabolic rate in the right frontal region during the CPT using PET.

The same authors recorded changes in blood oxygenation during reading aloud (Fallgatter, Müller & Strik, 1998) and during performance of the WCST (Fallgatter & Strik, 1998), both again at prefrontal positions. For the reading-aloud task subjects were asked to read an easy article from a sports magazine in normal pace. As a control condition a non-verbal picture observation task was introduced. The results showed a marked increase of HbR in prefrontal regions of both hemispheres, while HbO tended to decrease during the reading task.

No blood oxygenation changes were found during the picture observation task, indicating that the effects of the reading task are due to language-related efforts but not to visual perception. Using the WCST Fallgatter and Strik revealed a significant relative increase in HbO at the left as well as at the right frontal optode position. The average HbR concentration showed a slight decrease for both hemispheres during performance of the WCST, which was not significant.

Summarizing their above findings in different activation paradigms, distinct activation patterns become evident: The bilateral increase of HbO during the WCST clearly differs from the bilateral frontal hypo-oxygenation during the reading task, and also from the right lateralized frontal activation during the Continuous Performance Test. In conclusion of these findings the authors note that „the method (NIRS) is capable not only to detect massive blood oxygenation changes due to cerebral ischemia but also the more subtle changes during physiological brain activation“ (Fallgatter, Müller & Strik, 1998, p. 218).

In their series of NIRS measurements in healthy volunteers Hoshi, Tamura and coworkers (Hoshi et al., 1994; Hoshi and Tamura 1993a; Hoshi and Tamura, 1993b; Tamura, Hoshi & Okada, 1997) have encountered unexpected results, which challenge the phenomenon of over-compensation, i.e. an increase in HbO and HbT and a decrease of HbR under cognitive activation. Hoshi and Tamura (1993a) found that only subjects who had difficulty in solving a mathematical problem showed the typical Hb response. This mismatch between neuronal activation and blood flow was detected first in volunteers above 45 years of age, but subsequently also in about 27% of the young subjects (Hoshi et al., 1994; see also Table 1-1). Following this unexpected result the question arises whether neuronal activation is always accompanied by increasing blood flow and oxygen consumption (Tamura et al., 1997). Hoshi et al. (1994, p. 132) conclude that these unexpected results might be related to the problem-solving strategy used by these subjects and/or to the difficulty of the task: „When changes in neuronal demand are slight because of easy mental tasks for subjects, changes in oxygen extraction fraction might be sufficient to compensate. Alternatively, glycolysis might compensate for the increase in neuronal demand“. However, one of these subjects with unexpected results, in whom also PET was recorded, only solved 22 of the 30 calculations correctly. Thus, the conclusion that neuronal demand was reduced due to easy task demand can be doubted, at least for this subject.

In the recent publication of Schroeter et al. (2002), NIRS was used in an event-related design during a Stroop color-word matching task (see Table 1-1). Event-related designs, which allow a differentiation of the brain‘s response to separate events (in this case congruent color-word pairs, incongruent color-word pairs and neutral in terms of presenting only ‚XXXX‘

instead of words), also have the advantage of providing much shorter response latencies than block-designs. The results of their study showed that the interference inherent to the Stroop task led to specific brain activation in the lateral prefrontal cortex in both hemispheres (F3/4, F7/8, FC3/4). This is in accordance with fMRI (Leung, Skudlarski, Gatenby, Peterson & Gore, 2000) and PET (Taylor, Kornblum, Lauber, Minoshima & Koeppe, 1997) studies.

In conclusion of the above studies, a typical, activation-induced NIRS response pattern can be described, with an increase of HbO and a decrease of HbR over areas most probably activated by the stimulus. HbT, which is gained by summing up the concentration changes of HbO and HbR, is generally reported to have the same response direction as HbO, due to the fact that the changes in HbO generally are bigger in amplitude than the changes in HbR. There is only limited data available for the intracellular NIRS parameter Cyt-Ox, which, unless specifically analysed did not show a marked change related to brain activation. Functional activation studies using a motor task consistently showed a clear response lateralisation, i.e. a significantly stronger response over the sensorimotor cortex area contralateral to the hand performing the motor task. In NIRS functional activation studies using a cognitive task such as mental arithmetics, the Stroop task, the Wisconsin Card Sorting Test, letter fluency and the Continuous Performance Test have showed increased activation of the frontal region as reflected by an increase of HbO and a decrease of HbR during these tasks relative to rest. It has to be noted however, that in most early studies only the frontal brain region was investigated. In this regard, a recent multi-site study using an event-related approach (Schroeter et al, 2002) earns special consideration as the incongruent trials of the Stroop task led to a markedly stronger hemodynamic response only in the lateral prefrontal cortex. There is some evidence for task difficulty and the problem-solving strategy used by the subject to have a response-modifying effect. This however needs further examination, together with the combined analysis of overt behavior (e.g. number of correct answers) and NIRS response.

2.5.2 Response patterns in normal elderly subjects and in different patient groups

2.5.2.1 Normal Aging

There is evidence for changes in the cerebral oxygen metabolism related to normal aging gathered in PET studies (Marchal et al., 1992; Eustache et al., 1995). Reviewing earlier PET studies to assess cerebral oxygen consumption ($CMRO_2$) in healthy aging, Eustache et al. came to the conclusion that “It is now widely accepted that, between the ages of 20 and 70 years,

there is a linear (about 5-6% per decade) decrease in $CMRO_2$. The cerebral cortex is involved preferentially, but no lobar or gyral predominance has been consistently observed“ (Eustache et al., 1995, p. 869).

The number of studies investigating age-related changes in cerebral brain oxygenation measured by NIRS is very limited. The often-cited study by Hock, Müller-Spahn, Schuh-Hofer, Hofmann, Dirnagl and Villringer (1995) is the only report of age-related changes in cerebral oxygen metabolism during cognitive activation and thus will be described in more detail below. A recent study by Mehagnoul-Schipper et al. (2002) (see also section 2.3.3), reported age-dependent decreases in HbR and HbO as measured by NIRS during performance of a motor task.

In the study of Hock et al. (1995) 12 healthy young (mean age: 28 ± 4 years) and 17 healthy elderly subjects (mean age: 52 ± 10 years) were included in the study. They were all carefully screened including EEG, ECG, lab, as well as a physical and neurological examination. All subjects were healthy and free of medication. Changes in HbO, HbR and HbT were recorded over the left frontal brain during rest and activation, using a calculation task as activation paradigm. It needs to be highlighted that the calculation task was not a uniform task but the difficulty was adapted to the calculating abilities of the subject, thus a sufficient brain activation should have been reached.

While performing the calculation task, the young subjects showed an increase in HbO and HbT and a decrease in HbR (mean arbitrary units ± S.D: HbO = 2.36 ± 1.07, HbR = -0.11 ± 0.48, HbT = 2.24 ± 1.13). The elderly subjects showed a similar response pattern, however they had a significantly lower mean increase in HbO and HbT, while HbR did not differ from the young (mean arbitrary units: HbO = 1.21 ± 1.38, HbR = -0.50 ± 0.72, HbT = 0.72 ± 1.42). When the HbO and HbT values were plotted against age, significant correlations were obtained for both parameters (HbO – age: r = -0.43; HbT – age: r = -0.57). These results support the hypothesis of an age-dependent decline in cerebral hemoglobin oxygenation, at least for HbO and HbT. Looking at the changes in HbO, HbR and HbT from an individual subject’s perspective, interindividual qualitative differences could be observed: Whereas all young subjects showed an increase in HbO, two elderly subjects (12%) showed a decrease during brain activation. Three young subjects (25%) exhibited an increase in HbR compared to only one elderly subject (6%). As for HbT all young subjects showed an increase whereas 6 elderly individuals (35%) showed a decrease under brain activation. This latter observation is interpreted by the authors as an index for not only quantitative but also qualitative differences

between the two age groups, thus, they point out, the reduction of HbO and HbT in the elderly group cannot be a mere consequence of measuring less brain tissue due to a possible age-related brain atrophy. However, we need to keep in mind that concentration changes in HbT are the summation of changes in HbO and HbR and thus, smaller increases in HbO coupled with slightly bigger decreases in HbR by definition lead to smaller values of HbT which equals the total brain blood volume. The observation that all young subjects showed an increase in HbO whereas more elderly subjects showed a decrease in HbR needs further consideration: As only the left frontal position was investigated, and only one mental task was performed, we cannot conclude as to which of the two parameters is more specific. We might assume that HbO reflects a state of arousal with a satisfactory high sensitivity whereas HbR is more specific in that it is a better reflection of oxygen consumption.

2.5.2.2 Depression

In an attempt to throw light on the neurobiological basis of hypofrontality in depression, changes in HbO and HbR were examined with NIRS in 9 elderly patients with major depression and 10 control subjects matched for age, sex, education and MMSE score (Matsuo, Kato, Fukuda & Kato, 2000). Alterations of HbO and HbR were measured at the left frontal forebrain during rest and during a verbal repetition task, a verbal fluency task, a hyperventilation test and a paper-bag breathing test. During the verbal fluency test, HbO significantly increased and HbR significantly decreased in control subjects, whereas no significant change could be observed in depressive patients. During the hyperventilation test however both groups showed a significant decrease in HbO accompanied with a significant increase in HbR. Thus the lack of activation in the left forebrain in the depressed group during the verbal fluency test is not due to an altered vasodilator response.

In a follow-up study by the same group (Matsuo, Kato & Kato, 2002) the number of patients was increased, now including also younger patients with major depression (MD) as well as bipolar disorder (BD) patients (14 patients with MD, 11 patients with BD not specified, 21 controls). The three groups were matched in age, sex, and education. Following the same methodology as described above, the results of the study revealed significant differences between the control and MD group, and between the control and the BD group in HbO during the verbal fluency test: During the verbal fluency test, only control subjects showed an increase in HbO, although only at the trend level, and a significant decrease in HbR. There was no significant change in either MD or BD patients during the verbal fluency test. This result could

not be explained by differences of performance in the verbal fluency test, because there was no difference in the number of words produced among these three groups.

2.5.2.3 Schizophrenia

Applying the same methodology as in their study in healthy subjects (Fallgatter and Strik, 1997), Fallgatter and Strik (2000) report results of nine schizophrenic patients (DSM-IV; n=3 disorganized, n=3 catatonic, n=3 paranoid) obtained with NIRS during the CPT. In contrast to the previous study, no hemispheric activation effects were found in schizophrenic patients. The schizophrenic patients displayed a trend for higher HbR levels in the left frontal region, irrespective of functional activation status, i.e. at rest as well as under performance of the CPT. The absence of this lateralized activation could not be attributed to a worse performance since number of omission errors and false alarms, as well as reaction times were not different from the control group.

2.5.2.4 Alzheimer's Disease (AD)

In their first experiment Hock and coworkers (1997a; see also section 1.3.4), investigated 19 elderly healthy volunteers (67 ± 10 years) and 19 patients with probable moderate AD (71 ± 10 years; diagnosis according to NINCDS-ADRDA) using a letter fluency task. One pair of optodes was placed to cover the left parietal cortex. The results (mean values) revealed a typical response pattern for the elderly healthy subjects during the verbal fluency test, i.e. increases in HbO and HbT in parallel to a smaller decrease in HbR. Patients with AD showed significant decreases in HbO as well as HbT, coupled with a small decrease in HbR. The NIRS variables in the AD group did not correlate neither with the MMSE score nor with the verbal fluency test score (correct responses). As Hock et al. pointed out however, large interindividual differences in the NIRS variables were detected over the left parietal position as follows: 4 of the 19 healthy elderly subjects (21%) showed a decrease in HbO as opposed to 15 of the 19 AD patients (79%). 6 of the healthy elderly subjects (32%) and 3 of the AD patiens (16%) presented an increase in HbR, and a decrease of HbT was observed in 7 healthy controls (37%) versus in 17 AD patients (89%).

To examine simultaneous changes in the left parietal and left frontal cortex during the performance of the verbal fluency task, additional NIRS measurements were conducted in another group of 8 healthy elderly subjects and 10 patients with moderate AD (same groups as reported in section 2.3.4). As illustrated in Figure 5, the healthy elderly control group showed increases in HbO and HbT in both frontal and parietal cortex during the verbal fluency test, the changes between activation and rest being slightly more pronounced at the left frontal position In contrast, the group of AD patients showed marked decreases in HbO and HbT over the left parietal cortex, and, at the same time, increases in HbO and HbT in the frontal cortex.

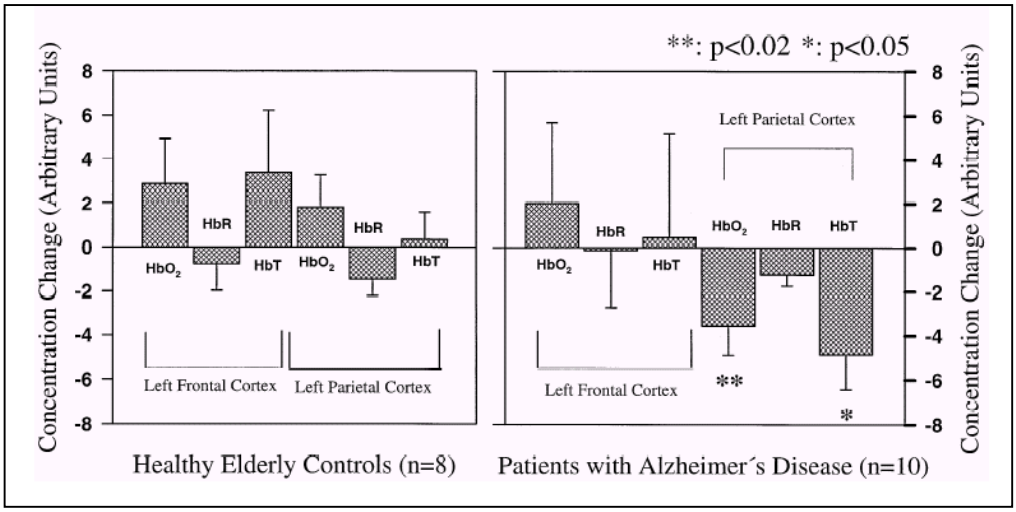


Figure 5: Results of NIRS measurements of Hock et al. (1997a, p. 297) comparing healthy elderly subjects with patients with probable moderate AD.

For the observation of decreases in HbO and HbT in the parietal cortex of AD patients together with a 'normal', though smaller response than healthy controls, over the frontal cortex during cognitive activation with the verbal fluency task the authors list three possible interpretations (Hock et al., 1997a, b): First, there may be a reduction of hemoglobin oxygenation in degenerating brain areas during cognitive activation which is in favour of other healthier brain regions due to an altered functional brain organization. This interpretation would be in line with observations by Grady et al. (1993) who described an additional frontal activation in AD patients during performance of an object recognition task. Secondly, the mechanism of neurovascular coupling may be altered during neurodegeneration, which may lead to an abnormal regulation of the brain miscrovasculature with increased vasoconstriction leading to regional reduced supply with oxygen during activation of brain function. Thirdly,

anatomical changes during neurodegeneration may affect the optical properties of the brain (enlargement of the subarachnoid space, brain atrophy, changes in white/grey matter ratio).

In a study of Fallgatter, Roesler, Sitzmann, Heidrich, Mueller and Strik (1997) ten patients with AD (according to DSM-IV and NINCDS/ADRDA criteria) were compared to a group of ten young healthy control subjects. Two verbal fluency tests were performed while changes in HbO and HbR (activation-rest) were recorded at the left frontal and right frontal positions in all study participants: a letter fluency task and a category fluency task. In both groups, the relative concentrations of HbR decreased significantly during the letter fluency task and the relative concentrations of HbO increased as a trend, but not during the category fluency. Calculating for hemispheric differences, the control subjects showed a marked hemispheric effect during the letter fluency task, in that their relative changes of HbO were more pronounced in the left frontal than the right frontal region. The patients with AD did not show this hemispherical asymmetry and performed worse on the letter fluency task: "It appears, in fact, that DAT patients are characterized by a loss of this physiological hemispheric lateralization and, instead, show a global activation involving right hemispheric regions in addition to the left hemispheric response observed in controls" (Fallgatter et al, 1997, p. 70).

2.6 Summary

Near-infrared spectroscopy (NIRS) is a non-invasive optical technique to measure changes in cerebral hemoglobin oxygenation in the outer 1-3 cm of the brain cortex. It makes use of the fact that brain activity is associated with changes in optical properties of brain tissue. As biological tissue is relatively transparent to light in the near-infrared range between 700–900 nm, this "optical window" can be used to measure concentration changes of several endogenous chromophores, e.g. oxygenated and deoxygenated hemoglobin, which have different absorption spectra for light at a specific wavelength in the near-infrared range. During increased brain activity and by means of the "neurovascular coupling" between neuronal activity and vascular response, there is an increase in local cerebral blood flow. This increase in cerebral blood flow however is exceeding the increase in oxygen consumption and thus leads to an increase in intravascular haemoglobin oxygenation during brain activity. Therefore, when the NIRS measuring optode is located over an area in which cerebral blood flow increases during brain activity, a localized increase in the concentration of oxygenated hemoglobin (HbO) and a decrease in deoxygenated hemoglobin (HbR) is seen.

Studies combining PET and fMRI with NIRS were able to demonstrate that concentration changes in HbO and total hemoglobin (HbT) correspond well to rCBF increases as measured by PET, whereas concentration changes in HbR highly correlate with signal intensity increases measured by fMRI.

NIRS has a spatial resolution in the range of several cm^3 and thus is clearly behind PET and fMRI. However, it has a high temporal resolution and the potential of measuring metabolic as well as vascular events simultaneously. It does not require large expensive equipment nor an exogenous contrast medium, it is easy to handle and portable and thus can be used also in clinical populations.

In the past ten years NIRS has been widely used in functional brain activation studies including several types of activation such as motor activity, visual activation, and performance of cognitive tasks. In NIRS functional activation studies it has been consistently shown that the NIRS-parameters HbO and HbR exhibit typical responses to brain activation: an increase of HbO and a decrease of HbR over the brain areas most probably activated by the stimulus.

There are only very limited functional NIRS data in normal elderly subjects and different patient groups, and the studies conducted used only one or two optode positions. The study of Hock et al. (1995) in healthy elderly vs. healthy young subjects is the only report of age-related changes in cerebral hemoglobin oxygenation using a cognitive activation paradigm. In Alzheimer's Disease only 2 NIRS functional activation studies have been published, and both used a verbal fluency activation paradigm. They suggest a reduction of cerebral hemoglobin oxygenation in the left parietal cortex compared to normal activity in the left frontal cortex (Hock et al., 1997a), and, measuring over the left and right frontal cortex, a global activation involving the right frontal cortex in addition to the left-frontal response observed in controls.

3 Investigating brain-behaviour and -ApoE relationships with specific consideration of the verbal fluency task- evidence from neuroimaging studies

The investigation of brain-behavior relationships has generally followed two major routes: 1) detailed neuropsychological assessment of specific cognitive functions including clinical case studies and 2) „on-line“ assessment of cognitive processes with the use of functional brain imaging techniques. With the development of high-resolution techniques such as fMRI, it became possible to very precisely localize the neural correlates of task performance and thus this route has recently become most prominent.

In the first part of this chapter, the Verbal fluency task will be presented as a sensitive measure of frontal lobe functioning, not only in neuropsychological practice, but also recently in functional activations studies using PET and fMRI. The neural correlates of a verbal fluency task as investigated with functional activation studies will be outlined, and the question of how task performance during the scan relates to brain activation will be addressed.

In the second part, the Apolipoprotein E (ApoE) genotype, thought to be a major genetic susceptibility factor for AD, will be introduced and its importance in modulating the brain response in normal elderly subjects will be investigated.

3.1 The Verbal fluency task - a sensitive measure of frontal lobe function

The purpose of the test is to evaluate the spontaneous production of words within a limited amount of time. There are two versions of Verbal fluency tasks: the letter fluency or „phonemic“ fluency and the category fluency or „semantic“ fluency.

The letter fluency is a well established test with a long tradition in neuropsychology, going back to the Thurstone Word Fluency Test (Thurstone & Thurstone, 1962). The most widely used version is the one developed by Benton (Benton, 1968), in which subjects orally generate words beginning with letters (e.g. F, A, and S) with 60 seconds for each letter. The category fluency is a measure of semantic memory as subjects are asked to generate words from certain semantic categories (e.g. animals, fruits, clothing). Performance on a verbal fluency task relies on efficient organization of verbal retrieval and further involves short-term memory (in keeping track of the words already said), ability to initiate and maintain word production set, cognitive flexibility (in rapidly shifting from one word to the next within the selected category), as well as response inhibition capacity. These processes are commonly considered as aspects of „executive“ functioning, which are subserved by the frontotemporal region, particularly left frontal area, as demonstrated in clinical and functional activation studies (Mitrushina, Boone & D'Elia, 1999).

It is nowadays generally accepted that in neurodegenerative disorders such as AD, performance on letter fluency tasks dissociate from category fluency tasks (see Warburton et al., 1996). In studies performed by Monsch and colleagues (Monsch et al., 1992; Monsch et al., 1997) it could be shown that the category fluency test is superior over the letter fluency test in differentiating AD patients from normal controls. Both tests require attention, initiation, retrieval and working memory, however it is assumed that letter fluency is more dependent on the phonologically based word store, whereas category fluency is dependent on access to intact representations in semantic memory. This argument is consistent with the notion that AD patients suffer from a significant deterioration in the structure of semantic knowledge (Monsch, 2001).

Neuropsychological research has revealed a large number of characteristic features of early-stage AD that may serve as guidelines in the search for preclinical cognitive markers. In short, tests that assess verbal episodic memory (e.g. recalling a list of words) and semantic memory (e.g. category fluency) were shown to be most sensitive and specific in distinguishing AD patients in early stages from normal controls (Monsch, 2001). A thorough review of this

field of research as well as the analysis on preclinical markers from the neuropsychological perspective in the BASEL study is provided in the habilitation thesis of PD. Dr. Andreas Monsch (2001), and will not be discussed here.

3.2 PET and fMRI functional activation studies investigating the neural substrates of verbal fluency tasks

3.2.1 Studies with PET

Tests of verbal fluency have become a prominent activation paradigm in functional imaging, specifically to test the function of the frontal lobes. verbal fluency tasks have a long tradition in PET functional activation studies, and they have lately also been used in fMRI and NIRS.

There is vast and consistent evidence from PET functional activation studies in young and middle-aged adults that the performance of a letter fluency task is strongly associated with the left prefrontal cortex (Benton, 1968; Carlsson, Wendt & Risberg, 2000; Elfgren & Risberg, 1998; Frith, Friston, Liddle & Frackowiak, 1991a, b; Friston, Frith, Liddle & Frackowiak, 1993; Milner, 1964; Warburton et al., 1996; Warkentin & Passant, 1993). In addition to the activation of the left prefrontal area, a number of studies have also found an involvement of the temporal lobe with some of the studies having found a rCBF increase (e.g. Warkentin & Passant, 1993) and others a symmetrical bilateral rCBF decrease (e.g. Frith et al., 1991a).

The results of PET studies using some form of a semantic fluency task (Klein, Milner, Zatorre, Meyer & Evans, 1995; Warburton et al. 1996) suggest that common neural substrates are involved: the left prefrontal cortex together with left parietal and inferotemporal regions. Klein et al. (1995) investigated whether the search process in word generation, i.e. phonological vs. semantic search, activate similar regions in bilingual young subjects. As a result, they discovered a broad activation of the left inferior and dorsolateral frontal cortex together with activations of the left parietal and inferotemporal regions irrespective of the search requirement. Warburton et al. (1996) examined word retrieval (verbs and nouns) in four experiments, contrasting it to four different control states (rest, listening to words, silent repetition of pseudowords, verb-noun comparison). In summary of their findings they conclude that word retrieval is the product of a distributed and predominantly left-lateralized neural network involving particularly left inferior temporal and parietal regions as well as the left prefrontal cortex. The studies of Klein et al. and Warburton et al. both propose that the involvement of the left parietal and temporal lobe reflect semantic processing.

3.2.2 Studies with fMRI

With high-resolution fMRI becoming available, it became possible to more precisely localize the brain regions involved during performance of a verbal fluency task. One of the first groups to compare the findings obtained with PET as described above was Phelps, Hyder, Blamire and Shulman (1997). Following the methodology of Frith et al. (1991b), they investigated three task conditions: repeating words, generating antonyms, and generating words (letter fluency). 11 healthy subjects were included in their study, however, due to movement artifacts, data from only six subjects could be analysed. The results obtained with fMRI by Phelps and colleagues (1997) were in very good agreement with the Frith study: During performance of letter fluency compared to simply repeating words, there was increased activation in the left dorsolateral prefrontal cortex and in the left anterior cingulate (Brodmann Area [BA] 45, 24, and 32; for an illustration of BAs see Appendix I, Figure I-1). The fMRI study however showed an additional area of activation around the left superior frontal gyrus (BA 8), which was interpreted in relation with different behavioural components of verbal fluency in their task.

Trying to overcome the problems with movement artifacts resulting from overt speech during the scanning procedure, covert response paradigms of verbal fluency have been developed (Paulesu et al., 1997; Schlösser et al., 1998; Smith et al., 1999, 2002). Paulesu et al. (1997), by contrasting letter fluency to semantic fluency in six young male normal volunteers, found that in comparison to rest both tasks activated the anterior triangular portion of the left inferior frontal gyrus (BA 45) and the left dorsomedial thalamus. Significant temporal cortex activation was found only in four of the six subjects in the superior and medial temporal gyri and particularly during the phonemic fluency task. Smith et al. (1999, 2002) found activation of the left inferior frontal lobe (BA 44, 45), the exastriate areas of the occipital lobe bilaterally (BA 18, 19), inferomedial areas of the posterior temporal lobe (BA 37), and the left premotor cortex (BA 6) together with a significant deactivation bilaterally in the temporoparietal and superior parietal areas and in the right premotor region (see Figure 6 for illustration) during visual letter fluency vs. a baseline visual monitoring task in cognitively intact, middle-aged women at high and low risk for AD (see section 3.4.1 for further details with respect to ApoE-ε4 genotype). Schlösser et al. (1998) obtained whole brain fMRI from six male and six female young normal volunteers and found a broadly consistent pattern of activation increase in the left prefrontal cortex and right cerebellum together with significant decreased responses in mesial and dorsolateral parietal cortex bilaterally. There were no gross differences in the pattern of

activation between male and female subjects.

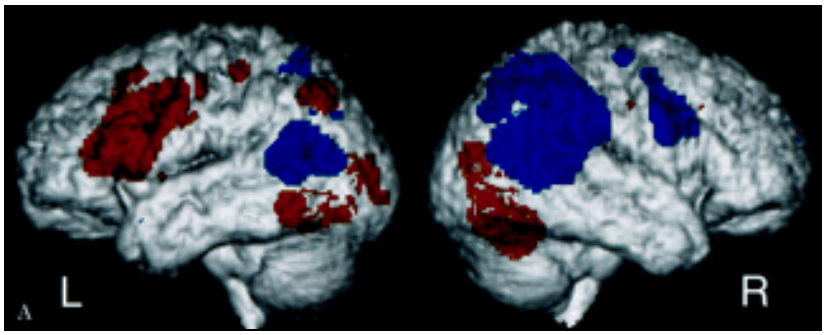


Figure 6: Task activation maps comparing baseline and letter fluency for the low-AD risk group (n=17). Areas of increased activation are in red, areas of decreased activation in blue (taken from Smith et al., 2002, p. 1199).

These studies are all consistent with earlier PET observations, in that they consistently showed activation of the left dorsolateral prefrontal cortex. However, they have precluded direct measurement of task performance during image acquisition.

Using a novel overt verbal fluency design that allowed for performance measurement during image acquisition, the subjects in the study of Abrahams et al. (2003) performed a letter fluency task vs. a baseline word-repetition task. During the tasks the subject was presented with a letter cue every 6 seconds and responded overtly with a single word in a 4-second quiet period. The 18 adult healthy volunteers (mean age: 56.7 ± 12.1 years) were clearly capable of performing the letter fluency task as they on average produced 42.6 ± 1.8 different words from a total of 45 possible responses. Task performance did not correlate with age. The cortical regions of significant activation during the letter fluency task included extensive regions of the left and right middle frontal gyrus (BAs 46 and 9), left inferior frontal gyrus (areas 44 and 45), left and right anterior cingulate gyrus (BAs 32 and 24) and left medial prefrontal cortex (area 6). Correlational analyses of BOLD changes and task performance showed no significant results.

3.2.3 Verbal fluency in patients with Alzheimer's Disease

To date, brain imaging in patients with Alzheimer's disease has primarily been performed in the context of the diagnostic process and/or to better understand the neural correlates of the cognitive deficits in Alzheimer's disease. *Functional* imaging techniques have surprisingly been performed to a very little extent and the number of available publications is very limited. Furthermore, the studies differ in terms of imaging techniques, activation paradigm, and

dementia severity, and thus are hardly comparable. Disease severity was found to correlate with rCBF and oxygen metabolism (Rapoport, 1991; Tohgi et al., 1998). In this section we will focus on PET and SPECT studies in Alzheimer's disease and on the neural correlates of a verbal fluency task in AD.

Measuring CBF and glucose metabolism in so called „resting brain", i.e. without a stimulus-induced cerebral activation, many researchers have found reductions in blood flow and glucose utilization in the parieto-temporal association cortex, with the frontal cortex being relatively spared in mild stages of the disease (for reviews see Heiss, Pawlik, Holthoff, Kessler & Szelies, 1992; Lee, Mintun, Buckner & Morris, 2003; Mayberg, 1994; Matsuda, 2001; Rapoport, 1991).

As summarized in Table 3-1, the majority of imaging studies on verbal fluency in Alzheimer's disease have investigated patients' resting blood flow and/or glucose metabolism and correlated these measures with the performance in verbal fluency tasks assessed outside the scan (Hirono et al., 2001; Keilp, Gorlyn, Alexander, Stern & Prohovnik, 1999; Kitabayashi et al., 2001; Pasquier, Lebert, Grymonprez & Petit, 1995). Only Warkentin and Passant (1997) have applied a functional imaging methodology, investigating the rCBF in patients with AD during rest as well as during performance of a letter fluency task. Overall, these data suggest that the relationship between regional cerebral blood flow/regional cerebral glucose metabolism and verbal fluency in Alzheimer's disease is comparable to what has been observed in healthy volunteers: letter fluency scores were significantly correlated with the left prefrontal blood flow/glucose metabolism, whereas category fluency scores in addition correlated with left temporal flow/glucose metabolism. In the functional imaging study of Warkentin and Passant (1997), the patients with AD during performance of a letter fluency task had significant activation in the Broca's area only, whereas the remaining frontal areas, particularly prefrontal, unlike normal subjects showed no flow increases. Unfortunately, the AD sample of Warkentin and Passant is not specified: neither the diagnostic classification system nor the severity of the disease is indicated but only the duration of illness (which for the sample of 17 patients varied between 1 and 10 years).

Table 3-1: PET/SPECT studies on neural substrates of verbal fluency in Alzheimer's Disease

Authors	Study Design	Results
Hirono et al. (2001)	Resting [^{18}F]deoxyglucose PET 57 patients with probable DAT (mean MMSE: 19.1±3.4); Category fluency	Significant positive correlations between fluency scores and glucose metabolism in : -left superior frontal gyrus (r=0.457) -left inferior frontal gyrus (r=0.417) -left and right anterior cingulate gyrus (r=0.475, r=0.424) -left inferior temporal gyrus (r=0.441)
Keilp et al. (1999)	Resting [^{133Xe}]rCBF 25 patients with probable DAT (mean MMSE: 16.7±4.6); 24 elderly controls (mean MMSE: 28.6±1.8); Category and letter fluency	Left/right parietal rCBF values: DAT< controls Left/right frontal rCBF values: no difference between groups DAT group: primary predictor of letter fluency score = left frontal rCBF
Kitabayashi et al. (2001)	Resting SPECT 25 patients with probable DAT(mean MMSE: 17.7±4.7) Category and letter fluency	-Letter fluency score was significantly correlated with the left dorsolateral prefrontal rCBF (r=.463) -Category fluency score was significantly correlated with the left dorsolateral prefrontal (r=.439) and left temporal rCBF (r=.514)
Pasquier et al. (1995)	Resting SPECT 7 patients with probable DAT (mean MMSE: 23.1±5.2); 7 patients with dementia of frontal lobe type (DFT; mean MMSE: 23.9±4.4); both groups matched for gender, years of education, age, MMSE; Category and letter fluency	Frontal SPECT values: DFT < DAT Parietal SPECT values: no difference between groups No correlation between frontal SPECT and fluency measures Category and letter fluency: no difference between groups
Warkentin & Passant (1997)	[^{133Xe}]rCBF during rest and during performance of a letter fluency task 15 patients with dementia of frontal lobe type (DFT; mean duration of illness: 4.5 years; mean age: 62 years); 17 patients with AD (mean duration of illness: 4 years; mean age: 67 years) 22 healthy volunteers (mean age: 36 years)	AD patients: -Rest: regional flow pathology in parietal and temporal areas, frontal areas normal activation. -Activation: significant activation of Broca's area, no significant changes in other frontal areas, e.g. DLPFC, as in healthy volunteers

3.3 Correlation between task performance and brain response

To date there are only few reports on how performance in a verbal fluency task during functional activation correlates with the corresponding brain response, and the relationships between the two seem to be complex.

Elfgren and Risberg (1998) have investigated whether the cognitive strategy during the performance of a verbal fluency task has an influence on the frontal blood flow increase. They measured rCBF in twenty healthy young volunteers during performance of a letter fluency test vs. a baseline control task. Splitting the group according to the reported strategy during the letter fluency task yielded two subgroups: one with a „pure“ verbal strategy vs. one with a „mixed“ strategy, i.e. a verbal strategy combined with a visual search strategy. Their interesting main finding was that subjects reporting a verbal strategy not only produced significantly more words during the letter fluency task but also showed significant flow increases in the left dorsolateral prefrontal cortex while subjects with a mixed strategy showed no significant flow increases.

Measuring rCBF during rest and during a letter fluency task in 49 healthy volunteers, Warkentin and Passant (1993) have investigated whether the subject's performance was related to frontal lobe activation. They have divided the sample into „low“, „medium“, and „high“ performers. The high performers on average showed a trend towards highest frontal flow increase, however also the low and medium performing group revealed frontal flow increases, suggesting that task performance alone is not a critical factor for frontal lobe activation.

In one of the earliest PET studies of letter fluency, Parks et al. (1988) found negative correlations between verbal fluency scores and PET normalized metabolic rates over the entire cortex which was in contradiction to their hypothesis. They interpreted this finding in line with the notion of verbal fluency as „effortless responding“ in analogy to the relatively minor physical exertion of a proficient swimmer as opposed to that of the novice in swimming the length of a pool: „The novice must expend more energy to swim slower because his/her technique may not be as efficient as that of the expert „ (Parks et al., 1988, p. 572).

In the study of Abrahams et al. (2003), as outlined above, BOLD changes were not significantly correlated with task performance, however, with the design of their overt paradigm and the excellent average task performance, the variability might have been too small to detect a significant correlation.

Increased task demand seems to be related to greater cerebral activation as demonstrated

recently by Fu et al. (2002), who have investigated „easy“ vs. „hard“ letters in an overt fMRI letter fluency experiment. Although both conditions elicited the typical pattern of activation seen in letter fluency tasks, the hard letters were associated with greater activation of the left anterior cingulate (BA 32). This greater activation might be related to a greater engagement of arousal and stress responses by highly demanding tasks.

In summary of these findings, there are at least two possible scenarios for the relationship between task performance and brain activation: 1) a positive correlation and 2) a negative correlation:

1) A positive correlation: higher task performance is related to greater brain activation in the corresponding brain area needed for task performance. As shown by Elfgren and Risberg (1998), subjects using a verbal strategy, which was related with better performance than the mixed strategy, showed significant activation of the left dorsolateral prefrontal cortex, i.e. the area known to be involved in the performance of a letter fluency task. In other words, subjects able to bring the brain areas critical for successful performance of a cognitive task on-line, show better task performance.

2) A negative correlation: low task performance is associated with higher brain activation. For healthy normal subjects a negative correlation could be interpreted in terms of the „novice vs. expert“ metaphor, as done by Parks et al. (1988). Although the novice needs to invest more effort to solve a task than the expert, his performance is yet lower. In contrast to Elfgren and Risberg (1998), the correlation between letter fluency score and brain activation was not restricted to a specific brain area but was seen over frontal, parietal and temporal cortices of both hemispheres. Thus, using the novice-vs. expert metaphor, the novices had an increased cerebral activation across the whole cortex.

Finally, the difficulty of a task seems to have a modifying effect on brain activation (see also chapter 2, section 2.4.1), a generally accepted notion which has received further support from a recent investigation of Fu et al. (2002).

3.4 The Apolipoprotein E (ApoE) Genotype

The search for preclinical markers of AD gained an important boost by the detection that certain genotypes of Apolipoprotein E (ApoE) may serve as major genetic susceptibility factors for AD (Monsch, 2001).

The apolipoproteins represent a group of proteins that share their presence on plasma

lipoproteins (chylomicrons, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL)) that serve as the ferries for cholesterol, triglycerides and phospholipids. While several studies have investigated a variety of genes encoding apoAIV, ApoA, ApoB and ApoCIII, the effect of the ApoE gene has been studied most intensively (Smith, 2002). The gene coding for ApoE is located on the long arm of chromosome 19. Three primary alleles, ApoE-ε2, ApoE-ε3, and ApoE-ε4, determine an individual's ApoE status. As each of us inherits one apoE allele from each parent, there are six possible genotypes; ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4. In western European and white American populations, the allele frequencies for ε2, ε3 and ε4 are ~8, 78 and 14%, respectively (Smith, 2002). In contrast to the allele frequency the carrier frequency is often reported, especially with regard to the ApoE-ε4 allele: In the general population, 25 - 35% of healthy individuals have at least one ApoE-ε4 allele (Mahley, 1988) compared to approximately 60% of AD patients (Schmechel, Saunders, Strittmatter, Crain, Hulette, Joo et al., 1993). The relative risk of AD increases about 2.5 times for individuals who are heterozygous for the ApoE-ε4 allele and approximately eight times for homozygous individuals (Corder et al., 1993; van Duijn, de Knijff, Cruts, Wehnert, Havekes, Hofman et al., 1994). Furthermore, AD patients who are homozygous for ApoE-ε4 (ε4/ε4) have a significantly earlier onset of dementia than ε4 heterozygotes and individuals without an ε4 allele (Corder et al., 1993; Strittmatter et al., 1993; see Monsch (2001) for a more detailed review).

3.4.1 Brain correlates of ApoE-ε4 in the normal elderly

While the ApoE-ε4 allele has been identified as a major genetic susceptibility factor for AD, yet little is known *in vivo* about how the ApoE genotype modulates the brain in aging and in AD. One strategy to elucidate the relationship between ApoE genotype and normal and pathological aging has been to implement brain imaging techniques in subjects at genetic risk for AD. In this section, brain imaging studies in relation to ApoE genotype in normal elderly subjects will be presented.

Brain imaging studies which have been conducted in relation with the ApoE genotype, have in the majority of cases used two different brain imaging techniques:

- Volumetric MRI to measure potential hippocampal damage
- [^{18}F]deoxyglucose PET to measure cerebral metabolism

Table 3-2 provides an overview of design and results of these studies.

Table 3-2: Overview of studies investigating brain correlates of ApoE-ε4 using volumetric MRI and/or PET in elderly subjects

	Authors	Study Design	Results
Cross-sectional Studies	Small et al. 1995	29 subjects with AAMI* and a family history of AD, 19 ε4- 12 ε4+	Left/right parietal metabolism: ε4+ < ε4-, parietal asymmetry: ε4+ > ε4-, MRI: no difference in subjective atrophy ratings between groups
	Soininen et al. 1995	32 healthy subjects, 18 ε4-/-, 10 ε4+/-, 4 ε4+/+	Hippocampal volume asymmetry left < right: ε4+/+ < ε4+/- <ε4-/-
	Schmidt et al. 1996	214 population-based normal subjects, 175 ε4- 39 ε4+	No significant differences in hippocampal/parahippocampal volumes, no significant white matter differences
	Tohgi et al. 1997	54 healthy subjects, 40 ε4- 14 ε4+	Right hippocampus: ε4+ < ε4-, ε4-subjects: right hippocampus > left
	Jack et al. 1997	125 cognitively normal subjects, 95 ε4- 30 ε4+	ε4+ subjects slightly smaller hippocampal volumes, however not significant
	Reiman et al. 1998	33 subjects with a familiy history of AD, 22 ε4-/-, 11 ε4+/+	ε4 homozygotes slightly smaller hippocampal volumes, however not significant
	Jernigan et al. 2001	43 healthy subjects, 22 ε4- 21 ε4+	No significant difference in hippocampal volumes, nor in any cortical volume
Longitudinal Studies	Small et al. 2000	2-year follow-up of 20 subjects with mild memory complaints at baseline, 10 ε4- 10 ε4+	ε4+ subjects: significant decline in the inferior parietal and temporal cortices ε4- subjects: significant decline primarily in frontal cortex
	De Leon et al. 2001	3-year follow-up of 25 healthy subjects, 17ε4- 8 ε4+	Baseline: metabolism lateral temporal lobe, superior temporal gyrus: ε4+ < ε4- Follow-up: interaction ε4 x cognitive decline: declining ε4+ subjects significant reduction metabolism lateral temporal lobe

* AAMI = Age-associated memory impairment

To start with, it becomes evident that most of these studies have been performed cross-

sectionally, and the number of longitudinal studies is yet very limited. Furthermore, with the exception of the studies of Schmidt et al. (1996) and Jack et al. (1997) the sample size of subjects, especially subjects with an ε4 allele, was very small. Thus, these results must be interpreted with caution.

In the cross-sectional study of Schmidt et al. (1996) in addition to MRI and ApoE genotyping a detailed neuropsychological test battery was applied. It was found that the 39 ε4 carriers had significantly reduced scores on measures of memory and learning compared to the non-carriers (Bäumler's Lern- und Gedächtnistest; LGT-3), a difference which could not be attributed to potential confounders such as age, educational status, mood status, concomitant medications or diseases, and vascular risk factors. The ε4 carriers did not differ from non-carriers in neuropsychological measures of attention and speed (Alterskonzentrationstest, Trail Making Test Form B), visuopractical skills (Purdue Pegboard Test), and conceptualization (Wisconsin Card Sorting Test). As summarized in Table 3-1, the difference in memory and learning abilities between ε4 carriers and non-carriers was not reflected on the level of MRI. This lack of association was however not further investigated „It is a common radiological observation that in neurodegeneration, morphological cerebral abnormalities occur only late in the disease and are frequently proceeded by functional changes" (Schmidt et al., 1996, p. 297).

In the study of Jack et al. (1997) ε4-positives did show a trend toward smaller hippocampal volumes than ε4-negatives, this trend however did not reach statistical significance. The absence of a significant association between ApoE genotype and hippocampal volume was additionally confirmed by logistic regression analysis, in which no significant interaction between hippocampal volume and ApoE genotype was found. For the authors this finding was unexpected, and one possible explanation they give is the fact that their subjects, on average, were between a decade and a decade and a half older than the nondemented subjects investigated in earlier trials (mean age ε4-negatives: 78.8 ± 6.98 years, ε4-positives: 80.1 ±5.86 years). Thus, based on the generally accepted notion that intersubject anatomic variability increases with advancing age, especially in olders subjects, the ε4 effect on hippocampal volumes was small enough to have been overwhelmed by the much greater effect of intersubject anatomic variability. In fact, the studies of Small et al. (1995) and Tohgi et al. (1997), which found significant differences in hippocampal volume in relation to the ε4 allele, had subjects with a mean age below 60 years (Small et al: mean age ε4-negatives: 55.5 ±12.0 years, ε4-positives: 56.4 ± 10.3 years; Tohgi et al: mean age ε4-negatives: 59.0 ±11.0 years, ε4-positives: 58.8 ± 8.0 years).

In replication of their cross-sectional findings (Small et al., 1995), Small et al. (2000) observed lower metabolic rates in ε4 carriers for the inferior parietal and the lateral temporal cortices as well as for the posterior cingulate at baseline. At the 2-year follow-up, analyses of the PET data revealed significant glucose metabolic decline of 5% in the inferior parietal and lateral temporal cortices in ε4 carriers, whereas the ε4 non-carriers showed significant decline in the frontal cortex, which according to the authors, is consistent with normal aging. Moreover, in subjects at genetic risk for AD, it was found that lower baseline metabolism predicted future cognitive decline measured 2 years later. These results are in line with the observations of De Leon et al. (2001): At baseline, ε4 carriers had significantly lower glucose metabolism in the neocortical temporal lobe than non-carriers. At the 3-year follow-up, among those subjects with cognitive decline, ε4 carriers showed further marked reductions in the same area, suggesting that progressive ε4-related hypometabolism may underlie the known ε4 carriers increased susceptibility for AD.

In addition to the investigation of resting metabolism and brain anatomy, functional imaging has been used to further elucidate the differences between individuals at high vs. low risk for AD. Smith et al. (1999) compared the functional MRI scans of 14 women at high risk to develop AD (defined as having at least one ApoE-ε4 allele and a first degree relative with clinical AD; mean age = 51.8 ± 5.1 years) with 12 low risk individuals (mean age = 53.0 ±. 6.2 years) while they performed confrontation naming and letter fluency tasks. The high-risk group demonstrated significantly reduced activation in the bilateral temporal regions during both activation tasks. These results however need to be taken with some caution as unfortunately task performance during the scan was not assessed. Thus, differences in task performance between groups might have contributed to the differences in activation. In a follow-up study of the same group (Smith et al., 2002), the same methodology was used in an increased sample of women at high and low risk for AD (N = 38, 21 high-risk vs. 17 low-risk subjects incl. the 26 subjects described above). The results of this study using a bigger sample size revealed significantly increased left parietal activation for the high-risk group during the letter fluency task, an observation which at a trend-level was also found in their initial investigation. No difference was found in the frontal region, the area known to be similarly activated in a letter fluency task. Taken together, the authors interpreted their findings in relation to a disruption of functional circuits involving the left parietal lobe in individuals at increased risk for AD.

3.5 Summary

With the development of high-resolution brain-imaging techniques it became possible to precisely localize the neural correlates of cognitive functioning, and thus better understand the brain-behaviour relationships.

A simple and frequently used functional activation paradigm is the verbal fluency task. The verbal fluency task involves cognitive processes commonly considered as aspects of executive functioning. Verbal fluency tasks can be subdivided into phonemic or letter fluency tasks (e.g. words starting with a F, A, or S) and category or semantic fluency tasks (e.g. food, animals, tools).

PET studies investigating the brain areas engaged in the performance of a verbal fluency task have consistently shown activation of the left frontotemporal area. Recent fMRI studies have more precisely identified the patterns of activation during the performance of a letter fluency task: They have predominantly found activation of the left frontal cortex (including Broca's area, premotor cortex and dorsolateral prefrontal cortex), inferomedial areas of the posterior temporal lobe together with variable deactivation bilaterally in temporoparietal areas. Activation of the right prefrontal cortex was also reported. In these functional activation studies the elderly non-demented population has not been systematically represented, and there is to date only one functional imaging study using the verbal fluency Task in Alzheimer's Disease patients.

Positive as well as negative correlations between task performance during the brain scan and brain response have been reported. While a positive correlation has been found specifically in the cortical area of activation (i.e. the left prefrontal cortex in a letter fluency task), the negative correlation between task performance and brain response seems to be distributed globally across the entire cortex.

Although it is generally well accepted that the ApoE-ε4 allele acts as a major genetic susceptibility factor for AD, the evidence coming from resting-brain imaging studies in relation to the ApoE genotype in normal elderly subjects is controversial. There is some evidence that ApoE-ε4 carriers have a tendency towards smaller hippocampal volumes, reduced temporal glucose metabolism, and hippocampal asymmetry, however, given the fact that most of the reports have applied a cross-sectional design and that the sample size in these positive studies was relatively small, these reports have to be taken with caution.

Only a few data from functional activation studies in individuals at high vs. low risk for AD are available, suggesting a disruption of functional circuits involving the left parietal lobe

in individuals at increased risk for AD. Again, these studies used small sample sizes. Furthermore, they did not control for task performance during the scan, which might have had a confounding effect on the measured brain response.

4 Methods

The data reported in this doctoral thesis were recorded in the context of the "Basel Study on the Elderly (BASEL)" project. The BASEL project was planned and carried out as a multidisciplinary project incorporating several disciplines, of which, for the purposes of this thesis, near-infrared spectroscopy, neuropsychology and neurobiology (ApoE genotype) were chosen for combined analyses. The contributors to these data were the Basel Memory Clinic and NeuroPsychologieZentrum of the University Hospital of Basel (Prof. Dr. Hannes B. Stähelin & Prof. Dr. Andreas Monsch), the Institute of Physiology of the University of Basel (Prof. Dr. Uwe Otten) and the Psychiatric University Hospital of Basel (Prof. Dr. Franz Müller-Spahn & Prof. Dr. Christoph Hock). The project was approved by the Ethics Committee of the University Hospital of Basel, and written informed consent was obtained from all study participants.

4.1 The BASEL project: an overview

When recruiting the participants of The BASEL project, the authors could revert to an existing database of elderly subjects who had taken part in the longitudinal "Basel studies I-III", which began in 1959 with a cohort of approximately 6,400 subjects who were at that time employees of the pharmaceutical industry in Basel (Monsch, 2001). The last mortality follow-up in 1990 examined 1,931 individuals who had last been seen in 1972. This sample of individuals comprised the recruitment base of the current study. An overview of the study population and the flow of examinations in the BASEL project is provided in Figure 7.

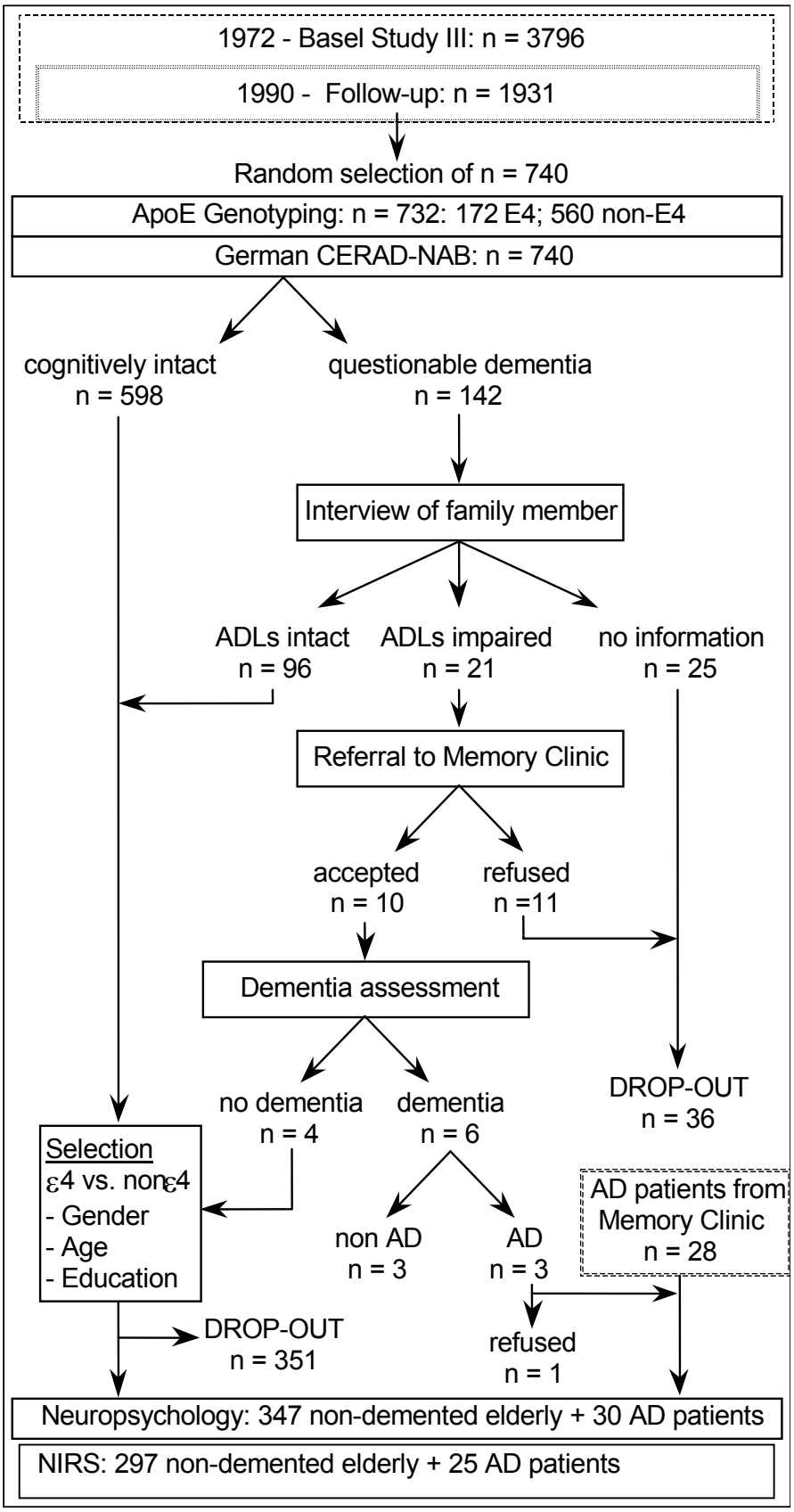


Figure 7: Overview of the BASEL project (reprinted with permission from Monsch, 2001)

4.2 Screening examinations

After subjects had signed informed consent, a thorough screening examination was performed to safeguard against the inclusion of demented subjects in the baseline sample of healthy elderly subjects.

The screening examination consisted of a clinical interview to assess the past and current medical history; the administration of the German version of the CERAD (Consortium to Establish a Registry for Alzheimer's Disease; Morris et al., 1989) neuropsychological assessment battery (CERAD-NAB) and the clock drawing test (CDT); and a blood drawing for ApoE genotyping. The clinical interviews, CERAD-NAB and CDT administrations were carried out by trained psychology students under the supervision of experienced clinical psychologists. The decision, whether a study subject could be considered as "cognitively intact" as opposed to presenting with a "questionable dementia" was based on a comparison of individual test results with a sample of 85 optimally healthy controls, who had not participated in the Basel Study III (see Monsch, 2001 for a detailed description of the procedure). Study participants with a questionable dementia were contacted by a trained gerontologist who carried out a semi-structured telephone interview on activities of daily living (ADL) with these subjects and a significant other (usually the spouse). The objective of this interview was to assess whether the low score on the CERAD-NAB would reflect a relatively new decrease and whether it would impact on the subject's daily life. If this was the case, the subject was asked to give permission that his/her family physician would be contacted to initiate detailed neuropsychological examination at the Memory Clinic.

4.3 Cross-sectional main examinations

On average 5.7 ± 2.7 months after the screening examination, all cognitively intact individuals (i.e. as determined in the screening phase) returned to the study centre for a detailed neuropsychological assessment as well as for a near-infrared spectroscopy examination. As displayed in Figure 5, these individuals were grouped according to the presence and absence of at least one ApoE-ε4 allele and were pairwise matched according to age (± 4 years), education (± 4 years) and gender.

4.3.1 Neuropsychological assessment

The selection of the neuropsychological test battery was made to cover several criteria relevant for the aims of the BASEL project: The tests needed to be able to detect subtle

cognitive changes (i.e. they needed to be challenging and broad); they needed to be appropriate for not only quantitative but also for qualitative analyses; they had to be suitable for repeated administration (longitudinal approach); and they had to be internationally compatible. For the cross-sectional correlation between neuropychological test performance and near-infrared spectroscopic data as reported in this thesis, not the whole test battery but a selection of most commonly used tests was chosen as displayed in Table 4-1. The neuropsychological assessments were carried out by trained psychology students under the supervision of experienced clinical psychologists.

Table 4-1: Selection of tests from the neuropsychological test battery used in the BASEL project (German versions)

Premorbid IQ
-Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B; Lehrl, 1993)
General intellectual functioning
-WAIS-R: Similarities, Block Design, Vocabulary (Tewes, 1991)
Language/Executive Functions
-Boston Naming Test (Kaplan, Goodglass & Weintraub, 1983)
-Category fluency (Newcombe, 1969), i.e., one minute each of animals, tools, and food
-Phonemic fluency (Benton & Hamsher, 1989), i.e., one minute each of S-, O-, and G-words
Memory
-California Verbal Learning Test (Delis et al., 1987)
Executive functions
-Trail Making Test A and B (Army Individual Test Battery, 1944)
-Modified Wisconsin Card Sorting Test (Nelson, 1976)

4.3.1.1 Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B; Lehrl, 1993)

The multiple-choice vocabulary word test is a measure of general verbal intelligence, especially for the use in patients with (transient) organic brain syndrome. Developed in a psychiatry environment, the test aims at gathering information about the premorbid intelligence of a patient in a short period of time. The MWT-B consists of 37 lines of five words each. Each line consists of one semantically correct word and four fictitious pseudowords. The score is obtained by adding up the number of correctly recognized words.

4.3.1.2 Wechsler Adult Intelligence Scale (WAIS-R; Tewes, 1991)

The WAIS-R is one of the most frequently used measures in neuropsychological test batteries, providing information about the overall level of intellectual functioning, and the presence or absence of significant intellectual disability (Lezak, 1995, Spreen & Strauss, 1998). The WAIS-R is composed of 11 subtests, 6 verbal and 5 performance-oriented. Of these, three subscales were chosen of which the Vocabulary and the Similarities subtest cover verbal intelligence whereas the Block Design test belongs to the performance tests. The Vocabulary subtest requires the subject to provide definitions of words. On the Similarities subtest, the subject is asked to explain in what way two things are alike. On the Block Design subtest, the subject is asked to construct replicas of constructions made by the examiner using red and white blocks.

4.3.1.3 Boston Naming Test (Kaplan, Goodglass & Weintraub, 1983)

The purpose of this test is to assess the ability to name pictured objects (Spreen & Strauss, 1998), which requires several stages of information processing: a perceptual analysis of the visual features of the object, semantic access of the underlying conceptual representation, the lexical retrieval of the appropriate name for the object, encoding the articulatory program and finally correct articulation of the name (Mitroshina et al., 1999; Nicholas, Obler, Albert & Goodglass, 1985,). With the complexity of processes involved, it is evident that several brain structures are responsible for object naming, of which e.g. the anterior part of the left inferior temporal lobe has been identified for naming animals and the posterior portions of the of the left inferior temporal lobe for naming tools (Damasio, Grabowski, Tranel, Hichwa & Damasio, 1996), but also focal left frontal regions have been suggested to be responsible for the ability to access the sound form of the word (Whatmough, Chertkow, Murtha & Hanratty, 2002).

The original test by Kaplan, Goodglass and Weintraub (1983) contains 60 items. In the BASEL project a subset of 45 items not contained in the CERAD-NAB screening battery was used. For the purpose of this thesis, only the number of spontaneously given correct responses were analysed.

4.3.1.4 Category Fluency (Newcombe, 1969)

In the category fluency test the subjects is asked to name as many different words as he or she can think of in one minute from the semantic categories „food“, „animals“ and „tools“, respectively. The test is scored by calculating the number of correctly named words, the number of perseverations and the number of errors. For our analyses the number of total correct words

across all three categories were entered into the database.

4.3.1.5 Phonemic Fluency (Benton & Hamsher, 1989)

In the phonemic fluency test, the subjects are prompted to name as many words starting with a given letter (e.g. in this case „S“, „G“ and „O“) as they can think of in one minute, respectively. Thus, this fluency test is also labeled „letter fluency“. The test is scored by calculating the number of correctly named words, the number of perseverations and the number of errors.

4.3.1.6 California Verbal Learning Test (CVLT; (Delis et al., 1987)

The CVLT assesses multiple aspects of the strategies and processes involved in learning and remembering verbal material (Spreen & Strauss, 1998), The learning material consists of two shopping lists, presented to the subject as the „Monday“ (List A) and the „Tuesday“ list (List B). Each list contains 16 items from four categories each. Administration begins by evaluating a subject's ability to recall the items of List A by reading the items to the subject over five sequential trials. The subjects are instructed to recall as many items as possible after each trial. Following trial 5, subjects are presented with List B, an interference list with 16 items from categories different from those in List A, and required to recall as many items as possible from List B. Immediately after List B subjects are again asked to recall the words from List A (short delay free recall) and are subsequently cued with each of the four categories from List A (short delay cued recall). After a 20-minute delay, long delay free recall and long delay cued recall of List A and finally long delay recognition of List A items are tested (Monsch, 2001). From this procedure, the CVLT provides multiple parameters, from which the following were chosen for data analysis:

- Number of correctly recalled words, perseverations, and intrusions across all 5 learning trials
- Number of correctly recalled words, perseverations, and intrusions in the short delay free recall
- Number of correctly recalled words, perseverations, and intrusions in the long delay free recall
- Number of hits, false alarms, correct rejections, and omissions in the long delay recognition.

- Recognition discriminability (%): Percentage of correctly identified and correctly rejected words from the total words presented:

  (hits + correct rejections)/44 x 100

Recent functional activation studies of episodic memory have consistently shown that retrieval processes are related to the activation of the right prefrontal cortex whereas the mesial temporal lobes and the left frontal lobe are associated with encoding (Fletcher, Shallice & Dolan, 1998; Fletcher, Shallice, Frith, Frackowiak & Dolan, 1998; Lapage, Habib & Tulving, 1998; Saykin et al., 1999). In a functional activation study of verbal memory, Johnson, Saykin, Flashman, McAllister and Sparling (2001) found a high positive correlation between the recognition discriminability and the fMRI signal increase to familiar words in the right prefrontal cortex.

4.3.1.7 Modified Wisconsin Card Sorting Test (Nelson, 1976)

The widely used Wisconsin Card Sorting Test (WCST) is considered a measure of executive function in that it requires strategic planning, organized searching, the ability to utilize feedback to shift „cognitive sets“, goal-oriented behavior, and the ability to modulate impulsive responding (Spreen & Stauss, 1998). In her modification of the WCST Nelson (1976) removed the response cards which shared more than one attribute with a stimulus card, thus eliminating ambiguity and simplifying the task for the patient. The test consists of a 48-card pack with the four stimulus cards set up as in the WCST, i.e. triangle, star, cross, and circle. The subject's task is to choose categories by receiving feedback from the examiner who informs the subject whether each choice is correct or not, until the subject has achieved a run of six correct responses. Then, the patient is told that the rule has changed and is instructed to find a different sorting rule. This procedure is continued until the maximal set of six categories are achieved or the cards are used up (Lezak, 1995). The test is scored by calculating the number of correct sorts, the number of obtained categories, and the total number of errors, which is composed of the number of perseverative errors and nonperseverative errors.

4.3.1.8 Trail Making Test A and B (Army Individual Test Battery, 1944)

The Trail Making Test is a popular and widely used test of speed of visual search, attention, mental flexibility, and motor function. Specifically, Part B (see below) assesses the ability to alternate between sets of stimuli and thus can be used as a measure of executive functioning (Mitrushina et al., 1999). The objective of the test is to connect, by making pencil

lines, randomly arranged numbers on a page in proper order (Part A), and to connect numbers and letters in alternating order (Part B). The subject is instructed to solve this task as fast as possible, without lifting the pencil from the paper. For both parts, scoring is expressed in terms of the time required to complete Part A and B, respectively, as well as the number of errors commited. We generated the quotient B/A as a specific measure of executive functioning, taking into account each subjects‘ individual motor speed: The lower the quotient B/A, the better the ability of a subject to shift between cognitive sets.

4.3.2 Near-infrared spectroscopy (NIRS)

Using NIRS cerebral hemoglobin oxygenation changes during brain activation were measured in 322 study subjects (see Figure 7, p. 47). The neuropsychological examination usually was performed in the morning, whereas the NIRS examination took place in the early afternoon (around 2:00 p.m.) of the same day. The NIRS examination was conducted at the Psychiatric University Hospital of Basel, which is located near to the Basel Memory Clinic/University Hospital where the neuropsychological assessment was performed. The study participants were offered a free taxi to the Psychiatric Hospital, where they were received by the NIRS study staff. Due to logistical reasons, not all study participants could pass the neuropsychological examination and the NIRS on the same day. These subjects were excluded from the cross-sectional statistical analysis of NIRS data (see section 4.4.1 for more details).

Finally, it needs to be pointed out that the participation in the NIRS examination was independent from the participation in the BASEL project, i.e. each subject was free to withdraw his consent to participate in the study at any time.

4.3.2.1 NIRS data recording and analysis

A multichannel system combining six commercially available NIRS devices (NIRO-500, Hamamatsu Photonics) was used to non-invasively measure changes in cerebral hemoglobin oxygenation over the left and right frontal, parietal and temporal cortex simultaneously during performance of either a verbal fluency task (letter fluency, modified after Benton and Hamsher, 1989) or a computerized labyrinth test (modified according to the Nürnberger Altersinventar; Oswald und Fleischmann, 1986). The NIRS variables obtained in this study are oxygenated hemoglobin (HbO), deoxygenated hemoglobin (HbR), and cytochrome oxidase (Cyt-Ox). The total hemoglobin (HbT) is obtained by summing up the concentration changes of HbO and HbR (HbT = HbO + HbR). Assuming that hematocrit is constant, the changes in HbT can be used as

an indicator of alterations in cerebral blood volume (Cope & Delpy, 1988).

The NIRO 500 system uses the difference of absorption spectra of HbO, HbR, and Cyt-Ox in the near infrared. The measurements were performed in the reflection mode. Light from the laser diodes (wavelengths 775, 825, 850, 904 nm) is guided through a fibre optic bundle, the end of which (the first optode: admission of near-infrared light) is placed strictly at the same site on the head in each subject to avoid effects of optode positioning. Optode pairs were placed over the left and right frontal, left and right parietal and left and right temporal cortex according to the international EEG 10-20 system (see Appendix I, Figure I-2) by a very well-experienced EEG technician (left frontal: Fp1-F3, right frontal: Fp2-F4, left parietal: T5-P3, right parietal: T6-P4, left temporal: T3-C3, right temporal: T4-C4). The interoptode distance was 4 cm.

Spatial resolution: It has been estimated that the brain volume measured corresponds to a banana-shaped figure beneath the two optodes of an optode pair with an estimated penetration depth of near-infrared light into brain tissue of a few centimetres (Hock et al., 1997a; Störmer, Müller-Spahn & Hock, 1998). Light source: The laser diodes are pulsed at 1.9 kHz, thus every pulse has a duration of 100 ns. Light detector: The reflected light is conducted by an optical fibre to a photomultiplier-based optical detection system and the number of photons at each wavelength is compared with the light output of the lasers (Cope & Delpy, 1988). The analysis procedure converts the obtained optical densities to concentrations of the chromophores HbO, HbR and Cyt-Ox, expressed in micromoles per liter times the optical pathlength ($\mu mol \times 1^{-1} \times cm$), using Beer-Lambert's law and an algorithm developed by Wray, Cope, Delpy, Wyatt, and Reynolds (1988).

Temporal resolution: NIRS recordings between the 17-Nov-1997 and 04-Dec-1997 (n=20) were made by using a sampling time for each photon count of 1 second, thereafter, for the majority of recordings (n=302), a sampling time of 2 seconds was used. The pathlength was estimated by multiplying the interoptode distance (4 cm) with the (age-dependent) differential pathlength factor (DPF) for the adult head (5.93; van der Zee et al., 1992). All values were averaged and described as mean arbitrary units. Assuming that this pathlength estimate is correct, these "arbitrary concentration units" correspond to micromolar.

Verbal fluency task: Subjects were sitting in a convenient position with eyes closed throughout the whole examination that included a period of rest (5 min), a period of cognitive activation (2 min, verbal fluency task, see below), another period of rest (10 min) and another period of cognitive activation (2 min, verbal fluency task). For the verbal fluency task (letter fluency, modified after Benton and Hamsher, 1989) subjects were asked to generate as many words as they could think of beginning with e.g. the letters F and A within 60 s allowed for

each letter (different letters were used for the second period of activation). The score used for analysis was the total number of correct responses.

Labyrinth test: This test consisted of a computerized version of the labyrinth test according to the Nürnberger Altersinventar (Oswald und Fleischmann, 1986). All subjects received adequate instructions about the test and the use of the mouse. Subjects with reduced vision and/or hemisymptomatic subjects were activated with the verbal fluency task. The sequence and duration of rest and activation periods was the same as with the verbal fluency task: After a 5-min period of rest, the first 2-min period of activation, followed by a second 10-min period of rest and finally another 2-min period of activation occurred. Unfortunately, and in contrast to the verbal fluency task, no data on the subjects' overt task performance was collected with the labyrinth test. Therefore, it is not possible to draw conclusions about the involvement of a subject in this task, as could be operationalized by the quality and/or speed of his/her performance.

Statistical analysis: The change of the relative chromophore concentrations for HbO, HbR, and Cyt-Ox, i.e. from the resting to the activation state, was calculated resulting to so called "delta values": A mean value for the *first* period of rest as well as a mean value for the *first* period of activation was calculated for each chromophore respectively (*Note: the second period of rest and activation did not enter the analysis. It was introduced for the purposes of a separate analysis which will not be discussed here*). The time windows for both means were 120 s. The difference was calculated by subtracting the prestimulation mean value from the mean during the activation period (delta = mean $\text{concentration}_{\text{activation}}$ − mean $\text{concentration}_{\text{rest}}$). The period between the end of the resting state and the start of the activation state, i.e. the instruction period, was omitted from the calculation of delta values, as this period cannot be assigned neither to the rest nor to the activation state (see Appendix I, Figure I-3 for a graphical display). One methodological drawback needs to be considered here: the NIRS technique, as applied in our study, could not provide absolute baseline concentrations of HbO, HbR, and Cyt-Ox. This would have required the exact quantification of the optical pathlength of the near-infrared light in brain tissue in each experiment and for each wavelength, and, in addition, the quantitation of the loss due to scatter (Hock et al., 1997). Therefore, the measurements using the current technique, i.e. as presented above, only refer to *changes* in absolute concentrations starting from an estimated baseline level.

4.3.3 ApoE

The configuration of subjects' ApoE alleles was determined in a polymerase chain reaction (PCR) analysis at the laboratory of PD Dr. André Miserez at the University Hospital, Basel.

4.4 Population

4.4.1 Subject disposition

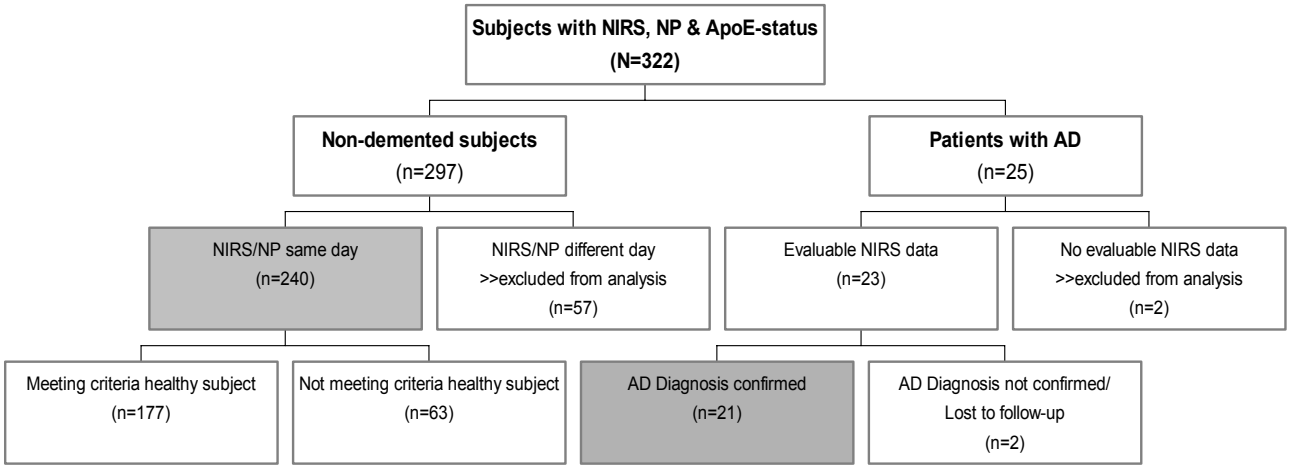


Figure 8: Subject disposition of study sample

As outlined in Figure 8, 297 non-demented elderly subjects and 25 patients with probable AD participated in this study. With regard to the non-demented elderly subjects it was aimed at performing the NIRS and neuropsychological assessment on the same day. However, this could not be achieved due to several reasons (e.g. personal reasons of study subjects, logistical reasons). Therefore, as in this thesis the main focus is on the association of brain hemoglobin oxygenation and neuropsychology measures, only non-demented elderly subjects were selected who had both assessments on the very same day, resulting in a sample of 240 subjects.

The AD patients first had their neuropsychological assessment, followed by the NIRS examination a couple of days later (maximum: 10 days in one subject, mean: 4.6 days). Of the 25 patients with AD, two patients had no evaluable NIRS data. From the remaining 23 patients with a diagnosis of probable Alzheimer's Disease, only those patients were entered into the database for who the diagnosis of probable AD could be confirmed in at least one follow-up neuropsychological examination at least one year after the date of diagnosis, resulting in a total of 21 evaluable patients.

4.4.1.1 Non-demented elderly subjects

A spin-off of the BASEL project was to conduct a normative study for the newly developed German version of the CERAD-NAB to be used in German-speaking Memory

Clinics. In order to be enrolled in the normative sample, subjects were selected if they did not meet a list of exclusion criteria summarized in Table 4-2. Subjects were additionally excluded if their native language was not German and if they had less than eight years of formal education. Based on these criteria, 74% (n=177) of our subjects belonged to the normative sample, and will be labelled „healthy subjects“. The remaining 26% (n=63) were excluded from the CERAD-NAB normative sample for reasons listed in Table 4-3. However, for the analysis of the relationship between NIRS, NP and ApoE-status, these subjects remained in our study sample. At the time of neuropsychological assessment (i.e. on average 5.7 months later) the same exclusion criteria were applied again to capture potential changes in health status which might have occurred in the meantime. Fortunately, there was no such change in any subject, so that the constitution of the sample with regard to the criterion „healthy subject“ remained constant.

Table 4-2: Exclusion criteria for non-demented elderly subjects being part of the normative CERAD-NAB study

A. Clinical findings

1. Severe hearing, visual, or language disturbance affecting the ability to complete neuropsychological testing.
2. Sensory deficits (i.e., not able to have a conversation and/or not able to read a newspaper without magnifying glasses).
3. Motor deficits that lead to problems in everyday life (paresis, essential tremor, dyskinesia, etc.).
4. Severe systemic diseases (heart, lungs, kidney, endocrine, gastrointestinal).
5. Continuous light to intense pain.
6. Current psychiatric problems (depression, psychosis, etc.).

B. Medical History

1. CNS diseases (e.g., Parkinson's disease, Huntington's disease, multiple sclerosis, Stiff-man syndrome, epilepsy, encephalitis, meningitis, etc.).
2. Diseases or events during life that most likely negatively impact(ed) CNS activity, specifically:
   - Head injury with loss of consciousness for more than 5 minutes;
   - Brain surgery;
   - General anesthesia within the last three months;
   - Psychiatric disorders requiring hospitalization;
   - Intake of potent psychoactive drugs (excluding minor tranquilizers);
   - Insulin-dependent diabetes;
   - Alcohol abuse (i.e., > 80g pure alcohol per day);
   - Poisoning with substances that are toxic to the CNS.
3. Cerebrovascular diseases (e.g., transient ischemic attack (TIA), stroke).
4. Generalized atherosclerosis.

Table 4-3: Reasons for the exclusion of subjects from the normative CERAD-NAB sample (multiple reasons possible)

Reason for exclusion	Number of subjects
Native language other than German	2
Less than 8 years of education	2
Medical reason	
Loss of consciousness (> 5 minutes)	21
Actual depression	10
Systemic disease	10
Cerebrovascular incident	8
Diabetes (requiring insulin therapy)	6
Migraine/daily headaches	4
Suspected parkinsonism	3
Sensory deficits	2
History of meningitis	1
General anasthesia within last 3 months	1

4.4.1.2 Patients with AD

For the purpose of the cross-sectional investigation including NIRS only patients with a mild probable AD according to DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, 4th edition, American Psychiatric Association, 1994) were selected (for more information on the diagnostic procedure see Monsch, 2001). In addition to disease severity, only patients were included who had no current comorbid depressive disorder (assessed with the DSM-IV questionnaire for depression, Kühner, 1997). In our patient sample the diagnosis of probable AD was confirmed in at least one detailed follow-up neuropsychological examination at least one year after the date of diagnosis.

Most patients were living with their spouse (60%), while some lived on their own (20%; no data available from the remaining 20%). At the medical screening examination the patients indicated to have no severe disease other than Alzheimer's and at the time of examination felt in good health. Specifically, none of the patients indicated to suffer from migraine or headaches, diabetes, dizziness, feeling weak or falling. None of the patients indicated to have

current or past epilepsy, strokes in the past, or past diagnosis of depression and/or schizophrenia which required hospitalization. All patients reported to be non-smokers and drinking less than 80 g pure alcohol per day (see also Table 4-2, p. 59).

13 patients (62%) were receiving pharmacological treatment with acetylcholinesterase inhibitors (11 patients Exelon®, 2 patients Aricept®), and one patient was taking Gingko capsules. Psychotropic medication was prescribed to a total of 5 patients, either to treat sleeping problems (1x lorazepam, 1x oxazepam, 1x zolpidem) and/or to treat symptoms of anxiety and/or depression (1x sertralin, 1x citalopram, 1x melitracen/flupentixol).

4.4.2 Demographics

Table 4-4: Key subject demographics and MMSE scores

	Non-demented elderly subjects			
	Healthy (n=177)	Not Healthy (n=63)	Total (n=240)	AD Patients (n=21)
Age				
Mean ± SD	71.8 ±6.89	73.5 ± 7.32	72.3 ± 7.03	76.2 ± 6.37
Min, Max	54, 88	56, 90	54, 90	57, 86
Gender				
Males/Females	129/48	42/21	171/69	9/12
Years of Education				
Mean ± SD	12.6 ±3.33	11.9 ± 3.30	12.4 ± 3.33	11.8 ± 2.59
Min, Max	7, 20	4, 20	4, 20	8, 19
Handedness				
Left/right/ambidexter	14/158/5	6/56/1	20/214/6	0/21/0
MMSE score				
Mean ± SD	28.6 ± 1.33	28.2 ± 1.68	28.5 ± 1.44	25.3 ± 2.08
Min, Max	25, 30	21, 30	21, 30	22, 29

As depicted in Table 4-4, the non-demented elderly subjects covered quite a broad age range (54-90 years), with age showing a normal distribution around a mean of 72.3 ± 7.03 years. Substantially more male subjects were included in the non-demented group, which was due to the fact that a substantial part of subjects was recruited from the earlier „Basel studies“, in which former employees of the Basel pharmaceutical industry were included (see also section 4.1). Male individuals had received significantly more years of total education than their female counterparts (13. 2 ± 3.21 vs. 10.5 ±2.84 years; Z=-6.07; p<0.000). Non-demented males and females did not differ with respect to age (72.1 ± 6.93 vs. 72.8 ± 7.28; Z=-1.148; p=0.25) and MMSE (28.5 ± 1.47 vs. 28.5 ± 1.37; Z=-0.544; p=0.59).

The 63 subjects who met at least one of the exclusion criteria listed in Table 4-2 did not significantly differ from the remaining 177 individuals with respect to age, gender, years of education, handedness, and MMSE score (all p-values > 0.05).

The patients with AD were significantly older than the non-demented subjects (Z=-2.95; p=.003), and not surprisingly had lower MMSE scores (Z=-6.08; p<.001), but they did not differ with respect to years of education (Z=-.491; p=.624). As shown in Table 4-4, the group of AD patients contained a larger portion of female subjects. All AD patients were right-handed.

4.4.2.1 Demographics by NIRS activation paradigm

During the course of the study two different NIRS activation paradigms were used to measure changes in cerebral brain tissue oxygenation: In 119 non-demented subjects and 18 patients with AD a letter fluency task was applied, as opposed to 121 cognitively intact subjects and 3 AD patients who were activated with a computerized labyrinth test (see section 4.3.2.1 for more details on the tasks). The allocation of subjects to the NIRS activation paradigm was not randomized. Assuming that the changes in cerebral brain blood oxygenation, especially with regard to the brain-topographical activation, will vary depending on the administered cognitive task, these two paradigms will be analysed separately. Table 4-5 displays the subject demographics for non-demented subjects per activation paradigm.

Table 4-5: Subject demographics and MMSE scores of non-demented elderly subjects per activation paradigm

	Non-demented elderly subjects		
	Letter fluency task (n=119)	Labyrinth test (n=121)	Comparison*
Age			
Mean ± SD	74.6 ± 6.29	70.0 ± 6.97	p<.001
Min, Max	57, 90	54, 89	
Gender			
Males/Females	70/49	101/20	p<.001
Years of Education			
Mean ± SD	11.7 ± 3.21	13.0 ± 3.31	p<.001
Min, Max	7, 20	4, 20	
Handedness			
Left/right/ambidexter	8/108/3	12/106/3	p=.670
MMSE score			
Mean ± SD	28.4 ± 1.41	28.6 ± 1.47	p=.460
Min, Max	25, 30	21, 30	

**Age, years of education, MMSE: Mann-Whitney U; gender, handedness: Chi-Quadrat test*

The non-randomized allocation to the activation paradigm led to significant differences in age, gender, and years of education: Subjects activated with the letter fluency task were significantly older, and had less years of total education. The difference in education is partly due to the fact that there were substantially more males who were activated with the labyrinth test (however, when the females are removed from the analysis, this difference still remains highly significant).

4.4.3 Results of ApoE-Genotyping

The results of the ApoE-genotyping are displayed in Table 4-6. The proportion of ε4 -positives and -negatives was evenly distributed across both subgroups of elderly subjects who had either NIRS-activation paradigm, and also across patients with AD, with 45% of both non-demented elderly subjects and AD patients having had at least one ApoE-ε4 allele. Please note that the even distribution of ε4 carriers and non-carriers is due to the fact that our study participants were selected according to this criteria, thus allowing to compare subjects at high vs. low risk for AD (see Monsch, 2001 for more details).

Table 4-6: Results of ApoE-Genotyping

	Non-demented elderly subjects					
	Letter fluency (n=119)	Labyrinth test (n=121)	Total (n=240)		AD-Patients (n=20)*	
ε2/ε2	0	1	1	(0.4%)	0	(0%)
ε2/ε3	12	11	23	(9.6%)	1	(5%)
ε2/ε4	11	8	19	(7.9%)	1	(5%)
ε3/ε3	53	56	109	(45.4%)	10	(50%)
ε3/ε4	41	39	80	(33.3%)	7	(35%)
ε4/ε4	2	6	8	(3.3%)	1	(5%)
ε4	54	53	107	(45%)	9	(45%)
Non-ε4	65	68	133	(55%)	11	(55%)

**No ApoE-genotyping available for one AD patient*

4.5 Data management and quality control

All neuropsychological test data were inspected by doctoral candidates working in the BASEL project. A group of psychology students who had not administered the tests entered the data into a FileMaker 5.0 database. A final, independent group of psychology students inspected the entered data for correctness.

The NIRS delta-values were transferred into an excel spreadsheet and thereafter merged into an SPSS-datafile together with the neuropsychological, ApoE-genotype, and medical history data. To control for possible merging errors, the whole merging procedure was

performed twice with verification upon first and second merging. A 100% manual data check was performed for the variables „date of examination“ (NIRS, NP), „age“, „gender“, „years of education“, „NIRS activation paradigm“, „performance in NIRS activation“. For all other variables at least a 10% manual data check was performed.

4.6 Statistical methods

Descriptive and inferential statistical tests were calculated using the *Statistical Package for the Social Sciences* (SPSS) version 10. A detailed description of the key statistical tests is provided in section 5 together with the study objectives and hypotheses.

Normal distribution of all variables was tested using the Kolmogorov-Smirnov and/or the Lilliefors test, and parametric statistics were applied only for variables with normal distribution (e.g. NIRS parameters, NIRS task performance). In addition, to test for potential differences between groups, parametric tests were only used if the criterion of homogeneity of variances was fulfilled, otherwise nonparametric tests (e.g. Mann-Whitney U) were performed. Furthermore, to test for potential differences between groups with markedly different sample sizes (e.g. AD patients vs. non-demented elderly subjects; healthy vs. non-healthy subjects), nonparametric tests were preferred.

Unless otherwise specified, statistical tests were two-tailed and performed at the 5% significance level. In case of multiple univariate comparisons under an exploratory strategy, i.e. when no a priori research hypotheses were formulated (e.g. differences in neuropsychological test performance between healthy and non-healthy subjects), inferential statistics were tested at an alpha level of 1%. This procedure was preferred because the more restrictive Bonferroni alpha correction procedure was deemed to be too conservative when performing exploratory analyses.

Figures in text and tables were presented displaying three significant figures (i.e. .XXX, X.XX, XX.X).

Missing values: As with every typical data set, information may be missing for some variables or for some cases. For the NIRS data reported in this thesis there was a substantial number of missing values, which was due to the fact that some subjects had no evaluable NIRS measures on one or more optode positions due to several sources of artifacts (e.g. movement artifacts, strong hair, damaged optodes). We initially planned to replace missing values using multiple imputation methods. After a first data inspection and being aware of the high variance

in the NIRS data however, we decided to not replace missing values but instead use pairwise or listwise deletion of missing data: The conservative method of listwise deletion is accomplished by deleting from the sample any cases that have missing data on any variables in the data set. By using pairwise deletion of missing data, all available cases for a particular variable are entered in the analysis, which across several variables with missing values may lead to different case numbers per variable. Whereas listwise deletion seems to be a simple solution, it has a major disadvantage: It excludes a large fraction of the original sample and thus maximises the standard error as less information is utilized. In the recent book by Allison (2002) on missing data for quantitative applications in the social sciences, the author prefers pairwise deletion for variables which are "missing completely at random" (MCAR) and defines these as follows: "The data on Y are said to be missing completely at random (MCAR) if the probability of missing data on Y is unrelated to the value of Y itself or to the values of any other variables in the data set" (Allison, 2002, p. 3). When this assumption is satisfied for all variables, the author concludes that the set of individuals with complete data can be regarded as a simple random subsample from the original set of observations. According to the above, the NIRS variables in our data set can be considered as MCAR, and in this case and following Allison's recommendation, in general the method of pairwise deletion was preferred.

5 Study objectives and hypotheses

The primary objectives of this study are to investigate the brain response measured with functional NIRS in a large sample of non-demented elderly subjects and in a homogenous group of patients with mild-stage AD, and to analyse the relationship between the NIRS response and 1) sociodemographic factors such as age, years of formal education, and gender; 2) the ApoE genotype (ε4 vs. non-ε4); and 3) neuropsychological test performance. In the first part, these primary objectives will be investigated in our large sample of non-demented elderly subjects, and in the second part in our patients with AD.

Whenever appropriate and possible, hypotheses-driven analyses will be preferred to exploratory analyses.

5.1 Non-demented elderly subjects

5.1.1 Response on NIRS

1. Can the „typical“ hemoglobin oxygenation response pattern, i.e. an increase in HbO and a decrease in HbR under cognitive activation be replicated in our sample of non-demented elderly subjects? As outlined in chapter 2, there is vast evidence for a typical, activation-induced NIRS response pattern with an increase of HbO and a decrease of HbR over areas most probably activated by the stimulus. Concentration changes of HbT, which are gained by summing up the concentration changes of HbO and HbR, are generally reported to have the same response direction as HbO, due to the fact that the changes in HbO generally are bigger in amplitude than the changes in HbR.

   Statistical analysis: Graphical display of mean (± S.E.M) concentration changes of HbO, HbR, and HbT across all optode positions and separate for each activation paradigm (letter fluency vs. labyrinth test)

a) Do the NIRS parameters exhibit a pattern of general activation across all optode positions or of specific activation? Based on earlier findings (Kleinschmidt et al., 1996; Schroeter et al. 2002), the increase of oxygen consumption (HbR) is more localized than the increase in blood flow (HbO).

   Statistical analysis: One-sample t-tests for the total sample of non-demented elderly subjects (N=240) calculating whether the changes in HbO, HbR, HbT and Cyt-Ox during activation vs. rest are significantly different from zero (= no change). Two-tailed t-tests performed at the 1% significance level.

b) Do the two activation paradigms (letter fluency vs. labyrinth test) differ in their elicited NIRS response? Do they differ in brain-topography?

   Statistical analyses:

   - Separate one-sample t-tests for the sample of subjects activated with the verbal fluency task (n=119) and the group of subjects who performed the labyrinth test (n=121) calculating whether the changes in HbO, HbR, and HbT during activation vs. rest are significantly different from zero (= no change). Two-tailed t-tests performed at the 1% significance level.

- Repeated measures ANOVA with within-factors „NIRS parameter“ (4 levels: HbO, HbR, HBT, Cyt-Ox) and „topography“ (6 levels: left and right frontal, parietal and temporal), and between-factor „paradigm“ (letter fluency vs. labyrinth test). Listwise deletion of missing values was used for within factors.

2. Investigate subgroups of subjects:

a) Do the healthy subjects in our study population differ from the non-healthy population in terms of their NIRS response? Do non-healthy subjects exhibit a greater variability in their hemodynamic brain response due to various CNS pathology? Do the non-healthy subjects show smaller cerebral hemoglobin oxygenation changes due to less activation of brain tissue?

Statistical analyses:

- Descriptive statistics of all NIRS parameters for both groups
- Two-tailed Mann-Whitney U-Tests at alpha 1% testing the null hypotheses that the two groups do not differ in NIRS parameters HbO, HbR, and HbT.

b) Taking the example of subjects activated with the verbal fluency task: Can the sample be divided into subjects showing a typical response pattern („responders“) vs. subjects who do not show a typical response pattern („non-responders“)? Is the response pattern restricted to specific optode positions, e.g. the left frontal position?

Statistical analyses:

- Descriptive statistics for NIRS responders at the left frontal position
- ANOVA comparing left-frontal responders with left-frontal non-responders in the dependent variables HbO and HbR to test the null hypothesis that the two groups do not differ in their hemodynamic brain response across all positions.

3. Relationship between brain oxygenation and task performance:

a) As is known from PET and fMRI studies, the performance of a verbal fluency task is highly associated with the activation of the left frontal and frontotemporal cortex, we would expect a stronger correlation between the NIRS cerebral hemodynamics and task performance at the frontal optode positions (with left being bigger than right), a minor correlation at the left temporal position, and no correlation at the parietal and right temporal position. If the correlation between task performance and brain response is positive (i.e. HbO: positive correlation and HbR: negative correlation) and indeed is limited to specific areas like the frontal positions and to a minor extent the

left temporal position, this could be interpreted in line with Elfgren and Risberg (1998): Subjects able to bring the brain areas critical for successful performance on-line, show better task performance.

Statistical analysis: Correlation analyses testing the research hypothesis that Pearson correlation coefficients for the relationship between task performance and hemoglobin oxygenation are significantly different from zero for the left and right frontal and the left temporal position while the correlation coefficients for all other positions are not different from zero. As task performance was assessed only with the verbal fluency task, only this subgroup of subjects will be used for the analysis (n=119).

b) Do left-frontal responders show a better NIRS task performance than left-frontal non-responders?

Statistical analysis: One-tailed ANOVA comparing left-frontal responders with left-frontal non-responders in the NIRS task performance. In agreement with the hypothesis put forward in a), we hypothesize that subjects with a typical NIRS response at the left frontal position show better task performance than left-frontal non-responders.

5.1.2 NIRS, sociodemographic factors and ApoE genotype

1. Do the sociodemographic factors „age“, „years of education“, and „gender“ and the ApoE genotype (ε4 vs. non-ε4) correlate with concentration changes of HbR and HbT? Specifically, can we replicate the findings of Hock et al. (1995), who found a significant negative correlation between age and concentration changes in HbT at the left frontal position in non-demented elderly subjects?

   Statistical analysis: Multiple linear regression analyses (stepwise backwards) with the independent variables “age”, “years of education”, “gender”, and “ApoE genotype”, for the dependent variables HbR (left and right frontal position) and HbT (all optode positions). The criteria for probability of F to enter were .05 and the backward criteria for F to remove .10. The total sample of non-demented elderly subjects (N=240) was used for these analyses.

2. Functional activation studies using a letter fluency task have reported a disruption of functional circuits involving the left parietal lobe in ApoE-ε4 carriers vs non-carriers. We sought to find out whether this finding can be replicated in our sample and with

NIRS. The research hypothesis was tested that ApoE-ε4 carriers present significantly lower values in HbT at the left parietal optode position than ApoE-ε4 non-carriers.

Statistical analysis: One-tailed t-test for the dependent variable HbT at the left parietal position and presence of ApoE-ε4 allele as the independent variable. The sample of subjects activated with the verbal fluency task was chosen for this analysis (n=119).

3. Do subjects showing the typical NIRS response pattern differ from non-responders with respect to sociodemographic factors and ApoE genotype?

   Statistical analysis: ANOVA for the dependent variables "age" and "years of education"; Chi-Square for the variables "gender" and "ApoE status".

5.1.3 NIRS and Neuropsychological Test Performance

1. We assume that the performance during the NIRS letter fluency task significantly and highly correlates with the performance of the NP fluency task as both tasks measure the same construct.

   Statistical analysis: Pearson correlation analysis testing the research hypothesis that the Pearson correlation coefficient for the relationship between the NIRS letter fluency and the NP letter fluency task is significantly different from zero. For this and all subsequent analyses only the subjects activated with the verbal fluency task were entered (n=119).

2. Describe the neuropsychological test performance of our non-demented elderly subjects activated with the verbal fluency test (n=119).

a) Compare the test results of our sample with normative data for the respective neuropsychological test and data obtained in the BASEL project: Do our subject show normal scores?

b) Do the non-healthy subjects differ from the healthy subjects in our study sample with regard to test performance?

   Statistical analyses:

   - Descriptive statistics of neuropsychological and NIRS test performance
   - Multiple Mann Whitney U-Tests comparing neuropsychological test performance of healthy subjects activated with the verbal fluency task (n=88) with the respective scores of non-healthy subjects (n=31).

3. Investigate the predictive value of NIRS for the performance in a specific neuropsychological test: How well can NIRS predict neuropsychological test performance? Furthermore, if age and the ApoE genotype (ε4 vs. non-ε4) are included in the set of predictors, does the proportion of explained variability increase?

   Statistical analysis: Multiple regression analyses (stepwise backwards) with the NIRS parameters HbO and HbR at each optode position as well as age and ApoE-ε4-status as predictor variables and the following neuropsychological tests as dependent variables:

   - WAIS-R Vocabulary: total correct
   - Category Fluency: total correct
   - Phonemic Fluency: total correct
   - CVLT: correctly recalled List A trials 1-5
   - TMT B/A

   The dependent variables fulfilled the criterion of normal distribution (all Kolmogorov-Smirnov $p \geq .05$), as did the predictor variables HbO and HbR at each position. The criteria for probability of F to enter were .05 and the backward criteria for F to remove .10. The sample of non-demented elderly subjects activated with the verbal fluency test (n=119) was used for these analyses.

4. Based on the hypotheses that left-frontal responders show a better NIRS task performance than left-frontal non-responders and that there are positive correlations between NIRS task performance and performance in the NP letter fluency task (cf. Section 5.1.1), we hypothesize that left-frontal NIRS responders show better performance in other neuropsychological tests of executive functioning (i.e. NP phonemic fluency, TMT B and TMT B/A, WCST) than the respective non-responders. Although a positive relationship between verbal fluency and general intellectual functioning, especially verbal intelligence, has been reported by a few groups (e.g. Mitrushina et al., 1999), we expect the difference between left-frontal responders and non-responders only to be minor and not of statistical significance. Furthermore, left frontal responders should not differ substantially from left frontal non-responders with regard to verbal memory performance (e.g. CVLT), as the neural network involved in explicit verbal memory involves additional structures (e.g. the medial temporal lobe including hippocampus and amygdala).

   Statistical analyses:

- Mean neuropsychological test scores of left-frontal NIRS responders (n=47) vs. left-frontal non-responders (n=48)
- One-sided Mann Whitney U-Tests testing the research hypothesis that left-frontal NIRS responders exhibit significantly better performance in tests of executive functioning, i.e. phonemic (letter) and category fluency, WCST total correct categories and WCST number of perseverative errors, time needed in the TMT-B as well as the quotient TMT B/A.
- Two-sided Mann Whitney U-Tests comparing left-frontal NIRS responders with left-frontal non-responders in tests of general intellectual functioning (MWT-B, WAIS-R Vocabulary, Similarities, Block design), language (Boston Naming), and verbal memory (CVLT learning List A, correctly recalled words after short and long delay, recognition discriminability).

5.2 Patients with mild-stage Alzheimer's Disease (AD)

5.2.1 Response on NIRS

1. Do AD patients show the typical hemoglobin oxygenation response pattern during performance of a cognitive task?

   Statistical analysis:

   - Graphical display of mean (± S.E.M) concentration changes of HbO, HbR, and HbT across all optode positions. *Note:* In our sample of 21 patients with AD, 18 were activated with the letter fluency task, and 3 with the labyrinth test described in section 4.3.2.1

a) Do AD patients show a reduced hemodynamic response? In resting-brain studies of cerebral blood flow and glucose utilization AD patients showed most pronounced decreases in the parietal and temporal cortices in contrast to age-matched controls. This evidence however cannot be directly transferred to our data as we were using a functional paradigm. In the functional NIRS study of Hock et al. (1997a), patients with *moderate* AD showed marked reductions over the left parietal cortex whereas the hemodynamic response over the left frontal cortex revealed the typical hemoglobin oxygenation response pattern. In conclusion of the above, we might expect a reduced hemodynamic response in the parieto-temporal cortex together with a spared response over the frontal area.

   Statistical analysis:

   - One-sample t-tests calculating whether the changes in HbO, HbR, and HbT during activation vs. rest are significantly different from zero (= no change). Two-tailed t-tests performed at the 1% significance level.
   - Mann Whitney-U tests at the 1% significance level comparing the NIRS data of our mild-stage AD patients activated with the verbal fluency task (n=18) with the data of our non-demented elderly subjects (n=119). One-tailed tests for the parietal and temporal positions, two-tailed tests for both frontal positions.

2. Relationship between brain oxygenation and task performance:

a) Evidence from resting-brain imaging studies suggest that the relationship between regional cerebral blood flow or regional cerebral glucose metabolism and verbal

fluency in Alzheimer's disease is comparable to what has been observed in healthy volunteers, i.e. fluency scores are significantly correlated with the left prefrontal blood flow/glucose metabolism.

Statistical analysis: Correlation analyses testing the research hypothesis that Spearman correlation coefficients for the relationship between task performance and hemoglobin oxygenation are significantly different from zero for the left frontal and the left temporal position while the correlation coefficients for all other positions are not different from zero. Spearman's rho was preferred to the Pearson correlation coefficient due to the small sample size (n=18).

b) Can our sample of AD patients be divided in „NIRS-responders" and „NIRS-non-responders", and do left-frontal responders show a better NIRS task performance than left-frontal non-responders?

Statistical analysis: Mann Whitney-U test comparing left-frontal responders with left-frontal non-responders in the NIRS task performance.

5.2.2 NIRS and Neuropsychological Test Performance

1. Are the brain-behavior relationships of mild AD patients distorted?

a) The performance in the NIRS letter fluency task significantly correlates with the performance of the NP fluency task as both tasks measure the same construct.

Statistical analysis: Pearson correlation analysis testing the research hypothesis that the Pearson correlation coefficient for the relationship between the NIRS letter fluency and the NP letter fluency task is significantly different from zero. For this analysis only the patients activated with the verbal fluency task were entered (n=18).

b) Describe the neuropsychological test performance of our patients with AD (n=21), and compare the test results with our sample of non-demented elderly subjects activated with the verbal fluency task (n=119)

Statistical analysis: Mann Whitney-U tests at the 1% significance level.

c) Based on our observations in the sample of non-demented elderly subjects, we hypothesize that left-frontal NIRS responders show better performance in other tests of executive functioning as well as in tests of general intellectual functioning than the respective non-responders.

Statistical analyses:

- Descriptive statistics of neuropsychological test scores of left-frontal NIRS responders (n=7) vs. left-frontal non-responders (n=8)
- One-sided Mann Whitney U-Tests at the 5% significance level testing the research hypothesis that left-frontal NIRS responders exhibit significantly better performance in tests of executive functioning (i.e. phonemic fluency, time needed in the TMT-B as well as the quotient TMT B/A; total correct BNT), and tests of general intellectual functioning (WAIS-R Similarities, WAIS-R Block design).

6 Results

6.1 Investigation of NIRS response in non-demented elderly subjects

6.1.1 Is there a "typical" NIRS response?

Figure 9 shows the mean changes (activation – rest) in cerebral hemoglobin oxygenation measured with NIRS over the left and right frontal, left and right parietal, and left and right temporal cortex in our sample of non-demented elderly subjects (N=240) during performance of either a verbal fluency task (n=119) or a computerized labyrinth test (n=121).

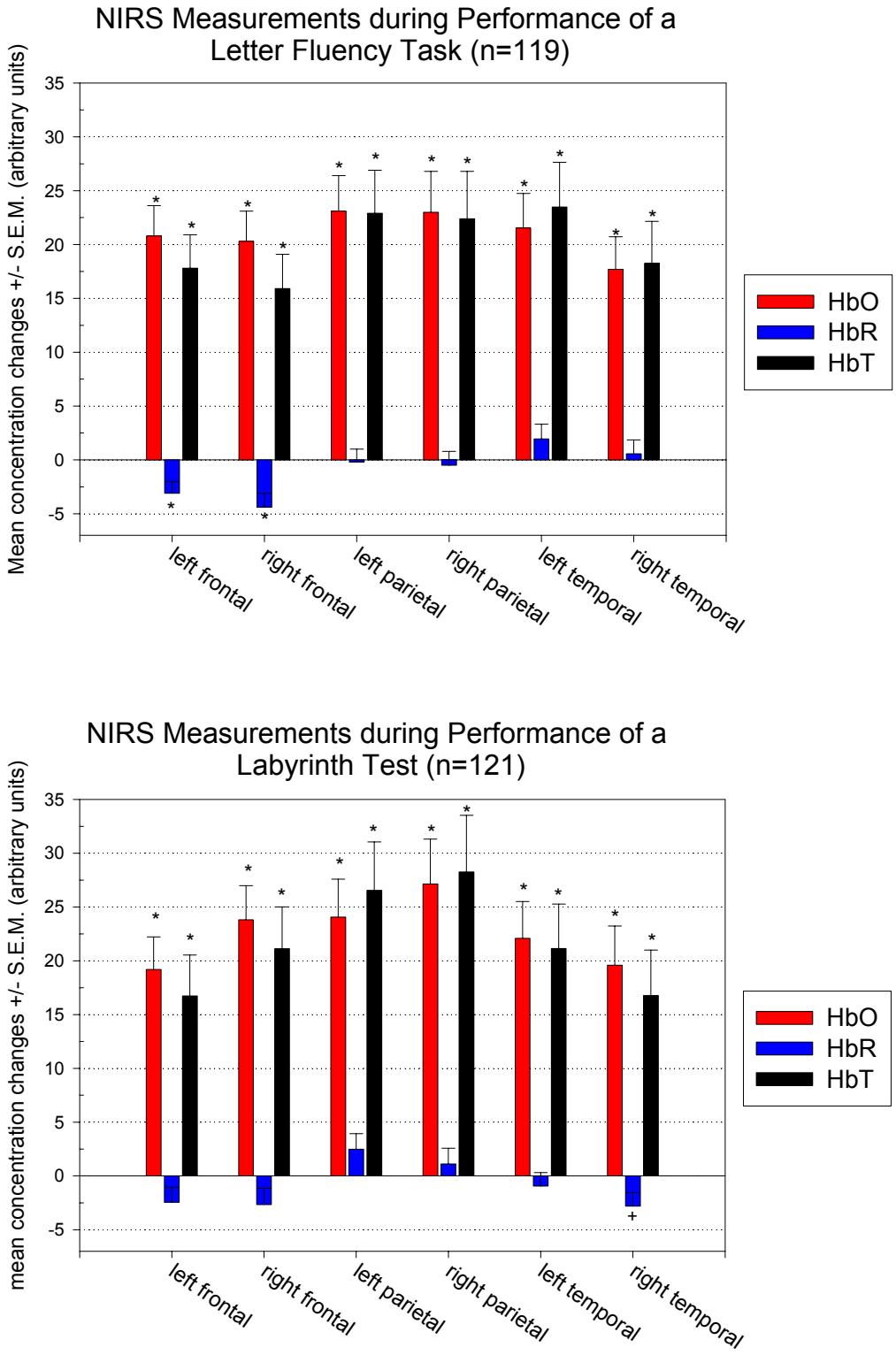


Figure 9: Mean concentration changes ± S.E.M. in HbO, HbR, and HbT over the left and right frontal, parietal and temporal cortex using either a letter fluency task as cognitive activation paradigm or a computerized labyrinth test in non-demented elderly subjects.
* = $p<.01$; $^{+}$ = $p<.05$

The mean concentration changes showed a strong and highly significant increase in the concentration change of oxygenated hemoglobin across all optode positions during performance of either cognitive task (all p-values <.001). Total hemoglobin, HbT, used as an indicator of alterations in cerebral blood volume, also showed significant increases at all optode positions and for both activation tasks (all p-values <.001). As illustrated in Figure 9, in contrast to the strong increase of HbO and HbT, there was a substantially smaller and localized decrease in HbR in both paradigms. Changes in the NIRS parameter cytochrome oxidase were in the negative direction except for the right temporal position (mean = .471, SD = 4.79), and were very small: Across both paradigms there was a significant decrease in Cyt-Ox at the right parietal position only (mean = -.770, SD = 3.58), $t(176) = -2.86$, p = .005.

Comparing the two activation tasks with each other, differences in the pattern of reduction in HbR became evident as illustrated in Figure 9: In the verbal fluency task the reduction in HbR was most pronounced over the frontal cortex (left frontal position: $t(94) = -2.66$, p = .009; right frontal position: $t(99) = -3.29$, p = .001) and only minimally and not significantly reduced over the parietal cortex (left parietal position: $t(91) = -.177$, p =. 860; right parietal position: $t(81) = -.410$, p = .683). Measurements over the temporal cortex showed positive mean values for HbR, i.e. the concentration of reduced hemoglobin was smaller under activation with the verbal fluency task as compared to rest, which was however not significant (left temporal position: $t(96) = 1.43$, p = .155; right temporal position: $t(92) = .445$, p = .658). This brain-topographical pattern of reduction in HbR could not be found for the labyrinth test, where there was a trend towards reduction in HbR only over the right temporal cortex, $t(80) = -2.45$, p = .028. The reductions over the frontal cortex and the left parietal cortex were only slight (left frontal position: $t(98) = -1.74$, p = .085; right frontal position: $t(101) = -1.76$, p = .081; left parietal position: $t(96) = 1.71$, p = .091).

When the two activation paradigms are taken together (N=240; data not shown), the changes in deoxygenated hemoglobin were significant only at the left and right frontal position (left frontal position: $t(193) = -3.01$, p = .003; right frontal position: $t(201) = -3.49$, p = .001), with all other positions showing no significant change from baseline (all p-values ≥.224).

A repeated-measures ANOVA with the within-subject factors „topography“ (6 levels: left and right frontal, left and right parietal, and left and right temporal) and „NIRS parameter“ (3 levels: HbO, HbR, HbT), and the between-subject factor „activation paradigm“ (verbal fluency vs. labyrinth test) did not reveal a significant difference between the two activation paradigms with regard to their brain-topographical characteristics of all three NIRS parameters (Interaction: $F_{(10,94)}=1.50$; p=0.15). Not surprisingly, the main factor „NIRS parameter“ showed

a significant effect ($F_{(2,102)}$=45.12; p<0.000), and the main factor „topography“ a trend ($F_{(5,99)}$=2.28; p=0.06). The interaction of „topography“ and „NIRS parameter“ also showed a trend ($F_{(10,94)}$=1.90; p=0.05). It must be noted that subjects with missing values in one or more optode position(s) did not enter the ANOVA („listwise deletion of missing values“, see section 4.6, p. 66; remaining n=105).

As is apparent from Figure 9 (p. 78), there is considerable interindividual variance in the NIRS data. In Table 6-1 descriptive statistics for all NIRS parameters are compiled, separately for each activation paradigm and for each optode position. Looking at the dispersion statistics of HbO and HbR not only reveals a marked variance, but also the existence of subjects with extreme negative and positive values. Comparing the two activation paradigms, the variability is more pronounced for the labyrinth test, especially with regard to HbR at the frontal and parietal optode positions. The descriptive statistics for the NIRS parameter Cyt-Ox showed up to 3000 times smaller mean values compared to the concentration changes of HbO. Finally, as illustrated by the number of valid cases, there is a substantial number of missing values, which is due to the fact that some subjects had no evaluable NIRS measures on one or more optode positions. In detail, in the verbal fluency task only 50/119 subjects (42%) and in the labyrinth test 55/121 subjects (46%) had evaluable NIRS data across all optode positions.

Table 6-1: Descriptive statistics for all NIRS parameters at every position, displayed per activation paradigm (verbal fluency vs. labyrinth test)

		Valid N	Missing	Mean	SD	Q1	Median	Q3	Min	Max
HbO_lf	verbal fluency	95	24	20.8	27.6	3.32	16.0	35.1	-36.6	102
	labyrinth test	99	22	19.2	30.1	3.47	14.0	29.2	-48.0	123
HbR_lf	verbal fluency	95	24	-3.05	11.2	-10.1	-2.16	4.19	-41.3	24.1
	labyrinth test	99	22	-2.46	14.1	-7.83	-1.43	6.18	-69.5	34.9
HbT_lf	verbal fluency	95	24	17.8	29.8	-.478	14.1	34.9	-52.5	113
	labyrinth test	99	22	16.7	37.8	-3.57	11.2	29.8	117	135
Cyt_lf	verbal fluency	95	24	-.051	3.63	-1.31	-.241	1.08	-11.5	11.9
	labyrinth test	99	22	-.789	3.81	-2.27	-.330	1.09	–18.7	11.7
HbO_rf	verbal fluency	100	19	20.3	28.3	1.53	15.2	35.4	-59.4	116
	labyrinth test	102	19	23.8	32.1	6.56	19.5	36.0	-43.3	178
HbR_rf	verbal fluency	100	19	-4.37	13.3	-11.5	-3.83	3.89	-39.9	43.8
	labyrinth test	102	19	-2.66	15.3	-8.16	-2.43	4.48	-104	35.2
HbT_rf	verbal fluency	100	19	15.9	32.3	-5.03	16.5	28.1	-92.4	128
	labyrinth test	102	19	21.1	39.0	-1.08	16.7	37.1	-147	154
Cyt_rf	verbal fluency	100	19	-.129	5.53	-2.48	-.434	1.60	-19.2	21.3
	labyrinth test	102	19	-.008	4.84	-1.18	.175	1.54	-36.0	15.7
HbO_lp	verbal fluency	92	27	23.1	31.7	3.92	19.2	38.8	-51.7	150
	labyrinth test	97	24	24.1	34.8	2.22	16.1	45.3	-38.4	186
HbR_lp	verbal fluency	92	27	-.217	11.8	-4.52	.411	4.80	-33.0	39.5
	labyrinth test	97	24	2.48	14.3	-5.94	.300	6.15	-31.2	78.1
HbT_lp	verbal fluency	92	27	22.8	38.2	3.62	18.1	38.8	-84.7	165
	labyrinth test	97	24	26.5	44.3	1.42	16.4	46.5	-53.6	264
Cyt_lp	verbal fluency	92	27	-.437	4.18	-2.35	-.719	.882	-12.3	20.6
	labyrinth test	97	24	-.766	4.34	-1.24	-.159	1.15	-32.3	7.16
HbO_rp	verbal fluency	82	37	23.0	34.5	.098	16.4	38.4	-31.0	193
	labyrinth test	95	26	27.2	40.5	-2.77	19.4	37.5	-35.5	195
HbR_rp	verbal fluency	82	37	-.525	11.6	-7.69	-.847	5.19	-25.0	43.0
	labyrinth test	95	26	1.11	14.2	-7.20	-1.65	4.60	-17.2	66.2
HbT_rp	verbal fluency	82	37	22.4	39.9	-1.87	14.6	39.2	-40.0	222
	labyrinth test	95	26	28.3	51.4	-5.09	18.0	38.5	-37.4	256
Cyt_rp	verbal fluency	82	37	-.955	3.11	-2.38	-.590	.637	-11.0	8.78
	labyrinth test	95	26	-.610	3.96	-2.22	-.724	1.10	-24.5	10.4
HbO_lt	verbal fluency	97	37	21.5	31.7	3.09	15.9	37.8	-37.8	143
	labyrinth test	89	32	22.1	32.3	-.913	18.3	38.6	-45.3	105
HbR_lt	verbal fluency	97	22	1.95	13.4	-5.62	.184	6.59	-30.6	63.4
	labyrinth test	89	32	-.940	11.8	-6.41	-2.17	3.95	-41.0	40.9
HbT_lt	verbal fluency	97	22	23.5	41.0	.633	17.0	37.5	-56.9	168
	labyrinth test	89	32	21.1	38.9	-1.21	14.9	42.0	-86.2	129
Cyt_lt	verbal fluency	97	22	-1.25	7.95	-2.18	-.424	1.35	-62.9	9.40
	labyrinth test	89	32	.167	2.81	-1.20	-.036	.998	-6.61	12.6
HbO_rt	verbal fluency	93	26	17.7	29.1	1.19	14.4	32.6	-41.5	127
	labyrinth test	81	40	19.6	32.8	-.236	18.8	32.1	-44.9	193
HbR_rt	verbal fluency	93	26	-.567	12.3	-7.07	-.268	6.34	-29.2	42.7
	labyrinth test	81	40	-2.81	11.2	-9.73	-2.68	4.66	-44.0	33.0
HbT_rt	verbal fluency	93	26	18.3	37.5	-3.87	15.8	27.8	-67.9	160
	labyrinth test	81	40	16.8	38.0	-5.48	14.2	37.3	-88.9	193
Cyt_rt	verbal fluency	93	26	-.205	5.60	-1.75	0.023	2.24	-21.0	14.9
	labyrinth test	81	40	1.25	3.52	-.907	1.31	3.24	-9.62	12.5

6.1.2 Healthy vs. non-healthy subjects

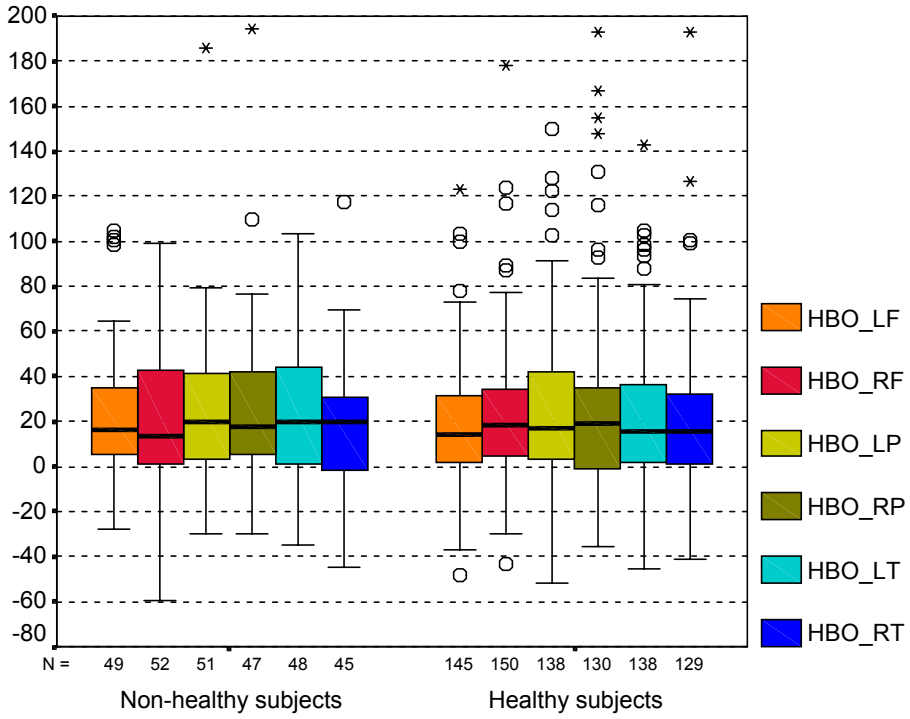
The marked interindividual variability in the NIRS data might partly be due to the fact that one quarter of our study sample was composed of non-healthy subjects, i.e. subjects with either current sensory or motor deficits, severe systemic disease, or a psychiatric or neurologic disorder; and/or a medical history of a CNS disease or events negatively affecting CNS activity (see Tables 4-4 and 4-5), which might be associated with a substantially greater variability in the NIRS data.

Descriptive statistics for the group of healthy (n=177) and non-healthy (n=63) subjects as well as the results of the multiple Mann-Whitney U-Tests are displayed in Table 6-2. Again, as some subjects had no evaluable NIRS measures on one or more optode positions, the number of valid cases per group, NIRS parameter and optode position is listed. As a result, the two groups do not systematically differ in their cerebral hemodynamics: Only for the parameter Cyt-Ox at the left frontal position, the healthy subjects showed a trend towards lower concentration changes during cognitive activation (Z=-2.51, p=.012). Testing all NIRS parameters of the two groups for homogeneity of group variance revealed equal group variance for all NIRS parameters. Finally, as can be seen from the boxplots for HbO and HbR in Figure 10, both groups had a comparable number of outliers and extreme values.

Table 6-2: Descriptive statistics of all NIRS parameters displayed for healthy vs. non-healthy subjects. P-values are taken from multiple Mann-Whitney U-Tests

		Valid N	Mean	SD	Median	Min	Max	p-value
HBO_LF	Non-healthy subjects	49	24.5	30.5	16.2	-27.8	105	
	Healthy subjects	145	18.5	28.2	14.2	-48.0	123	.436
HBR_LF	Non-healthy subjects	49	-4.13	12.7	-2.75	-41.3	18.0	
	Healthy subjects	145	-2.29	12.7	-1.43	-69.5	34.9	.673
HBT_LF	Non-healthy subjects	49	20.4	33.5	17.4	-52.7	113	
	Healthy subjects	145	16.2	34.3	11.6	-117	135	.355
CYT_LF	Non-healthy subjects	49	-1.20	3.71	-.761	-9.01	10.0	
	Healthy subjects	145	-.165	3.72	-.132	-18.7	11.9	.012
HBO_RF	Non-healthy subjects	52	22.4	33.6	13.6	-59.4	99.0	
	Healthy subjects	150	22.0	29.1	18.2	-43.3	178	.824
HBR_RF	Non-healthy subjects	52	-3.81	14.9	-4.62	-33.0	35.2	
	Healthy subjects	150	-3.40	14.2	-3.25	-104	43.8	.423
HBT_RF	Non-healthy subjects	52	18.6	41.0	15.2	-92.4	128	
	Healthy subjects	150	18.6	34.0	16.9	-147	154	.682
CYT_RF	Non-healthy subjects	52	.891	5.63	.204	-14.4	21.3	
	Healthy subjects	150	-.401	4.99	-.171	-36.0	19.8	.421
HBO_LP	Non-healthy subjects	51	25.6	34.9	19.6	-30.2	186	
	Healthy subjects	138	22.8	32.7	17.2	-51.7	150	.708
HBR_LP	Non-healthy subjects	51	3.21	19.0	-.887	-26.4	78.1	
	Healthy subjects	138	.414	10.2	.403	-33.0	39.5	.881
HBT_LP	Non-healthy subjects	51	28.8	48.9	17.4	-56.6	264	
	Healthy subjects	138	23.2	38.2	17.1	-84.7	165	.713
CYT_LP	Non-healthy subjects	51	-.615	6.29	-.317	-32.3	18.7	
	Healthy subjects	138	-.602	3.23	-.345	-13.3	20.6	.483
HBO_RP	Non-healthy subjects	47	26.1	36.4	17.3	-30.1	195	
	Healthy subjects	130	24.9	38.4	19.3	-35.5	193	.559
HBR_RP	Non-healthy subjects	47	-1.01	13.8	-2.72	-25.0	61.7	
	Healthy subjects	130	.846	12.8	-1.12	-18.9	66.2	.388
HBT_RP	Non-healthy subjects	47	25.1	46.4	12.6	-38.5	256	
	Healthy subjects	130	25.7	46.6	17.8	-40.0	222	.915
CYT_RP	Non-healthy subjects	47	-.648	3.67	-.678	-11.0	8.8	
	Healthy subjects	130	-.814	3.56	-.677	-24.5	10.4	.806
HBO_LT	Non-healthy subjects	48	25.0	32.8	19.5	-34.9	104	
	Healthy subjects	138	20.7	31.7	15.7	-45.3	143	.436
HBR_LT	Non-healthy subjects	48	1.06	13.9	-1.95	-22.1	45.6	
	Healthy subjects	138	.395	12.3	-.735	-41.0	63.4	.563
HBT_LT	Non-healthy subjects	48	26.1	42.5	17.4	-56.9	149	
	Healthy subjects	138	21.1	39.0	15.6	-86.2	168	.672
CYT_LT	Non-healthy subjects	48	.771	3.19	.015	-5.61	9.4	
	Healthy subjects	138	-1.04	6.76	-.615	-62.9	12.6	.045
HBO_RT	Non-healthy subjects	45	15.0	28.9	19.7	-44.9	15	
	Healthy subjects	129	19.8	31.5	15.8	-41.5	19.8	.549
HBR_RT	Non-healthy subjects	45	-2.53	13.7	-1.01	-44.0	-2.53	
	Healthy subjects	129	-.471	11.2	-.824	-31.6	-.471	.502
HBT_RT	Non-healthy subjects	45	12.5	39.4	11.1	-88.9	12.5	
	Healthy subjects	129	19.3	37.0	15.6	-55.1	19.3	.449
CYT_RT	Non-healthy subjects	45	1.06	6.37	.614	-21.0	1.06	
	Healthy subjects	129	.265	4.11	.564	-20.3	.265	.367

(a)



(b)

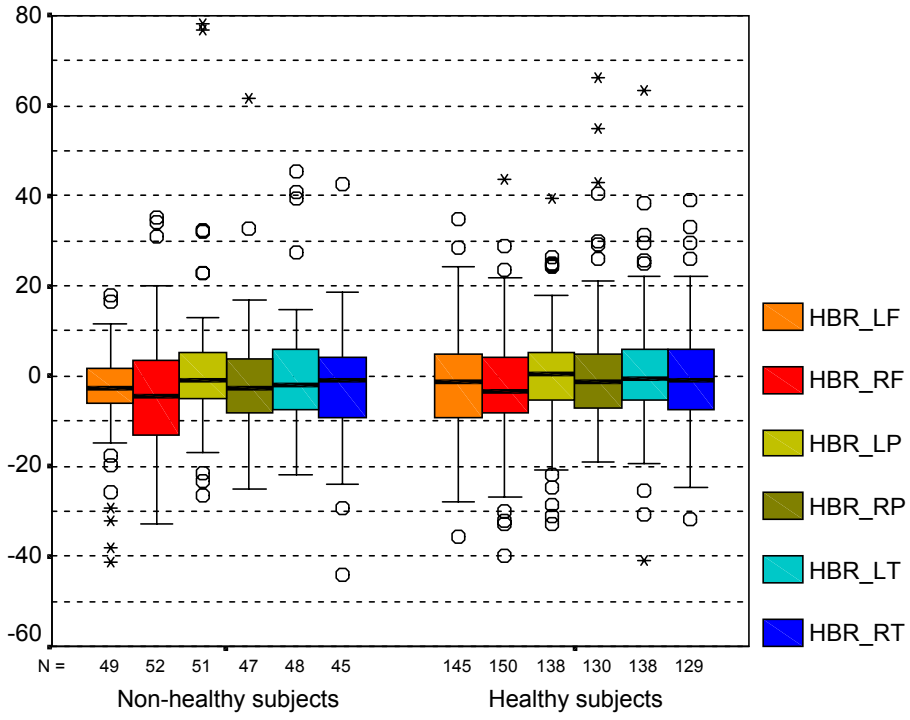


Figure 10: (a) Changes in HbO and (b) HbR across all positions in non-healthy vs. healthy subjects.
O = outliers (values between 1.5 and 3 box lengths from the upper or lower edge of the box)
✳ = extreme values (values more than 3 box lengths from the upper or lower edge of the box)

6.1.3 Analysis of NIRS responders vs non-responders, verbal fluency

As described earlier, there was a marked interindividual variance in hemoglobin oxygenation changes during brain activation. For example, of the 119 subjects activated with the verbal fluency task, 16 subjects (17%, based on valid N) showed a decrease in HbO and 35 subjects (37%) an increase in HbR at the left frontal position. There was a decrease of HbT in 24 subjects (25%) at the same position. This observation may give rise to the assumption that subgroups of subjects can be identified in relation to their NIRS response pattern, i.e. some subjects showing a typical response pattern whereas others do not.

In order to perform a classification into „responders“ and „non-responders“, the NIRS delta values were in a first step transferred into dichotomous variables as follows:

change HbO > 0 = 1; change HbO < 0 = 0
change HbR < 0 = 1; change HbR > 0 = 0.

Thus, a value of „0“ corresponds to an untypical response in the respective NIRS parameter, and a value of „1“ to a typical response, i. e. as extensively described in the literature. In a second step, *and for each optode position*, all subjects were classified as responders or non-responders according to the following rule:

Responder = change HbO = 1 & change HbR = 1
Non-responder = change HbO = 1 & change HbR = 0 or
change HbO = 0 & change HbR = 1 or
change HbO = 0 & change HbR = 0.

Because HbT equals the sum of concentration changes of HbO and HbR, it was decided not to enter it into the response definition.

In Table 6-3 the number of responders and non-responders is presented. Obviously, the two categories are evenly distributed in both frontal positions (left frontal: 49.5% NIRS responders vs. 50.5% NIRS non-responders; right frontal: 50% each), whereas the portion of non-responders is markedly greater at the parietal (left parietal: 33.7% NIRS responders vs. 66.3% NIRS non-responders; right parietal: 39% NIRS responders vs. 61% NIRS non-responders) and temporal positions (left temporal: 34% NIRS responders vs. 66% NIRS non-responders; right temporal: 32% NIRS responders vs. 68% NIRS non-responders). The

relatively high number of missing values is not due to the response definition but, as mentioned earlier, due to the fact that many subjects had no evaluable NIRS measures on one or more optode positions.

Table 6-3: Number of responders and non-responders in the verbal fluency task (n=119)

Position	Responders	Non-Responders	Missing values
Left frontal	47	48	24
Right frontal	50	50	19
Left parietal	31	61	27
Right parietal	32	50	37
Left temporal	33	64	22
Right temporal	30	63	26

In addition to categorizing responders and non-responders for each position separately, we were interested to know how many subjects show the typical response pattern across all positions. Applying the above outlined response criteria, the following picture emerges:

- Responders left and right frontal: n=30
- Responders left and right frontal and left and right parietal: n=7
- Responders across all positions: n=2

In other words, 30 subjects (25%) activated with the verbal fluency task showed an increase in HbO and a simultaneous decrease in HbR over both frontal positions. When the parietal positions are included, the number of subjects with a typical response pattern decreases to 7, and finally, across all positions, only 2 of the 119 subjects (neglecting missing values) activated with the verbal fluency task qualify as responders.

As a consequence of this finding, we asked whether frontal responders do also show the strongest activity on the frontal positions, i.e. whether they show a rather specific left frontal activity. To address this question, descriptive statistics for the subgroup of responders at the left frontal position were calculated (Table 6-4). As a result, left frontal responders not only show activity on the left frontal position, but also on the other positions. In detail, the increase in HbO for this group seems to be even more pronounced on the parietal positions. With regard to HbR however, the left frontal position indeed shows the strongest decrease. (Note: By definition, this group has only positive values for HbO and only negative values for HbR).

Table 6-4: Descriptive statistics for responders at the left frontal position

	Valid N	Mean	SD	Min	Max
HbO_lf	47	27.6	24.4	.040	101
HbO_rf	42	28.3	27.6	-12.8	116
HbO_lp	38	34.0	34.0	-13.3	150
HbO_rp	29	36.2	33.9	-13.8	131
HbO_lt	43	29.6	34.8	-19.4	143
HbO_rt	39	22.6	30.1	-41.5	118
HbR_lf	47	-9.67	8.32	-41.3	-.270
HbR_rf	42	-8.54	12.9	-32.8	34.1
HbR_lp	38	.490	11.4	-24.6	32.3
HbR_rp	29	-2.54	11.3	-25.0	40.4
HbR_lt	43	.530	11.9	-30.6	45.6
HbR_rt	39	-1.09	11.8	-24.9	42.7

Comparing left frontal responders to left frontal non-responders (Figure 11) revealed significantly stronger increases in HbO for the responders at the right frontal, and the left and right parietal position (right frontal: $F(1,86)=9.48$, $p=.003$; left parietal: $F(1,77)=9.40$, $p=.003$; right parietal: $F(1,68)=15.2$, $p<.001$). There was also a trend for left frontal responders to have stronger increases in HbO at the left frontal ($F(1,93)=5.78$, $p=.018$) and the left temporal position ($F(1,84)=4.96$, $p=.029$). Left frontal responders had significantly stronger reductions in HbR at the left and right frontal position (left frontal: $F(1,93)=49.2$, $p<.001$; right frontal: $F(1,86)=8.46$ $p=.005$). In other words, left frontal responders not only differed from non-responders in their cerebral hemodynamic activity at the left frontal position, but also at other positions:

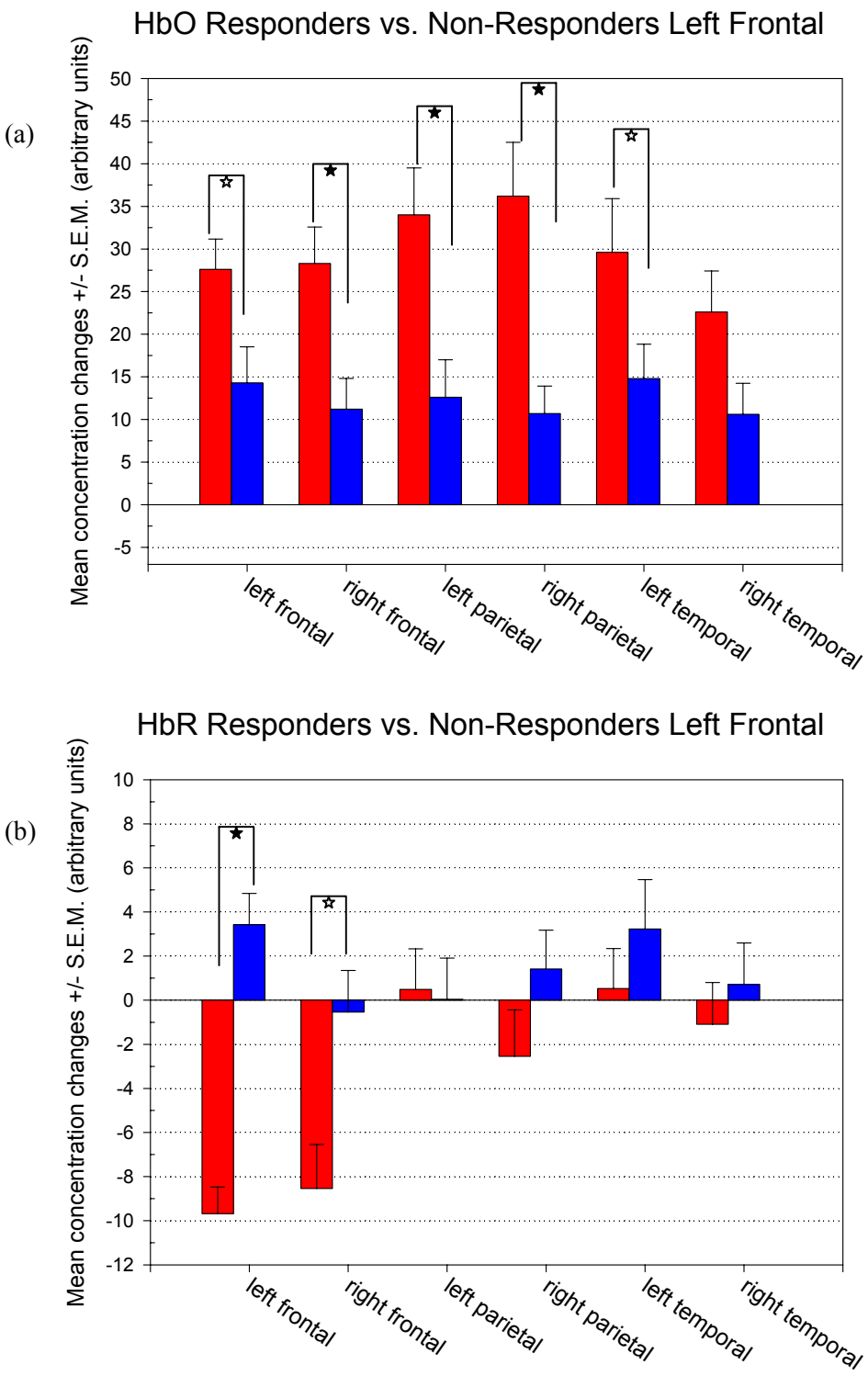


Figure 11: Changes in (a) HbO and (b) HbR for subjects who at the left frontal position either showed a typical NIRS response pattern („responders“, red) or not („non-responders“, blue)

6.1.4 Relationship between NIRS cerebral hemodynamics and NIRS task performance

Subjects who performed the verbal fluency task (n=119) produced on average 52.8 words (SD=14, range=24 – 88, median=52.0) during the two activation periods (see section 4.3.2.1), with the task performance being normally distributed (Kolmogorov-Smirnov and Lilliefors both $p>0.05$).

The correlation matrix provided in Table 6-5 as well as the scatterplots in Figure 11 show numerically small but statistically significant correlations between NIRS task performance and concentration changes of HbR at the left frontal and the left temporal position, as well as between concentration changes of HbO and the right parietal position. There were no significant correlations between any Cyt-Ox parameter and task performance, interestingly however, the correlation coefficients all point in the negative direction.

Table 6-5: Pearson correlation coefficients NIRS parameter and NIRS task performance

	Frontal		Parietal		Temporal	
Left	HbO:	.040	HbO:	.096	HbO:	-.058
	HbR:	-.292**	HbR:	-.070	HbR:	-.297**
	Cyt-Ox:	-.122	Cyt-Ox:	-.110	Cyt-Ox:	-.057
Right	HbO:	.030	HbO:	.288*	HbO:	.071
	HbR:	-.197	HbR:	.165	HbR:	-.104
	Cyt-Ox:	-.142	Cyt-Ox:	-.208	Cyt-Ox:	-.092

*=p≤.01; **=p≤.005

The correlations for HbR are in the negative direction, i.e. subjects with a higher task performance had a higher reduction in deoxygenated hemoglobin. In contrast, the correlation coefficient for HbO at the right parietal position is positive: the more words a subject produced the stronger was the increase in oxygenated hemoglobin (Figure 12).

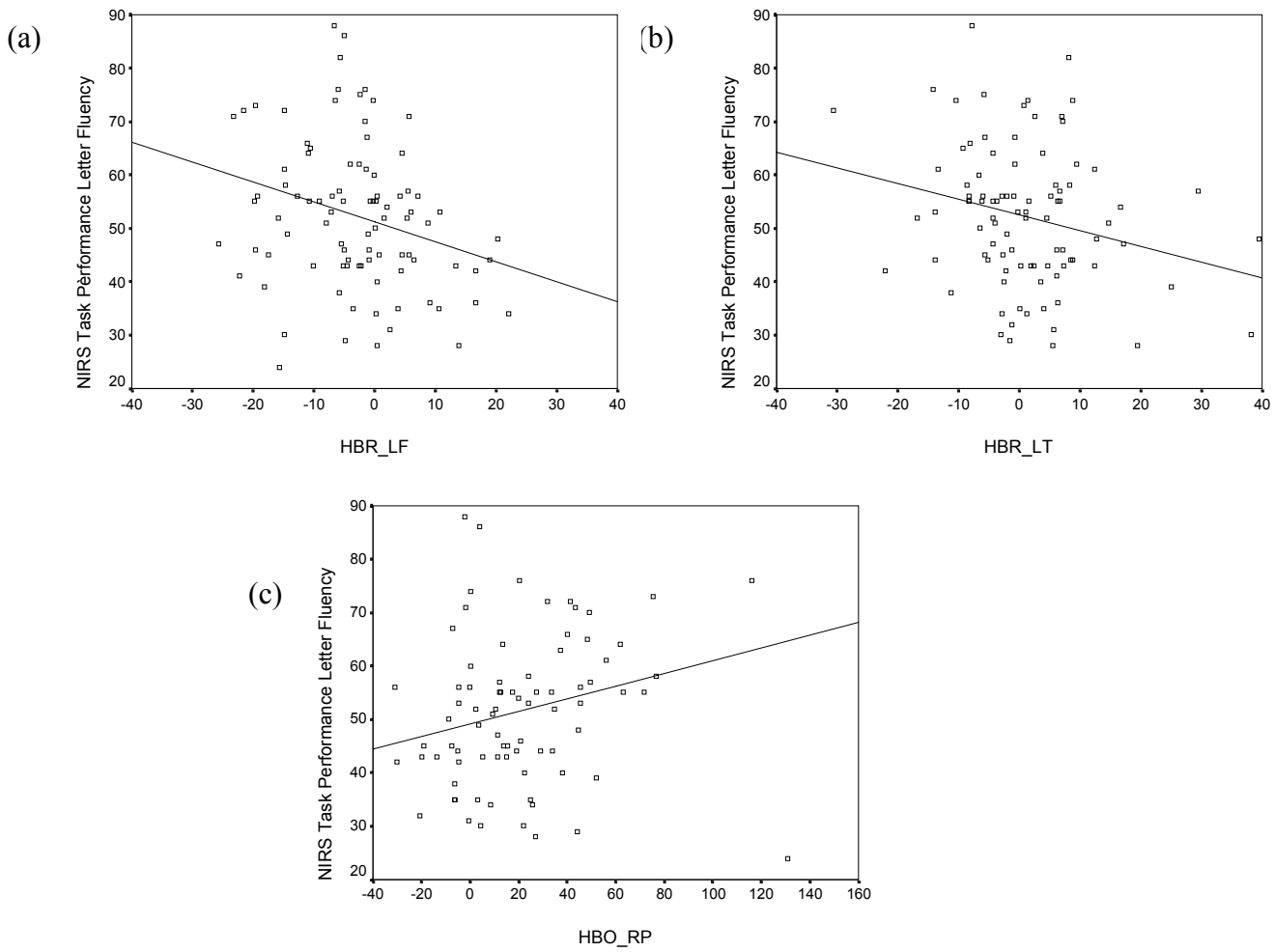


Figure 12: Scatterplots of statistically significant correlations between NIRS task performance and concentration changes of (a) HbR at the left frontal position (HBR_LF), (b) HbR at the left temporal position (HBR_LT), and (c) HbO at the right parietal position (HBO_RP)

In addition to investigating the relationship between NIRS task performance and brain activation as a continuous measure, we asked whether the subjects with a typical NIRS response at the left frontal (i.e. covering the area known to be most associated with the performance of a letter fluency task) differ from the respective non-responders in that they show better task performance.

Task performance for responders and non-responders at every optode position is displayed in Table 6-6. A comparison of mean task performance between left frontal responders and left frontal non-responders revealed a highly significant difference ($F_{(1,90)}$ =7.56; p=.004, one-tailed). This difference was not found for all other optode positions, however there was a trend for better task performance for right frontal responders ($F_{(1,96)}$ =2.01; p=.080, one-tailed), left parietal responders ($F_{(1,89)}$ =1.94; p=.084, one-tailed) and left temporal responders ($F_{(1,92)}$ =1.82; p=.091, one-tailed). Right parietal and right temporal responders did not differ from the respective non-responders (both $F \leq .390$).

Table 6-6: Mean (SD) correct words NIRS verbal fluency NIRS responders vs. non-responders

	Responders	Non-Responders
Left frontal	56.4 (14.6)	48.6 (12.2)
Right frontal	53.8 (14.4)	49.9 (12.6)
Left parietal	55.7 (13.4)	51.4 (14.3)
Right parietal	53.1 (13.6)	51.1 (14.9)
Left temporal	54.4 (13.0)	50.6 (13.1)
Right temporal	52.2 (15.6)	50.6 (13.0)

6.1.5 Summary

The inspection of *mean concentration changes* of oxygenated and reduced hemoglobin in our sample of non-demented elderly subjects (N=240) during performance of either the verbal fluency task or the labyrinth test disclosed a „typical“ NIRS response pattern as described by many earlier reports: an increased concentration of oxygenated hemoglobin (HbO) together with a decrease in reduced hemoglobin (HbR) over areas most probably activated by the stimulus. The mean concentration changes of total hemoglobin (HbT) were in the same direction as for oxygenated hemoglobin, i.e. there was an increase in total hemoglobin at all optode positions and for both activation tasks. Concentration changes in the NIRS parameter cytochrome oxidase (Cyt-Ox) were minimal and generally in the negative direction, i.e. a decrease under brain activation compared to rest.

The results of one-sample t-tests showed that whereas concentration changes of HbO and HbT exhibited a pattern of general activation across the whole brain during activation with either task, the decrease in HbR was restricted to the frontal positions only (N=240). Furthermore, when the two activation paradigms were contrasted to each other, this frontal decrease of HbR was observed for the sample of subjects activated with the verbal fluency task (n=119) only, whereas the subjects performing the labyrinth test (n=121) showed a trend towards reduction of HbR at the right temporal optode position. A repeated measures ANOVA with the within-subject factors „topography“ and „NIRS parameter“ and the between-subject factor „activation paradigm“ did not reveal a significant difference between the two activation paradigms with regard to their brain-topographical characteristics of all three NIRS parameters. However, this result must be interpreted with caution as only a relatively small sample size was included in this analysis (n=105) due to missing values in one or more optode positions.

However, although the mean concentration changes of HbO, HbR and HbT in our unique sample of 240 non-demented elderly subjects revealed a typical NIRS response pattern, there were large interindividual differences in hemoglobin oxygenation changes during brain activation.

Investigating whether the marked interindividual variability in the NIRS data was due to the non-healthy subjects in our study sample, i.e. subjects with various CNS pathology (i.e. as defined by our inclusion/exclusion criteria), the NIRS data of our group of healthy non-demented elderly subjects (n=177) were compared with the ones of our non-healthy non-demented elderly subjects (n=63). The results of Mann-Whitney U-Tests were in agreement with the null hypothesis that the two groups do not differ in their cerebral hemodynamics.

Moreover, the two groups had comparable group variance for each NIRS parameter and a comparable number of outliers and extreme values.

The observation of the marked interindividual difference in the NIRS data led us to classify subjects showing the typical NIRS response for both HbO and HbR, i.e. so-called NIRS responders, and contrast them to subjects not showing the typical NIRS response, i.e. NIRS non-responders. This classification was done for each optode position and particularly for subjects activated with the verbal fluency task as this task had proved to be a reliable and well-examined brain activation paradigm. Frequency counts revealed that the two categories were evenly distributed in both frontal positions, and that the portion of non-responders was markedly greater at the parietal and temporal positions. Descriptive statistics of HbO and HbR at all optode positions for the subgroup of left-frontal NIRS responders illustrated that this group also had NIRS activity on the other optode positions, in other words, they did not show a specific left-frontal activity. This was specifically true for the parameter HbO, where the activity for left-frontal responders was maximal at the right parietal position. Multiple ANOVAS comparing left-frontal NIRS responders with left-frontal NIRS non-responders in the dependent variables HbO and HbR at all optode positions were calculated. Left-frontal responders not only showed a marked higher increase in HbO at the right frontal position and a trend towards higher increase at the left frontal and the left temporal position, but also had significantly higher increases in HbO at both parietal positions. At the same time, they had a significantly stronger reduction in HbR not only at the left frontal but also at the right frontal position. In short, left frontal responders not only differed from left-frontal non-responders in their cerebral hemodynamic activity at the left frontal position, but also at other positions.

The relationship between cerebral hemoglobin oxygenation and task performance during the NIRS verbal fluency task was investigated by performing Pearson correlation analyses. We hypothesized that the correlation coefficients for the relationship between task performance and hemoglobin oxygenation would be significantly different from zero for the left and right frontal and the left temporal position, i.e. areas known to be highly associated with the performance of a verbal (letter) fluency task, while the correlation coefficients for all other positions would not be different from zero. In line with our hypothesis, we found numerically small but statistically significant correlations between NIRS task performance and concentration changes of HbR at the left frontal and the left temporal position, i.e. subjects with a higher task performance had a stronger reduction in deoxygenated hemoglobin. However, and in contrast to our expectations, there was also a statistically significant correlation for the relationship between HbO at the right parietal position and NIRS task performance. In addition to investigating the relationship

between NIRS task performance and cerebral hemoglobin oxygenation as a continuous measure, and in line with the hypothesis put forward above, we asked whether subjects with a typical NIRS response at the left frontal position (i.e. covering the area known to be most associated with the performance of a letter fluency task) show better task performance than the respective non-responders. The results of the one-tailed ANOVA was in good agreement with our hypothesis: Left-frontal NIRS responders performed highly significantly better than left-frontal non-responders.

6.2 NIRS, Sociodemographic factors and ApoE genotype in non-demented elderly subjects

6.2.1 Sociodemographic factors and ApoE-genotype as predictors of NIRS response?

The question whether the sociodemographic factors age, years of education, and gender, and the ApoE-genotype (ε4 vs. non-ε4) might have a predictive value for the changes in cerebral brain hemodynamics as measured by NIRS, was investigated using multiple linear regression analyses (stepwise backwards) in the total sample of non-demented elderly subjects (N=240). The factors „age“ and „years of education“ are continuous variables whereas the factors „gender“, and „ApoE-genotype“ are binary nominal or so-called dichotomous variables. As dependent variables the NIRS parameter HbR at the left and right frontal position and the variable HbT at all optode positions were chosen.

In a first step the predictor variables were tested for possible intercorrelations. Only the variables „gender“ and „years of education“ showed a numerically small but significant intercorrelation ($\chi 2$=57.8; p<.001; r= -.362), with males having had markedly more years of education than females (mean: 13.2 ± 3.21 vs. 10.5 ± 2.84).

The results of the multiple regression analyses for HbR and HbT at the left frontal position, displayed in Table 6-6 and 6-7, clearly show no predictive value, neither of any of the sociodemographic variables outline above, nor of ApoE-genotype (ε4 vs. non-ε4). Although the beta coefficient for ApoE-ε4 in Table 6-6 is highly significant, it only explains about 5% of the variability of HbT. The results for the NIRS parameter HbR right frontal and HbT at all other positions were very similar, with the predictor variables explaining less than 4% of the variability.

Table 6-7: Results of stepwise backwards regression analysis for HbR at the left frontal position and the predictor variables age, years of education, gender and ApoE-genotype (ε4 vs. non-ε4)

Step	Variables entered	R	Adjusted R^2	β	t	p	Variables removed
1		.088	-.013				
	Gender			-.067	-.858	n.s.	
	Age			.041	.562	n.s.	
	ApoE			.039	.534	n.s.	
	Education			-.048	-.613	n.s.	
2		.079	-.009				
	Age			.037	.513	n.s.	
	Education			-.045	-.581	n.s.	
	Gender			-.071	-.912	n.s.	ApoE
3		.070	-.006				
	Education			-.049	-.629	n.s.	
	Gender			-.070	-.908	n.s.	Age
4		.053	-.002				
	Gender			-.053	-.731	n.s.	Education

Table 6-8: Results of stepwise backwards regression analysis for HbT at the left frontal position and the predictor variables age, years of education, gender and ApoE-genotype (ε4 vs. non-ε4)

Step	Variables entered	R	Adjusted R^2	β	t	p	Variables removed
1		.260	.048				
	Gender			-.118	-1.56	n.s.	
	Age			.092	1.30	n.s.	
	ApoE			.194	2.72	.007	
	Education			-.153	-2.02	.045	
2		.243	.044				
	Gender			-.118	-1.56	n.s.	
	ApoE			.185	2.60	.010	
	Education			-.161	-2.13	.035	Age
3		.217	.037				
	ApoE			.195	2.74	.007	
	Education			-.120	-1.68	.094	Gender

Against our expectations, age did not correlate with any of the NIRS parameters at any position. As an example, Figure 13 represents a scatterplot for the relationship between age and changes in total hemoglobin measured at the left frontal position, for which, in the study of Hock et al. (1995), a significant negative correlation with age was found.

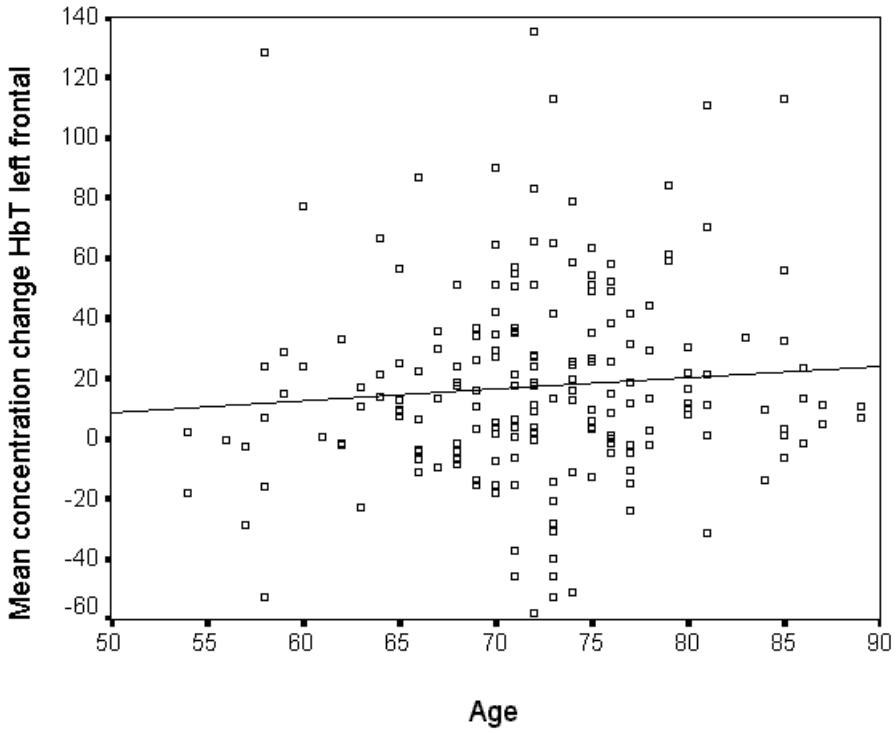


Figure 13: Scatterplot for correlation between age and concentration changes of HbT at the left frontal position for the sample of non-demented elderly subjects and both paradigms (valid cases = 194). Partial correlation = .094

Classifying subjects according to their age in subgroups with „young old“ (54-63 years, n=24), „old old“ (64-80 years, n=190), and „oldest old“ (81-90 years, n=26) did not reveal differences in NIRS characteristics between groups. In detail, the extreme groups of „young old“ vs. „oldest old“ subjects did not differ in any NIRS parameter at any position (see Table II-1 Appendix). The same was true when only subjects who were activated with the letter fluency task during NIRS were compared.

Subjects with an ApoE-ε4-allele (n=133) did not differ from subjects without ApoE-ε4-allele (n=107) with respect to age, years of education, and MMSE score (ANOVA: all $p \geq .083$).

To test whether ApoE-ε4 carriers present significantly lower values in HbT at the left parietal optode position than ApoE-ε4 non-carriers, a t-test was performed. The mean

concentration changes of HbT at the left parietal position were 21.8 (± 35.5) for ApoE-ε4 carriers (n=46) and 23.9 (± 41.1) for non-carriers (n=46). The result of the one-sided t-test revealed no significant differences between the two groups (t=-.253, p=.401). The marked interindividual variance in concentration changes of HbT within both groups is illustrated in Figure 14.

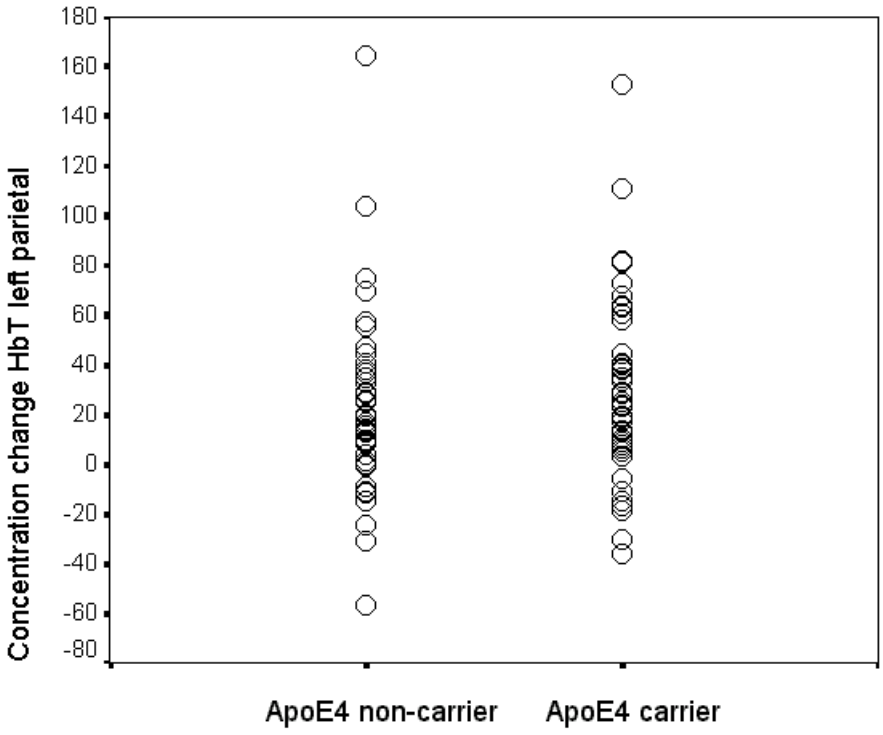


Figure 14: Concentration change of HbT at the left parietal position in relation to ApoE-ε4 status for subjects with verbal fluency task (46 subjects in each group)

6.2.2 Differences between NIRS responders and non-responders in the verbal fluency task with regard to sociodemographic factors and ApoE-genotype

Subjects with a typical NIRS response (responders) at the left frontal position (n=47) did not differ from subjects who did not show a typical NIRS response (non-responders, n=48) with regard to age and years of education (mean age: responders = 74.2 ± 5.75, non-responders: 74.5 ± 6.79, $F_{(1,93)}$ = .057; p = .812; mean years of education: responders = 11.9 ± 2.99, non-responders: 11.7 ± 3.58; $F_{(1,93)}$ = .484, p = .833). The same is true for non-responders and responders at all other positions (right frontal, left and right parietal, left and right temporal): there was no difference with respect to age and years of education (all p≥.092).

Furthermore, NIRS responders did not differ from non-responders with respect to gender

and ApoE-genotype (ε4 vs. non-ε4), as revealed by Chi-Square tests (all p≥.220) and illustrated for the left frontal position in Tables 6-8 and 6-9.

Table 6-9: Contingency table with gender and NIRS response type left frontal position

		NIRS Response Type Left Frontal		
		Responder	Non-Responder	Total
Gender	Male	31	28	59
	Female	16	20	36
	Total	47	48	95

$\chi 2$ = .587, p = .444

Table 6-10: Contingency table with ApoE-genotype (ε4 vs. non-ε4) and NIRS response type left frontal position

		NIRS Response Type Left Frontal		
		Responder	Non-Responder	Total
Presence of ApoE-ε4 allele	No ε4 allele	25	27	52
	At least one ε4 allele	22	21	43
	Total	47	48	95

$\chi 2$ = .090, p = .765

6.2.3 Summary

Multiple regression analyses revealed that the sociodemographic factors „age“, „years of education“, and „gender“, and the ApoE genotype (ε4 vs. non-ε4) did not correlate with concentration changes of deoxygenated hemoglobin HbR nor with changes of total hemoglobin HbT. Specifically, in our sample of non-demented elderly subjects age was not correlated with any of the NIRS parameters at any optode position.

As functional activation studies using a letter fluency task had reported a disruption of functional circuits involving the left parietal lobe in ApoE-ε4 carriers vs. non-carriers, we investigated whether this finding could be replicated in our sample and with NIRS. The research hypothesis was tested that ApoE-ε4 carriers (n=46) have significantly lower values in HbT at the left parietal optode position than ApoE-ε4 non-carriers (n=46). The result of the one-tailed t-test demonstrated that the two groups did not differ in their cerebral brain

hemodynamics.

Subjects who showed the typical NIRS response pattern during the verbal fluency task, NIRS responders, did not differ from the respective NIRS non-responders with regard to age, years of education, gender and ApoE-genotype.

6.3 NIRS and neuropsychology in non-demented elderly subjects

A great advantage in the search of brain-behaviour relationships in our study is the fact that half of our non-demented elderly subjects during NIRS were activated with a verbal letter fluency task, a task which had also been assessed during the extensive neuropsychological assessment on the same day. Theoretically, a strong positive correlation between the two tasks would mean that they measure the same aspects of brain function. The scatterplot in Figure 15, illustrating the relationship between the subjects‘ performance in the NIRS letter fluency vs. the subjects‘ performance in the NP letter fluency, clearly shows that the two tasks have a strong positive correlation.

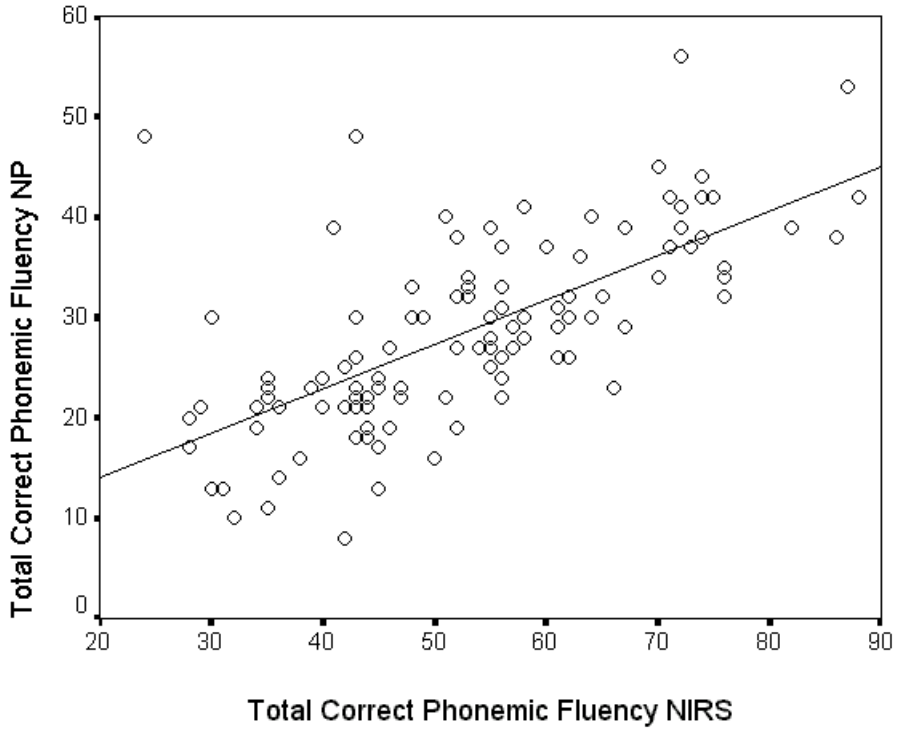


Figure 15: Correlation between performance in NIRS phonemic fluency and NP phonemic fluency. r =.671, p<.001

In other words, we have good reason to believe that the brain activity measured during the NIRS letter fluency task is to a great deal reflected in the neuropsychological assessment of letter fluency performance.

This correspondence cannot be established for the NIRS labyrinth test, as unfortunately no performance measures were assessed while subjects were performing the labyrinth test. Therefore, in this section, correlating neuropsychological measures of brain function with

cerebral hemoglobin oxygenation measures, the subgroup of subjects activated with the NIRS letter fluency task will be focused.

6.3.1 Test performance of non-demented elderly subjects

Descriptive statistics of neuropsychological and NIRS test performance are displayed in Table 6-11. Comparing our subjects‘ test scores with normative data of the respective tests and data obtained in the BASEL project, indicates that our subjects are within the normal ranges of elderly non-demented individuals (for normal ranges see Monsch, 2001; Mitrushina et al., 1999; Spreen & Strauss, 1998).

Table 6-11: Neuropsychological test performance of non-demented elderly subjects activated during NIRS with the verbal fluency task

	Valid N	Mean	SD	Min	Max
NIRS Letter Fluency	116	52.8	14.0	24	88
MWT-B: correct words	119	32.1	3.58	14	37
WAIS-R Vocabulary: total correct	105	21.6	5.46	6	31
WAIS-R Similarities: total correct	116	24.7	5.30	6	32
WAIS-R Block design: total correct	119	23.6	7.69	6	45
BNT: total spontaneous correct	115	39.2	4.15	25	45
Category Fluency: total correct	117	50.4	11.9	24	88
Phonemic Fluency: total correct	117	28.8	9.20	8	56
CVLT: correctly recalled List A trials 1-5	119	50.6	8.96	28	69
CVLT: correctly recalled short delay	119	10.3	2.95	0	16
CVLT: correctly recalled long delay	119	11.0	2.76	2	16
CVLT: Recognition discriminability (%)	119	95.0	5.74	65.9	100
WCST: total correct categories	108	5.23	1.23	0	6
WCST: perseverative errors	108	2.40	3.11	0	19
TMT-A: time needed (s)	115	48.2	17.0	22	130
TMT-B: time needed (s)	115	135	64.5	60	435
TMT B/A	115	2.86	1.09	1.13	8.64

Table 6-12: Mean (SD) performance of healthy vs. non-healthy subjects activated during NIRS with the verbal fluency task

	Healthy subjects (n=88)	Not healthy subjects (n=31)	*Z*	p (2-tailed)
NIRS Letter Fluency	53.5 (14.0)	50.9 (14.0)		p=.452
MWT-B: correct words	31.9 (3.34)	32.6 (4.21)	-1.30	p=.193
WAIS-R Vocabulary: total correct	22.0 (5.26)	20.3 (5.87)	-1.23	p=.218
WAIS-R Similarities: total correct	24.7 (5.52)	24.6 (4.70)	-.591	p=.554
WAIS-R Block design: total correct	24.2 (7.50)	21.7 (8.02)	-1.34	p=.180
BNT: total spontaneous correct	39.2 (4.30)	39.4 (3.75)	-.077	p=.939
Category Fluency: total correct	51.9 (12.1)	46.2 (10.6)	-1.96	p=.050
Phonemic Fluency: total correct	29.0 (9.14)	28.0 (9.51)	-.669	p=.504
CVLT: correctly recalled List A trials 1-5	51.7 (8.31)	47.5 (10.1)	-2.06	p=.039
CVLT: correctly recalled short delay	10.5 (2.76)	9.55 (3.38)	-1.60	p=.111
CVLT: correctly recalled long delay	11.4 (2.46)	9.94 (3.30)	-2.55	p=.011
CVLT: Recognition discriminability (%)	95.6 (4.74)	93.3 (7.78)	-1.15	p=.249
WCST: total correct categories	5.41 (1.04)	4.93 (1.60)	-1.38	p=.168
WCST: perseverative errors	2.23 (2.77)	2.87 (3.89)	-.556	p=.578
TMT-A: time needed (s)	47.2 (16.6)	51.4 (18.2)	-1.06	p=.288
TMT-B: time needed (s)	124 (53.1)	169 (82.2)	-2.94	p=.003
TMT B/A	2.70 (.986)	3.36 (1.24)	-2.77	p=.006

Comparing the healthy subjects with the non-healthy subjects revealed highly significant differences between the two groups in part B of the Trail Making Test as well as in the quotient TMT B/A, i.e. in both aspects of the test which measure executive functioning (Table 6-12). Executive functioning is known to be highly vulnerable to deterioration resulting from brain pathology of different etiologies, and in this respect the TMT is known to be a sensitive measure, particularly useful in documenting cerebral dysfunction in mild traumatic brain injury and the differential diagnosis of dementia (Mitrushina et al., 1999). The differences in the CVLT (correctly recalled words across all five learning trials, correctly recalled words long-delay free recall) do show a strong trend for our healthy subjects to have a better verbal memory than the group of non-healthy volunteers. In addition, there is a trend for higher performance of our healthy subjects in the Category Fluency. The two groups however do not differ substantially in measures of general intellectual functioning and verbal intelligence.

In summary, even though there are subtle differences in neuropsychological test performance between healthy and non-healthy subjects, the test scores of the non-healthy subjects lie within the normal ranges of normal elderly individuals.

6.3.2 Do changes in cerebral hemodynamics predict neuropsychological test performance?

One way to explore the relationship between cerebral hemodynamics measured with NIRS and neuropsychological test performance is to ask whether the NIRS parameters can predict the performance in a specific neuropsychological test. This question was investigated for the following neuropsychological measures as dependent variables:

- WAIS-R vocabulary: total correct
- Category fluency: total correct
- Phonemic fluency: total correct
- CVLT: correctly recalled List A trials 1-5
- TMT B/A

A correlation analysis across all NIRS predictor variables not surprisingly revealed moderately strong correlations for HbO and HbR between the left and right hemisphere, as well as between frontal, parietal and temporal positions (see Table II-2, Appendix II).

In Table 6-13 the last step of every regression analysis is displayed for each dependent variable respectively. With the exception of the Category Fluency test, every regression model listed in Table 6-13 is highly significant - yet, as can be seen from the values of R^2 - the NIRS parameters in general only predict a low proportion of the variability (e.g. 27% of the WAIS-R Vocabulary test score).

For the quotient TMT B/A the NIRS variables HbO measured at the left and right frontal and at the left temporal position as well as HbR measured at the right frontal, right parietal and left temporal position are able to predict a subject's score in the TMT B/A to 42%. The standardized partial regression coefficients (β) indicate the relative importance of the predictor variables: For the TMT B/A the most important predictor variables are HbO at the left and right frontal position (fairly equal weight), and HbR at the right frontal and the left temporal position. The multiple regression equation for the expected score in the TMT B/A is:

$$y_{\text{TMT B/A}} = 3.04 + .036*\text{HbO\_lf} - .039*\text{HbO\_rf} + .088*\text{HbR\_rf} - .062*\text{HbR\_lt} - .022*\text{HbR\_rp} + .017*\text{HbO\_lt}$$

According to this equation, a reduction in HbR as well as an increase in HbO at the right frontal position is associated with a lower TMT B/A score, i.e. with a better performance in executive functioning. In contrast, increases in HbO at the left frontal and the left temporal as well as

reductions in HbR at the left temporal and right parietal position are associated with a higher test score and thus with lower abilities of executive functioning. Scatterplots for each predictor variable and the score in TMT B/A are compiled in Figure 1 Appendix II (Note: there is one subject with an extreme value, ID 5130, who could potentially have had an impact on the high multiple correlation. Therefore, the multiple regression analysis was calculated again with this subject excluded. The exclusion of the subject did not change the results of the regression analysis, not even to a minor extent).

With regard to the multiple regression results for the Phonemic Fluency and the WAIS-R Vocabulary, the NIRS parameter HbO at the right parietal position in both tests was associated with a higher test score as was a reduction in HbR at either frontal position.

To test whether age and ApoE-ε4 status increase the prediction of the above neuropsychological test scores, both variables were entered into the multiple regression analyses. As a result, age was kept in the last step of the regression equations (i.e. in addition to the NIRS variables outlined in Table 6-13) only for the Category Fluency and the CVLT list learning, and did not markedly increase the percentage of explained variability (Category Fluency: adjusted R^2 last step = .088, $Beta_{Age}$ = -.273, p = .020; CVLT correctly recalled List A trials 1-5: adjusted R^2 last step = .200, $Beta_{Age}$ = -.205, p = .065). Age did not contribute to the prediction of the TMT B/A, the Phonemic Fluency and the WAIS Vocabulary. The ApoE-ε4 status was removed from the regression equations of all five tests, with other words did not contribute to the prediction of any of the neuropsychological test scores.

Table 6-13: Summary of results of multiple regression analyses: Do NIRS parameters predict neuropsychological test performance?

WAIS-R: Vocabulary

Number of steps	Variables in last step	R^2 last step	Adjusted R^2 last step	β	t	p
9		.312	.270			
	HbO_lf			-.464	-3.91	<.001
	HbO_rp			.451	3.85	<.001
	HbR_rf			-.308	-2.47	.016
	HbR_lt			.265	2.26	.027

Category Fluency

Number of steps	Variables in last step	R^2 last step	Adjusted R^2 last step	β	t	p
12		.040	.026, n.s.			
	HbO_rp			.200	1.682	.097

Phonemic Fluency

Number of steps	Variables in last step	R^2 last step	Adjusted R^2 last step	β	t	p
10		.311	.280			
	HbO_rp			.511	4.19	<.001
	HbO_lf			-.219	-1.89	.063
	HbR_lf			-.199	-1.83	.072

CVLT: correctly recalled List A trials 1-5

Number of steps	Variables in last step	R^2 last step	Adjusted R^2 last step	β	t	p
8		.228	.168			
	HbO_lt			-.637	-3.47	.001
	HbO_rp			.386	2.75	.008
	HbR_rt			.351	2.68	.009
	HbR_rf			-.359	-2.518	.014
	HbR_lt			.423	2.33	.023

TMT B/A

Number of steps	Variables in last step	R^2 last step	Adjusted R^2 last step	β	t	p
7		.467	.416			
	HbR_rf			1.07	6.65	<.001
	HbO_rf			-1.02	-6.25	<.001
	HbO_lf			.909	6.03	<.001
	HbR_lt			-.762	-4.35	<.001
	HbO_lt			.487	2.99	.004
	HbR_rp			-.238	-2.39	.020

6.3.3 NIRS responders vs. non-responders: do they differ in neuropsychological test performance?

Mean neuropsychological test scores of NIRS responders and non-responders at the left frontal position are displayed in Table 6-14 (Tables for all other positions are listed in Appendix II, Tables II-3, II-4, II-5, II-6, II-7). As we hypothesized that responders at the left frontal position, i.e. the main area of activation during performance of a letter fluency task, show better performance in other measures of executive functioning, the Mann Whitney U comparisons were performed at the one-sided significance level for these.

Table 6-14: Mean (SD) performance of left-frontal NIRS responders vs. left-frontal non-responders

	Responders (n=47)	Non-Responders (n=48)	*Z*	p
Phonemic Fluency: total correct	32.3 (8.84)	25.0 (7.74)	-3.84	<.001*
Category Fluency: total correct	52.7 (12.6)	48.5 (10.7)	-1.36	.087*
WCST: total correct categories	5.22 (1.40)	5.35 (1.10)	-.124	.451*
WCST: perseverative errors	2.11 (3.35)	2.70 (3.00)	-1.44	.075*
TMT-B: time needed	118 (39.9)	156 (76.4)	-2.56	.005*
TMT B/A	2.58 (.772)	3.19 (1.36)	-2.17	.015*
BNT: total spontaneous correct	40.4 (3.53)	38.4 (4.19)	-2.40	.016
WAIS-R Block design: total correct	25.9 (7.15)	20.9 (7.39)	-3.18	.001
WAIS-R Similarities: total correct	26.3 (3.26)	22.9 (6.63)	-2.22	.026
WAIS-R Vocabulary: total correct	22.5 (4.74)	20.2 (6.08)	-1.73	.084
MWT-B: correct words	32.6 (2.90)	31.0 (4.30)	-1.89	.058
CVLT: correctly recalled List A trials 1-5	51.3 (8.31)	49.0 (8.78)	-1.29	.197
CVLT: correctly recalled short delay	10.7 (2.84)	9.79 (2.92)	-1.76	.078
CVLT: correctly recalled long delay	11.4 (2.67)	10.3 (2.74)	-1.87	.062
CVLT: Recognition discriminability (%)	95.0 (6.23)	95.1 (4.48)	-.732	.464

* = one-tailed p-values

As a result, the comparison of NIRS responders and non-responders at the left frontal position revealed markedly significant differences between the two groups in the NP letter fluency task, the TMT B time needed, and as a strong trend in the quotient of TMT B/A, with the responders consistently showing better performance in all of these measures. The left-frontal NIRS responders did not differ from the non-responders with regard to the obtained total

correct categories of the WCST and number of perseverative errors. The left-frontal NIRS responders further showed significantly better performance in the WAIS Block Design and as a strong trend better performance in the BNT. Left-frontal responders performed somewhat better in tests of verbal intelligence, particularly in the WAIS-R Similarities. There was no significant difference between left-frontal responders and non-responders in tests of verbal learning and memory.

For the remaining optode positions, i.e. the right frontal, left and right parietal and left and right temporal position, no systematic difference could be observed between NIRS responders and non-responders: At the right frontal and both temporal positions, NIRS responders did not differ from non-responders with respect to executive functioning, verbal intelligence and/or general intellectual functioning, and verbal learning and memory. Responders at the left parietal position exhibited significantly better performance in Category Fluency, and a strong tendency for less perseverative errors in the WCST than non-responders. At the right parietal position, a trend towards better performance of responders were seen for the quotient TMT B/A.

6.3.4 Summary

The analyses presented in this section investigated brain-behaviour relationships in 119 non-demented elderly subjects, compiling functional NIRS data using a verbal fluency paradigm with neuropsychological test data collected on the same day. The reason for choosing subjects on the NIRS verbal fluency paradigm only was the finding that the scores in the NIRS and NP Fluency Task showed a strong and highly significant correlation. In other words, we have strong evidence that the brain activity measured during NIRS is indeed a function of performing a verbal fluency task.

Descriptive statistics of the neuropsychological test data investigated in this study revealed that our sample's test scores were within the normal ranges of elderly non-demented individuals. The healthy subjects in our sample of subjects activated with the verbal fluency task (n=88) showed significantly better performance than the group of non-healthy subjects (n=31) in both aspects of the Trail Making Test which measures executive functioning, e.g. part B and the quotient TMT B/A They also showed a trend towards better performance in the number of correctly recalled words across all five learning trials as well as in the number of correctly recalled words after a long delay (CVLT).

Using multiple regression analysis we investigated the predictive value of NIRS for the performance in the WAIS-R Vocabulary, Category and Phonemic Fluency, CVLT correctly recalled words across all five learning trials, and the quotient TMT B/A. With the exception of

the Category Fluency, every regression model was highly significant. The NIRS parameters predicted 27% of the variability for the WAIS-R Vocabulary test score, 28% of the Phonemic Fluency, 17% of the CVLT learning, and 42% of the TMT B/A. For the quotient TMT B/A the most important predictor variables revealed to be concentration changes of HbO at the left and right frontal position and concentration changes of HbR at the right frontal position. The inclusion of the factors „age“ and „ApoE-ε4 status“ into the regression analyses did not markedly alter the prediction of the neuropsychological test scores.

Based on the expectations that left-frontal responders show a better NIRS task performance than left-frontal non-responders and that there are positive correlations between NIRS task performance and performance in the NP letter fluency task (cf. Section 5.1.1), we hypothesized that left-frontal NIRS responders show better performance in other neuropsychological tests of executive functioning than the respective non-responders. In agreement with our hypothesis, the left-frontal NIRS responders in our sample reached significantly better test scores in the Phonemic Fluency, the TMT B and as a trend in the quotient TMT B/A. In contrast to our expectations, the left-frontal NIRS responders also showed significantly better performance in the WAIS Block Design.

For the other optode positions, i.e. the right frontal, left and right parietal and left and right temporal position, no systematic difference could be observed between NIRS responders and non-responders.

6.4 NIRS in mild-stage Alzheimer's Disease (AD)

6.4.1 Do patients with AD show a reduced hemodynamic response?

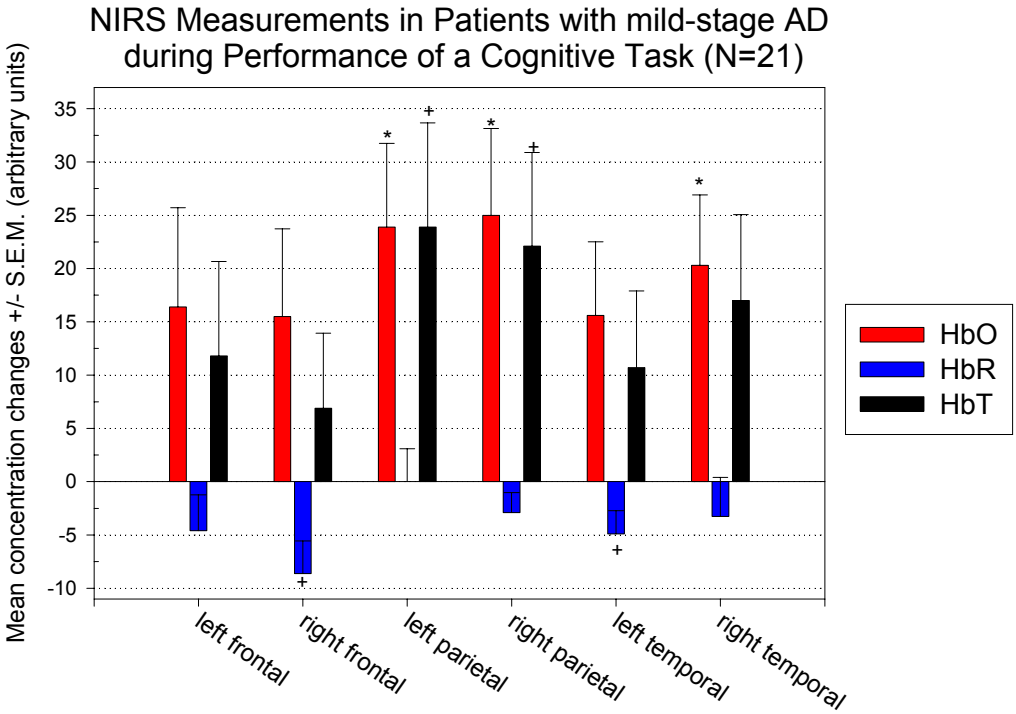


Figure 16: Mean concentration changes ± S.E.M. in HbO, HbR, and HbT over the left and right frontal, parietal and temporal cortex during performance of either a verbal (letter) fluency task (n=18) or a computerized labyrinth test (n=3) in patients with mild-stage AD.
* = p<.01; $^{+}$ = p<.05

Figure 16 shows the mean concentration changes (activation – rest) in cerebral hemoglobin oxygenation measured with NIRS over the left and right frontal, left and right parietal, and left and right temporal cortex in our sample of patients with Alzheimer's Disease (N=21) during performance of a cognitive task. Concentration changes of HbO showed a strong and highly significant increase at the left and right parietal and right temporal optode position (left parietal position: $t(16) = 3.06$, p = .008; right parietal position: $t(19) = 3.08$, p = .006; right temporal position: $t(17) = 3.07$, p = .007) together with a smaller increase at both frontal positions as well as at the left temporal position. HbT, used as an indicator of alterations in cerebral blood volume, showed a trend towards increases only at the parietal positions (left parietal position: $t(16) = 2.45$, p = .026; right parietal position: $t(19) = 2.52$, p = .021). There was a trend towards reductions of HbR at the right frontal and the left temporal position (right

frontal position: $t(15) = -2.81$, $p = .013$; left temporal position: $t(15) = -2.25$, $p = .040$). Small reductions in HbR could also be observed at all other positions with the exception of the left parietal position. Concentration changes in cytochrome oxidase were very small and not statistically different from zero for any position.

In comparison to our sample of non-demented elderly subjects (n=119) the patients activated with the verbal fluency task (n=18) overall did not show a reduced hemodynamic response as illustrated in Figure 17. Not surprisingly, the standard errors of the mean were substantially greater in the patient sample. The results of Mann Whitney-U tests for the comparison of HbO, HbR, and HbT between the non-demented elderly population and the sample of mild-stage AD patients (Table 6-15), showed no significant difference between the two groups. In particular, our expectation of a reduced hemodynamic response in the parieto-temporal cortex was not confirmed. In fact, the patients with AD on average showed a slightly stronger increase in cerebral blood volume at both parietal positions than the non-demented group. The patients showed the typical hemoglobin oxygenation response pattern at both frontal positions, with the concentration changes in HbR being slightly more pronounced than in the non-demented group.

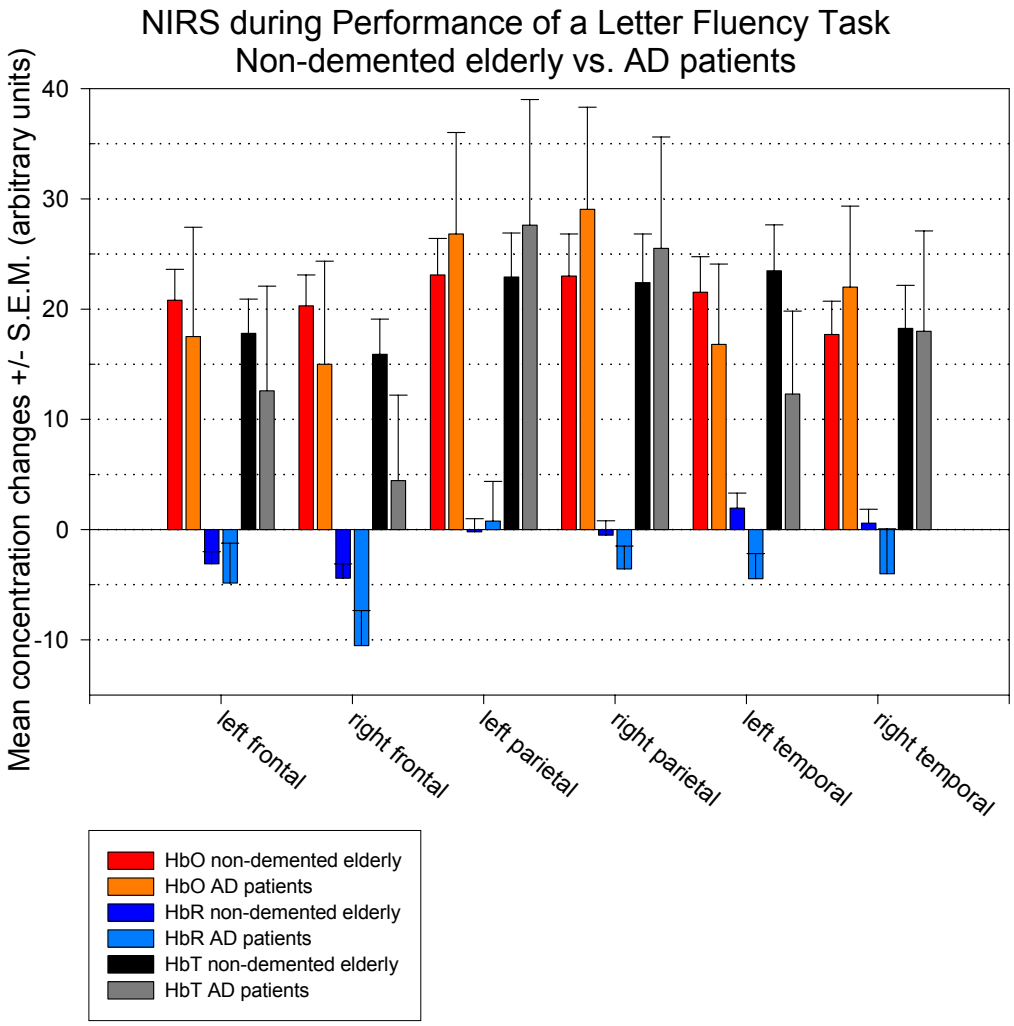


Figure 17: Mean concentration changes ± S.E.M. in HbO, HbR, and HbT in non-demented elderly subjects (n=119) and patients with mild-stage AD (n=18).

Table 6-15: Comparison of NIRS values between non-demented elderly subjects and patients with AD activated with the verbal fluency task

		Valid N	Mean	SD	Median	*Z*	p
HbO_lf	Non-demented	95	20.8	27.6	16.0	-.652	.514
	Patients with AD	14	17.5	37.1	14.2		
HbR_lf	Non-demented	95	-3.05	11.2	-2.16	-.498	.618
	Patients with AD	14	–4.84	13.5	–3.98		
HbT_lf	Non-demented	95	17.8	29.8	14.1	-.869	.385
	Patients with AD	14	12.6	35.5	10.5		
HbO_rf	Non-demented	100	20.3	28.3	15.2	-.043	.966
	Patients with AD	14	15.0	35.0	18.8		
HbR_rf	Non-demented	100	-4.37	13.3	-3.83	-1.60	.110
	Patients with AD	14	–10.5	11.9	–7.11		
HbT_rf	Non-demented	100	15.9	32.3	16.5	-.863	.388
	Patients with AD	14	4.45	29.0	10.7		
HbO_lp	Non-demented	92	23.1	31.7	19.2	-.495	.311*
	Patients with AD	14	26.8	34.5	21.2		
HbR_lp	Non-demented	92	-.217	11.8	.411	-.345	.365*
	Patients with AD	14	.764	13.5	–2.92		
HbT_lp	Non-demented	92	22.8	38.2	18.1	-.551	.291*
	Patients with AD	14	27.6	42.7	27.1		
HbO_rp	Non-demented	82	23.0	34.5	16.4	-.585	.280*
	Patients with AD	17	29.1	38.2	22.3		
HbR_rp	Non-demented	82	-.525	11.6	-.847	-1.06	.145*
	Patients with AD	17	–3.57	8.55	–4.73		
HbT_rp	Non-demented	82	22.4	39.9	14.6	-.315	.376*
	Patients with AD	17	25.5	41.7	20.5		
HbO_lt	Non-demented	97	21.5	31.7	15.9	-.594	.277*
	Patients with AD	15	16.8	28.2	15.6		
HbR_lt	Non-demented	97	1.95	13.4	.184	-1.99	.024*
	Patients with AD	15	–4.46	8.84	–4.85		
HbT_lt	Non-demented	97	23.5	41.0	17.0	-.799	.212*
	Patients with AD	15	12.3	29.1	10.2		
HbO_rt	Non-demented	93	17.7	29.1	14.4	-.694	.244*
	Patients with AD	16	22.0	29.3	23.3		
HbR_rt	Non-demented	93	-.567	12.3	-.268	-1.34	.090*
	Patients with AD	16	–4.01	16.4	–3.74		
HbT_rt	Non-demented	93	18.3	37.5	15.9	-.086	.466*
	Patients with AD	16	18.0	36.3	16.6		

*=one-tailed

6.4.2 Relationship between NIRS cerebral hemodynamics and NIRS task performance

The patients who performed the verbal fluency task (n=18) produced on average 44.2 words (SD=16.7, range=14 – 89, median=42.5) during the two activation periods (see section 4.3.2.1), significantly less than the non-demented elderly subjects (Z= -2.32, p = .021).

The results of the correlation analyses between NIRS parameters and NIRS task performance are displayed in Table 6-16. None of the correlation coefficients reached statistical significance, however there was a trend for a negative correlation between reductions in HbR at the left frontal position and the number of words produced in the fluency task (r_S = .524, p = .054): a strong reduction in HbR during activity compared to rest was associated with fewer words produced during NIRS. This finding for the group of AD patients is illustrated in Figure 18, together with the observations made in the non-demented subjects. Interestingly, with the exception of both parietal positions for which the correlation with task performance was close to zero, the directions of the correlations between changes in HbO and task performance were in the negative direction. There was a weak but non-significant correlation between the reduction in Cyt-Ox and task performance at the right parietal position (r_S = -.403 p = .109).

Table 6-16: Spearman Correlation Coefficients NIRS and NIRS task performance

	Frontal		Parietal		Temporal	
Left	HbO:	-.185	HbO:	.042	HbO:	-.374
	HbR:	.524	HbR:	.101	HbR:	.306
	Cyt-Ox:	-.306	Cyt-Ox:	-.154	Cyt-Ox:	.175
Right	HbO:	-.211	HbO:	.081	HbO:	-.172
	HbR:	-.055	HbR:	.271	HbR:	-.019
	Cyt-Ox:	.136	Cyt-Ox:	-.403	Cyt-Ox:	.239

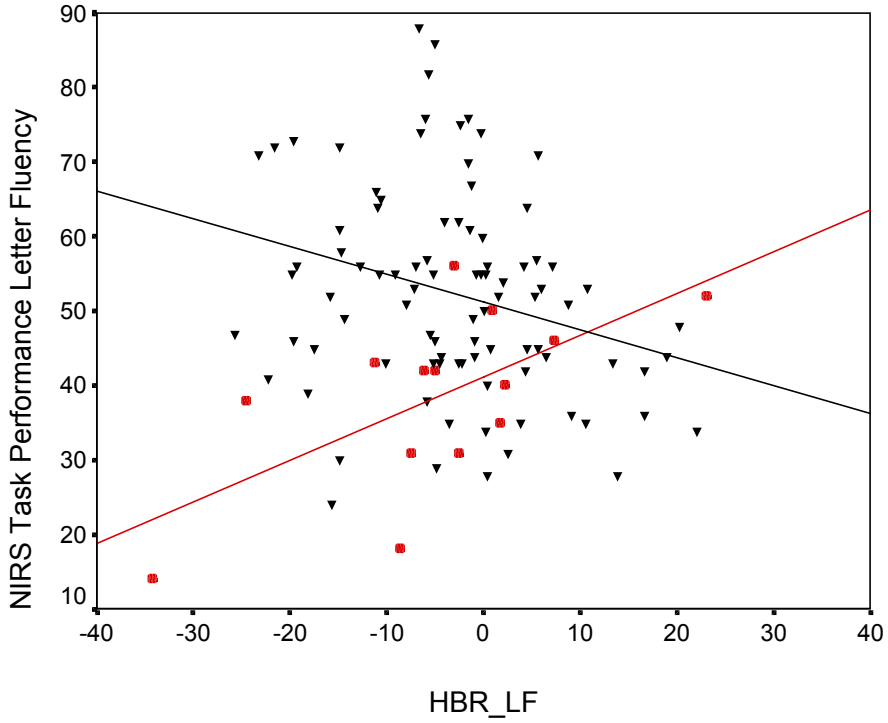


Figure 18: Scatterplot for correlation between NIRS task performance and concentration changes of HbR at the left frontal position.

▼ Non-demented elderly subjects

● Patients with AD

As in our group of non-demented elderly subjects left-frontal responders showed a highly significant better task performance than left-frontal non-responders, we were interested to find out whether this observation could be replicated in our sample of mild-stage patients with AD. An analysis of response type for the left frontal position revealed that out of the 15 patients with valid NIRS values at the left frontal position, 7 showed a typical NIRS response and 8 a non-typical response. The scores of both groups in the NIRS verbal fluency task are represented in Figure 19. The median values show a trend towards higher task scores for left-frontal non-responders (Z= -1.86, p = .064).

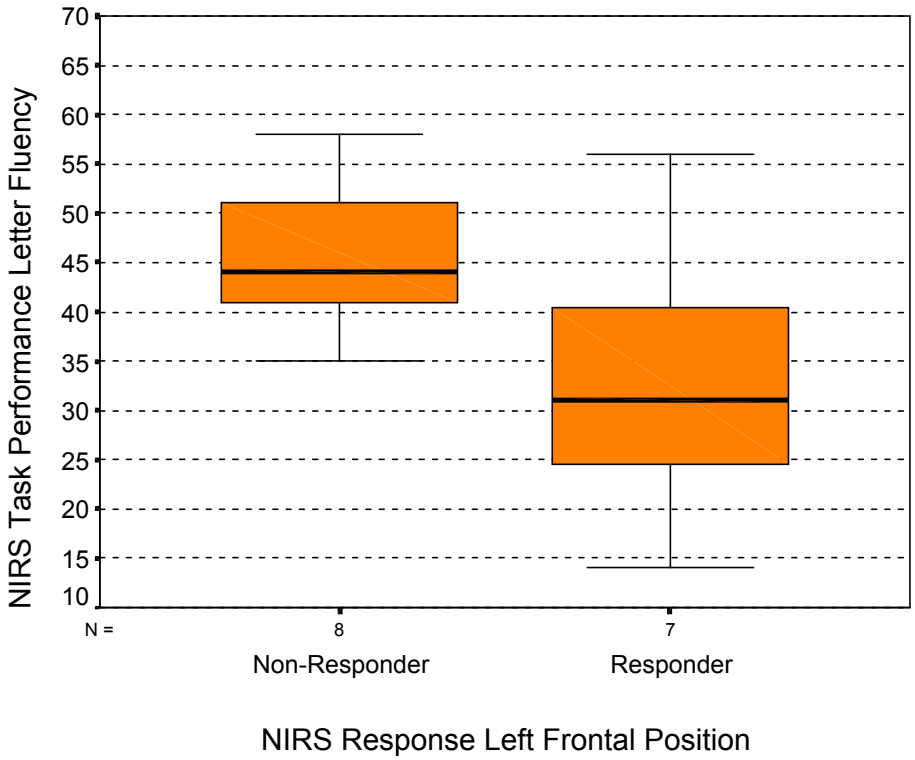


Figure 19: Scores in NIRS task performance of patients showing a typical NIRS response at the left frontal position („Responder“) vs. patients showing an untypical NIRS response („Non-Responder“)

6.5 NIRS and neuropsychology in mild-stage Alzheimer's Disease

6.5.1 Relationship NIRS – neuropsychology phonemic fluency

The result of the Pearson correlation analysis for the relationship between the performance in the NIRS letter fluency task and the score in the NP letter fluency task revealed a positive and significant correlation in our sample of patients with mild-stage AD as illustrated in Figure 20.

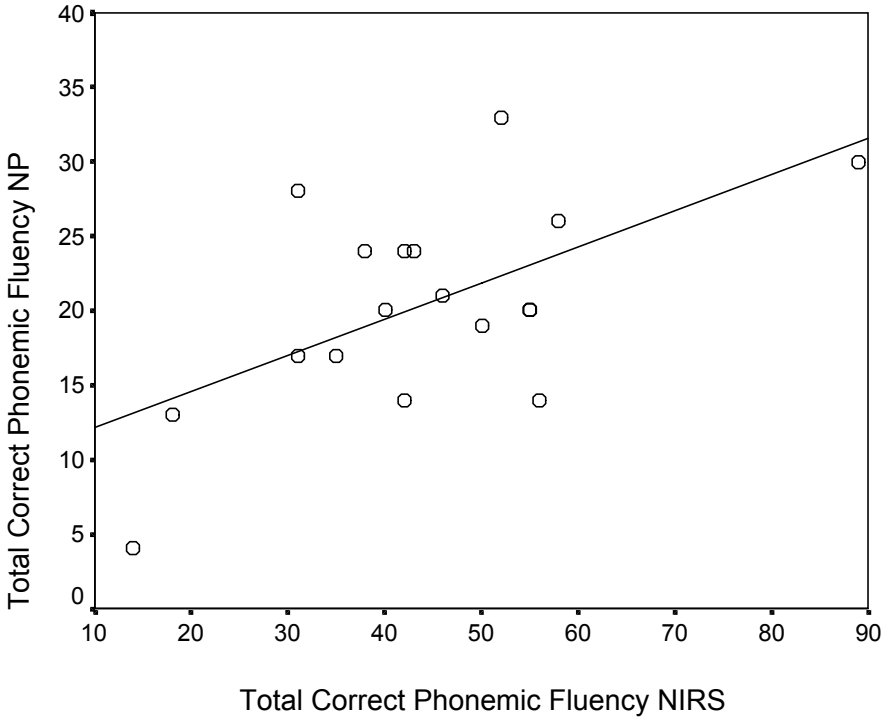


Figure 20: Correlation between performance in NIRS phonemic fluency and NP phonemic fluency. r =.591, p<.05

6.5.2 Neuropsychological test performance

The mean neuropsychological test scores of our patients with AD are displayed in Table 6-17 and in Table 6-18 in comparison to the scores achieved by our non-demented elderly subjects. With the exception of the MWT-B and the WAIS-R Similarities, the patient sample was markedly impaired in all tests of executive functioning, language, verbal learning and memory.

Table 6-17: Neuropsychological test performance of patients with mild-stage AD

	Valid N	Mean	SD	Min	Max
NIRS Letter Fluency	18	44.2	16.7	14	89
Phonemic Fluency: total correct	21	19.7	7.41	4	33
Category Fluency: total correct	21	33.1	12.2	10	59
WCST: total correct categories	18	4.17	1.79	1	6
WCST: perseverative errors	18	7.00	9.78	0	42.0
TMT-B: time needed (s)	18	256	126	119	617
TMT B/A	18	3.84	1.44	1.03	6.29
MWT-B: correct words	21	30.1	4.22	21	37
WAIS-R Vocabulary: total correct	19	16.8	5.86	2	27
WAIS-R Similarities: total correct	21	20.4	8.65	2	30
WAIS-R Block design: total correct	21	14.4	6.48	1	27
BNT: total spontaneous correct	21	30.0	8.54	8	42
CVLT: correctly recalled List A trials 1-5	21	28.5	8.64	17	48
CVLT: correctly recalled long delay	21	2.29	2.49	0	9

Table 6-18: Mean (SD) performance of patients with mild-stage AD (n=21) and non-demented elderly (n=119)

	AD Patients	Non-demented elderly	*Z*	p (2-tailed)
Phonemic Fluency: total correct	19.7 (7.41)	28.0 (9.20)	-3.96	p<.001
Category Fluency: total correct	33.1 (12.2)	50.4 (11.9)	-5.03	p<.001
WCST: total correct categories	4.17 (1.79)	5.23 (1.23)	-2.93	p=.003
WCST: perseverative errors	7.00 (9.78)	2.40 (3.11)	-2.89	p=.004
TMT-B: time needed (s)	256 (126)	135 (64.5)	-4.80	p<.001
TMT B/A	3.84 (1.44)	2.86 (1.09)	-3.20	p=.001
MWT-B: correct words	30.1 (4.22)	32.1 (3.58)	-2.19	p=.029
WAIS-R Vocabulary: total correct	16.8 (5.86)	21.6 (5.46)	-3.15	p=.002
WAIS-R Similarities: total correct	20.4 (8.65)	24.7 (5.30)	-2.17	p=.030
WAIS-R Block design: total correct	14.4 (6.48)	23.6 (7.69)	-4.89	p<.001
BNT: total spontaneous correct	30.0 (8.54)	39.2 (4.15)	-4.96	p<.001
CVLT: correctly recalled List A trials 1-5	28.5 (8.64)	50.6 (8.96)	-6.57	p<.001
CVLT: correctly recalled long delay	2.29 (2.49)	11.0 (2.76)	-7.05	p<.001

6.5.3 Do left-frontal NIRS responders show better neuropsychological test performance?

The mean neuropsychological test scores of left-frontal NIRS responders vs. left-frontal non-responders as well as the results of the multiple Mann Whitney-U Tests are displayed in Table 6-19. At an alpha of 5% there was no significant difference between the two groups.

Table 6-19: Mean (SD) performance of left-frontal NIRS responders vs. left-frontal non-responders

	Responders (n=7)	Non-Responders (n=8)	*Z*	p
Phonemic Fluency: total correct	17.7 (8.26)	21.8 (5.90)	-.931	.199*
Category Fluency: total correct	31.9 (12.2)	30.4 (11.8)	-.696	.536
WCST: total correct categories	4.43 (2.07)	3.71 (1.25)	-.977	.383
WCST: perseverative errors	8.57 (14.9)	8.00 (5.35)	-1.09	.318
TMT-B: time needed	223 (61.9)	210 (68.8)	≈.000	≈1.00*
TMT B/A	4.33 (1.20)	2.94 (1.30)	-1.28	.120*
BNT: total spontaneous correct	33.1 (7.12)	26.3 (10.1)	-1.33	.095*
WAIS-R Vocabulary: total correct	16.8 (8.18)	14.4 (4.08)	-.935	.366
WAIS-R Similarities: total correct	19.6 (10.1)	17.5 (9.24)	-.580	.310*
WAIS-R Block design: total correct	16.9 (5.98)	15.5 (5.42)	-.350	.399*
MWT-B: correct words	29.3 (5.22)	29.3 (3.66)	-.652	.536
CVLT: correctly recalled List A trials 1-5	27.0 (4.69)	25.0 (5.86)	-.406	.694
CVLT: correctly recalled long delay	2.29 (1.98)	1.00 (.760)	-.1.45	.189

*p = one-tailed

7 Summary and Discussion

7.1 Summary of background and purpose of the study

The data reported in this doctoral thesis were recorded in the context of the "Basel Study on the Elderly (BASEL)" project, an extensive multidisciplinary research project combining cross-sectional with longitudinal approaches in subgroups of individuals at lower and higher risks to develop AD. The analyses presented in this thesis focus on the cross-sectional aspect of the BASEL project, incorporating functional Near-Infrared Spectroscopy of the brain (NIRS), neuropsychology, and ApoE genotype data. The main objective of this thesis was to correlate cerebral hemoglobin oxygenation data obtained during functional NIRS with NIRS task performance and with selected neuropsychological tests, thus providing a better understanding of the brain-behaviour relationships in healthy elderly subjects and in patients with Alzheimer's Disease (AD).

We studied 240 non-demented elderly individuals selected from the project BASEL cohort (69 females, 171 males; mean age = 72.3 ± 7.03 years) and 21 patients with probable, mild Alzheimer's Disease (12 females, 9 males; mean age = 76.2 ± 6.37 years; mean MMSE = 25.3 ± 2.08) with multi-channel (left and right frontal, left and right parietal, left and right temporal) NIRS during performance of either a verbal (letter) fluency task (non-demented subjects: n=119; patients with AD: n=18) or a computerized labyrinth test (non-demented subjects: n=121; patients with AD: n=3). The patients with AD were significantly older than the non-demented elderly subjects but were comparable with respect to number of years of formal education. On the same day of the NIRS examination, the non-demented elderly subjects obtained a comprehensive neuropsychological assessment; the patients with AD had their NIRS examination on average 4.6 days after the neuropsychological assessment. In all study participants the ApoE genotype was determined using a polymerase chain reaction (PCR) analysis. The proportion of ApoE-ε4-positives and –negatives was evenly distributed in both study groups (non-demented elderly/patients with AD: 45% ApoE-ε4 positives, 55% ApoE-ε4 negatives).

As outlined in chapter 2, NIRS has recently gained increasing interest as a functional brain imaging technique. It is non-invasive, applicable at the bedside, and has low expense compared to more popular methods such as PET and fMRI. The method relies on a simple basic principle, the fact that brain activity is associated with changes in optical properties of brain

tissue. During increased brain activity and by means of the "neurovascular coupling" between neuronal activity and vascular response, there is an increase in local cerebral blood flow. This increase in cerebral blood flow however is exceeding the increase in oxygen consumption and thus leads to an increase in intravascular haemoglobin oxygenation during brain activity. Therefore, when the NIRS measuring optode is located over an area in which cerebral blood flow increases during brain activity, a localized increase in the concentration of oxygenated hemoglobin (HbO) and a decrease in deoxygenated hemoglobin (HbR) is seen.

The functional NIRS studies published so far have been predominantly conducted in healthy young volunteers and using a single-channel methodology. There are however only very limited functional NIRS data in normal elderly subjects and different patient groups, and the majority of studies conducted used only one or two optode positions. The study of Hock et al. (1995) in healthy elderly vs. healthy young subjects is the only investigation of age-related changes in cerebral hemoglobin oxygenation using a cognitive activation paradigm.

From the consistent finding of a typical, activation-induced NIRS response pattern, i.e. an increased concentration of HbO together with a decrease in reduced hemoglobin HbR over areas most probably activated by the stimulus, we sought to investigate whether this finding can be replicated in our unique sample of 240 non-demented elderly subjects. We were specifically interested in the NIRS response pattern during performance of the verbal fluency task, a well-established neuropsychological test and functional imaging activation paradigm, known to activate the left frontotemporal area of the brain (cf. chapter 3), and further investigated whether a specific NIRS response pattern relates to high task performance during the NIRS verbal fluency task. In a second step, incorporating sociodemographic factors such as age, gender, and years of formal education, and including a well-described risk factor for AD, the ApoE genotype, we investigated if and how these factors correlate with the NIRS data, and if they have a modifying effect on the brain response. In a third step, we sought to explore the brain-behaviour relationships of our non-demented elderly population by analysing the NIRS data in relation to the neuropsychological test data recorded on the same day.

Using the same research methodology, we investigated whether and to what extent our well-defined sample of patients with mild-stage probable Alzheimer's Disease (DSM-IV criteria) shows reductions in cerebral hemoglobin oxygenation and changes in brain-behavior relationships. As both study samples, non-demented elderly and patients with AD, had even distribution of ApoE-ε4 positives and –negatives, any differences in NIRS and brain-behaviour relationship between the two groups could not be attributed to the influence of ApoE genotype alone. Therefore, in the comparison of AD vs. non-demented elderly data, the ApoE genotype

was neglected.

7.2 NIRS response in non-demented elderly subjects

7.2.1 Do normal elderly subjects show a typical NIRS response?

Mean concentration changes of HbO, HbR and HbT obtained using NIRS in our sample of 240 non-demented elderly subjects revealed that elderly subjects on average show a typical hemoglobin oxygenation response pattern during cognitive activation as observed in young healthy volunteers (Hock et al., 1995; Hoshi et al., 1994; Hoshi and Tamura, 1993a; Fallgatter & Strik, 1997, 1998; Herrmann, Ehlis & Fallgatter, 2003; Villringer et al, 1993; Villringer et al., 1997; Schroeter, Zysset, Kupka, Kruggel & von Cramon, 2002): an increase of HbO and a decrease of HbR over the brain areas most probably activated by the stimulus. In detail, as we were applying a multi-channel approach, we found that increases in HbO were generic in that they occurred across all optode positions and for either activation task whereas significant reductions in HbR could only be found at the frontal positions and only for the verbal fluency task. Total hemoglobin, an indicator of changes in cerebral blood volume, also showed significant increases across all optode positions and for both activation tasks.

NIRS response during performance of a verbal (letter) fluency task: Our findings in non-demented elderly subjects and using NIRS are in good agreement with earlier PET functional activation studies in young and middle-aged adults that the performance of a letter fluency task is strongly associated with the left prefrontal cortex (Benton, 1968; Carlsson, Wendt & Risberg, 2000; Elfgren & Risberg, 1998; Frith, Friston, Liddle & Frackowiak, 1991a, b; Friston, Frith, Liddle & Frackowiak, 1993; Milner, 1964; Warburton et al., 1996; Warkentin & Passant, 1993). In addition to the strong activation of the left frontal cortex our non-demented elderly subjects also showed a strong activation of the right frontal area during performance of the letter fluency task. Activation of right frontal areas was also reported by Abrahams et al. (2003) in an fMRI study of letter fluency in a group of middle-aged normal subjects. This bilateral frontal activation could be an index of a compensatory re-allocation of cognitive resources which has been repeatedly observed in PET and fMRI studies on language and verbal memory encoding tasks that are strongly left-lateralized in young adults: older adults tend to show bilateral recruitment patterns (see Buckner, 2004 for a review).

7.2.2 Is the NIRS response observed in the elderly specific or generic?

Whereas concentration changes of HbO and HbT exhibited a pattern of general activation across the whole brain during performance of either cognitive task, the decrease in HbR was restricted to the frontal positions. Using one-sample t-tests we found that this frontal decrease in HbR only became significant for subjects activated with the verbal fluency task, whereas the subjects performing the labyrinth test showed a trend towards reduction in HbR at the right temporal optode position. Concluding from these findings we state that the NIRS response pattern is both: generic and specific: Cognitive activation – irrespective of the task requirements – leads to an increase of cerebral blood flow and blood volume across the whole brain. In other words, concentration changes of HbO reflect a state of arousal with a very high sensitivity. Reductions in HbR, rather an index of oxygen consumption, however are localized to areas most probably activated by the stimulus, and thus are specific. Our observations are in good agreement with earlier reports of Kleinschmidt et al. (1996), according to which the decrease in HbR strongly correlates with the signal intensity measured in BOLD fMRI, Hirth et al. (1996) and recently Schroeter et al. (2002), who found that reductions in HbR are more localized than increases in blood flow.

7.2.3 Investigating subgroups of the BASEL project

Although the mean concentration changes of HbO, HbR and HbT in our sample revealed a typical NIRS response pattern, there were large interindividual differences in hemoglobin oxygenation changes during brain activation. For example, at the left frontal position during performance of the verbal fluency task, 17% of subjects showed a decrease in HbO and 37% an increase in HbR. One source of variance could have been the fact that 26% of our study sample was composed of non-healthy subjects as defined by the inclusion/exclusion criteria of the BASEL project, i.e. subjects with either current sensory or motor deficits, severe systemic disease, a psychiatric or neurologic disorder; and/or a medical history of a CNS disease or events negatively affecting CNS activity in the past, all of which might be associated with a substantially greater variability in the NIRS data. Therefore, we compared the NIRS data of the non-healthy subjects (n=63) with the data of the healthy subjects (n=177). According to the results of Mann-Whitney U-Tests the two groups did not differ in their cerebral hemodynamics. They had comparable group variance for each NIRS parameter and a comparable number of outliers and extreme values. In conclusion, current neuropsychiatric findings and/or a medical history of CNS disease and/or events negatively affecting CNS activity in the past were not associated with a greater variability in the functional NIRS data. However, one major drawback

of our NIRS methodology needs to be mentioned at this point: the fact that absolute baseline concentrations of HbO and HbR could not be obtained. This would have required the exact quantification of the optical pathlength of the near-infrared light in brain tissue for each wavelength (see Hock et al., 1995). Therefore, since the absolute baseline value is not known, the measurements refer only to *changes* in absolute concentrations. Consequently, we cannot rule out that the two groups (non-healthy vs. healthy subjects) differed in their baseline values. Following the regression to the mean, if the non-healthy subjects' absolute baseline values were at a higher level, the probability for them to further increase during activation would be lower than for subjects who start off at a lower threshold.

7.2.4 Investigating "NIRS responders" versus "NIRS non-responders"

In the attempt to further elucidate the source of variance in the NIRS data, we further investigated our subject sample by classifying our subjects into "NIRS responders", i.e. subjects showing a typical NIRS response for HbO *and* HbR, and "NIRS non-responders", i.e. subjects showing an untypical NIRS response in either parameter. From the consistent findings of earlier PET and fMRI studies according to which the performance of a verbal fluency task is particularly related to the left frontal cortex, the NIRS response at the left-frontal optode position and the NIRS response during the performance of the verbal fluency task were of special interest. NIRS responders and non-responders were evenly distributed at both frontal positions, whereas the proportion of non-responders was markedly greater at the parietal and temporal positions. However, our data showed that the subgroup of left-frontal NIRS responders also had marked NIRS activity at the other optode positions – they did not show a specific left-frontal activity. In fact, left-frontal NIRS responders had the strongest increase in HbO at both parietal, left-temporal and right-frontal positions, whereas their maximum decrease in HbR was indeed at the left-frontal position. This finding is in agreement with what we observed earlier: concentration changes of HbO are a sensitive marker of brain activation but brain-topographically unspecific, whereas reductions in HbR are specifically localized to areas activated by the stimulus.

7.3 NIRS response in mild Alzheimer's Disease: preserved or reduced?

From earlier studies using PET and SPECT in mild AD, a characteristic pattern of reduced cortical metabolism and blood flow predominantly in the parieto-temporal cortex has emerged (for reviews see Matsuda, 2001; Rapoport, 1991). Using functional NIRS, marked reductions of HbO and HbT over the left parietal cortex were observed whereas the

hemodynamic response over the left frontal cortex revealed the typical hemoglobin oxygenation response pattern (Hock et al., 1997). On the basis of these findings we expected a reduced hemodynamic response in the parieto-temporal cortex together with a spared response over the frontal area.

In contrast to our expectations we found that our patients with mild AD showed a typical hemoglobin oxygenation response pattern during performance of a cognitive task compared to rest: increases in concentrations of HbO and decreases in HbR. In more detail, the strongest increases in cerebral blood flow were observed at both parietal positions and at the right temporal position, whereas the increases at both frontal positions were marked but statistically non-significantly different from zero. Concentrations of deoxygenated hemoglobin showed a strong trend towards reduction at the right frontal position and to a smaller degree at the left temporal position. Cerebral blood volume – concentration changes in HbT – increased particularly at the parietal positions and to a minor degree at the temporal and frontal positions. Finally, in comparison to our sample of non-demented elderly subjects (n=119) the patients activated with the letter fluency task (n=18) did not show a reduced hemodynamic response. The patients with AD were significantly older than the non-demented subjects and they contained a larger portion of female subjects, but they did not differ with respect to years of education. However, as will be discussed later, our findings in non-demented elderly showed that the sociodemographic factors “age” and “gender” do not have an effect on the NIRS response.

Our finding of preserved hemodynamic response during cognitive action in mild AD is in contrast to the report of Hock et al. (1997a), who found a reduced response over the left parietal cortex together with a preserved response over the left frontal cortex. This dissociation between left-frontal and left-parietal activity was interpreted in line with Grady et al (1993) who proposed a compensatory reallocation of cognitive resources by means of increased utilization of the frontal cortex during memory tasks due to an altered functional brain organization. However, the patients of Hock et al. were at the moderate stage of the disease, whereas our patients were at a very mild stage of AD and showed no reduction in NIRS response at any of the cortical positions measured. One possible explanation could be that our patients were just at the beginning of an altered brain organization, with the functional brain networks still being relatively intact.

The results of our NIRS study in patients with mild AD do not seem in line with the functional rCBF study of Warkentin and Passant (1997) who described a frontal dysfunction of their patients with probably mild to moderate AD during performance of a letter fluency task.

As reported by other groups, they found a regional flow pathology in the temporal and parietal areas bilaterally during rest while frontal and central areas seemed normal. During performance of the letter fluency task the AD patients had a significant activation in the Broca's area only, whereas - in contrast to the control subjects – the remaining frontal areas showed no significant changes. They concluded that the findings of a widespread frontal dysfunction in the AD patients could be the result not only of a primary frontal deficit, but also a dysfunction in any part of the system normally involved in this task. Altered brain functional connectivity was also proposed by Grady, Furey, Pietrini, Horwitz and Rapoport (2001) using a face memory task in patients with mild AD and found a functional disconnection between the prefrontal cortex and the hippocampus - a disruption of the distributed network that includes these two areas.

Another, more methodological contributor to our findings, could have been a different concentration level of HbO and HbR at baseline yet leading to similar delta values: If, as described in the literature, patients with mild AD have preserved resting blood flow over the frontal cortex and reduced resting blood flow over parieto-temporal areas, the regional flow over the frontal cortex is less likely to further increase during activation as compared to the parieto-temporal cortex. Consequently, the *changes* from rest to activation would turn out to be relatively smaller for the frontal cortex as compared to parietal and temporal areas as found in our study.

Atrophy and an altered mechanism of coupling between brain cell activity and blood flow may confound the interpretation of activation differences between non-demented and demented elderly subjects. In the early stages of AD there may be hemodynamic and metabolic abnormalities that are part of the neurodegenerative process and could affect the neurovascular coupling. Johnson et al. (2000) were the first to investigate the relationship between region-specific and whole-brain atrophy and BOLD fMRI activation effects in normal aging and AD. No significant correlations between region-specific or whole-brain atrophy and fMRI activation were observed among controls covering a broad age range. In contrast, the patients with mild AD showed a strong positive correlation between the degree of atrophy in the left inferior frontal gyrus and the magnitude of activation - greater atrophy was associated with greater activation - suggesting that compensatory activation may require a certain level of preservation of brain structure (Saykin et al., 2004). However, as Johnson et al. conclude, it remains yet unclear whether the compensatory response is a response at the neural level or at the vascular level.

Our results are partially in line with the findings of Fallgatter, Roesler, Sitzmann, Heidrich, Mueller and Strik (1997) who found a strong left-lateralized response in HbO in their

normal controls during performance of a letter fluency task. In contrast, their patients with AD showed increases in HbO at the left and right frontal positions. Patients and controls did not significantly differ in their concentration changes in HbR, which - according to our findings in a much larger sample and using a multi-channel NIRS approach - seems to be the more specific NIRS parameter. A methodological drawback of this study is the selection of the control group which consisted of healthy physicians and psychologists and was not matched to the patient group with regard to age and years of education. Furthermore, the patients of Fallgatter et al. were in a more progressed state of the disease, and like in many other imaging publications in AD, mild and moderately severe states were pooled together into the same sample without providing fundamental characteristics of the sample (e.g. performance in the ADAS-Cog which was part of the test battery).

A final consideration needs to be made with regard to acetylcholinesterase (AChE) inhibitor treatment which at the time of the investigation was taken by 62% of our patients (11 patients rivastigmine, 2 patients donepezil). Little is known about how AChE inhibitors affect functional brain systems. Single doses of cholinesterase inhibitors have been shown to normalize quantitative electroencephalographic patterns in patients with AD (Lanctot et al., 2003), and PET and SPECT studies have shown preserved or increased cerebral blood flow in AD patients treated with cholinesterase inhibitors (Matsuda, 2001; Mega et al., 2001). There are indications that AChE inhibitors exert regionally specific effects on frontal regions rather than a global effect on brain activity. In the recent study of Kaasinen et al. (2002) direct effects of donepezil and rivastigimine on AChE activity (after 3 and 5 months of treatment, respectively) were measured in the frontal, temporal, and parietal cortices of patients with mild to moderate AD. Treatment with donepezil reduced the AChE activity in the AD brain by 39% in the frontal, 29% in the temporal, and 28% in the parietal cortex, and the corresponding levels of inhibition for rivastigmine were 37%, 28% and 28%. Treatment with either AChE inhibitor led to significantly greater inhibition of AChE in the frontal cortex compared to the temporal cortex. According to Saykin (2004), a preferential frontal response could be related to the fact that the frontal cortex is relatively spared in the early stages of AD compared with temporal and parietal regions and therefore has greater structural integrity with which to support increased activation in response to pharmacological stimulation. Altogether, because the AChE inhibitor treatment was not standardized in our study and the sample is to small to allow for comparisons between treated and untreated patiens, we cannot rule out that AChE treatment did have a normalizing effect on the NIRS response.

7.4 Covert and overt behaviour: relationship between NIRS response and NIRS task performance

Functional imaging allows to investigate the relationship between „covert“ and „overt“ behaviour, i.e. between brain activity measured in the scan and task performance displayed during the scan. PET and fMRI studies have consistently shown that the performance of a verbal fluency task is highly associated with the activation of the left frontal and frontotemporal cortex (see section 7.2.1, p. 131). The evidence however is inconsistent for the relationship between brain response and performance in the verbal (letter) fluency task during the scan: A positive correlation between task performance and cerebral blood flow augmentations in the left prefrontal cortex was found (Elfgren and Risberg, 1998), as well as a negative correlations between fluency scores and normalized metabolised rates across the whole cortex (Parks et al., 1988). No significant correlations between frontal lobe activation and task performance was reported by Warkentin and Passant (1993) measuring rCBF, and by Abrahams et al. (2003) using BOLD fMRI. Using NIRS, no significant correlation between concentration changes of HbO in prefrontal areas and task performance was found by Herrmann, Ehlis and Fallgatter (2003).

7.4.1 Non-demented elderly subjects

On the basis of the above findings and for our sample of 119 non-demented elderly subjects activated with the letter fluency task we hypothesized a significant correlation between the NIRS cerebral hemodynamics and task performance particularly at the left frontal optode position. A minor correlation at the left temporal position, and no significant correlation at the parietal and right temporal position was expected. In line with our expectations, pearson correlation analyses revealed numerically small but statistically significant correlations between NIRS task performance and concentration changes of HbR at the left frontal and the left temporal position. In other words, subjects with a higher NIRS task performance had a stronger reduction in deoxygenated hemoglobin, the brain-topographically specific NIRS parameter. In contrast to our expectations, there was also a significant correlation for task performance and increases in oxygenated hemoglobin, an index of generic brain activation, at the right parietal optode position. The analysis of NIRS responders vs. NIRS non-responders confirmed the finding of a correlation between NIRS response and task performance: Subjects with a typical NIRS response pattern at the left frontal position performed significantly better in the NIRS letter fluency task than left-frontal non-responders. There were trends of better task

performance for responders vs. non-responders at the right frontal, left parietal and left temporal position, but only the left-frontal position showed a marked dissociation between responders and non-responders with regard to the performance achieved during the cerebral oxygenation measurements.

Our findings are in agreement with the observations of Elfgren and Risberg (1998) who found a positive correlation between brain activity in the left prefrontal cortex and task performance: Subjects able to bring the brain areas critical for successful performance in a cognitive task on-line, show better task performance. This interpretation is receiving additional support from our finding that particularly concentration changes in the specific NIRS parameter HbR are related to efficient task performance, whereas flow increases as a result of brain activation vs. rest play a minor role.

7.4.2 Patients with AD

A number of rCBF studies suggest that the relationship between regional cerebral blood flow and verbal fluency in Alzheimer's disease is comparable to what has been observed in healthy volunteers, i.e. fluency scores are significantly and positively correlated with the left prefrontal blood flow (Hirono et al., 2001; Keilp et al., 1999; Kitabayashi et al., 2001). However, these studies mostly used resting-brain imaging instead of functional imaging.

Compared to the sample of non-demented elderly subjects our patient sample with mild-stage AD performed significantly worse in the NIRS letter fluency task. Correlation analyses between NIRS response and NIRS task performance in our small patient sample revealed no statistically significant relationship, however there was a strong trend for a moderately *negative* correlation between reductions in HbR at the left frontal position during activity compared to rest and number of words produced during the NIRS letter fluency task. Also in the negative direction were the correlations between activity-related increases in HbO at both frontal and temporal positions and task performance. At both parietal positions the correlation with task performance was close to zero. The investigation of NIRS responders vs. NIRS non-responders in our patient sample confirmed the correlational results: The patients who showed an untypical NIRS response at the left-frontal position had a strong trend towards higher task performance than left-frontal NIRS responders.

Altogether, the relationship between covert and overt behavior in our homogenous sample of patients with mild AD proved to be completely different from what we found in the elderly non-demented group: The patients with AD could not make use of the left frontotemporal network required for an efficient task performance as did the group of cognitively intact elderly

subjects. This observation could be interpreted in agreement with the „novice vs. expert“ metaphor used by Parks et al. (1988): Although the novice needs to invest more effort to solve a task than the expert, his performance is yet lower. In the study of Hock et al. (1997), investigating left frontal and left parietal cerebral blood oxygenation during performance of a letter fluency task and in a small sample of patients with probable moderate AD, no correlations were found between NIRS and verbal fluency test scores. As mentioned earlier, their sample of patients was at the moderate stage of the disease, and they showed marked reductions in concentration changes of HbO and HbT over the left parietal cortex during brain activation as compared to rest.

A common notion in the relationship between brain response and task performance is the concept of “cognitive reserve”. It posits that “...compensatory or other forms of reserve factors, such as education level and intelligence, mitigate cognitive decline” (Buckner, 2004, p. 203). In aging and dementia, neural mechanisms of reserve may include the reorganization of brain pathways and recruitment of atypical brain pathways, such as e.g. the activation of (additional) frontal brain regions during performance in memory tasks (Buckner, 2004). Grady et al. (2003) have recently corroborated their earlier findings that patients with AD can make use of additional neural resources in the frontal cortex to compensate for losses attributable to the degenerative process of the disease. One of the novel contributions from their experiment was the finding that patients who had more activity in bilateral prefrontal areas performed better in tasks of semantic and episodic memory, in other words, recruitment of additional prefrontal areas was associated positively with better task performance. As attractive as the concept of compensatory recruitment may be, the findings of our study do not support it: First, our patients with AD were comparable in their cerebral hemoglobin oxygenation response pattern to our non-demented elderly subjects. Second, the activation of the frontal areas during performance of the letter fluency task (concentration changes in HbO) were negatively correlated with task performance.

7.5 Interactions of NIRS with sociodemographic factors and ApoE genotype in non-demented elderly subjects

Calculating multiple linear regression analyses we investigated whether the sociodemographic factors age, years of formal education, and gender, as well as a well-known risk factor for AD, the ApoE genotype (ε4 vs. non-ε4), act as predictors of NIRS response in the total sample of non-demented elderly subjects activated with either the letter fluency or the

computerized labyrinth test. We were specifically interested to find out whether we could 1) replicate the findings of Hock et al. (1995), who found a significant negative correlation between age and concentration changes in HbT at the left-frontal position and during performance of a calculation task, and 2) whether ApoE-ε4 carriers present significantly lower concentration changes in HbT at the left parietal position, which could be indicative of a disruption of functional circuits involving the left parietal lobe in ApoE-ε4 carriers.

The results of the multiple linear regression analyses were clear: Neither the sociodemographic factors nor the ApoE genotype acted as predictors of NIRS response: nor for the specific parameter HbR nor for the parameter HbT.

Against our expectations, in our sample of cognitively intact elderly subjects age did not correlate with any of the NIRS parameters at any position. This finding is in contrast to the report of Hock and colleagues who detected an effect of physiological aging on the activation-induced increase in cerebral hemoglobin oxygenation. The young subjects in their study demonstrated significantly higher concentration changes in HbO and HbT at the left frontal position than the elderly subjects, whereas both groups were comparable in their concentration changes of HbR. The negative correlations between age and HbT clearly were of linear nature, even for the subgroup of elderly subjects alone. In contrast to Hock et al. (1995) no young control subjects were included in our study, and a different activation paradigm was used. The calculation task used by Hock et al. might have been more challenging than our letter fluency task. Even though no group of young control subjects was included, the age range in our study sample (54 – 90 years) still was big enough to allow for a correlation with NIRS response. Interestingly, the "young old" in our sample (54-63 years, n=24) did not differ in their NIRS characteristics from the "oldest old" (81-90 years, n=26). In conclusion, in elderly cognitively intact subjects the calendar age is not the critical factor to determine brain activation during mental tasks Therefore, in following Hock et al.'s interpretations of their findings, we cannot support the hypothesis that there is „less activation" of brain tissue and thus less metabolic demand during brain stimulation as elderly subjects age, i.e. in young-old vs. old-old subjects, as might be assumed on the basis of a linear decrease in HbO and HbT with age.

Another important result of our study is the finding that the ApoE genotype (ε4 versus non-ε4) only explains a very small percentage of the NIRS response in normal elderly subjects, less than 5% in the case of concentration changes of HbT at the left frontal position. Specifically, ApoE-ε4 carriers did not differ from ApoE-ε4 non-carriers with regard to concentration changes of total hemoglobin at the left parietal optode position, the position of

interest in the context of ApoE-ε4 as preclinical marker of AD. In conclusion, the cross-sectional data of our study in a large sample of non-demented elderly subjects are not indicative of an altered cerebral hemoglobin oxygenation pattern of subjects at higher genetic risk to develop AD. This is in agreement with the findings of Schmidt et al. (1996), who, similar to our investigation, performed brain imaging and ApoE genotyping as well as a detailed neuropsychological test battery in a large sample of normal elderly subjects. The ε4 carriers in their sample had comparable hippocampal and parahippocampal volumes compared to non-carriers, and there were no significant white matter differences between the two groups. However, like most studies in this area of research, Schmidt and colleagues applied a resting-brain imaging methodology. There are only a few studies available to date which have investigated the influence of ApoE genotype on brain activation. These studies used small sample sizes and did not control for task performance during the scan, which might have had a confounding effect on the measured brain response. Altogether, even though the ApoE-ε4 allele plays a major role in AD symptomatology and AD progression once the disease has developed, the predictive value of ApoE-ε4 in cognitively normal elderly subjects seems irrelevant.

7.6 Brain-behaviour relationships: NIRS and neuropsychological test performance

With the cross-sectional design of the BASEL project, compiling functional NIRS and neuropsychological test data collected on the same day, we investigated brain-behaviour relationships in a study sub-sample of 119 non-demented elderly subjects activated with the verbal fluency paradigm during NIRS and in the sample of patients with mild AD. Verbal fluency tasks are well established in functional imaging and the brain areas involved in solving these tasks are well known from earlier PET and fMRI studies. Another major advantage of focussing on the NIRS verbal fluency task in our search for brain-behaviour relationships was the fact that verbal fluency was also assessed outside the imaging and within the scope of the neuropsychological test battery. As a vantage point, the NIRS verbal fluency score highly correlated with the NP fluency score, suggesting that the brain activity measured during NIRS was indeed a function of performing a verbal fluency task, and not only mere vocalisation artifact and noise.

7.6.1 Non-demented elderly subjects

The first route in examining brain-behaviour relationships led us to investigate whether

NIRS is a predictor of performance in selected neuropychological tests: 1) WAIS-R Vocabulary as a measure of general intellectual functioning, 2) Category and Phonemic Fluency as measures of language and executive functioning, 3) word list learning as measured in the California Verbal Learning Test (CVLT), and 4) the quotient of the time needed in the Trail Making Test Forms B over A (TMT B/A) as measure of executive functioning. With the exception of the Category Fluency, every regression model was highly significant. The inclusion of the factors „age“ and „ApoE-ε4 status“ into the regression analyses did not markedly alter the prediction of the neuropsychological test scores The NIRS parameters predicted 27% of the variability of the WAIS-R Vocabulary test score, 28% of the Phonemic Fluency, 17% of the CVLT learning, and 42% of the quotient TMT B/A. For the quotient TMT B/A the most important predictor variables were concentration changes of HbO at the left and right frontal position and concentration changes of HbR at the right frontal and the left temporal position: A reduction in HbR and an increase in HbO at the right frontal position was associated with better performance in executive functioning whereas increases in HbO at the left frontal position and decreases in HbR at the left temporal positions were correlated with lower abilities in executive functioning. In other words, and in the context of a typical activation-induced cerebral hemoglobin response pattern, activation of the right frontal cortex in cognitively intact elderly subjects during a verbal fluency task as measured with NIRS is a good predictor of performance in executive functioning - a measure which is well known to be associated with the frontal lobe.

Our second route in exploring brain-behaviour relationships followed the comparison of left-frontal NIRS responders with left-frontal NIRS non-responders. Based on the expectation that there are positive correlations between covert and overt behaviour, i.e. NIRS and NP task performance in the verbal fluency task, we hypothesized that left-frontal NIRS responders show predominantly better performance in neuropsychological tests of executive function, whereas they do not differ from left-frontal non-responders in tests of general intellectual functioning. The results of our study were largely in line with our hypothesis: left-frontal NIRS responders scored higher in tests of executive function: Phonemic Fluency, time needed in the TMT B and as a trend in the quotient TMT B/A. In contrast to our expectations, the two groups did not significantly differ in the Wisconsin Card Sorting Test (total correct categories, perseverative errors). Further, the subjects with a typical NIRS response at the left frontal position performed markedly better in the WAIS Block Design and slightly better in the Boston Naming Test and the WAIS Similarities. The markedly better performance of the responders in the WAIS Block Design Test might be related to the demands of the tasks, i.e. problem-solving under time

pressure, which is also a key element of the letter fluency task. In fact, in our sample of non-demented elderly subjects activated with the verbal fluency test, there was a significantly positive correlation between the performance in the Block Design and the WAIS Similarities and the Phonemic Fluency test.

A systematic dissociation in neuropsychological test performance of subjects with a typical, activation-induced cerebral hemoglobin oxygenation pattern from subjects who did not show a typical NIRS response could only be found at the left-frontal optode position, the cortical area known to be predominantly involved in the performance of a verbal fluency task. This observation of a relationship between brain activity and neuropsychological test performance corroborates our findings of covert and overt behaviour: Subjects able to bring the brain areas critical for successful performance in a cognitive task on-line, show better task performance.

7.6.2 Patients with AD

In comparison to the non-demented elderly subjects, the patients with AD on average performed significantly worse in tests of executive functioning, language, verbal learning and memory, but were relatively comparable in premorbid IQ measured with the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B). On the level of NIRS however, we found a preserved hemodynamic response during cognitive activation, and, like in the group of cognitively intact elderly subjects, found patients with a typical NIRS response pattern over the left-frontal cortex.

Based on the observations in the sample of non-demented elderly described above and applying the same research strategy, we investigated whether the left-frontal NIRS responders in the patient sample show better performance in tests of executive functioning. The results of Mann Whitney-U tests at the 5% alpha level revealed no significant difference between the two groups, however, as a weak trend, the left-frontal NIRS responders on average had *lower* scores in the Phonemic Fluency and in the TMT B/A which was in contradiction to our expectations but fits well with our data of covert vs. overt behaviour, i.e. a negative correlation between activation-induced hemodynamic changes in the left-frontal cortex and NIRS task performance. Altogether, our data are in favour of a distorted relationship between brain and behaviour in patients at a very mild stage of AD. However, given the relatively small sample size of our patient group, a more substantial investigation in a bigger patient sample is needed.

8 Conclusions for future research

Alzheimer's Disease (AD), a progressive neurodegenerative disorder, affects more than 5% of individuals over the age of 65 and increases up to 50% in those over 85. Adults over 65 years of age are the fastest growing segment of the population in Western industrialized countries. Thus, with the high prevalence rates indicated above, AD represents one of the most challenging public health problems of the near future. As of today, there is no means to prevent or cure AD once the disease has been diagnosed, i.e. at a stage at which significant pathological changes (loss of synapses, deposition of tangles and plaques) have already occurred. As a consequence, treatments aimed at preventing the disease will intervene too late at this stage. Therefore, the detection of preclinical markers of AD is an important objective in the search for the etiology of AD and would allow treatment of AD to be initiated as early as possible in the disease process when the least amount of neurodegeneration has taken place.

The identification of preclinical markers of AD is the primary objective of the "Basel Study on the Elderly (BASEL)" project, in which the data presented in this doctoral thesis were recorded. In the search for preclinical markers of AD, the BASEL project applies a research strategy which combines multidisciplinary cross-sectional with longitudinal approaches in subgroups of individuals at lower and higher risk to develop AD. Once some of these subjects develop overt AD, baseline comparisons would identify the preclinical markers of this sample. Near-Infrared Spectroscopy (NIRS) of the brain was used to measure cerebral hemoglobin oxygenation during cognitive activation in a unique sample of 297 healthy elderly subjects, evenly distributed for high vs. low risk for AD based on the presence or absence of at least one ApoE-ε4 allele, and in a homogenous sample of 21 patients with mild AD. The contribution of NIRS to the detection of preclinical markers of AD was not the focus of this thesis. However, with the data gathered from our cross-sectional investigations combining NIRS, neuropsychological and ApoE genotype data, we can draw some conclusions for future research.

The data presented in this thesis do not support the notion that cognitively normal elderly subjects at a higher risk to develop AD, i.e. ApoE-ε4 positives, show differences in cerebral hemoglobin oxygenation compared to those without an ApoE-ε4 allele. In fact, the ApoE genotype explained less than 5% of the NIRS signal, making the ApoE-ε4 allele a very unsuitable preclinical marker of cortical brain response in the normal elderly population. However, with regard to longitudinal analyses, it would be interesting to investigate the

baseline NIRS response of those ApoE-ε4-positives who convert to AD.

In comparison to our sample of non-demented elderly subjects, the patients with mild-stage AD did not show a reduced hemodynamic response during cognitive activation as compared to rest. NIRS proved to be sensitive to cognitive activation in both sample groups, however, our finding of comparable NIRS response between normal and demented individuals puts the specificity of the NIRS signal into question. NIRS has a spatial resolution in the range of several cm^3 and thus is clearly behind PET and fMRI. Another methodological drawback of NIRS as applied in our study is that absolute baseline values of the chromophores could not be measured. Thus, we cannot rule out that major baseline differences between normal and mildly-demented patients might have played a role. As a consequence, improvements of the NIRS technique would be desirable for the future.

The advantage of functional imaging is that it allows not only to investigate the brain in response to cognitive demand, but also to study the *relationships between covert and overt behaviour*, i.e. between brain response during the task and performance in the imaging task – or as stated by Sir Francis Bacon " A prudent question is one-half of wisdom". One of the most interesting findings of our analyses is that normal elderly subjects and patients with AD show clearly distinguishable profiles in their relationship between covert and overt behaviour: Cognitively normal elderly subjects under cognitive demand showed increased activation of those brain areas responsible for the performance of the task, and higher activation of these brain areas was associated with better task performance. In patients with mild AD, albeit the NIRS response pattern was comparable to the one of the normal elderly, task performance during the scan was negatively correlated with the brain response. The patients with AD could not make use of the cortical network required for an efficient task performance as did the group of cognitively normal elderly subjects. In the same direction were the results of our investigations of brain-behaviour relationships, relationships between brain response and performance in several selected neuropsychological tests collected in the same day. Subjects with a typical, activation-induced NIRS response at the left-frontal position (left-frontal NIRS responders) during performance of a verbal fluency task not only showed better task performance but also scored higher in neuropsychological tests of executive function. In contrast, left-frontal NIRS responders in the patient group showed a trend for lower scores in tests of executive functioning. Altogether, our data are in favour of a distorted relationship between brain and behaviour in patients at a very mild stage of AD. As a consequence for future functional imaging studies, the analysis of brain-behaviour relationships might add valuable benefit to the investigation of the brain response alone. This field of research might

also be interesting for future clinical drug trials in AD, in developing more sensitive outcome measures.

REFERENCE LIST

Abrahams, S., Goldstein, L.H., Simmons, A., Brammer, M.J., Williams, S.C., Giampietro, V.P., Andrew, C.M., & Leigh, P.N. (2003). Functional magnetic resonance imaging of verbal fluency and confrontation naming using compressed image acquisition to permit overt responses. *Hum Brain Mapp, 20,* 29-40.

Adelson, P.D., Nemoto, E., Colak, A., & Painter, M. (1998). The use of near infrared spectroscopy (NIRS) in children after traumatic brain injury: a preliminary report. *Acta Neurochir Suppl (Wien), 71,* 250-4.

Allison, P. (2002). *Missing Data.* Thousand Oaks, CA: Sage: Sage University Papers Series on Quantitative Applications in the Social Sciences.

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Mental Disorders 4th ed (DSM-IV).* Washington, DC.: American Psychiatric Association.

Army Individual Test Battery. (1944). *Manual of directions and scoring.* Washington D.C.: War Department, Adjudant General's Office.

Bailey, T.L., Rivara, C.B., Rocher, A.B., & Hof, P.R. (2004). The nature and effects of cortical microvascular pathology in aging and Alzheimer's disease. *Neurol Res, 26,* 573-8.

Barfield, C.P., Yu, V.Y., Noma, O., Kukita, J., Cussen, L.J., Oates, A., & Walker, A.M. (1999). Cerebral blood volume measured using near-infrared spectroscopy and radiolabels in the immature lamb brain. *Pediatr Res, 46,* 50-6.

Benton, A. (1968). Differential behavioral effects in frontal lobe disease. *Neuropsychologia,* 53-60.

Benton, A.L., & De Hamsher, K. (1989). *Multilingual Aphasia Examination.* Iowa City, Iowa: AJA Associates.

Borbély, A. (1991). *Das Geheimnis des Schlafs: neue Wege und Erkenntnisse der Forschung*. Frankfurt/M; Berlin: Ullstein.

Buchner, K., Meixensberger, J., Dings, J., & Roosen, K. (2000). Near-infrared spectroscopy--

not useful to monitor cerebral oxygenation after severe brain injury. *Zentralbl Neurochir, 61,* 69-73.

Buchsbaum, M.S., Nuechterlein, K.H., Haier, R.J., Wu, J., Sicotte, N., Hazlett, E., Asarnow, R., Potkin, S., & Guich, S. (1990). Glucose metabolic rate in normals and schizophrenics during the Continuous Performance Test assessed by positron emission tomography. *Br J Psychiatry, 156,* 216-27.

Buckner, R. (2004). Memory and Executive Function in Aging and AD: Multiple Factors that Cause Decline and Reserve Factors that Compensate. *Neuron, 44,* 195-208.

Cabeza, R., & Nyberg, L. (2000). Imaging cognition II: An empirical review of 275 PET and fMRI studies. *J Cogn Neurosci, 12,* 1-47.

Carlsson, I., Wendt, P.E., & Risberg, J. (2000). On the neurobiology of creativity. Differences in frontal activity between high and low creative subjects. *Neuropsychologia, 38,* 873-85.

Chance, B. (1993). NMR and time-resolved optical studies of brain imaging. *Adv Exp Med Biol, 333,* 1-7.

Chance, B., Villringer, A., Dirnagl, V., & Einhaupl, K.M. (1992 Aug). Optical imaging of brain function and metabolism. Garmisch- Partenkirchen, 21-22 October 1991. *J Neurol, 239,* 359-60.

Cherry, S.&.P.M. (1996). Imaging brain function with positron emission tomography. In Anonymous, (pp. 191-222). San Diego: Academic Press.

Cooper, C.E., Cope, M., Quaresima, V., Ferrari, M., Nemoto, E., Springett, R., Matcher, S., Amess, P., Penrice, J., Tyszczuk, L., Wyatt, J., & Delpy, D.T. (1997). Measurement of cytochrome oxidase redox state by near infrared spectroscopy. *Adv Exp Med Biol, 413,* 63-73.

Cooper, C.E., Cope, M., Springett, R., Amess, P.N., Penrice, J., Tyszczuk, L., Punwani, S., Ordidge, R., Wyatt, J., & Delpy, D.T. (1999 Jan). Use of mitochondrial inhibitors to demonstrate that cytochrome oxidase near-infrared spectroscopy can measure mitochondrial dysfunction noninvasively in the brain. *J Cereb Blood Flow Metab, 19,*

27-38.

Cooper, C.E., & Delpy, D.T. (1997). Introduction to a Discussion on Near-infrared spectroscopy and Imaging of Living Systems. *Philosophical Transactions: Biological Sciences, 352,* 647-648.

Cooper, C.E., Delpy, D.T., & Nemoto, E.M. (1998 Jun 15). The relationship of oxygen delivery to absolute haemoglobin oxygenation and mitochondrial cytochrome oxidase redox state in the adult brain: a near-infrared spectroscopy study. *Biochem J, 332 (Pt 3),* 627-32.

Cooper, C.E., Elwell, C.E., Meek, J.H., Matcher, S.J., Wyatt, J.S., Cope, M., & Delpy, D.T. (1996 Jan). The noninvasive measurement of absolute cerebral deoxyhemoglobin concentration and mean optical path length in the neonatal brain by second derivative near infrared spectroscopy. *Pediatr Res, 39,* 32-8.

Cooper, C.E., Matcher, S.J., Wyatt, J.S., Cope, M., Brown, G.C., Nemoto, E.M., & Delpy, D.T. (1994 Nov). Near-infrared spectroscopy of the brain: relevance to cytochrome oxidase bioenergetics. *Biochem Soc Trans, 22,* 974-80.

Cooper, C.E., & Springett, R. (1997). Measurement of cytochrome oxidase and mitochondrial energetics by near- infrared spectroscopy. *Philos Trans R Soc Lond B Biol Sci, 352,* 669-76.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., & Pericak-Vance, M.A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science, 261,* 921-3.

Damasio, H., Grabowski, T.J., Tranel, D., Hichwa, R.D., & Damasio, A.R. (1996). A neural basis for lexical retrieval. *Nature, 380,* 499-505.

De Blasi, R.A., Almenrader, N., & Ferrari, M. (1997). Brain oxygenation monitoring during cardiopulmonary bypass by near infrared spectroscopy. *Adv Exp Med Biol, 413,* 97-104.

de la Torre, J.C. (2004). Alzheimer's disease is a vasocognopathy: a new term to describe its

nature. *Neurol Res, 26,* 517-24.

de Leon, M.J., Convit, A., Wolf, O.T., Tarshish, C.Y., DeSanti, S., Rusinek, H., Tsui, W., Kandil, E., Scherer, A.J., Roche, A., Imossi, A., Thorn, E., Bobinski, M., Caraos, C., Lesbre, P., Schlyer, D., Poirier, J., Reisberg, B., & Fowler, J. (2001). Prediction of cognitive decline in normal elderly subjects with 2-[(18)F]fluoro-2-deoxy-D-glucose/poitron-emission tomography (FDG/PET). *Proc Natl Acad Sci U S A, 98,* 10966-71.

Delis, D., Kramer, J., Kaplan, E., & Ober, BA. (1987). *California Verbal Learning Test: Adult Version.* San Antonio, TX: The Psychological Corporation.

Duncan, A., Meek, J.H., Clemence, M., Elwell, C.E., Fallon, P., Tyszczuk, L., Cope, M., & Delpy, D.T. (1996). Measurment of Cranial Optical Path Length as a Function of Age Using Phase Resolved Near Infrared Spectroscopy. *Pediatr Res, 39,* 889-894.

Elfgren, C.I., & Risberg, J. (1998). Lateralized frontal blood flow increases during fluency tasks: influence of cognitive strategy. *Neuropsychologia, 36,* 505-12.

Eustache, F., Rioux, P., Desgranges, B., Marchal, G., Petit-Taboue, M.C., Dary, M., Lechevalier, B., & Baron, J.C. (1995). Healthy aging, memory subsystems and regional cerebral oxygen consumption. *Neuropsychologia, 33,* 867-87.

Fallgatter, A.J., Muller, T.J., & Strik, W.K. (1998). Prefrontal hypooxygenation during language processing assessed with near-infrared spectroscopy. *Neuropsychobiology, 37,* 215-8.

Fallgatter, A.J., Roesler, M., Sitzmann, L., Heidrich, A., Mueller, T.J., & Strik, W.K. (1997). Loss of functional hemispheric asymmetry in Alzheimer's dementia assessed with near-infrared spectroscopy. *Brain Res Cogn Brain Res, 6,* 67-72.

Fallgatter, A.J., & Strik, W.K. (1998). Frontal brain activation during the Wisconsin Card Sorting Test assessed with two-channel near-infrared spectroscopy. *Eur Arch Psychiatry Clin Neurosci, 248,* 245-9.

Fallgatter, A.J., & Strik, W.K. (2000). Reduced frontal functional asymmetry in schizophrenia during a cued continuous performance test assessed with near-infrared spectroscopy.

Schizophr Bull, 26, 913-9.

Fallgatter, A.J., & Strik, W.K. (1997). Right frontal activation during the continuous performance test assessed with near-infrared spectroscopy in healthy subjects. *Neurosci Lett, 223,* 89-92.

Fan, X.H., Asahara, T., Miyata, Y., Ohdan, H., Shibata, S., Yamamoto, H., Fudaba, Y., & Dohi, K. (1999 Nov). Diagnostic value of noninvasive near-infrared spectroscopy to assess liver viability of brain-dead donors. *Transplant Proc, 31,* 2922-3.

Fan, X.H., Asahara, T., Ohdan, H., Miyata, Y., Shibata, S., & Dohi, K. (2000). Nondestructive and real-time evaluation of liver viability in brain dead donor for liver transplantation using near-infrared spectroscopy. *Transpl Int, 13 Suppl 1,* S272-7.

Federico, P., Borg, S.G., Salkauskus, A.G., & MacVicar, B.A. (1994 Feb). Mapping patterns of neuronal activity and seizure propagation by imaging intrinsic optical signals in the isolated whole brain of the guinea-pig. *Neuroscience, 58,* 461-80.

Ferrari, M., De Blasi, R.A., Safoue, F., Wei, Q., & Zaccanti, G. (1993). Towards human brain near infrared imaging: time resolved and unresolved spectroscopy during hypoxic hypoxia. *Adv Exp Med Biol, 333,* 21-31.

Ferrari, M., Giannini, I., Carpi, A., & Fasella, P. (1983). Non-invasive near infrared spectroscopy of brain in fluorocarbon exchange-transfused rats. *Physiol Chem Phys Med NMR, 15,* 107-13.

Ferrari, M., Giannini, I., Sideri, G., & Zanette, E. (1985). Continuous non invasive monitoring of human brain by near infrared spectroscopy. *Adv Exp Med Biol, 191,* 873-82.

Ferrari, M., Williams, M.A., Wilson, D.A., Thakor, N.V., Traystman, R.J., & Hanley, D.F. (1995). Cat brain cytochrome-c oxidase redox changes induced by hypoxia after blood-fluorocarbon exchange transfusion. *Am J Physiol, 269,* H417-24.

Ferris, S.H. (2002). Clinical trials in AD: are current formats and outcome measures adequate? *Alzheimer Dis Assoc Disord, 16 Suppl 1,* S13-7.

Fletcher, P.C., Shallice, T., & Dolan, R.J. (1998). The functional roles of prefrontal cortex in episodic memory. I. Encoding. *Brain, 121,* 1239-48.

Fletcher, P.C., Shallice, T., Frith, C.D., Frackowiak, R.S., & Dolan, R.J. (1998). The functional roles of prefrontal cortex in episodic memory. II. Retrieval. *Brain, 121,* 1249-56.

Fox, P.T., Raichle, M.E., Mintun, M.A., & Dence, C. (1988). Nonoxidative glucose consumption during focal physiologic neural activity. *Science, 241,* 462-4.

Friston, K.J., Frith, C.D., Liddle, P.F., & Frackowiak, R.S. (1993). Functional connectivity: the principal-component analysis of large (PET) data sets. *J Cereb Blood Flow Metab, 13,* 5-14.

Frith, C.D., Friston, K., Liddle, P.F., & Frackowiak, R.S. (1991). Willed action and the prefrontal cortex in man: a study with PET. *Proc R Soc Lond B Biol Sci, 244,* 241-6.

Frith, C.D., Friston, K.J., Liddle, P.F., & Frackowiak, R.S. (1991). A PET study of word finding. *Neuropsychologia, 29,* 1137-48.

Fu, C.H., Morgan, K., Suckling, J., Williams, S.C., Andrew, C., Vythelingum, G.N., & McGuire, P.K. (2002). A functional magnetic resonance imaging study of overt letter verbal fluency using a clustered acquisition sequence: greater anterior cingulate activation with increased task demand. *Neuroimage, 17,* 871-9.

Giannini, I., Ferrari, M., Carpi, A., & Fasella, P. (1982). Rat brain monitoring by near-infrared spectroscopy: an assessment of possible clinical significance. *Physiol Chem Phys, 14,* 295-305.

Grady, C.L., Haxby, J.V., Horwitz, B., Gillette, J., Salerno, J.A., Gonzalez-Aviles, A., Carson, R.E., Herscovitch, P., Schapiro, M.B., & Rapoport, S.I. (1993). Activation of cerebral blood flow during a visuoperceptual task in patients with Alzheimer-type dementia. *Neurobiol Aging, 14,* 35-44.

Grady, C.L., McIntosh, A.R., Beig, S., Keightley, M.L., Burian, H., & Black, S.E. (2003). Evidence from functional neuroimaging of a compensatory prefrontal network in Alzheimer's disease. *J Neurosci, 23,* 986-93.

Gratton, G., Corballis, P.M., Cho, E., Fabiani, M., & Hood, D.C. (1995). Shades of gray matter: noninvasive optical images of human brain responses during visual stimulation. *Psychophysiology, 32,* 505-9.

Gratton, G., Goodman-Wood, M.R., & Fabiani, M. (2001 May). Comparison of neuronal and hemodynamic measures of the brain response to visual stimulation: an optical imaging study. *Hum Brain Mapp, 13,* 13-25.

Grinvald, A. (1992 Winter). Optical imaging of architecture and function in the living brain sheds new light on cortical mechanisms underlying visual perception. *Brain Topogr, 5,* 71-5.

Grinvald, A., Anglister, L., Freeman, J.A., Hildesheim, R., & Manker, A. (1984 Apr 26-May 2). Real-time optical imaging of naturally evoked electrical activity in intact frog brain. *Nature, 308,* 848-50.

Grinvald, A., Frostig, R.D., Siegel, R.M., & Bartfeld, E. (1991 Dec 15). High-resolution optical imaging of functional brain architecture in the awake monkey. *Proc Natl Acad Sci U S A, 88,* 11559-63.

Grinvald, A., Lieke, E., Frostig, R.D., Gilbert, C.D., & Wiesel, T.N. (1986). Functional architecture of cortex revealed by optical imaging of intrinsic signals. *Nature, 324,* 361-4.

Hasegawa, M., Houdou, S., Takashima, S., Tatsuno, M., Okuyama, K., & Suzuki, S. (1992). Monitoring of immature rabbit brain during hypoxia with near-infrared spectroscopy. *Pediatr Neurol, 8,* 47-50.

Hausser-Hauw, C., Rakotonanahary, D., & Fleury, B. (2000). [Obstructive-sleep apnea syndrome: brain oxygenation measured with near- infrared spectroscopy. Preliminary results]. *Neurophysiol Clin, 30,* 113-8.

Heiss, W.D., Pawlik, G., Holthoff, V., Kessler, J., & Szelies, B. (1992). PET correlates of normal and impaired memory functions. *Cerebrovasc Brain Metab Rev, 4,* 1-27.

Herrmann, M.J., Ehlis, A.C., & Fallgatter, A.J. (2003). Frontal activation during a verbal-fluency task as measured by near-infrared spectroscopy. *Brain Res Bull, 61,* 51-6.

Hill, D.K., & Keynes, R.D. (1949). Opacity changes in stimulated nerve. *Journal of Physiology, 108,* 278-281.

Hirono, N., Mori, E., Ishii, K., Imamura, T., Tanimukai, S., Kazui, H., Hashimoto, M.,

Takatsuki, Y., Kitagaki, H., & Sasaki, M. (2001). Neuronal substrates for semantic memory: a positron emission tomography study in Alzheimer's disease. *Dement Geriatr Cogn Disord, 12,* 15-21.

Hirth, C., Obrig, H., Villringer, K., Thiel, A., Bernarding, J., Muhlnickel, W., Flor, H., Dirnagl, U., & Villringer, A. (1996). Non-invasive functional mapping of the human motor cortex using near- infrared spectroscopy. *Neuroreport, 7,* 1977-81.

Hirth, C., Villringer, K., Thiel, A., Bernarding, J., Muhlnickl, W., Obrig, H., Dirnagl, U., & Villringer, A. (1997). Towards brain mapping combining near-infrared spectroscopy and high resolution 3D MRI. *Adv Exp Med Biol, 413,* 139-47.

Hock, C., Muller-Spahn, F., Schuh-Hofer, S., Hofmann, M., Dirnagl, U., & Villringer, A. (1995). Age dependency of changes in cerebral hemoglobin oxygenation during brain activation: a near-infrared spectroscopy study. *J Cereb Blood Flow Metab, 15,* 1103-8.

Hock, C., Stormer, R., Dursteler, K., Muller-Spahn, F., Ladewig, D., & Stohler, R. (1999). Heroin-induced deoxygenation of cerebral hemoglobin: monitoring by means of near-infrared spectroscopy. *Ann Neurol, 45,* 134-5.

Hock, C., Villringer, K., Heekeren, H., Hofmann, M., Wenzel, R., Villringer, A., & Muller-Spahn, F. (1997b). A role for near infrared spectroscopy in psychiatry? *Adv Exp Med Biol, 413,* 105-23.

Hock, C., Villringer, K., Muller-Spahn, F., Hofmann, M., Schuh-Hofer, S., Heekeren, H., Wenzel, R., Dirnagl, U., & Villringer, A. (1996). Near infrared spectroscopy in the diagnosis of Alzheimer's disease. *Ann N Y Acad Sci, 777,* 22-9.

Hock, C., Villringer, K., Muller-Spahn, F., Wenzel, R., Heekeren, H., Schuh-Hofer, S., Hofmann, M., Minoshima, S., Schwaiger, M., Dirnagl, U., & Villringer, A. (1997a). Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with Alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS)--correlation with simultaneous rCBF-PET measurements. *Brain Res, 755,* 293-303.

Holzschuh, M., Woertgen, C., Metz, C., & Brawanski, A. (1997). Dynamic changes of

cerebral oxygenation measured by brain tissue oxygen pressure and near infrared spectroscopy. *Neurol Res, 19,* 246-8.

Hoshi, Y. (2002). [Near-infrared optical imaging of human brain function--a novel approach to the brain and the mind]. *Seishin Shinkeigaku Zasshi, 104,* 381-93.

Hoshi, Y., Hazeki, O., Kakihana, Y., & Tamura, M. (1997). Redox behavior of cytochrome oxidase in the rat brain measured by near- infrared spectroscopy. *J Appl Physiol, 83,* 1842-8.

Hoshi, Y., Kobayashi, N., & Tamura, M. (2001). Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. *J Appl Physiol, 90,* 1657-62.

Hoshi, Y., Kosaka, S., Xie, Y., Kohri, S., & Tamura, M. (1998). Relationship between fluctuations in the cerebral hemoglobin oxygenation state and neuronal activity under resting conditions in man. *Neurosci Lett, 245,* 147-50.

Hoshi, Y., Mizukami, S., & Tamura, M. (1994). Dynamic features of hemodynamic and metabolic changes in the human brain during all-night sleep as revealed by near-infrared spectroscopy. *Brain Res, 652,* 257-62.

Hoshi, Y., Onoe, H., Watanabe, Y., Andersson, J., Bergstrom, M., Lilja, A., Langstrom, B., & Tamura, M. (1994). Non-synchronous behavior of neuronal activity, oxidative metabolism and blood supply during mental tasks in man. *Neurosci Lett, 172,* 129-33.

Hoshi, Y., & Tamura, M. (1993a). Detection of dynamic changes in cerebral oxygenation coupled to neuronal function during mental work in man. *Neurosci Lett, 150,* 5-8.

Hoshi, Y., & Tamura, M. (1993b). Dynamic multichannel near-infrared optical imaging of human brain activity. *J Appl Physiol, 75,* 1842-6.

Jack, C.R. Jr, Petersen, R.C., Xu, Y.C., O'Brien, P.C., Waring, S.C., Tangalos, E.G., Smith, G.E., Ivnik, R.J., Thibodeau, S.N., & Kokmen, E. (1998). Hippocampal atrophy and apolipoprotein E genotype are independently associated with Alzheimer's disease. *Ann Neurol, 43,* 303-10.

Jernigan, T.L., Archibald, S.L., Fennema-Notestine, C., Gamst, A.C., Stout, J.C., Bonner, J., &

Hesselink, J.R. (2001). Effects of age on tissues and regions of the cerebrum and cerebellum. *Neurobiol Aging, 22,* 581-94.

Jöbsis, F.F. (1977). Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science, 198,* 1264-7.

Johnson, S.C., Saykin, A.J., Flashman, L.A., McAllister, T.W., & Sparling, M.B. (2001). Brain activation on fMRI and verbal memory ability: functional neuroanatomic correlates of CVLT performance. *J Int Neuropsychol Soc, 7,* 55-62.

Jones, M., Berwick, J., Johnston, D., & Mayhew, J. (2001). Concurrent optical imaging spectroscopy and laser-Doppler flowmetry: the relationship between blood flow, oxygenation, and volume in rodent barrel cortex. *Neuroimage, 13,* 1002-15.

Kaasinen, V., Nagren, K., Jarvenpaa, T., Roivainen, A., Yu, M., Oikonen, V., Kurki, T., & Rinne, J.O. (2002). Regional effects of donepezil and rivastigmine on cortical acetylcholinesterase activity in Alzheimer's disease. *J Clin Psychopharmacol, 22,* 615-20.

Kampfl, A., Pfausler, B., Denchev, D., Jaring, H.P., & Schmutzhard, E. (1997). Near infrared spectroscopy (NIRS) in patients with severe brain injury and elevated intracranial pressure. A pilot study. *Acta Neurochir Suppl (Wien), 70,* 112-4.

Kaplan, E., Googlass, H., & Weintraub, S. (1983). *The Boston Naming Test* . Philadelphia: Lea & Febinger.

Kato, T., Kamei, A., Takashima, S., & Ozaki, T. (1993). Human visual cortical function during photic stimulation monitoring by means of near-infrared spectroscopy. *J Cereb Blood Flow Metab, 13,* 516-20.

Katoh, T., Esato, K., Gohra, H., Hamano, K., Fujimura, Y., Zempo, N., Nakashima, K., & Maekawa, T. (1997). Evaluation of brain oxygenation during selective cerebral perfusion by near-infrared spectroscopy. *Ann Thorac Surg, 64,* 432-6.

Keilp, J.G., Gorlyn, M., Alexander, G.E., Stern, Y., & Prohovnik, I. (1999). Cerebral blood flow patterns underlying the differential impairment in category vs letter fluency in Alzheimer's disease. *Neuropsychologia, 37,* 1251-61.

Kennan, R.P., Kim, D., Maki, A., Koizumi, H., & Constable, R.T. (2002). Non-invasive assessment of language lateralization by transcranial near infrared optical topography and functional MRI. *Hum Brain Mapp, 16,* 183-9.

Kerr, M.E., Marion, D., Orndoff, P.A., Weber, B.B., & Sereika, S.M. (1998). Evaluation of near infrared spectroscopy in patients with traumatic brain injury. *Adv Exp Med Biol, 454,* 131-7.

Kirkpatrick, P.J. (1997). Use of near-infrared spectroscopy in the adult. *Philos Trans R Soc Lond B Biol Sci, 352,* 701-5.

Kitabayashi, Y., Ueda, H., Tsuchida, H., Iizumi, H., Narumoto, J., Nakamura, K., Kita, H., & Fukui, K. (2001). Relationship between regional cerebral blood flow and verbal fluency in Alzheimer's disease. *Psychiatry Clin Neurosci, 55,* 459-63.

Kleinschmidt, A., Obrig, H., Requardt, M., Merboldt, K.D., Dirnagl, U., Villringer, A., & Frahm, J. (1996). Simultaneous recording of cerebral blood oxygenation changes during human brain activation by magnetic resonance imaging and near-infrared spectroscopy. *J Cereb Blood Flow Metab, 16,* 817-26.

Knapp, W.H., Dannenberg, C., Marschall, B., Zedlick, D., Loschmann, K., Bettin, S., Barthel, H., & Seese, A. (1996). Changes in local cerebral blood flow by neuroactivation and vasoactivation in patients with impaired cognitive function. *Eur J Nucl Med, 23,* 878-88.

Kühner, C. (1997). *Fragebogen zur Depressionsdiagnostik nach DSM-IV (FDD-DSM-IV).* Göttingen: Hogrefe.

Kurth, C.D., Liu, H., Thayer, W.S., & Chance, B. (1995). A dynamic phantom brain model for near-infrared spectroscopy. *Phys Med Biol, 40,* 2079-92.

Kutas, M., & Federmeier, K.D. (1998). Minding the body. *Psychophysiology, 35,* 135-50.

Kwong, K.K., Belliveau, J.W., Chesler, D.A., Goldberg, I.E., Weisskoff, R.M., Poncelet, B.P., Kennedy, D.N., Hoppel, B.E., Cohen, M.S., Turner, R., & et, a.l. (1992). Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci U S A, 89,* 5675-9.

Lanctot, K.L., Herrmann, N., & LouLou, M.M. (2003). Correlates of response to acetylcholinesterase inhibitor therapy in Alzheimer's disease. *J Psychiatry Neurosci, 28,* 13-26.

Lee, B.C., Mintun, M., Buckner, R.L., & Morris, J.C. (2003). Imaging of Alzheimer's disease. *J Neuroimaging, 13,* 199-214.

Lehrl, S. (1993). *Mehrfachwahl-Wortschatz-Intelligenztest: MWT-B.* Nürnberg: Perimed-spitta.

Lepage, M., Habib, R., & Tulving, E. (1998). Hippocampal PET activations of memory encoding and retrieval: the HIPER model. *Hippocampus, 8,* 313-22.

Leung, H.C., Skudlarski, P., Gatenby, J.C., Peterson, B.S., & Gore, J.C. (2000). An event-related functional MRI study of the stroop color word interference task. *Cereb Cortex, 10,* 552-60.

Lezak, M. (1995). *Neuropsychological Assessment.* New York: Oxford University Press.

MacVicar, B.A., & Hochman, D. (1991). Imaging of synaptically evoked intrinsic optical signals in hippocampal slices. *J Neurosci, 11,* 1458-69.

Magistretti, P.J., & Pellerin, L. (1999a). Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. *Phil. Trans. R. Soc. Lond. B,* 1155-1163.

Magistretti, P.J., & Pellerin, L. (1999b). Astrocytes couple synaptic actvity to glucose utilization in the brain. *News Physiol. Sci. 14,* 177-182.

Mahley, R.W. (1988). Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science, 240,* 622-30.

Maki, A., Yamashita, Y., Ito, Y., Watanabe, E., Mayanagi, Y., & Koizumi, H. (1995). Spatial and temporal analysis of human motor activity using noninvasive NIR topography. *Med Phys, 22,* 1997-2005.

Malonek, D., & Grinvald, A. (1996). Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: implications for functional brain mapping. *Science, 272,* 551-4.

Marchal, G., Rioux, P., Petit-Taboue, M.C., Sette, G., Travere, J.M., Le Poec, C., Courtheoux, P., Derlon, J.M., & Baron, J.C. (1992). Regional cerebral oxygen consumption, blood flow, and blood volume in healthy human aging. *Arch Neurol, 49,* 1013-20.

Matsuda, H. (2001). Cerebral blood flow and metabolic abnormalities in Alzheimer's disease. *Ann Nucl Med, 15,* 85-92.

Matsumoto, H., Oda, T., Hossain, M.A., & Yoshimura, N. (1996). Does the redox state of cytochrome aa3 reflect brain energy level during hypoxia? Simultaneous measurements by near infrared spectrophotometry and 31P nuclear magnetic resonance spectroscopy. *Anesth Analg, 83,* 513-8.

Matsuo, K., Kato, N., & Kato, T. (2002). Decreased cerebral haemodynamic response to cognitive and physiological tasks in mood disorders as shown by near-infrared spectroscopy. *Psychol Med, 32,* 1029-37.

Matsuo, K., Kato, T., Fukuda, M., & Kato, N. (2000). Alteration of hemoglobin oxygenation in the frontal region in elderly depressed patients as measured by near-infrared spectroscopy. *J Neuropsychiatry Clin Neurosci, 12,* 465-71.

Matthews, B., Siemers, E.R., & Mozley, P.D. (2003). Imaging-based measures of disease progression in clinical trials of disease-modifying drugs for Alzheimer disease. *Am J Geriatr Psychiatry, 11,* 146-59.

Mayberg, H.S. (1994). Clinical correlates of PET- and SPECT-identified defects in dementia. *J Clin Psychiatry, 55 Suppl,* 12-21.

Mayhew, J., Johnston, D., Martindale, J., Jones, M., Berwick, J., & Zheng, Y. (2001). Increased oxygen consumption following activation of brain: theoretical footnotes using spectroscopic data from barrel cortex. *Neuroimage, 13,* 975-87.

Mayhew, J., Zheng, Y., Hou, Y., Vuksanovic, B., Berwick, J., Askew, S., & Coffey, P. (1999). Spectroscopic analysis of changes in remitted illumination: the response to increased neural activity in brain. *Neuroimage, 10,* 304-26.

Mega, M.S., Cummings, J.L., O'Connor, S.M., Dinov, I.D., Reback, E., Felix, J., Masterman, D.L., Phelps, M.E., Small, G.W., & Toga, A.W. (2001). Cognitive and metabolic

responses to metrifonate therapy in Alzheimer disease. *Neuropsychiatry Neuropsychol Behav Neurol, 14,* 63-8.

Mehagnoul-Schipper, D.J., van der Kallen, B.F., Colier, W.N., van der Sluijs, M.C., van Erning, L.J., Thijssen, H.O., Oeseburg, B., Hoefnagels, W.H., & Jansen, R.W. (2002). Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. *Hum Brain Mapp, 16,* 14-23.

Mitrushina, M.N., Boone, K.B., & D'Elia, L.F. (1999). *Handbook of normative data for neuropysychological assessment.* New York: Oxford University Press.

Monsch, A.U. (2001). *In search for preclinical cognitive markers of Alzheimer's Disease.* Habilitationsschrift, Philosophisch-Historische Fakultät, Universität Basel.

Monsch, A.U., Bondi, M.W., Butters, N., Salmon, D.P., Katzman, R., & Thal, L.J. (1992). Comparisons of verbal fluency tasks in the detection of dementia of the Alzheimer type. *Arch Neurol, 49,* 1253-8.

Monsch, A.U., Seifritz, E., Taylor, K.I., Ermini-Funfschilling, D., Stahelin, H.B., & Spiegel, R. (1997). Category fluency is also predominantly affected in Swiss Alzheimer's disease patients. *Acta Neurol Scand, 95,* 81-4.

Morris, J., Heyman, A., Mohs, R., Hughes, J., van Belle, G., Fillenbaum, G., Mellits, E., & Clark C. (1989). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology, 39,* 1159-1165.

Murata, Y., Katayama, Y., Oshima, H., Kawamata, T., Yamamoto, T., Sakatani, K., & Suzuki, S. (2000). Changes in cerebral blood oxygenation induced by deep brain stimulation: study by near-infrared spectroscopy (NIRS). *Keio J Med, 49 Suppl 1,* A61-3.

Nelson, HE. (1976). A modified card sorting test sensitive to frontal lobe defects. *Cortex,* 313-324.

Newcombe F. (1969). *Missile wounds of the brain.* London: Oxford University Press.

Nicholas, M., Obler, L., Albert, M., & Goodglass, H. (1985). Lexical retrieval in healthy aging.

Cortex, 595-606.

Nielsen, H.B., Boesen, M., & Secher, N.H. (2001). Near-infrared spectroscopy determined brain and muscle oxygenation during exercise with normal and resistive breathing. *Acta Physiol Scand, 171,* 63-70.

Obrig, H., Hirth, C., Junge-Hulsing, J.G., Doge, C., Wolf, T., Dirnagl, U., & Villringer, A. (1996). Cerebral oxygenation changes in response to motor stimulation. *J Appl Physiol, 81,* 1174-83.

Obrig, H., & Villringer, A. (1997). Near-infrared spectroscopy in functional activation studies. Can NIRS demonstrate cortical activation? *Adv Exp Med Biol, 413,* 113-27.

Obrig, H., Wenzel, R., Kohl, M., Horst, S., Wobst, P., Steinbrink, J., Thomas, F., & Villringer, A. (2000). Near-infrared spectroscopy: does it function in functional activation studies of the adult brain? *Int J Psychophysiol, 35,* 125-42.

Ogawa, S., Tank, D.W., Menon, R., Ellermann, J.M., Kim, S.G., Merkle, H., & Ugurbil, K. (1992). Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci U S A, 89,* 5951-5.

Ohnishi, Y., Hayashi, Y., Shimizu, J., Koyama, Y., & Kuro, M. (1996). [Brain monitoring with near infrared spectroscopy during carotid endarterectomy]. *Masui, 45,* 1420-3.

Okada, E., Firbank, M., & Delpy, D.T. (1995). The effect of overlying tissue on the spatial sensitivity profile of near-infrared spectroscopy. *Phys Med Biol, 40,* 2093-108.

Okubo, Y., & Ogata, H. (1995). [Brain blood volume measured with near infrared spectroscopy increased after stellate ganglion block]. *Masui, 44,* 423-7.

Oswald, WD., & Fleischmann, U. (1986). *Nürnberger-Alters-Inventar.* Göttingen: Hogrefe.

Pardo, J.V., Fox, P.T., & Raichle, M.E. (1991). Localization of a human system for sustained attention by positron emission tomography. *Nature, 349,* 61-4.

Parks, R.W., Loewenstein, D.A., Dodrill, K.L., Barker, W.W., Yoshii, F., Chang, J.Y., Emran, A., Apicella, A., Sheramata, W.A., & Duara, R. (1988). Cerebral metabolic effects of a verbal fluency test: a PET scan study. *J Clin Exp Neuropsychol, 10,* 565-75.

Pasquier, F., Lebert, F., Grymonprez, L., & Petit, H. (1995). Verbal fluency in dementia of frontal lobe type and dementia of Alzheimer type. *J Neurol Neurosurg Psychiatry, 58,* 81-4.

Paulesu, E., Goldacre, B., Scifo, P., Cappa, S.F., Gilardi, M.C., Castiglioni, I., Perani, D., & Fazio, F. (1997). Functional heterogeneity of left inferior frontal cortex as revealed by fMRI. *Neuroreport, 8,* 2011-7.

Phelps, E.A., Hyder, F., Blamire, A.M., & Shulman, R.G. (1997). FMRI of the prefrontal cortex during overt verbal fluency. *Neuroreport, 8,* 561-5.

Phillips, J.M., Brennan, M., Schwartz, C.E., & Cohen, L.M. (2005). The Long-Term Impact of Dialysis Discontinuation on Families. *J Palliat Med, 8,* 79-85.

Pringle, J., Uystepruyst, C., Art, T., & Lekeux, P. (1998). Continuous and non-invasive study of brain oxygenation in the calf by near infrared spectroscopy. *Res Vet Sci, 65,* 239-44.

Quaresima, V., Springett, R., Cope, M., Wyatt, J.T., Delpy, D.T., Ferrari, M., & Cooper, C.E. (1998). Oxidation and reduction of cytochrome oxidase in the neonatal brain observed by in vivo near-infrared spectroscopy. *Biochim Biophys Acta, 1366,* 291-300.

Rapoport, S.I. (1991). Positron emission tomography in Alzheimer's disease in relation to disease pathogenesis: a critical review. *Cerebrovasc Brain Metab Rev, 3,* 297-335.

Reiman, E.M., Uecker, A., Caselli, R.J., Lewis, S., Bandy, D., de Leon, M.J., De Santi, S., Convit, A., Osborne, D., Weaver, A., & Thibodeau, S.N. (1998). Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. *Ann Neurol, 44,* 288-91.

Saykin, A.J., Johnson, S.C., Flashman, L.A., McAllister, T.W., Sparling, M., Darcey, T.M., Moritz, C.H., Guerin, S.J., Weaver, J., & Mamourian, A. (1999). Functional differentiation of medial temporal and frontal regions involved in processing novel and familiar words: an fMRI study. *Brain, 122,* 1963-71.

Saykin, A.J., Wishart, H.A., Rabin, L.A., Flashman, L.A., McHugh, T.L., Mamourian, A.C., & Santulli, R.B. (2004). Cholinergic enhancement of frontal lobe activity in mild

cognitive impairment. *Brain, 127,* 1574-83.

Schlosser, R., Hutchinson, M., Joseffer, S., Rusinek, H., Saarimaki, A., Stevenson, J., Dewey, S.L., & Brodie, J.D. (1998). Functional magnetic resonance imaging of human brain activity in a verbal fluency task. *J Neurol Neurosurg Psychiatry, 64,* 492-8.

Schmechel, D.E., Saunders, A.M., Strittmatter, W.J., Crain, B.J., Hulette, C.M., Joo, S.H., Pericak-Vance, M.A., Goldgaber, D., & Roses, A.D. (1993). Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A, 90,* 9649-53.

Schmidt, H., Schmidt, R., Fazekas, F., Semmler, J., Kapeller, P., Reinhart, B., & Kostner, G.M. (1996). Apolipoprotein E e4 allele in the normal elderly: neuropsychologic and brain MRI correlates. *Clin Genet, 50,* 293-9.

Schmidt, M.E. (2003). Neuroimaging studies in psychopharmacology . In René Spiegel (Ed.), *Einführung in die Psychopharmakologie, 4. Auflage.* (pp. 207-226). Bern; Stuttgart; Toronto: Hans Huber .

Schroeter, M.L., Zysset, S., Kupka, T., Kruggel, F., & Von Cramon, D.Y. (2002). Near-infrared spectroscopy can detect brain activity during a color- word matching Stroop task in an event-related design. *Hum Brain Mapp, 17,* 61-71.

Schwartz-Bloom, R.D. (1999). Optical imaging of living brain slices. *Methods, 18,* 183-4.

Shadid, M., Hiltermann, L., Monteiro, L., Fontijn, J., & Van Bel, F. (1999). Near infrared spectroscopy-measured changes in cerebral blood volume and cytochrome aa3 in newborn lambs exposed to hypoxia and hypercapnia, and ischemia: a comparison with changes in brain perfusion and O2 metabolism. *Early Hum Dev, 55,* 169-82.

Sharpe, M.A., & Cooper, C.E. (1997). Nitric oxide reacts with mitochondrial cytochrome c. *Biochem Soc Trans, 25,* 407S

Skov, L. (1996). Noninvasive brain monitoring with near infrared spectroscopy in newborn infants. *Dan Med Bull, 43,* 277-84.

Slosman, D.O., Ludwig, C., Zerarka, S., Pellerin, L., Chicherio, C., de Ribaupierre, A., Annoni, J.M., Bouras, C., Herrmann, F., Michel, J.P., Giacobini, E., & Magistretti, P.J. (2001).

Brain energy metabolism in Alzheimer's disease: 99mTc-HMPAO SPECT imaging during verbal fluency and role of astrocytes in the cellular mechanism of 99mTc-HMPAO retention. *Brain Res Brain Res Rev, 36,* 230-40.

Small, G.W., Ercoli, L.M., Silverman, D.H., Huang, S.C., Komo, S., Bookheimer, S.Y., Lavretsky, H., Miller, K., Siddarth, P., Rasgon, N.L., Mazziotta, J.C., Saxena, S., Wu, H.M., Mega, M.S., Cummings, J.L., Saunders, A.M., Pericak-Vance, M.A., Roses, A.D., Barrio, J.R., & Phelps, M.E. (2000). Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A, 97,* 6037-42.

Small, G.W., Mazziotta, J.C., Collins, M.T., Baxter, L.R., Phelps, M.E., Mandelkern, M.A., Kaplan, A., La Rue, A., Adamson, C.F., Chang, L., & et, a.l. (1995). Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. *JAMA, 273,* 942-7.

Smith, C.D., Andersen, A.H., Kryscio, R.J., Schmitt, F.A., Kindy, M.S., Blonder, L.X., & Avison, M.J. (1999). Altered brain activation in cognitively intact individuals at high risk for Alzheimer's disease. *Neurology, 53,* 1391-6.

Smith, C.D., Andersen, A.H., Kryscio, R.J., Schmitt, F.A., Kindy, M.S., Blonder, L.X., & Avison, M.J. (2002). Women at risk for AD show increased parietal activation during a fluency task. *Neurology, 58,* 1197-202.

Smith, J.D. (2002). Apolipoproteins and aging: emerging mechanisms. *Ageing Res Rev, 1,* 345-65.

Soininen, H., Partanen, K., Pitkanen, A., Hallikainen, M., Hanninen, T., Helisalmi, S., Mannermaa, A., Ryynanen, M., Koivisto, K., & Riekkinen, P. Sr. (1995). Decreased hippocampal volume asymmetry on MRIs in nondemented elderly subjects carrying the apolipoprotein E epsilon 4 allele. *Neurology, 45,* 391-2.

Spreen, O., & Strauss, E. (1998). *A Compendium of Neuropsychological Test: Administration, Norms, and Commentary.* New York: Oxford University Press.

Springett, R., Newman, J., Cope, M., & Delpy, D.T. (2000). Oxygen dependency and precision of cytochrome oxidase signal from full spectral NIRS of the piglet brain. *Am J Physiol Heart Circ Physiol, 279,* H2202-9.

Stepnoski, R.A., LaPorta, A., Raccuia-Behling, F., Blonder, G.E., Slusher, R.E., & Kleinfeld, D. (1991). Noninvasive detection of changes in membrane potential in cultured neurons by light scattering. *Proc Natl Acad Sci U S A, 88,* 9382-6.

Störmer, R., Müller-Spahn, F., & Hock, C. (1998). Multichannel near infrared spectroscopy - data evaluations by means of time series analysis. *SPIE Proceedings (The International Society for Optical Engineering), 3194,* 9-17.

Strangman, G., Boas, D.A., & Sutton, J.P. (2002). Non-invasive neuroimaging using near-infrared light. *Biol Psychiatry, 52,* 679-93.

Talairach, J., & Tournoux, P. (1988). *Co-planar Stereotaxic Atlas of the Human Brain.* Stuttgart: Thieme.

Tamura, M., Hoshi, Y., & Okada, F. (1997). Localized near-infrared spectroscopy and functional optical imaging of brain activity. *Philos Trans R Soc Lond B Biol Sci, 352,* 737-42.

Tamura, M., Nemoto, M., Sato, C., & Hoshi, Y. (2001). [Optical imaging of brain function]. *No To Shinkei, 53,* 140-50.

Tateishi, A., Maekawa, T., Soejima, Y., Sadamitsu, D., Yamamoto, M., Matsushita, M., & Nakashima, K. (1995). Qualitative comparison of carbon dioxide-induced change in cerebral near-infrared spectroscopy versus jugular venous oxygen saturation in adults with acute brain disease. *Crit Care Med, 23,* 1734-8.

Taylor, S.F., Kornblum, S., Lauber, E.J., Minoshima, S., & Koeppe, R.A. (1997). Isolation of specific interference processing in the Stroop task: PET activation studies. *Neuroimage, 6,* 81-92.

Tewes, U. (1991). *Hamburg-Wechsler-Intelligenztest für Erwachsene - Revision (HAWIE-R).* Bern, Stuttgart: Huber.

Thurstone, L.L., & Thurstone, T.G. (1962). *Primary mental abilities.* Chicago: Science Research Associates.

Tohgi, H., Takahashi, S., Kato, E., Homma, A., Niina, R., Sasaki, K., Yonezawa, H., & Sasaki, M. (1997). Reduced size of right hippocampus in 39- to 80-year-old normal subjects

carrying the apolipoprotein E epsilon4 allele. *Neurosci Lett, 236,* 21-4.

Tohgi, H., Yonezawa, H., Takahashi, S., Sato, N., Kato, E., Kudo, M., Hatano, K., & Sasaki, T. (1998). Cerebral blood flow and oxygen metabolism in senile dementia of Alzheimer's type and vascular dementia with deep white matter changes. *Neuroradiology, 40,* 131-7.

Toronov, V., Webb, A., Choi, J.H., Wolf, M., Michalos, A., Gratton, E., & Hueber, D. (2001). Investigation of human brain hemodynamics by simultaneous near-infrared spectroscopy and functional magnetic resonance imaging. *Med Phys, 28,* 521-7.

van der Zee, P., Arridge, S.R., Cope, M., & Delpy, D.T. (1990). The effect of optode positioning on optical pathlength in near infrared spectroscopy of brain. *Adv Exp Med Biol, 277,* 79-84.

van der Zee, P., Cope, M., Arridge, S.R., Essenpreis, M., Potter, L.A., Edwards, A.D., Wyatt, J.S., McCormick, D.C., Roth, S.C., Reynolds, E.O., & et, a.l. (1992). Experimentally measured optical pathlengths for the adult head, calf and forearm and the head of the newborn infant as a function of inter optode spacing. *Adv Exp Med Biol, 316,* 143-53.

van Duijn, C.M., de Knijff, P., Cruts, M., Wehnert, A., Havekes, L.M., Hofman, A., & Van Broeckhoven, C. (1994). Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. *Nat Genet, 7,* 74-8.

van Rossem, K., Garcia-Martinez, S., De Mulder, G., Van Deuren, B., Engelborghs, K., Van Reempts, J., & Borgers, M. (1999). Brain oxygenation after experimental closed head injury. A NIRS study. *Adv Exp Med Biol, 471,* 209-15.

Villringer, A. (1997). Functional neuroimaging. Optical approaches. *Adv Exp Med Biol, 413,* 1-18.

Villringer, A., & Chance, B. (1997). Non-invasive optical spectroscopy and imaging of human brain function. *Trends Neurosci, 20,* 435-42.

Villringer, A., & Dirnagl, U. (1995). Coupling of brain activity and cerebral blood flow: basis of functional neuroimaging. *Cerebrovasc Brain Metab Rev, 7,* 240-76.

Villringer, A., Planck, J., Hock, C., Schleinkofer, L., & Dirnagl, U. (1993). Near infrared

spectroscopy (NIRS): a new tool to study hemodynamic changes during activation of brain function in human adults. *Neurosci Lett, 154,* 101-4.

Villringer, A., Planck, J., Stodieck, S., Botzel, K., Schleinkofer, L., & Dirnagl, U. (1994). Noninvasive assessment of cerebral hemodynamics and tissue oxygenation during activation of brain cell function in human adults using near infrared spectroscopy. *Adv Exp Med Biol, 345,* 559-65.

Villringer, K., Minoshima, S., Hock, C., Obrig, H., Ziegler, S., Dirnagl, U., Schwaiger, M., & Villringer, A. (1997). Assessment of local brain activation. A simultaneous PET and near- infrared spectroscopy study. *Adv Exp Med Biol, 413,* 149-53.

Warburton, E., Wise, R.J., Price, C.J., Weiller, C., Hadar, U., Ramsay, S., & Frackowiak, R.S. (1996). Noun and verb retrieval by normal subjects. Studies with PET. *Brain, 119 (Pt 1),* 159-79.

Warkentin, S., & Passant, U. (1993). Functional activation of the frontal lobes. Regional cerebral blood flow findings in normals and in patients with frontal lobe dementia performing a word fluency test. *Dementia, 4,* 188-91.

Warkentin, S., & Passant, U. (1997). Functional imaging of the frontal lobes in organic dementia. Regional cerebral blood flow findings in normals, in patients with frontotemporal dementia and in patients with Alzheimer's disease, performing a word fluency test. *Dement Geriatr Cogn Disord, 8,* 105-9.

Watanabe, A., Kato, N., & Kato, T. (2002). Effects of creatine on mental fatigue and cerebral hemoglobin oxygenation. *Neurosci Res, 42,* 279-85.

Watanabe, E. (1996). [Clinical application of functional mapping; the present state of the art and its future prospects: (series 4) noninvasive functional mapping of the brain with near-infrared spectroscopy]. *No Shinkei Geka, 24,* 409-14.

Whatmough, C., Chertkow, H., Murtha, S., & Hanratty, K. (2002). Dissociable brain regions process object meaning and object structure during picture naming. *Neuropsychologia, 40,* 174-86.

Wickramasinghe, Y.A., Rolfe, P., Palmer, K., & Spencer, S.A. (1995). Investigation of

neonatal brain cytochrome redox by NIRS. *Brain Res Dev Brain Res, 89,* 307-8.

Wobst, P., Wenzel, R., Kohl, M., Obrig, H., & Villringer, A. (2001). Linear aspects of changes in deoxygenated hemoglobin concentration and cytochrome oxidase oxidation during brain activation. *Neuroimage, 13,* 520-30.

Wolf, T., Lindauer, U., Obrig, H., Dreier, J., Back, T., Villringer, A., & Dirnagl, U. (1996). Systemic nitric oxide synthase inhibition does not affect brain oxygenation during cortical spreading depression in rats: a noninvasive near-infrared spectroscopy and laser-Doppler flowmetry study. *J Cereb Blood Flow Metab, 16,* 1100-7.

Wray, S., Cope, M., Delpy, D.T., Wyatt, J.S., & Reynolds, E.O. (1988). Characterization of the near infrared absorption spectra of cytochrome aa3 and haemoglobin for the non-invasive monitoring of cerebral oxygenation. *Biochim Biophys Acta, 933,* 184-92.

Wyatt, J.S. (1993). Near-infrared spectroscopy in asphyxial brain injury. *Clin Perinatol, 20,* 369-78.

Wyatt, J.S. (1992). Near infrared spectroscopy. Investigation and assessment of perinatal brain injury. *Biol Neonate, 62,* 290-4.

Wyatt, J.S., Edwards, A.D., Azzopardi, D., & Reynolds, E.O. (1989). Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury. *Arch Dis Child, 64,* 953-63.

Zaramella, P., Freato, F., Amigoni, A., Salvadori, S., Marangoni, P., Suppjei, A., Schiavo, B., & Chiandetti, L. (2001). Brain auditory activation measured by near-infrared spectroscopy (NIRS) in neonates. *Pediatr Res, 49,* 213-9.

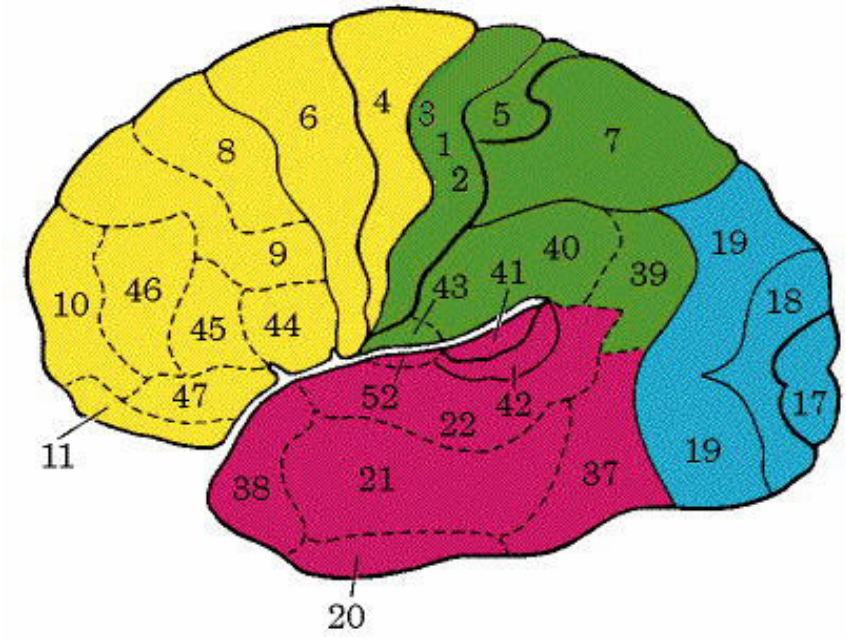
Zhang, G., Katz, A., Alfano, R.R., Kofinas, A.D., Kofinas, D.A., Stubblefield, P.G., Rosenfeld, W., Beyer, D., Maulik, D., & Stankovic, M.R. (2000). Brain perfusion monitoring with frequency-domain and continuous-wave near-infrared spectroscopy: a cross-correlation study in newborn piglets. *Phys Med Biol, 45,* 3143-58.

Appendix I

Appendix II

Appendix I

(a)



(b)

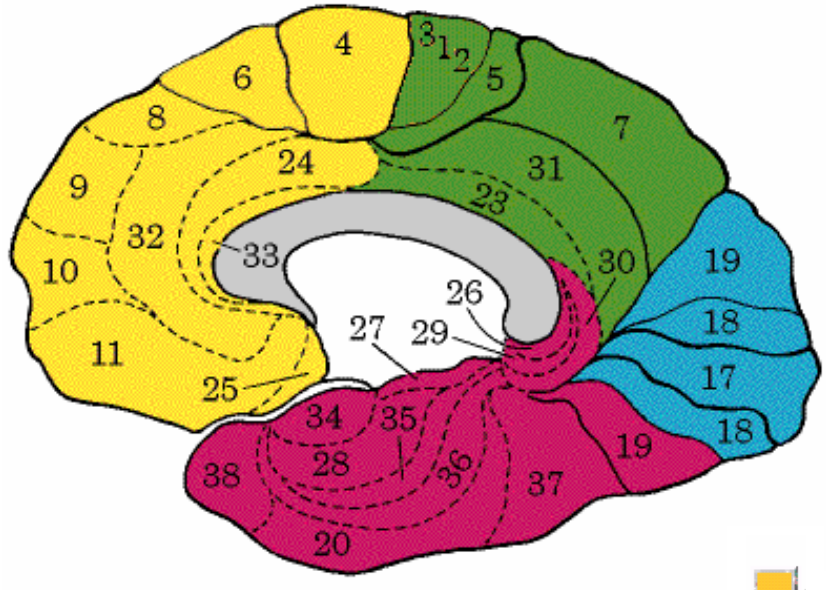


Figure I-1: Brodmann Areas. (a) Sagittal view, (b) Mid-sagittal view
Taken from http://www.umich.edu/~cogneuro/jpg/Brodmann.html

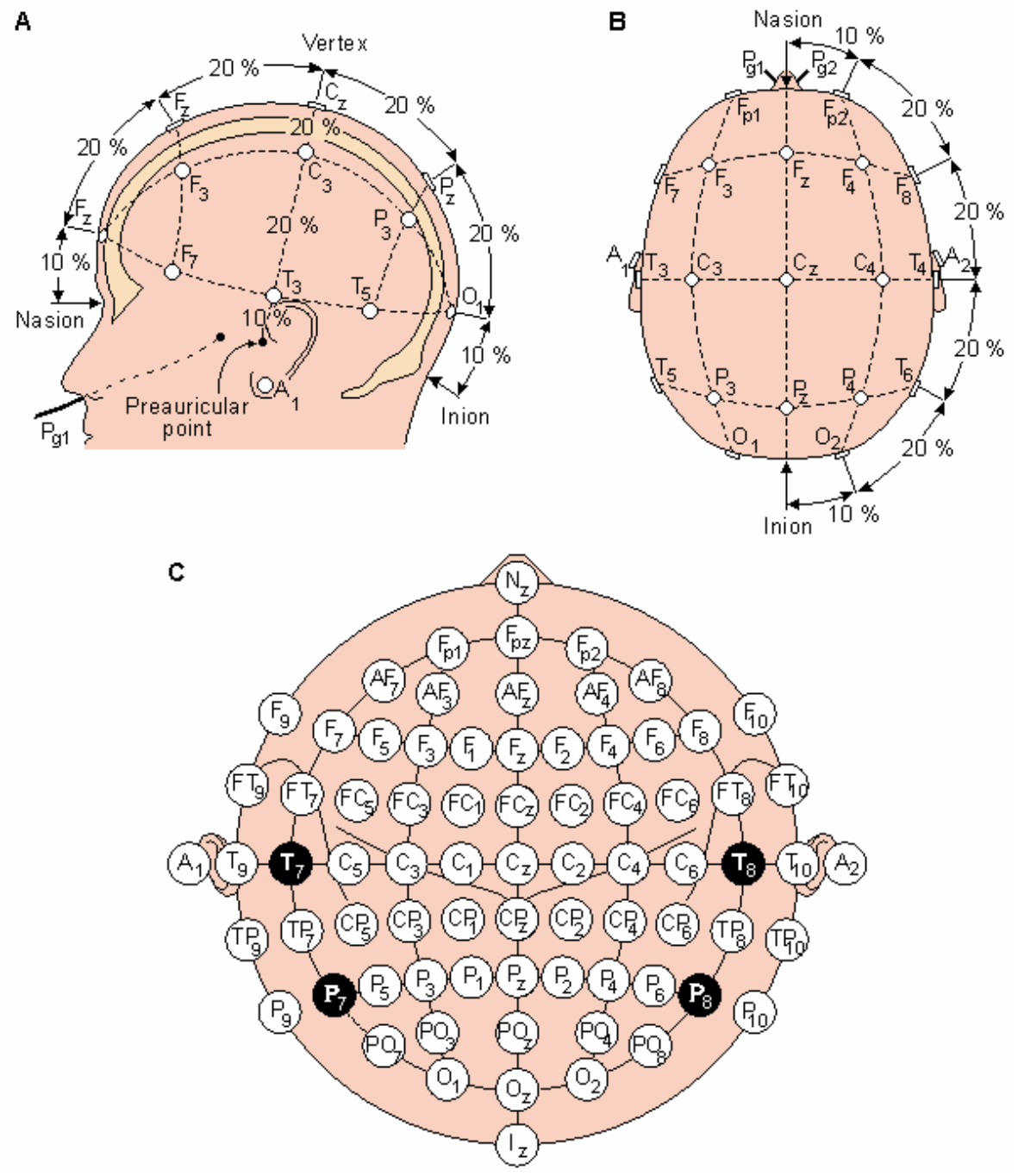


Figure I-2: The international 10-20 system for electrode positioning. (A) View from the left. (B) vertex view. (C) Location and nomenclature of the intermediate 10% electrodes, as standardized by the American Electroencephalographic Society.
Taken from http://butler.cc.tut.fi/~malmivuo/bem/bembook/13/13.htm

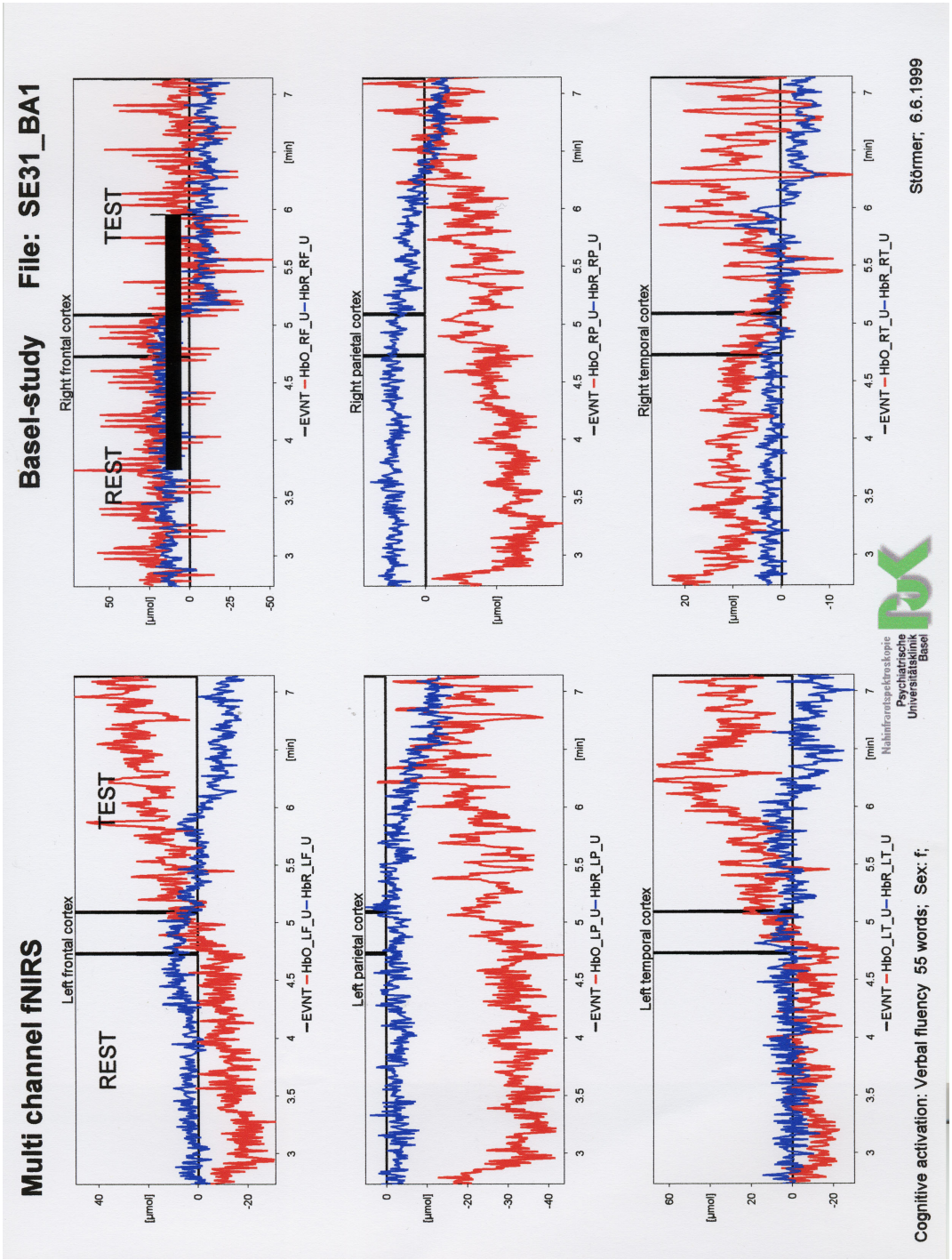


Figure 1-3: Sample time course of the NIRS parameters HbO (red) and HbR (blue) at the left and right frontal, left and right parietal, and left and right temporal optode position of a non-demented elderly subject performing the verbal fluency task. Two event markers (black vertical lines) indicate the end of the resting period (first marker) and the time at which the subject starts performing the verbal fluency task (second marker).

Appendix II

Table II-1: NIRS values „young old“ (54-63 years) versus „oldest old“ (81-90 years) subjects

		N	Mean		Min	Max	***F***	p
HBO_LF	young old	21	12.7	30.5	-35.6	99.7		
	oldest old	22	22.7	30.7	-12.2	104.6	1.15	.291
HBR_LF	young old	21	-2.06	11.1	-32.1	28.4		
	oldest old	22	-.85	10.2	-29.2	16.6	.136	.714
HBO_RF	young old	20	14.8	21.5	-26.1	55.2		
	oldest old	21	16.4	32.8	-40.5	99.0	.034	.855
HBR_RF	young old	20	-2.96	8.82	-17.8	19.7		
	oldest old	21	-5.02	17.1	-39.9	43.8	.232	.633
HBO_LP	young old	21	16.7	23.5	-38.4	52.3		
	oldest old	20	22.1	44.7	-33.4	185.7	.243	.625
HBR_LP	young old	21	2.13	8.06	-8.97	24.8		
	oldest old	20	5.78	27.9	-31.2	78.1	.330	.569
HBO_RP	young old	21	17.8	31.1	-35.5	93.0		
	oldest old	16	32.0	47.4	-6.17	194.5	1.20	.281
HBR_RP	young old	21	-.46	10.1	-13.5	25.9		
	oldest old	16	6.50	21.8	-20.1	61.7	1.67	.204
HBO_LT	young old	18	13.2	28.7	-39.1	70.8		
	oldest old	21	27.6	31.5	-17.0	102.6	2.19	.147
HBR_LT	young old	18	1.37	11.8	-12.9	29.5		
	oldest old	21	5.73	17.8	-13.9	63.4	.782	.382
HBO_RT	young old	16	11.2	22.4	-20.5	46.1		
	oldest old	20	17.1	34.8	-44.9	126.5	.345	.561
HBR_RT	young old	16	-.74	9.86	-18.3	14.4		
	oldest old	20	-.77	15.9	-44.0	39.1	.001	.995

Table II-2: Intercorrelations (*r*) of NIRS parameters HbO and HbR across all optode positions and for subjects activated during NIRS with the verbal fluency task (N=119)

	HbO_lf	HbR_lf	HbO_rf	HbR_rf	HbO_lp	HbR_lp	HbO_rp	HbR_rp	HbO_lt	HbR_lt	HbO_rt
HbR_lf	.003										
HbO_rf	.658	-.092									
HbR_rf	-.310	.477	.094								
HbO_lp	.382	-.294	.451	-.269							
HbR_lp	-.159	-.033	-.013	.195	.417						
HbO_rp	.448	-.308	.497	-.275	.577	-.115					
HbR_rp	-.143	.195	-.106	.308	-.163	.027	.339				
HbO_lt	.445	-.182	.363	-.092	.568	.094	.490	-.095			
HbR_lt	.007	.112	-.034	.448	.124	.363	.012	.050	.578		
HbO_rt	.169	-.240	.146	-.174	.340	.045	.294	-.209	.402	.166	
HbR_rt	-.070	.187	-.009	.353	-.051	.233	-.173	-.005	.258	.486	.566

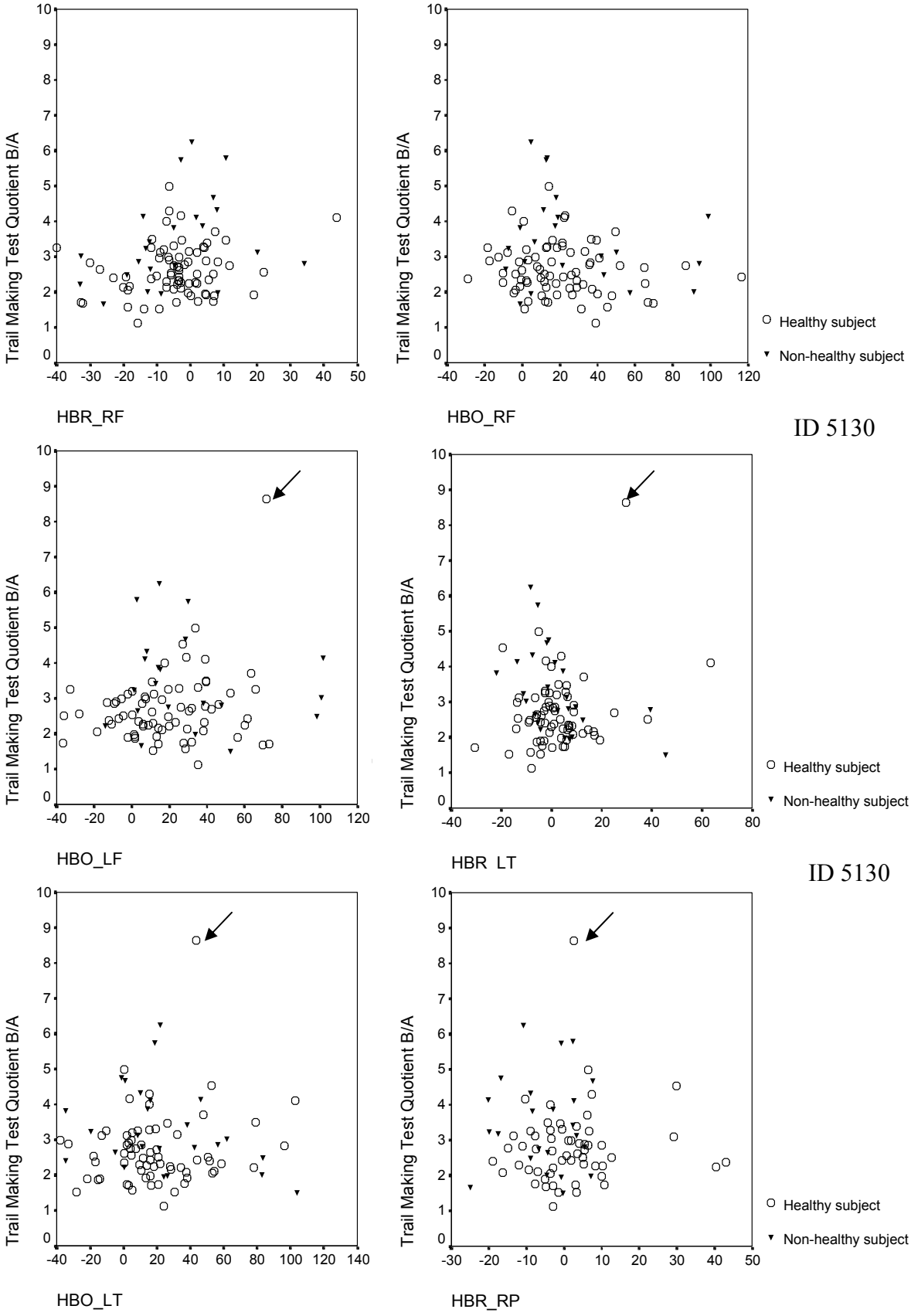


Figure II-1: Scatterplots for partial correlations between TMT B/A and the NIRS parameters which, as revealed by multiple regression analysis, significantly contribute to the prediction of the TMT B/A performance.

Table II-3: Mean (SD) performance of right-frontal NIRS responders vs. right-frontal non-responders

	Responders (n=50)	Non-Responders (n=50)	*Z*	p
Phonemic Fluency: total correct	29.3 (9.68)	27.0 (8.16)	-1.36	.174
Category Fluency: total correct	52.4 (11.9)	48.8 (11.5)	-1.17	.241
WCST: total correct categories	5.27 (1.39)	5.24 (1.20)	-.673	.501
WCST: perseverative errors	2.05 (3.33)	2.74 (3.14)	-1.40	.163
TMT-B: time needed	131 (59.0)	145 (68.8)	-1.17	.241
TMT B/A	2.70 (.872)	2.94 (1.02)	-.982	.326
BNT: total spontaneous correct	39.3 (3.67)	39.4 (4.23)	-.409	.682
WAIS-R Block design: total correct	23.6 (7.49)	22.8 (7.52)	-.621	.534
WAIS-R Similarities: total correct	24.7 (5.79)	24.2 (5.07)	-.793	.428
WAIS-R Vocabulary: total correct	21.9 (5.75)	21.3 (5.47)	-.553	.580
MWT-B: correct words	31.8 (3.43)	31.8 (4.07)	-.208	.835
CVLT: correctly recalled List A trials 1-5	51.7 (9.65)	49.1 (7.53)	-1.69	.092
CVLT: correctly recalled short delay	10.7 (3.01)	9.84 (2.89)	-1.78	.075
CVLT: correctly recalled long delay	11.4 (2.86)	10.5 (2.71)	-1.77	.077
CVLT: Recognition discriminability (%)	95.9 (4.49)	94.1 (7.26)	-1.18	.238

Table II-4: Mean (SD) performance of left-parietal NIRS responders vs. left-parietal non-responders

	Responders (n=31)	Non-Responders (n=61)	*Z*	p
Phonemic Fluency: total correct	29.8 (10.0)	28.5 (8.91)	-.993	.321
Category Fluency: total correct	56.4 (12.0)	48.6 (10.8)	-2.92	.004
WCST: total correct categories	5.48 (.935)	5.17 (1.38)	-1.04	.297
WCST: perseverative errors	1.22 (1.63)	2.93 (3.58)	-2.47	.013
TMT-B: time needed	134 (69.4)	136 (64.0)	-.328	.743
TMT B/A	2.72 (1.05)	2.93 (1.19)	-.730	.466
BNT: total spontaneous correct	39.6 (3.83)	39.3 (3.97)	-.361	.718
WAIS-R Block design: total correct	25.8 (8.73)	22.8 (7.32)	-1.76	.078
WAIS-R Similarities: total correct	24.6 (6.20)	24.6 (5.20)	-.364	.716
WAIS-R Vocabulary: total correct	22.3 (6.94)	21.6 (4.99)	-.904	.366
MWT-B: correct words	31.8 (5.03)	32.1 (2.77)	-1.01	.314
CVLT: correctly recalled List A trials 1-5	50.3 (7.61)	51.1 (9.30)	-.566	.571
CVLT: correctly recalled short delay	10.7 (2.61)	10.2 (3.12)	-.831	.406
CVLT: correctly recalled long delay	11.6 (2.52)	10.9 (2.99)	-.816	.414
CVLT: Recognition discriminability (%)	95.9 (4.49)	94.1 (7.26)	-.841	.400

Table II-5: Mean (SD) performance of NIRS right-parietal responders vs. right-parietal non-responders

	Responders (n=32)	Non-Responders (n=50)	*Z*	p
Phonemic Fluency: total correct	30.7 (9.37)	26.7 (9.52)	-1.76	.078
Category Fluency: total correct	54.5 (11.4)	48.7 (12.2)	-1.96	.050
WCST: total correct categories	5.50 (1.14)	5.30 (1.13)	-1.16	.247
WCST: perseverative errors	1.40 (1.75)	2.59 (2.96)	-1.67	.095
TMT-B: time needed	122 (54.5)	151 (76.2)	-1.87	.062
TMT B/A	2.71 (1.16)	3.18 (1.22)	-2.17	.030
BNT: total spontaneous correct	40.4 (3.07)	39.0 (4.41)	-1.01	.311
WAIS-R Block design: total correct	24.6 (8.56)	23.5 (8.48)	-.752	.452
WAIS-R Similarities: total correct	24.6 (5.10)	23.9 (6.29)	-.170	.865
WAIS-R Vocabulary: total correct	21.1 (6.26)	21.7 (5.70)	-.232	.817
MWT-B: correct words	32.2 (4.16)	31.7 (3.69)	-.901	.367
CVLT: correctly recalled List A trials 1-5	49.5 (8.93)	50.1 (8.14)	-.423	.672
CVLT: correctly recalled short delay	10.8 (2.76)	9.74 (2.69)	-1.65	.099
CVLT: correctly recalled long delay	11.3 (3.18)	10.7 (2.61)	-1.12	.263
CVLT: Recognition discriminability (%)	95.7 (4.23)	94.4 (6.56)	-.795	.427

Table II-6: Mean (SD) performance of left-temporal NIRS responders vs. left-temporal non-responders

	Responders (n=33)	Non-Responders (n=64)	*Z*	p
Phonemic Fluency: total correct	28.6 (7.92)	27.6 (8.96)	-.767	.443
Category Fluency: total correct	50.8 (10.5)	50.3 (13.0)	-.203	.443
WCST: total correct categories	5.42 (1.21)	5.18 (1.33)	-.739	.460
WCST: perseverative errors	2.19 (3.67)	2.47 (2.59)	-1.24	.215
TMT-B: time needed	141 (55.0)	130 (54.5)	-1.23	.218
TMT B/A	3.05 (1.24)	2.78 (1.06)	-1.08	.280
BNT: total spontaneous correct	40.2 (3.05)	39.1 (4.27)	-.873	.383
WAIS-R Block design: total correct	24.8 (7.82)	23.2 (7.23)	-.831	.406
WAIS-R Similarities: total correct	24.3 (4.83)	24.7 (5.40)	-.823	.411
WAIS-R Vocabulary: total correct	20.2 (6.17)	22.3 (4.93)	-1.60	.109
MWT-B: correct words	31.2 (3.35)	32.3 (3.78)	-1.85	.065
CVLT: correctly recalled List A trials 1-5	49.9 (8.66)	50.5 (8.98)	-.210	.834
CVLT: correctly recalled short delay	10.4 (2.67)	10.2 (3.14)	-.115	.909
CVLT: correctly recalled long delay	11.0 (2.49)	11.0 (2.84)	-.015	.988
CVLT: Recognition discriminability (%)	95.2 (4.32)	94.5 (6.89)	-.233	.816

Table II-7: Mean (SD) performance of right-temporal NIRS responders vs.right-temporal non-responders

	Responders (n=30)	Non-Responders (n=63)	*Z*	p
Phonemic Fluency: total correct	29.1 (10.2)	27.9 (9.28)	-.311	.756
Category Fluency: total correct	50.7 (11.6)	50.7 (12.2)	-.217	.828
WCST: total correct categories	5.42 (1.10)	5.35 (1.18)	-.222	.824
WCST: perseverative errors	1.66 (1.85)	2.45 (2.62)	-1.13	.258
TMT-B: time needed	133 (51.0)	136 (67.9)	-.285	.776
TMT B/A	2.85 (1.19)	2.88 (1.20)	-.142	.887
BNT: total spontaneous correct	39.5 (3.93)	39.5 (4.11)	-.176	.860
WAIS-R Block design: total correct	24.8 (9.37)	23.4 (6.72)	-.346	.730
WAIS-R Similarities: total correct	24.5 (5.57)	24.7 (5.00)	-.091	.928
WAIS-R Vocabulary: total correct	21.2 (6.51)	21.9 (4.44)	-.235	.814
MWT-B: correct words	32.0 (2.90)	32.0 (3.93)	-.542	.588
CVLT: correctly recalled List A trials 1-	49.2 (9.96)	51.4 (8.78)	-1.23	.217
CVLT: correctly recalled short delay	10.4 (3.15)	10.4 (3.08)	-.186	.852
CVLT: correctly recalled long delay	10.4 (3.04)	11.2 (2.79)	-.945	.345
CVLT: Recognition discriminability (%)	94.6 (6.79)	95.0 (5.92)	-.050	.960