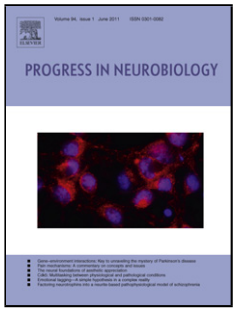

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Functional magnetic resonance imaging as a dynamic candidate biomarker for Alzheimer's disease

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Contents

Abstract

During the last two decades, imaging of neural activation has become an invaluable tool for assessing the functional organization of the human brain in vivo. Due to its widespread application in neuroscience, functional neuroimaging has raised the interest of clinical researchers in its possible use as a diagnostic biomarker. A hallmark feature of many neurodegenerative diseases is their chronic non-linear dynamic and highly complex preclinical course. Neurodegenerative diseases unfold over years to decades through clinically silent and asymptomatic stages of early adaptive, compensatory to pathophysiological (i.e. actively neurodegenerative) and decompensatory mechanisms in the brain – phases that are increasingly being considered as critical for primary and secondary preventive and therapeutic measures.

Emerging evidence supports the concept of a potentially fully reversible functional phase that may precede the onset of micro- and macrostructural and cognitive decline, a potentially late-stage ”neurodegenerative” phase of a primary neurodegenerative disorder. Alzheimer’s disease serves as an ideal model to test this hypothesis supported by the neural network model of the healthy and diseased brain. Being highly dynamic in nature, brain activation and neuronal network functional connectivity represent not only candidate diagnostic but also candidate surrogate markers for interventional trials. Potential caveats of functional imaging are critically reviewed with focus on confound variables such as altered neurovascular coupling as well as parameters related to task- and study design.

1. Introduction

1.1. Why do we need next level *functional dynamic biomarker* development?

-BOX START

Definition and rationale of "functional dynamic biomarkers"

Single neurochemical biomarkers can be considered as 'unidimensional' biomarkers since they mostly indicate individual molecular aspects of pathophysiology, However, modern approaches in biomarker development (e.g. proteom based marker), increasingly attempt to account for the complexity of pathological changes related with neurodegenerative diseases. Functional dynamic biomarkers are 'multidimensional' as they are proposed to integrate a broad range of molecular and physiological processes which converge to brain function on a large-scale system level. This approach allows to track non-linear dynamic states of functionality within cognitively relevant neuronal systems and to capture effects of disease modifying as well as of symptomatic compounds on these functionally relevant systems. Functional dynamic biomarkers do not depend on single pathophysiological processes involved in neurodegeneration (e.g. oxidative stress, synaptotoxicity or apoptosis) but rather reflect the interaction of the sum of all pathophysiological events at a given timepoint with physiological large-scale functional systems. It can be argued that higher dimensionality of markers may come at the cost of their specificity. This concern needs to be addressed in clinical studies specifically aiming to determine diagnostic sensitivity and specificity of such markers. Current findings, however, suggest that complex large-scale system markers may indeed provide promising levels of diagnostic specificity if appropriate analysis methods are being applied to neuroimaging data.

-END OF BOX

A number of core feasible diagnostic biomarkers from various methods and modalities have been developed and systematically validated in recent years for diagnostic use in neurodegenerative disorders, primarily in Alzheimer's disease (AD). Large-scale international controlled multi-centre programs (such as ADNI) have validated neurochemical core feasible CSF based biomarkers, such as total tau (t-tau), hyperphosphorylated tau (p-tau) and amyloid

beta$_{1-42}$ protein (Aβ_{1-42}), which accurately discriminate between healthy control subjects and patients suffering from incipient and established clinical AD with high sensitivity and specificity (Blennow et al., 2009; Hampel et al., 2010a; 2010b). To date, manual hippocampus volumetry is the best established and validated macrostructural neuroimaging core biomarker for AD, with matured confirmation from meta-analyses and large-scale controlled international multicenter trials (e.g. Teipel et al., 2010a). Ultimately, positron emission tomography (PET) using radioligands suited for detection of regional cerebral metabolism (^{18}FDG-PET) (Karow et al, 2010) or fibrillar amyloid deposition in the brain (e.g. Pittsburgh Substance B – PiB-PET) have been established as useful diagnostic markers in a large number of studies in MCI subjects and patients with AD (Rabinovici and Jagust, 2009).

All of these established core feasible "first stage" biomarkers will soon find their way (to some degree) into clinical practice, mainly and ideally providing supportive diagnostic information relevant to the clinical diagnosis of early AD. Furthermore, the use of biomarkers for clinical diagnostics is encouraged by the newly proposed revised AD research criteria (Dubois et al., 2010) and current international diagnostic revision initiatives that will soon publish their modified guidelines and recommendations. Even high standard national guidelines (e.g. the German S3 guidelines on dementia) recommend the use of biomarkers in ambiguous cases with tricky and difficult differential diagnoses. Moreover, they seem to have an emerging role as independent co-primary or secondary outcome endpoints in an increasing number of clinical trials investigating potential disease modifiers for AD, supporting evidence for beneficial effects of tested compounds on pathophysiologically relevant pathways and mechanisms in AD (Hampel et al., 2010a; 2010b).

However, with AD being a disease featuring chronic non-linear dynamic multifactorial etiology and multifaceted pathophysiology with potentially converging molecular mechanisms, each of these biomarkers can only depict a fraction of disease associated events at a given time point in cross-sectional investigations (Jack et al., 2009). For example, there is

ample evidence that changes of CSF $A\beta_{1-42}$ levels and changes of fibrillar Aβ-levels in the brain represent early events of AD pathology which have apparently passed their highest levels of disease dynamics before or at the clinical onset of dementia, after which they don't seem to correlate anymore with further cognitive decline (Hardy and Higgins, 1992; reviewed in Rabinovici and Jagust, 2009). On the other hand, when used in clinical trials on Aβ-targeting compounds in AD, Aβ markers may accurately indicate effects on Aβ-metabolism but these biological effects do in turn not necessarily translate into a favourable clinical outcome. The reasons for this are still unresolved. A recent example for this dilemma has been involuntarily provided by the immediate stop of two large phase III confirmatory trials on semagacestat, a gamma-secretase inhibitor (http://newsroom.lilly.com/releasedetail.cfm?releaseid=499794): despite evidence for effective inhibition of Aβ production, which had raised high hopes for an effective disease-modifying therapy of AD, patients treated with semagacestat actually deteriorated significantly faster than patients using placebo. The lessons learned from such unfortunate cases are twofold: 1.) "unidimensional" markers indicating only single and potentially fragmented pathomechanisms may not likely suffice indicating or predicting clinically relevant effects in interventional trials, which includes pharmacological, as well as non-pharmacological therapeutic interventions (cognitive or physical training are examples for non-pharmacological interventions; see e.g. Buschert et al., 2010) 2.) Because the human brain displays a hypercomplex functional organization allowing for substantial molecular and structural damage before cognitive deficits become apparent, dynamic and sensitive markers of brain functionality are clearly required in order to capture effects of the disease (early diagnosis; prediction of outcome) and to indicate therapeutic effects which actually have a functional relevance (as opposed to a mere pathophysiological role) to the diseased brain and thus represent true surrogate markers (Hampel et al., 2010a).

Although detectable structural changes may precede dementia diagnosis, *structural* imaging biomarkers (e.g. hippocampus volumetry) show only a linear and predominantly unidirectional decline during the course of ongoing neurodegeneration. Some interventional studies, albeit not in neurodegenerative diseases, found substantial increases in hippocampal volumes after short-term physical exercise programs (Pajonk et al., 2010). However, the biological basis (e.g. increased neuropil vs. increased increased blood vessel volume) as well as the physiological relevance of these results are entirely unclear, especially as hippocampal volume increases were not associated with an improvement of episodic memory function. While structural decline may correlate well with neuronal loss, structural improvement may be associated with various factors, most of which are not related to increased neuronal count or to cognitive function. Moreover, the effects of structural decline on cognitive function may likely be diluted by various functional-adaptational processes on the one hand and be artificially aggravated by independent impairments in remote efferent or afferent processing pathways on the other hand. Altogether, these factors represent limitations of structural markers when compared with functional dynamic biomarkers as dynamic surrogate endpoints. This might be subject to change with the emergence of structural imaging methods which could detect subtle structural changes of functionally relevant subregions of the hippocampus (as well as of other brain areas) on a microsopic level.

Cumulating evidence suggests that aberrant brain activation and abnormal neuronal network interconnectivity may precede cognitive deficits, metabolic brain changes and structural atrophy by years or even by decades, which has led to the development of novel hypotheses and concepts for the pathophysiology and pathogenesis of neurodegenerative diseases (Hampel et al., 2010b; Palop et al., 2006). These concepts basically distinguish between potentially fully reversible early functional stages, characterized by aberrant neuronal function and activity, which may play an adaptive or maladaptive role, and later partly or totally non-reversible neurodegenerative stages of the disease with pathophysiological events ultimately

leading to regional and cell-type specific neuronal loss and micro- to macrostructural damage (Palop and Mucke, 2009; Palop et al., 2006) (Figure 1). The presumed potential reversibility of functional alterations in this hypothetical framework is supported by observed large fluctuations of cognitive function and clinical symptoms in patients with neurodegenerative diseases which cannot be entirely explained by neurodegeneration alone (Palop et al., 2006). Moreover, it is a generally accepted view that only neuronal loss is definitely irreversible across most parts of the brain, except for a few small and circumscribed brain regions which display adult neurogenesis (Gross, 2000). Any pathophysiological processes temporally preceding neuronal loss may thus be potentially reversible, - at least in theory. Findings of functional network alterations even in very young adults (starting at 20y of age) at genetic risk to develop AD later in life support the hypothesis that aberrant neuronal network function may precede apparent molecular amyloid pathophysiology (Filippini et al., 2009).

Based on these hypotheses and concepts, biomarkers capturing subtle functional changes of brain function (functional dynamic biomarkers) may therefore be best suited to detect disease-relevant and characteristic features or "signatures" during earliest preclinical stages, far before neurochemical, structural or cognitive changes come into measurable effect.

In the following sections we give an overview of the physiological bases underlying functional activation neuroimaging. We will then review findings in healthy aging and cognitive decline within the context of MCI and dementia syndromes and finally discuss findings on the diagnostic power of individual paradigms of functional activation neuroimaging.

1.2 Methods for imaging brain functional activation

The term "functional activation" is used to denote relative neuronal activation levels, generally driven by intrinsic activity or induced by cognitive tasks, by sensory stimulation or

by motor action. Neuroimaging methods that are used to assess functional activation, however, only capture indirect measures of neuronal activity. Methods based on electroencephalography (EEG) and magnetoencephalography (MEG), however, represent exceptions to this rule as they measure direct electric and magnetic correlates of neuronal and synaptic activity. The main principle of these *indirect* functional neuroimaging methods is based on reactive dynamic changes of blood flow and oxygenation due to neuronal and synaptic activity. Early functional imaging methods in humans were based on nuclear imaging using special radio-labelled tracers which allowed quantifying either task-related changes of regional cerebral blood volume (CBV) and flow (CBF) using Xenon-133and later H_2O-PET, or levels of regional cerebral glucose uptake using ^{18}FDG-PET. Since the 1990ies, the emerging method of functional magnetic resonance imaging (fMRI) has gained wide use and popularity in functional brain research using an even more indirect measure of neuronal activity: the so called blood oxygenation level dependent (BOLD) signal. To date, the vast majority of functional neuroimaging studies are in fact based on fMRI derived methods instead of nuclear or metabolic neuroimaging which is justified on several characteristic advantages of fMRI: First, fMRI does not rely on the application of radioligands to create signal contrast, since it is based on deoxygenated haemoglobin (dHb) as an endogenous contrast agent. Because of that, fMRI is entirely non-invasive and does not bear the risk of allergic reactions to exogeneous contrast agents nor does it expose the subject to ionizing radiation. Second, modern clinical MRI devices (basically all of which are technically capable of performing fMRI) are massively distributed nowadays thus providing a very high level of availability for clinical diagnostics, as well as for clinical and basic neuroimaging research. Finally, fMRI provides superior spatial and temporal resolution compared with nuclear imaging techniques, allowing to detect more subtle dynamic changes and to delineate functional changes more precisely within and between small distinct anatomical structures.

Since this review will focus on fMRI studies, the fundamental principles of the BOLD signal will be briefly explained in order to facilitate the understanding of the strengths, as well as of potential caveats of fMRI as a promising method to provide next level candidate diagnostic tools and potential early functional surrogate biomarkers for brain disorders, particularly primary neurodegenerative disorders.

1.3. The BOLD Signal

The human brain is estimated to consist of 100 billions of neurons with a number of synapses in the range of quadrillions providing a vast and complex network of over 100 trillion connections. Although the generation of neuronal electrical action potentials is considered to be relatively energy-efficient, electrochemical synaptic transmission accounts for a major part of brain's total energy demand. It is estimated that approximately half of the brain's energy production is utilized for the direct generation of electrophysiological function (Siesjo, 1978, Ames, 2000). Because the brain does not possess any meaningful energy stores (in contrast to many other organs), its function critically depends on uninterruptible substrate (glucose) and oxygen supply. The brain's excessively and disproportionately increased energy demand is reflected by a total of 20% of body oxygen consumption despite the mass of the brain constituting only a total of 2% of the bodies mass. This demand is further supported by a luxury blood perfusion that constitutes ~15% of total cardiac output, (Siesjo, 1978). Performing high-field MRI gradient-echo sequences in rodents, Ogawa et al. (1990) observed peculiar decreases of the MRI signal that were constrained to blood vessels. This observation was explained by magnetic field inhomogeneities (susceptibility artefacts) due to intravasal deoxygenated haemoglobin (dHb), which possesses paramagnetic properties (Brooks et al., 1975). Dependent on the oxygenated or deoxygenated state, the Fe+ atom of the haemoglobin changes between a low- and a high spin state, the latter having paramagnetic properties and thus acting as an endogenous contrast agent. With specific, highly sensitive MRI sequences,

local changes of this blood oxygen dependent (BOLD) signal can be measured over time reflecting the local concentration of dHb and its fluctuations over time.

Neuronal activity is associated with an immediate extraction of oxygen from the supplying vessels. This is leading to a short and small initial increase of dHb, after which a large increase of local CBF is initiated leading to substantial inflow of oxygenated Hb that exceeds the actual rate of oxygen extraction and decrease of dHb concentration, ultimately causing an increase of BOLD signal compared with the baseline (Fox et al., 1988; Fox and Raichle, 1986).

In a series of experiments, Logothetis and colleagues investigated the interrelations between BOLD signal changes and neuronal activity by combining fMRI with microelectrode recording, which is capable of obtaining electrical signals from small neuronal assemblies (Logothetis et al., 2001; reviewed in Logothetis, 2002). Using this combined approach, local field potentials (LFP) which reflect dendritic synaptic input signaling were found to be the best correlate of transient BOLD signal responses in monkey visual cortex. Another noteworthy finding from this series of studies was the observation that the BOLD signal had significantly more variability than electrophysiologically recorded neuronal activity.

Altogether, current knowledge on basic physiological principles of fMRI underscores the fact that the BOLD signal is not necessarily explained solely by neuronal activity and that possibly independent factors may also modulate the cerebrovascular response to neuronal activation. This constraint is of particular relevance when interpreting fMRI studies that compare subjects of different ages or when trying to find a physiological interpretation of fMRI activation differences between diagnostic groups.

1.4 Physiology and pathophysiology of neurovascular coupling

Although not fully understood, several physiological mechanisms involved in the regulation of CBF in response to neuronal activity have been identified. Cumulative findings suggest

that neurons, glia cells and cells of vascular vessels interact with each other in a site- and activity-dependent manner, forming a 'neurovascular unit' (Iadecola, 2004). While increased energy demand due to neuronal and synaptic activity suggests itself as a driving factor for reactive increases in local CBF in order to increase supply with oxygen and glucose, research demonstrates that local glucose and oxygen consumption driven by neuronal activity are by far not as pronounced as necessary in order to metabolically drive activity-induced hyperemia. However, subtle changes in cellular NAD/NADH ratio, as well as in the lactate/pyruvate ratio appear to serve as sensitive 'energy sensors', with potential relevance to CBF (Mintun et al., 2004; Ido et al., 2001) . Moreover, astrocytes are spatially linked with the vascular walls of arterioles and capillaries and can regulate the diameter and resistance of these vessels via perivascular release of vasoactive factors, among which are adenosine, K+, nitric oxide (NO) and prostaglandins (Harder et al., 2002; Paulson and Newman, 1987). Release of glutamate or GABA from neurons was found to activate intracellular calcium signalling pathways in nearby astrocytes, which in turn release vasoactive factors at the site of vessel contact and influence vessel resistance (Zonta et al., 2003; Idecola and Nedergaard, 2007). Moreover, neuronal activity in general and – neuronal activity of interneurons in particular – appear to influence vascular resistance in a direct, neurotransmitter-mediated way (Mathiesen et al., 1998). Beside glutamate and GABA, dopamine and acetylcholine have been identified as vasoactive neurotransmitters as well (Krimer et al., 1998; Scremin et al., 1973). Another important aspect of neurovascular coordination is based on intramural signalling, which denotes a release of vasoactive substances from upstream endothelial cells in response to increased generalized stress levels due to downstream increases of local CBF (Iadecola, 2004).

One essential and consistent finding on neurovascular coupling is that no single CBF-influencing mechanism is sufficient to explain the entire CBF response and that blocking of any single of these mechanisms alone cannot entirely abolish neurovascular coupling. Instead,

a coordinated and concerted interplay of *all* involved cell types – neurons, interneurons, astrocytes, endothelial cells, pericytes etc – seems to be required (Iadecola, 2004; Girouard and Iadecola, 2006).

Deposition of Aβ fibrils in extracellular perivascular space as well as within blood vessel walls is a frequent finding in AD: according to neuropathological studies, between 82-98% of all AD patients show signs of vascular amyloid deposition (cerebral amyloid angiopathy – CAA) (Jellinger, 2002). At the same time, a large proportion of patients with AD do also have cerebral microbleedings, which are associated with CAA (De Reuck et al., 2011) and pronounced microvascular changes with decreased density and structural abnormalities causing regional metabolic and blood-brain barrier dysfunctions (Jellinger, 2002). Co-localized with sites of greatest amounts of neurodegeneration, the basement membrane of capillaries in the cerebral cortex of AD patients is prominently thickened, mostly due to increased collagen deposition (Mancardi et al., 1980) and deposition of amorph Aβ fibrils (reviewed in Farkas and Luiten, 2001). Beside these pathological alterations associated with insoluble *fibrillar* Aβ, experimental administration of *soluble* Aβ monomers in experimental settings consistently showed massive abolishment of functional cerebrovascular autoregulation, reduced functial hyperemia and caused a fundamental shift of vascular reactivity in favour of vasconstricting stimuli, even in the absence of fibrillar plaque deposits (Crawford et al., 1998; Niwa et al., 2000; Niwa et al., 2001; Niwa et al., 2002; Park et al., 2004).

A study in patients with AD and those suffering from vascular dementia (VaD) using transcranial Doppler revealed markedly impaired vasoreactivity in both patient groups compared with healthy controls (Vicenzini et al., 2007). In a longitudinal follow-up study, AD patients with highest impairments of the "breath-holding index" (BHI), a measure of vasoreactivity using Doppler ultrasonography, displayed the highest rate of cognitive decline

over time (Silvestrini et al., 2006). However, some studies based on ^{15}O-PET could not replicate impaired vasoreactivity in AD patients when applying hypercapnia (Nagata et al., 1997; Kuwagara et al., 1992).

Taken together, some of these findings have led to the assumption that deficits in resting-state or in task-related increases of CBF in AD patients may be a consequence of deteriorated neurovascular coupling (Girouard and Idaecola, 2006). However, in how far reduced regional CBF in AD - which is regularly observed in resting state as well as during various cognitive tasks - reflects deficits in neuronal activation or alterations of neurovascular coupling, or both, is not yet fully understood. In any case, current evidence strongly suggests that neurovascular coupling may be independently affected by AD-related pathomechanisms and may thus independently affect outcome in CBF- and fMRI studies.

1.5. Interactions between neurovascular coupling, CBF and the BOLD signal

Hypercapnia is a strong stimulus to increase CBF. Several studies experimentally modulating baseline CBF using various levels of hypercapnia in anesthesized animals found a substantial reduction of the dHb response during functional activation that correlated with the level of baseline CBF (Huppert et al., 2009; Jones et al., 2005).

Handwerker and colleagues (2007) asked the question, whether observed differences in BOLD signal change between older and younger subjects across various tasks was due to differences in neuronal activity or due to differences in vascular reactivity (neurovascular coupling). They applied a simple visual task as well as a breath-holding task that served as a hypercapnia paradigm, testing for vascular reactivity. They found reduced task-related BOLD signal changes in the older group compared with young subjects in various cortical regions. These changes remained significant, even after correcting for the differences in BOLD response in the hypercapnia task. In contrast, applying a sensorimotor task and a breath-holding task, Riecker and colleagues (2003) found that reduced BOLD responses in the

sensorimotor cortex due to the motor task were mostly explained by reduced BOLD responses due to impaired vasoreactivity.

These apparently divergent results suggest that age-related changes in neuronal activity and in neurovascular coupling may be region-dependent. Indeed, by combining several tasks (cognitive, motor and breath-holding), Kannurpatti and colleagues (2010) found that vasoreactivity was differentially expressed across various brain regions and that correction for hypercapnia-related BOLD signal change explained the differences between older and young subjects in the motor task but not the task-related differences in brain areas subserving cognitive tasks.

These results demonstrate that reduced BOLD activity in older subjects cannot solely be explained by altered neurovascular coupling and that alterations of neurovascular coupling may be region-specific.

2. Functional dynamic imaging biomarkers in aging, MCI and AD

2.1. Task related *activation* as functional *dynamic* biomarker

In order to better understand the nature of altered brain functional activation, which may provide a new generation of truly "dynamic" biomarkers in neurodegenerative diseases, it is important to first assess possible changes of brain activation during normal aging. All the more as age is the largest single risk factor for neurodegenerative disorders and particularly for sporadic AD, with a near exponential increase of dementia incidence and prevalence after the age of 60 years (Jorm and Jolley, 1998). Parts of the frontal cortex have been suggested as particularly sensitive to aging and to show largest age-related structural and functional decline compared with other areas of the brain (Tisserand and Jolles, 2003). Examining structural MRI data across six different samples, with more than 800 subjects in total, the superior and inferior frontal gyri along with superior parts of the temporal lobe demonstrated highest rates of age-related cortical thinning with other cortical brain regions being less affected (Fjell et

al., 2009). With extensive evidence pointing to parts of the frontal lobe as prime locations for age-dependent *structural* decline, the next question to answer was whether task-related *functional* properties during 'frontal' cognitive tasks were altered as well.

The anterior parts of the frontal lobe (prefrontal cortex – PFC) are considered as critical morphological substrate of various cognitive functions, including executive functions, working and episodic memory, planning and monitoring (Stuss and Knight, 2002). Task-related activation of the PFC is consistently found during various cognitive tasks, including working and episodic memory (Fletcher and Henson, 2001).

In an attempt to find a common denominator that would unify the findings of age-related changes of brain activation, the so called "HAROLD" (hemispheric asymmetry reduction in older adults) model was proposed (Cabeza, 2002) suggesting that PFC activity during various cognitive tasks is generally less lateralized in older individuals compared with younger subjects. A large body of functional imaging studies supports the HAROLD model with age-dependent additional contralateral activation mostly being observed in working and episodic memory tasks (Backman et al., 1997; Madden et al., 1999; Grady et al., 2001; Logan et al., 2002; Morcom et al., 2002; Reuter-Lorenz et al., 2000).

The functional relevance of a greater contralateral PFC recruitment was interpreted as a result of compensatory co-activation in an attempt to make up for reduced functional processing efficiency. An alternative interpretation of the HAROLD phenomenon has been proposed to be aging-induced "dedifferentiation", an inability to direct cortical activation to focal areas relevant for a given cognitive process and instead inefficiently activating broader or additional cortical areas, including those in the contralateral hemisphere, none of which does contribute to a better task performance (Li and Lindenberger, 1999). In order to elucidate which of those two alternative explanations were more accurate, Cabeza et al. (2002) compared task related brain activation during an episodic memory task between a group of young healthy subjects, a group of low-performing older subjects and a group of high-performing elderly subjects.

While young and low-performing older subjects showed a similar predominant task-related activation of the right PFC, the high performing elderly revealed bilateral activation of the PFC (Cabeza et al., 2002). Accordingly, Wierenga et al. (2008) could demonstrate that in a verbal retrieval task, additional activation in the right inferior frontal gyrus (IFG), a homologue of the left-sided Broca's area, was significantly correlated with better task-accuracy in elderly subjects.

In summary, evidence supports the concept of overrecruitment and bilateral PFC recruitment as an indicator of increased neurocognitive effort that in some cases may be related with improved cognitive task performance in elderly subjects. However, while these findings are strongly suggestive of a compensatory role, the mere descriptive and correlational nature of studies that provided these data, does not allow for ruling out alternative explanations.

In an attempt to investigate whether age-related effects on brain functional activation are task-dependent, task-independent, or both, and colleagues (2004) applied a battery of different cognitive tasks (working memory, visual attention and episodic retrieval) to young and elderly healthy subjects during fMRI measurement. In line with the previously reported results from various independent studies, Cabeza and colleagues (2004) found reduced occipital activation as well as increased PFC activation across all tasks when compared with younger controls. Additionally, in the working memory task, elderly displayed the HAROLD phenomenon, while in the episodic memory task elderly subjects also showed a hippocampal activation deficit in comparison with the young group. In summary, these findings support the notion that deterioration of sensory processing as well as PFC overactivation are fundamental, task-independent age-related effects, while other aging-dependent differences of brain activation may be specific to the involved cognitive processes and tasks.

Episodic memory deficits are among the first symptoms of cognitive deterioration in AD which is a consequence of early involvement of the hippocampus in AD-related pathology

(Squire, 1992). Therefore, a large number of functional imaging studies used episodic memory tasks in order to reveal functional abnormalities in the hippocampus related to AD. In verbal as well as in visual episodic memory tasks, AD patients consistently show substantial activation deficits during encoding in the hippocampus as well as in other cortical areas (Small et al., 1999; Kato et al., 2001; Rombouts et al., 2000; Schroder et al., 2001; Sperling et al., 2003; Machulda et al., 2003). A meta-analysis on functional imaging studies of episodic memory in AD using activation likelihood estimation (ALE) revealed a high consistency across the included studies with regards to activation deficits in the medial temporal lobe in AD patients compared with controls. However, AD patients showed also increased activity levels in areas of the ventral prefrontal cortex, which may be interpreted as an attempt to recruit compensatory mechanisms (Schwindt and Black, 2008).

Findings in subjects with mild cognitive impairment (MCI), are largely bimodal with some MCI subjects displaying *higher* activation increases in the hippocampus (Hamalainen et al., 2007; Dickerson et al., 2005) (Figure 2) and other MCI having *smaller* task-related activation increases relative to controls (Machulda et al., 2003). When comparing cognitively more impaired MCI subjects with those that are less cognitively impaired, Celone et al., (2006) reported task-related hippocampal hyperactivation in less impaired MCI and task-related activation deficits in more impaired MCI subjects. Taken together, these findings support the concept of a continuum between healthy subjects with no genetic burden representing the low end of the spectrum, demented AD patients the upper end, and cognitively intact subjects at (genetic) risk for AD as well as subjects displaying minor cognitive deficits (MCI) being in between. Across this continuum, hippocampal task-related activation responds in a non-linear fashion with significantly overshooting activity in preclinical and early clinical stages and a subsequent abnormal decline of activation during clinical stages (Figure 1).

These conceptual notion on the course and progression of activation dynamics of the hippocampus is strikingly supported by recent findings from a longitudinal study (O'Brien et al., 2010): Elderly individuals with slight cognitive deficits at baseline developed a significant decline in hippocampal activation over the course of a 2-year follow up and the rate of activation decline correlated with the rate of cognitive decline. Further support comes from a number of studies in cognitively intact subjects at genetic risk for AD, reporting consistent task-related hyperactivation in the medial temporal lobe (exemplary: Johnson et al., 2006; Bookheimer et al., 2000).

Beside episodic memory, fMRI has been applied to various other sensory and task modalities, including olfactory stimulation (Förster et al., 2010), working memory (e.g. Bokde et al., 2010a) or various visual tasks (e.g. Bokde et al., 2010b; Bokde et al., 2006; Prvulovic et al., 2002; for review see Wierenga and Bondi, 2007). However, since the greater number of studies use episodic memory tasks, this cognitive domain seems the best investigated functional system during the clinical AD dementia and MCI disease stages to date using functional neuroimaging methods.

2.2. Task related *deactivation* as functional dynamic biomarker

Cognitive tasks generally induce a significant increase of activity in brain areas subserving various aspects of the task, which is the fundamental basis of functional activation imaging. However, a consistent pattern of cortical regions has been identified to show a *reduction* of activity during the task when compared to a baseline. These so called task-induced deactivations (TID) typically occur in areas of the default mode network (DMN), which shows coherent intrinsic slow-wave oscillatory activity in the absence of apparent attention-demanding tasks (Raichle et al., 2001). The DMN itself is comprised of a number of core regions including medial parietal (posterior cingulated cortex; PCC), medial prefronal (medial PFC; mPFC) and lateral parietal (lP) cortex. The hippocampus is also reported to be

functionally and structurally associated with the DMN (Teipel et al., 2010b). Typically, the different regions of the DMN show not only structural but significant levels of functional connectivity as well, a measure for synchronous, time-correlated neuronal activity across several spatially remote cortical areas. At the same time, activity in the DMN areas is highly anti-correlated with activity in areas outside the DMN, which are involved in active task processing (Fox et al., 2005; Fransson, 2005; Greicius, et al., 2003; Raichle et al., 2001). The DMN has been suggested to have a role in intrinsic thought processing, monitoring, and mind-wandering, all of which represent typically unconstrained cognitive processes in the absence of tasks demanding attentional focus and effort. Moreover, the typical deactivations observed within the DMN across a large number of different cognitive tasks may support a reallocation of cognitive resources towards tasks demanding constrained processing and thus represent an endogenous mechanism of cognitive control. Accordingly, TIDs within the DMN are often linearly correlated with task difficulty and with the amount of cognitive effort necessary to successfully perform these tasks (McKiernan et al., 2003). A few functional neuroimaging studies analysed the association of age with TIDs in the DMN. Lustig and colleagues (2003) applied a semantic classification task to young and healthy elderly subjects as well as to AD patients. Of note, compared with young controls, the healthy elderly subjects showed a smaller magnitude of TID particularly in medial parietal areas (PCC) and this finding was even more pronounced in AD patients also extending to further areas of the DMN. In order to test for possible interactions between age and task-difficulty, Persson and collegues (2007) used several verbal tasks with parametrically graded difficulty levels. They found that TIDs during the cognitively least demanding task was comparable between young and healthy elderly subjects and that the magnitude of TIDs increased with higher difficulty level in young subjects but not in the seniors. These results have been interpreted as a possible correlate for age-related impairments of cognitive control required to redirect attention away from task-irrelevant processes that comes into effect with higher task demands.

TIDs are of particular interest not only because they show differences between diagnostic and age groups but also because they significantly correlate with task-performance: Persson and colleagues (2007) reported that the amount of TIDs was associated with faster reaction times in the difficult task conditions. Congruently, Daselaar and colleagues (2004) found that the magnitude of TIDs during perceptual as well as during semantic encoding among several cortical regions, including the medial parietal cortex, accurately predicted correct retrieval of memorized items. Similar findings were reported from Miller and colleagues (2008), who showed that successful performance in an episodic memory task was strongly correlated with the magnitude of TID in the posteromedial cortex and that elderly subjects who performed poor on the memory task also showed reduced TIDs in this cortical area.

Because dysfunction of episodic memory is one of the first clinical symptoms to occur in predementia stages of AD and because of its substantial topographical overlap with the hippocampal episodic memory network, the DMN is a particularly promising target to detect functional aberrations in the preclinical and clinical course of AD (Vincent et al., 2006; Buckner et al., 2008; Buckner et al., 2005; Raichle et al., 2001). In AD, areas of the DMN, and most prominently the posteromedial parts of it (PCC), show abnormal failure for task-induced deactivations across various cognitive tasks (Greicius et al., 2004; Lustig et al., 2003; Pihlhajäkki et al., 2008).

2.3. Resting state activity as functional dynamic biomarker

Another excellent setting to test the DMN is the 'resting state paradigm', during which subjects are lying awake with their eyes closed or opened and with no specific task to be performed during the functional imaging measurement. The resting-state-paradigm is founded on the observation that the brain generally displays high levels of activity during rest (Fox and Raichle, 2007; Buzsaki et al., 2004), a fact that is also mirrored by constantly high levels of resting state brain energy consumption, which are only marginally (~5%) further elevated

during cognitive activity (Raichle, 2010). The resting state activity would not be of high interest, if it were not predictive of the performance in various active tasks, including sensory and attention (Boly et al., 2007; Fox et al., 2007; Fox et al., 2006; Weissmann et al., 2006). Another reason to focus on resting state activity is the finding that areas with highest levels of resting activity (largely resembling areas of the DMN) are at the same time among the first locations in the brain to develop pathophysiological changes typically associated with AD (Buckner et al., 2005; Buckner et al., 2009). Lifelong high levels of resting state activity coincides with predilection sites for AD-related changes. The intrinsic activation in resting-state networks can be assessed by the amount of BOLD signal fluctuations, by the extent and magnitude of resting state components identified with hypothesis-free methods such as the independent component analysis (ICA) (Calhoun et al., 2001) and by the temporal coherence of the BOLD signal between remote areas, which is a measure of functional connectivity. The latter can be based on either ICA derived or hypothesis-driven set regions of interest (ROIs) (Fox and Raichle, 2007).

Resting state-related intrinsic activation within the DMN is consistently found to be reduced in older subjects compared with young controls (Damoiseaux et al., 2008). Koch and colleagues (2010) found in elderly subjects a significantly reduced magnitude of resting state activity in the PCC - a central posterior node of the DMN - when compared with young control subjects. However, inter-connectivity between different nodes of the DMN was not significantly affected by age (Koch et al., 2010). This finding points to age-dependent activation deficits in some areas of the DMN but to a largely preserved functional network integrity within the DMN.

Using the ICA approach, reduced resting-state co-activation within the DMN was found in AD patients most prominently in the medial parietal cortex (PCC) and in the hippocampus (Greicius et al., 2004). In MCI, resting state co-activation of DMN core nodes was reduced in comparison with healthy controls, including frontal and medial parietal areas and bilateral

inferior/lateral parietal regions. Moreover, functional connectivity between the PCC and the hippocampus in the resting state was significantly smaller in MCI patients (Sorg et al., 2007). Using a hypothesis-driven region of interest (ROI)-based approach, reduced resting state functional connectivity between the hippocampus, the PCC and other areas of the DMN was found in AD patients, with somewhat differing results between various studies, which may be attributed to methodological issues and patient selection (Allen et al., 2007; Wang et al., 2006; Wang et al., 2007). Functional connectivity between core DMN regions was also significantly reduced in cognitively intact elderly subjects harbouring increased fibrillar Aβ deposition in the brain, as revealed by combined resting state fMRI and amyloid-PET imaging (Hedden et al., 2009).

2.4. Detection power of dynamic functional biomarkers: lessons from genetic imaging

The long asymptomatic preclinical course of AD calls for biomarkers which may be able to detect preclinical AD pathophysiology in the absence of overt clinical and cognitive symptoms (see also Trojanowski and Hampel in this issue). In order to test for the hypothesis that functional brain activation and neuronal network connectivity patterns are altered in preclinical AD, numerous fMRI studies have been performed in cognitively intact subjects with increased genetic risk to develop AD. Since the ApoE4 genotype is the single most prominent genetic risk factor for sporadic AD, groups with asymptomatic ApoE4 carriers and non-carriers have been compared in studies using various cognitive activation paradigms, including episodic and working memory tasks. A majority of these studies have found increased task-related functional activation across various brain areas and across different cognitive tasks in ApoE4 carriers compared with non-carriers (Bookheimer et al., 2000; Smith et al., 2002; Bondi et al., 2005; Fleisher et al., 2005; Han et al., 2007; Filippini et al., 2009). However, this finding is not consistent across the literature since some fMRI studies found indices of reduced task-induced functional activation in ApoE4 carriers (Mondadori et al.,

2007; Trivedi et al., 2006; Lind et al., 2006). A possible reason for this discrepancy could be due to effects from specific task-design properties. Another probable reason may be an interaction of the ApoE genotype with other genetic factors, e.g. as reflected by family history of AD as well as with age. Fillipini and colleagues (2009) found significantly increased resting state network interconnectivity within the DMN in young ApoE4 carriers (20-35y), while in older asymptomatic ApoE4 carriers resting state functional connectivity within the DMN was reduced (Sheline et al., 2010).

A general limitation of genetic imaging that is based on genotypes with discrete increase of risk to develop AD later on is that longitudinal follow up is mandatory in order to determine which of the subjects at risk actually developed dementia. However, performing fMRI measurements on mutation carriers from families with autosomal dominant early onset AD (ADAD) overcomes this important limitation as such mutation carrying individuals are at 100% risk to develop AD. An fMRI study comparing preclinical mutation carriers (mutations in the APP and PSEN1 gene) with mutation-free individuals from families with familial AD (FAD) failed to find any increased task-related brain activation in the mutation carriers (Ringman et al., 2010). On contrary, mutation carriers showed lower activation in medial prefrontal areas compared with the non-carriers during a novelty task. The presented data however did not allow interpreting whether the observed difference between groups was due to lower task-related activation or due to increased task-induced deactivation in the mutation carriers. In addition to this finding, ApoE4 positive individuals from this same subject group showed increased task-related activation across various brain regions compared with individuals without the ApoE4 allele (Ringman et al., 2010). Braskie and colleagues (2010) found that young amyloid precursor protein (APP) and presenilin 1 (PSEN1) mutation carriers (average age: 31 years) showed abnormal increases of brain activation during an episodic memory encoding task only as they approached the estimated age at onset of disease but not at earlier age. In another study, Mondadori and colleagues (2006) applied an episodic memory

as well as a working memory task in two PSEN1 mutation carriers from ADAD families and compared resulting brain activation patterns with those from a control group. While the younger of the two PSEN1 mutation carriers (20y old) showed substantial increases in task related brain activation when compared with the control group, the older mutation carrier (45y) had significantly decreased task-related activation. However, these differences pertained only to the episodic memory task but not to the working memory task.

Because of probable interactions of chosen cognitive tasks with genotype and underlying sets of brain areas subserving tested cognitive tasks, resting state fMRI appears to be a more robust candidate biomarker for use in genetic imaging than functional activation imaging based on cognitive tasks. Filippini and colleagues (2009) found increased resting state coactivation within the DMN in young healthy ApoE4 carriers (20-35y) compared with non-carriers. In contrast to this finding, older subjects (mean age > 58y) showed significantly reduced DMN resting state functional connectivity (Sheline et al., 2010). In summary, resting state fMRI data fully supports the model that abnormally increased network connectivity at young age is followed by subsequent abnormal decrease of network interconnectivity at older ages in ApoE4 carriers. Data from functional activation imaging during cognitive tasks is generally in line with this concept but with less consistent findings.

3. The diagnostic power of fMRI biomarkers

Several studies have tested and quantified the diagnostic value of various fMRI paradigms (activation / deactivation / resting state) in Alzheimer's dementia. Studies focusing on resting state fMRI are summarized in table 1.

Li and colleagues (2002) determined the COSLOF index (coefficients of spontaneous low frequency) of the hippocampus in elderly control subjects and AD patients. Spontaneous low frequency fluctuations of the BOLD signal were recorded during resting state fMRI. Calculation of the cross-correlation coefficients between all components of the slow

frequency oscillations from all voxels within the hippocampus revealed a significant difference between AD and control subjects. While no values were presented for the area under receiver operating characteristics (ROC) curve, a true-positive correct classification of AD patients in 80% of cases at an accepted rate of 10% false-positives were reported.

In an other study, testing the goodness to fit on components of the DMN that were detected by ICA applied to resting-state fMRI data, AD patients and healthy elderly controls could be correctly categorized with a sensitivity of 85% and a specificity of 77% (Greicius et al. 2004).

Koch and colleagues, (2010b) applied both, region-of-interest (ROI) based interconnectivity analyses as well as ICA based analyses of the magnitude of co-activation in analyzing the diagnostic value of resting state fMRI. While both approaches revealed moderate results when used separately, a combination of both methods within a multivariate approach resulted in a diagnostic accuracy of 97% (sensitivity 100%, specificity 95%) in discriminating AD patients from healthy subjects. This method was however less accurate in categorizing MCI subjects, which was proposed to be caused by the heterogeneous etiology of the MCI diagnosis (among others).

Fleisher and colleagues (2009) directly compared the diagnostic value between patterns of functional activation during a cognitive task and resting state connectivity in the same sample of subjects. They included cognitively intact elderly subjects which were separated by their individual risk to develop AD. The risk stratification was based on family history in combination with ApoE4 status (positive ApoE4 status *and* positive family history for AD vs. negative ApoE4 status *and* negative family history). As cognitive paradigm, they used a face-name association task that is well established in the literature to activate the hippocampus and to deactivate areas of the DMN. The low-risk subjects showed substantially stronger TIDs during encoding in the medial parietal cortex as well as bilaterally in the lateral parietal

cortex, all of which are core areas of the DMN. The high-risk subjects showed abnormally increased functional connectivity between most areas of the DMN, with exception of the medial parietal cortex, which showed significantly reduced functional connectivity with other DMN areas. A comparison between the two methods revealed that resting state functional connectivity discriminated between the two risk groups with a superior effect size (3.35) compared with task-related functional activation during memory encoding (1.39).

By dividing the brain into 116 anatomical regions of interest and calculating pairwise correlation coefficients between resting state fMRI signal from these regions, Chen and colleagues (2011) could separate amnestic MCI from healthy control groups with a diagnostic sensitivity of 93% and a specificity of 90%, while AD patients could be differentiated from the non-AD group (amnestic MCI and healthy controls) with a sensitivity of 85% and a specificity of 80%. Given the fact that a high proportion of subjects with MCI has underlying AD pathology, the lower diagnostic accuracy in separating AD from MCI vs. separating MCI from healthy controls does not surprise.

Complex properties of large-scale neuronal networks (such as 'small-world-metrics') can be assessed using graph analytical methods. Applying this method to resting-state-fMRI data of AD patients and elderly controls, Supekar and colleagues (2008) demonstrated a loss of small-world properties in AD patients, characterized by a significantly lower clustering coefficient, especially in the left and right hippocampus, which indicated disrupted local connectivity. This clustering coefficient distinguished AD participants from the controls with a sensitivity of 72% and specificity of 78%.

A commentary by James B. Rowe in this special issue specifically addresses the current state of the art as well as the most interesting developments in the field of complex network

analysis research in neuroimaging. Further extensive information on this important topic can also be retrieved from Rowe (2010) and from Seghier et al. (2010).

Using a combination of features (such as volumetric data, functional activation data and others), selecting those features based on correlation based filters (Yu and Liu, 2003) and feeding selected features to a Random Forrests classifier (Breiman, 2001), Tripoliti and collegues (2007) were able to correctly classify a sample of AD patients and healthy elderly controls with a specificity and sensitivity of 96-98%. The authors claim that a specific advantage of this method is constituted by the fact that it does not depend on the type of fMRI task and that any fMRI paradigm (activation task, resting state) can be used in this approach.

4. FMRI biomarkers for classification (differential diagnosis) of primary neurodegenerative and dementia disorders

Only very little work has been done on the value of fMRI for classification (differential diagnosis) of primary neurodegenerative or dementia disorders. Some primary neurodegenerative diseases, such as frontotempolar dementia (FTD) and AD may have overlapping clinical phenotypes, especially at the beginning of the dementia syndrome, which sometimes makes it difficult for the clinician to make a diagnostic decision based on the clinical picture alone. Using fMRI, Zhou and colleagues (2010) assessed intrinsic brain activity during resting state in FTD and in AD patients. Looking at two different neuronal networks, they found an almost inverted picture in FTD compared with the AD patients: while connectivity within the so called 'salience network' (frontoinsular, cingulate, striatal, thalamic and brainstem regions) was attenuated and connectivity in the DMN increased in the FTD group, the opposite picture was present in AD patients with strongly reduced connectivity in the DMN and increased connectivity in the frontal salience network. These results are highly promising and should encourage further fMRI based head-to-head

comparisons of AD with other neurodegenerative diseases as well as with psychiatric disorders such as affective disorders (major depression) or schizophrenia.

5. Discussion

In this review we have extracted and summarized results of available functional neuroimaging studies in order to answer the central question whether functional activation imaging may be a potentially suitable biomarker in neurodegenerative diseases.

Taken together, the reported findings indicate that functional activation imaging is a method that can serve as a basis to assess alterations of brain function and functional neuronal network properties, which in turn may serve as truly *dynamic* diagnostic or even detection biomarkers. A very large number of functional activation imaging studies have been performed in AD patients to date, however, only a handful of fMRI studies have actually computed the diagnostic detectionpower of the applied fMRI paradigm and the used analytical methods. Studies where this has been performed, however, allow for two general conclusions:

1. Resting state fMRI captures intrinsic brain activity and properties of resting state neuronal networks, based on which diseased and healthy subjects can be separated with high diagnostic accuracy.
2. A combination of different computational methods (e.g. Koch et al., 2010b) and a combination of different features (Tripoliti et al., 2007) may prove clearly superior in their diagnostic value compared with single-method, single-region, or single-feature approaches.

Some issues which are regarded as drawbacks to fMRI in general are high intra- and interindividual variability, dependence of the results on individual effort, and length of

measurement. While currently all these points are correct for functional activation imaging using active cognitive tasks, they don't apply to resting state fMRI, which requires minimal scanning time (~10 min), no cognitive effort or demand on the subject, and which appears to provide substantial test-retest-reliability (Meindl et al., 2010).

In addition to these features, rs-fMRI targets intrinsic brain activity that seems to be of much higher relevance to brain disease and brain function than previously assumed (Raichle, 2010). While the DMN may be of relevance to various brain disorders, its overlap with episodic memory network is of particular value to detect early changes in AD.

In summary, we conclude that rs-fMRI appears to be a very promising candidate diagnostic detectionbiomarker when compared with other fMRI paradigms. However, its stability, reliability and diagnostic value are based on a limited number of studies and thus need validation in larger trials.

Nevertheless, a combination of rs-fMRI with active sensory or cognitive paradigms would have the benefit to specifically test for processing capacity and for compensatory mechanisms within and across distinct neuronal subsystems, depending on the specific diagnostic question. This approach would be helpful when therapeutic effects of compounds in clinical trials need to be evaluated. Symptomatic as well as disease-modifying drugs could indeed improve processing capacity of certain subsystems on a precognitive level, that cannot be captured using behavioural tests but that could become evident from changes in task-related activation patterns (Bokde et al., 2009). This is of special interest for fast compound labelling in early stages of drug development, where decisions on whether or not to further support the development of a certain compound need to be evaluated before starting large phase II / III studies. Moreover, fMRI activation patterns may predict the therapeutic response and cognitive effect from compounds and reveal which physiological pathways are actually affected by a compound (Bentley et al., 2009).

When active fMRI tasks are being performed with diagnostic purposes, then they should generally be combined with rs-fMRI. The need for this combination comes from the pathophysiology of neurovascular coupling: of importance, the fMRI signal reflects synaptic and neuronal activity but it is also modulated by changes in baseline CBF and vasoreactivity, both of which can be independently influenced by the underlying disease as well as by various exogeneous factors such as consumption of caffeine, drugs, or various medications (for review see Lindauer et al., 2010). In order to scale the hemodynamic response in accordance to baseline blood flow or vasoreactive capacity, it has been suggested to combine BOLD fMRI experiments with additional measures of baseline cerebral perfusion (e.g. using arterial spin labelling MRI) and/or with measures of cerebrovascular vasoreactivity (e.g. breath-holding task). However, breath-holding, although being supported by a large number of studies in subjects of wide age-range, has certain disadvantages that result from individual differences in lung capacity or from the amount of subjective air hunger during breath holding. An increasing body of evidence suggests that amplitude and spatial distribution of low-frequency BOLD fluctuations measured with resting state fMRI, correlate exceptionally well with hypercapnic breath-holding response (Biswal et al., 2007; Kannurpatti et al., 2010b). These results are in line with earlier findings that resting-state low-frequency BOLD oscillations are sensitive to systemic fluctuations in CO_2 (blood CO2-levels), which in turn are an accepted indicator of vascular sensitivity (Wise et al., 2004). Therefore, information on subject-specific vascular sensitivity is reflected by the resting-state BOLD fluctuation amplitude (RSFA) that can easily be obtained from resting-state-fMRI (Kannurpatti and Biswal, 2008). First applications in subjects of different ages have already shown that RSFAs are as much useful as hemodynamic scaling factors obtained from breath-holding tasks in order to obtain neuronally derived functional activation, devoid of age-(or disease-)related modulation of vasoreactivity (Kannurpatti et al., 2010a).

Another problem with the assessment of fMRI in general and with rs-fMRI in particular is the impact of "noise" or signals of non-neuronal origin. Pulse and respiration have both been found to introduce artefacts into the rs-fMRI signal, mostly because of overlapping frequency ranges. It is therefore recommended to co-register these physiological parameters and use them as co-regressors when analysing rs-fMRI (Birn et al., 2006).

An important question pertains to the potential diagnostic usability of fMRI in subjects at risk to develop AD. However, neither the functional relevance of activation changes found in young at risk subjects is known (compensatory, aberrant, dedifferentiated?), nor their value in supporting a prediction of later conversion to AD. In order to resolve this imponderability, longitudinal and follow-up study designs are mandatory, testing which individual activation- and connectivity patterns may actually correspond with later cognitive decline or with the development of other pathological changes related with AD.

As with all candidate biomarkers, fMRI findings in AD do also need validation from clinicopathological studies providing final proof for the accuracy of diagnosis. Another way is to combine fMRI with other AD markers in order to have best possible in vivo diagnostic certainty and to enrich the diagnostic groups with "true" cases and exclude possibly preclinical cases from the control groups (Hampel et al., 2010b). This particularly applies to presymptomatic at-risk groups, where stratification based solely on single genetic risk markers such as ApoE4 may not be sufficient to characterize individual risk to develop AD.

From a conceptual perspective, fMRI findings of very early hyperactivation and hyperconnectivity may represent a beginning adaptive functional state of the brain in response to a variety of possible early risk factors (genetic, epigenetic, metabolic, energetic etc.), which exert subtle and additive negative effects on brain function. Alternatively, aberrantly increased activity and connectivity – especially in very young subjects - may be a mere

consequence of a misalignment of cognitive demand and cognitive activation, which represents an inefficient way to use neuronal resources. Regardless if aberrant neuronal activity is caused by adaptation, by reduced efficiency, by misalignment or by overstraining, it may have a role in driving pathophysiological processes independent of its cause, , e.g. by increasing local Aβ levels, which in turn – depending on the dose – may negatively affect synaptic function (Palop and Mucke, 2010). Although hippocampal atrophy may be evident as early several years before clinical onset of dementia, recent studies suggest, that functional brain activation abnormalities are already present in young individuals, several decades before a possible onset of dementia (Filippini et al., 2009). These findings imply that neurodegenerative events including macrostructural atrophy are generally preceded by chronic changes of brain functionality and brain responsiveness. Being probably among the earliest events in the course of preclinical and presymptomatic stages of neurodegenerative diseases, is a prerequisite for the assessment of brain function and of brain functional networks becoming very early dynamic detection, diagnostic and/or prognostic biomarkers. However, this may only then be the case if further longitudinal or prospective observational studies succeed to assign abnormal functional activation patterns (a functional brain endophenotype) to later cognitive decline or to clinical conversion (Bokde et al., 2009b) Most likely such a course would require several decades and may be substantially accelerated by selectively recruiting risk-stratified subjects just a few years before estimated onset of cognitive or clinical dementia symptoms.

6. Conclusion

Functional activation in the brain and functional connectivity of resting-state and memory networks represent a new generation of *dynamic* candidate biomarkers that reflect non-linear pathologically induced changes across the life-span and across various stages of preclinical and clinical progression in neurodegenerative disorders. The current state of fMRI as a tool to

provide candidate dynamic diagnostic and surrogate biomarkers in AD is very promising, however, needs further rigorous and stepwise validation. Larger and highly enriched samples are warranted, for which controlled international multi-centre studies with standardized high-level protocols and sophisticated paradigms are a feasible way that has been demonstrated successfully using classical *static* core feasible biomarkers (i.e. CSF core biomarkers or assessment of whole brain or hippocampal volume). Analysis of genetic material and body fluids such as CSF and plasma for additional biomarker tests should be routinely implemented in fMRI studies in order to enrich the samples with true diagnostic cases, as well as co-registration of physiological signals (e.g. respiration). Ultimately, confirmation of predictive value can only be achieved by prospective longitudinal studies and long-term population based observation.

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Figure Legends

Figure 1

Putative model of functional dynamic markers during the course of Alzheimer's disease (AD). Functional activation is increased early in the course of AD, already at presymptomatic stages, probably reflecting states of compensatory adaptation or misalignement of functional brain activity. With advancing age and with advance of pathophysiological disease-related processes, functional activation declines (stage of decompensation) and ultimately reaches abnormally low levels during clinical stages (late MCI and AD). Most consistent results supporting this model are from resting state fMRI studies showing aberrant increases of coactivation and/or interconnectivity across regions of the default mode network (DMN) during early and adaptive ("functional") stages of AD and concomitant decline to abnormally disrupted network activity and functional connectivity the more the time of onset of clinical symptoms is approaching.

Figure 2

Representative findings of substantially enlarged cortical activation areas in patients with mild cognitive impairment (MCI) (lower row) compared with healthy controls (HC) (upper row) during three different levels of a visual working memory task. Cortical activation maps are obtained from BOLD signal changes during encoding vs. baseline for easy (load 1: blue coded maps), middle (yellow coded maps) and difficult (red coded maps) task conditions. During all three difficulty levels, MCI subjects show substantially larger activation areas across a wide range of posterior (parietal, occitipital) cortical areas, while dorsolateral prefrontal areas are mostly overactivated during the easiest and the middle task load conditions in MCI subjects when compared with healthy controls (courtesy of D.P., unpublished data).

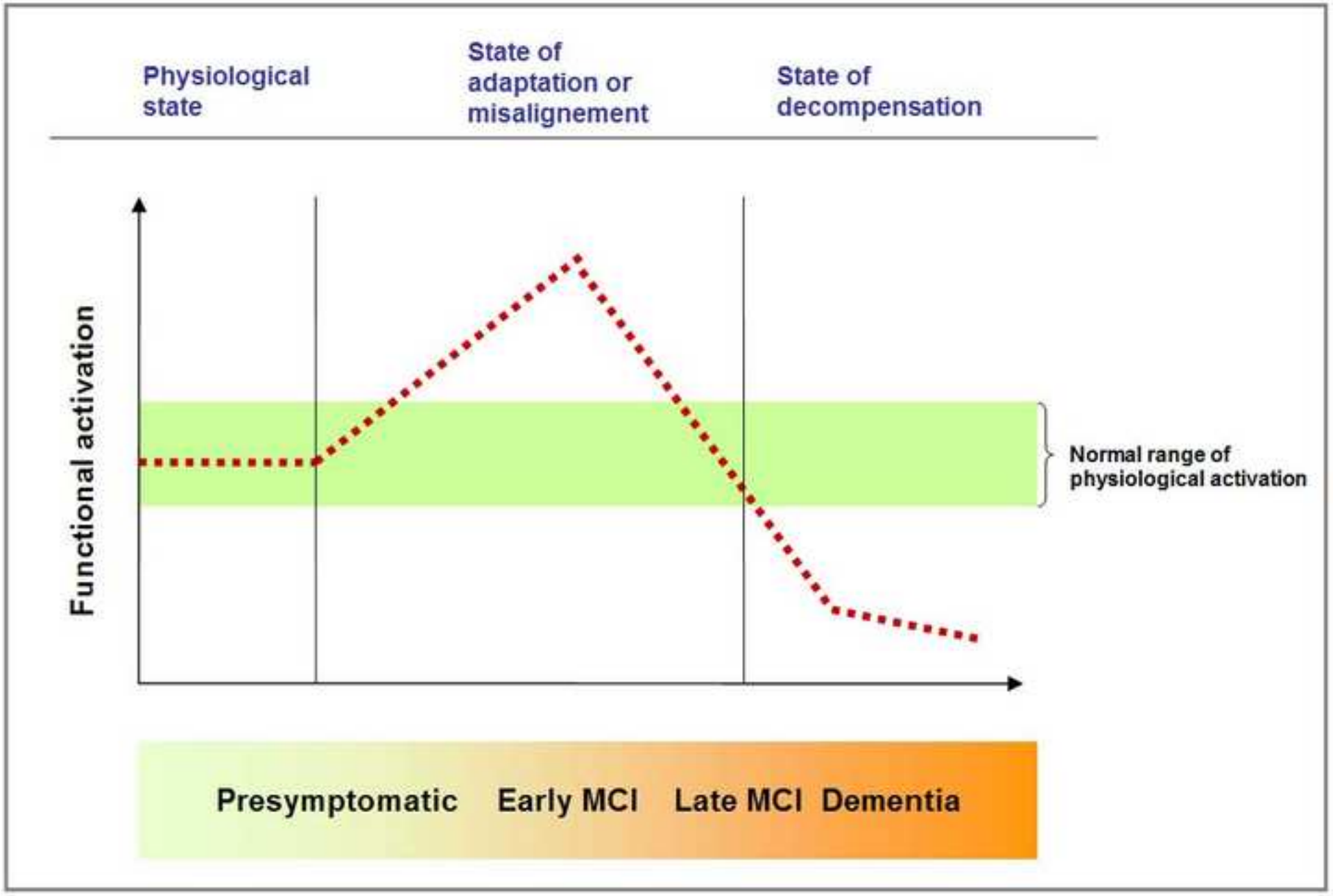
Table 1

Study	**fMRI candidate biomarker**	**Patients and Subjects**	**Diagnostic value**
Li et al. (2002)	rs-fMRI (COSLOF-Index)	AD (N=14) vs. HC (N=13)	Sensitivity: 80% Specificity: 90%
Greicius et al. (2004)	rs-fMRI - ICA components of the DMN	AD (N=13) vs. HC (N=13)	Sensitivity: 85% Specificity: 77%
Koch et al. (2010b)	rs-fMRI - Combined functional connectivity (ROI based) & coactivation magnitude (ICA-based)	AD (N=15) vs. HC (N = 21)	Sensitivity: 100% Specificity: 95%
Chen et al. (2011)	rs-fMRI - ROI-based pairwise product moment coefficients	AD (N=20) vs. Non-AD group (=pooled aMCI and HC (N=35))	Sensitivity: 85% Specificity: 80%
Supekar et al. (2008)	rs-fMRI - Small world network properties (clustering coefficient)	AD (N=21) vs. HC (N=18)	Sensitivity: 72% Specificity: 78%

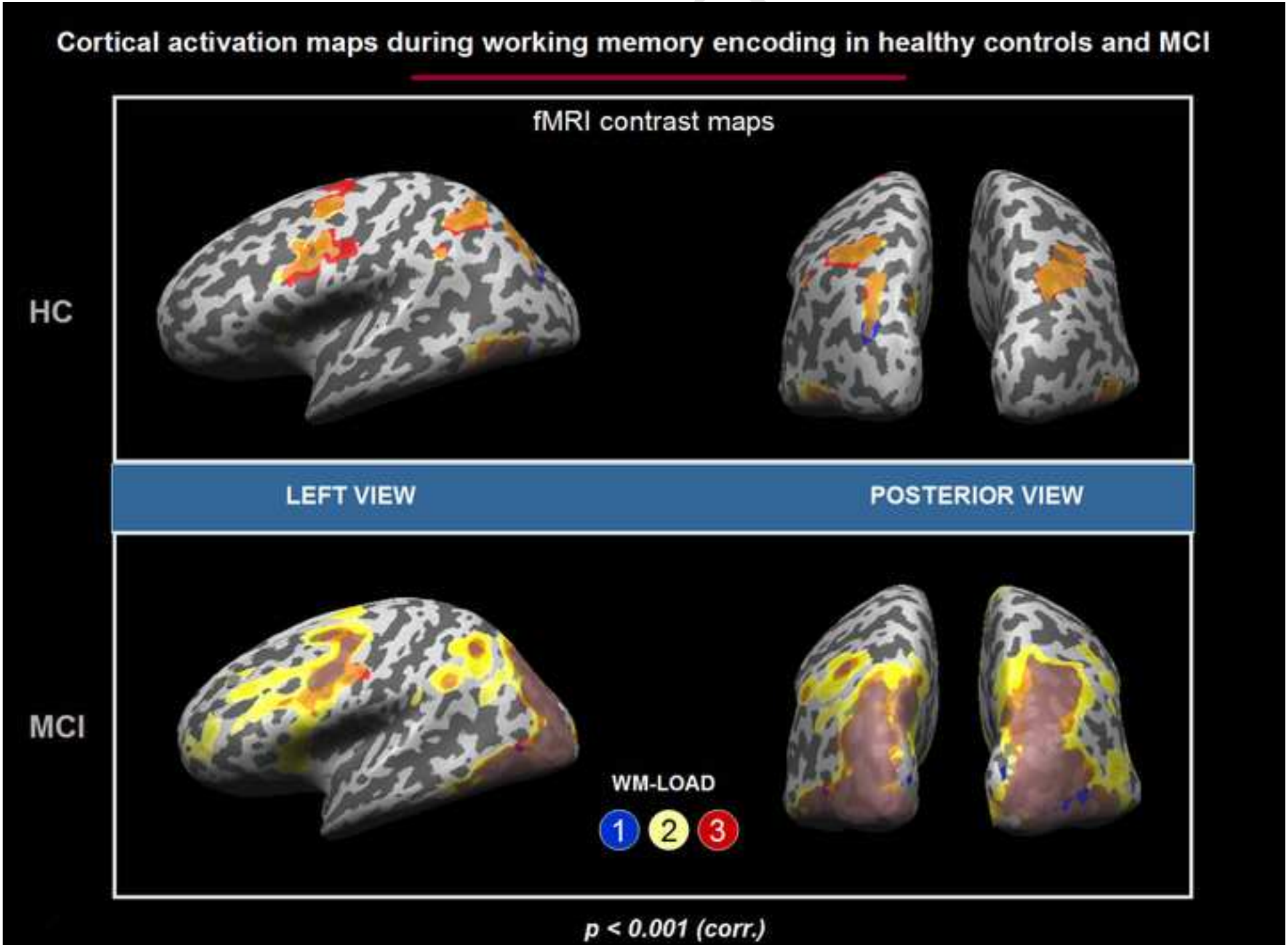
Table 1 Legend:

Overview of studies that assessed the diagnostic value of resting state fMRI (rs-fMRI) candidate biomarkers. Only values from AD patients and healthy controls (HC) are reported in the table, as they were consistently reported across all studies. Some studies also incorporated an additional group with subjects suffering from mild cognitive impairment (MCI) but these results are not reported here. All studies mentioned in table 1 are discussed in more detail in section 3 (The diagnostic power of fMRI biomarkers).

Figure(s)


Physiological state
State of adaptation or misalignement
State of decompensation
Functional activation
Normal range of physiological activation
Presymptomatic
Early MCI
Late MCI
Dementia

Figure(s)


Cortical activation maps during working memory encoding in healthy controls and MCI
fMRI contrast maps
HC
LEFT VIEW
POSTERIOR VIEW
MCI
WM-LOAD
1
2
3
p < 0.001 (corr.)

Abbreviation list:

AD: Alzheimer's disease

BOLD: Blood oxygenation dependent signal

CAA: Cerebral amyloid angiopathy

CBF: Cerebral blood flow

dHB: Deoxygenated haemoglobin

FMRI: Functional magnetic resonance imaging

LFP: Local field potential

PET: Positron emission tomography

ApoE: Apolipoprotein E

Research Highlights:

Functional neuroimaging captures non-linear states of brain function; Functional dynamic markers of default mode and memory networks have high diagnostic accuracy in the detection of AD; Functional dynamic biomarkers are promising candidate surrogate markers and may improve rapid compound labelling in AD in early drug development stages.