Neural substrates of spatial awareness and balance in stroke patients.

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I hereby declare that the thesis entitled “Neural substrates of spatial awareness and balance in stroke patients”, submitted in fulfillment of the requirements for the doctoral degree, is my own work and that, to the best of my knowledge and belief, it contains no material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning. I have used only the sources and aids indicated and any contribution made to this work by others is explicitly acknowledged in the thesis.

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Luca Ticini,

March 2009

To my Family & my Friends

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Abstract

This doctoral thesis attempts to investigate the anatomical and functional consequences of brain lesions in patients with disorders of spatial awareness and balance. It attacks the following problems central to this endeavour.

The first problem is that of the disorder of body orientation in the coronal (roll) plane of space known as ‘pusher syndrome’. Stroke patients with such disorder have a dysfunction of the upright body position and experience their body as oriented upright when actually tilted nearly 20 degrees towards the ipsilesional side. Typically, posterior thalamic strokes and, infrequently, lesions of the postcentral gyrus and insula provoke ‘pusher syndrome’. This argues for a primary role of these neural structures in our control of upright body posture. In pusher patients, very little - if nothing - is known about additional functional or metabolic abnormalities outside the areas of irreversible damage, which may contribute to the disorder. In the experiments presented in this thesis, I investigated 19 stroke patients

with thalamic or with extra-thalamic lesions, showing versus not showing ‘pusher syndrome’. We asked whether these patients show additional functional or metabolic abnormalities outside the areas of brain lesion. In order to do so, we used fluid-attenuated inversion-recovery (FLAIR) imaging and diffusion-weighted imaging (DWI) to investigate the structural damage and perfusion-weighted imaging (PWI) to depict the malperfused but structural intact tissue. We found that those pusher patients with thalamic lesions did not demonstrate additional dysfunctional brain areas. In those pusher patients with extra-thalamic lesions, the regions of abnormal perfusion were restricted in the structurally intact inferior frontal gyrus, middle temporal gyrus, inferior parietal lobule and parietal white matter. The results point out that the anatomical network composed by the posterior thalamus, the insula, postcentral gyrus and the malperfused areas mentioned above, is critical to perceiving gravity and controlling human upright body orientation.

The second problem is that of characterizing the neural correlates of ‘visual extinction’. Visual extinction is a disorder of bilateral distribution of attention and a typical manifestation of left hemispatial neglect. It is identified if the patients dismiss contralesional events when simultaneously presented with an ipsilesional stimulus. Earlier researches found that subcortical lesions of the basal ganglia, thalamus, internal capsule and of the periventricular white matter, as well as damage or inactivation of the right temporoparietal junction (TPJ) evoke visual extinction. The aim of this experiment was to investigate whether extinction in patients with subcortical damages may be explained by dysfunction of structurally intact cortical structures. In thirteen spatial neglect patients, showing versus not showing visual extinction, I investigated the functional or metabolic abnormalities associated with basal ganglia strokes. As for the first experiment, we used FLAIR imaging and DWI to study the structural damage and PWI to describe malperfused tissues. The results indicate that lesions of subcortical structures contribute to the appearance of

visual extinction through elicited dysfunction of the structurally intact TPJ. Our results endorse a direct relationship between subcortical damage and cortical malfunction and they show that, also in the case of subcortical damage, the normal functioning of the TPJ area plays a decisive role in the attentional network involved in detecting of visual stimuli under rivalry.

The third investigation represents a new methodological approach to lesion analysis on patients with ‘spatial neglect’. Neglect is an impairment of spatial attention in the transverse (yaw) plane, common after right hemisphere strokes. Patients with this disorder typically fail to report stimuli presented on the contralesional (left) side. In the last years, a lively controversy has animated the investigations on the neural correlates of spatial neglect. As a matter of fact, there are at least three cortical locations claimed to be involved in neglect: the right inferior parietal lobule (IPL), the right temporo-parietal junction (TPJ), the right superior temporal gyrus (STG) and, to some extent, the dorsolateral frontal cortex. However, neglect is not only

typically associated with cortical lesions, but it also evident after subcortical lesions of the putamen, pulvinar and caudate nucleus. In addition to these findings, further investigations proposed that spatial neglect may be the consequence of white matter fibres' disruption. In this experiment we aimed at demonstrating the actual involvement of cortical and subcortical structures in a large group of 140 stroke patients showing versus not showing spatial neglect. In order to do so, we used a statistical voxelwise lesion-behaviour mapping (VLBM) approach combined with the Juelich probabilistic cytoarchitectonic atlas of the human white matter fibre tracts. In patients with spatial neglect, our results indicate that the lesions are largely associated with damage of the grey instead of the white matter. Nevertheless, in accordance with prior connectivity studies, we found that the damage of right perisylvian white matter connections – the superior longitudinal fasciculus, the inferior occipitofrontal fasciculus, and the superior occipitofrontal fasciculus – is, to some extent, also associated with spatial neglect.

Abbreviations

AF	Arcuate fasciculus
CT	Computed tomography
DTI	Diffusion tensor imaging
DWI	Diffusion-weighted imaging
FLAIR	Fluid-attenuated inversion-recovery
fMRI	Functional magnetic resonance imaging
MSR	Maximum signal reduction
MRI	Magnetic resonance imaging
PWI	Perfusion-weighted imaging
PET	Positron emission tomography
PWI	Perfusion-weighted imaging
TPJ	Temporo-parietal junction
TTP	Time-to-peak
SLF	Superior longitudinal fasciculus
IOF	Inferior occipito-frontal fasciculus
SOF	Superior occipito-frontal fasciculus

1. Introduction

This thesis is concerned with disorders of spatial awareness and balance in stroke patients. Within this context, I specifically aimed at understanding the mechanisms underlying dysfunction of spatial perception and processing in the coronal (roll) and transverse (yaw) planes. In order to have a comprehensive view of the work presented here, first of all, we need to consider that our brain is constituted of a number of different anatomical structures and areas; each one of these it is supposed to be specialized for one or more specific tasks, like the processing of sensory stimuli (e.g. visual, tactile, auditory, etc.) or the planning and execution of motor processes. The earliest demonstrations of this 'functional specialization' date back already to the last decades of the XIX century. In particular, two popular names - among others - are usually associated with functional localization: Pierre Paul Broca and Korbinian Brodmann. In 1861, Broca presented a patient with a large brain lesion of the left frontal regions that could understand

language but was not able to produce any structured language. Broca concluded that this was an evidence for a straightforward association between the lesion in a specific area and the disorder: “The lesion in the frontal lobe had been the cause of the loss of speech”. While Broca made a first ground-breaking step towards the understanding of functional specialization, in 1905, Brodmann fractioned the cortex into 52 areas based upon their cortical cytoarchitecture, which would also reflect the different functions of brain districts. In plain English, he subdivided the cerebral cortex based upon visual differences in cell layering and cell types. Notwithstanding, after more than a century, this parcellation and the nomenclature accompanying each area (e.g. BA [Brodmann Area] 17) are still extensively utilized. Still nowadays, numerous scientists dedicate their researches to brain’s functional specialization, in order to deepen the relationships between structure and function (i.e. assigning activations of confined brain areas to specific functions). In the last two decades, this kind of investigation has been conducted in a more

comprehensive manner and with increasing less difficulty. As a matter of fact, a significant assistance has been given by non-invasive magnetic resonance imaging (MRI) techniques. For example, structural MRI has deepened the work of Broca allowing the identification of circumscribed brain regions that, when damaged, determine specific dysfunctions. Otherwise, through detecting the local increase in oxygen consumption that accompany neural activity, functional magnetic resonance imaging (fMRI) has allowed to measure and visualise *in vivo* the brain activity of subjects performing a task or perceiving a stimulus. Although of extreme interest and extensive use, fMRI studies have directed most of the research towards the understanding of grey matter's functional specialization. As a result, a detailed study of subcortical white matter structures and of their importance in brain processes has been primarily restricted to *post-mortem* studies. However, recent developments in MRI-based diffusion tensor tractography (Catani et al., 2002; 2005, 2007; Makris et al., 2005, 2007; Mori et al., 2005; Upadhyay et al., 2008)

have changed this trend, permitting the visualization *in vivo* of subcortical white matter fascicles in healthy subjects, as well as the disruption of white matter connectivity in stroke patients. This novel perspective has opened brand new possibilities to conduct investigations that take into account the whole of brain networks (consisting of a framework of distinct cortical and subcortical regions, connected by long-range white matter fibre tracts) and not only parts of them (e.g. [He et al., 2007]). Obviously, also the neural processes that assist our ability to plan, control and direct action in space (the topic of the present thesis) require the coordinated activity of large-scale brain networks as well as the convergence of information from multiple sensory sources, including vestibular, visual, and somatosensory. Inevitably, being able to discern the cortical regions and white matter pathways that are determinant for space and balance perception is a rather difficult problem, in a brain composed of numerous cortical areas and a complicated network of white matter fibres. As a matter of fact, a lot

has been written and different methodologies have been applied without reaching a firm and decisive conclusion. The approach that this PhD project has taken is to deepen the investigation of spatial awareness defects in groups of stroke patients, by using (i) structural MRI in order to visualize and define brain lesions and (ii) perfusion MRI in order to detect areas of dysfunction that may accompany the strokes. These two techniques, which I used extensively in the present work, need a brief introduction. First of all, we shall consider that an increase of precision in the description and localization of focal brain lesions has been witnessed in the last two decades, mostly thanks to the rising power of neuroimaging techniques that allow reproducing the fine details of brain's structures. Modern investigations on stroke patients commonly define brain lesions by means of structural MRI. Among the different kind of images that MRI can provide, diffusion-weighted magnetic resonance imaging (DWI) and fluid attenuated inverse recovery imaging (FLAIR) are most often employed to identify the lesioned tissue in the acute and subacute

phase of a stroke. Although structural MRI allows locating the lesions, frequently it is rather difficult to determine with a reasonable degree of precision which structure -when lesioned- causes a disorder. Indeed, lesions may encompass different functional modules and white matter tracts. In order to overcome this limitation, I used a method called 'group lesion mapping' (Rorden and Karnath, 2004). The word 'group' indicates that the process of lesion mapping requires the employment of a group of stroke patients with a specific disorder in which we identified the lesion's extension. The localization of the centre of overlapping that characterizes these lesions is compared with an appropriate group of patients without the disorder (controls). Of course, in order to conduct a group analysis, the differences among the individual brain's anatomical structures need to be reduced. This can be accomplished by using a spatial normalization process implemented in software such as SPM (http://www.fil.ion.ucl.ac.uk/spm/), which automatically transforms the image of individual brains to match with a standard stereotaxic space. In plain

English, the individual brain volumes are modified such that the anatomy of each single patient is comparable to the other. When the anatomy of each subject is comparable, it is possible to superimpose the individual lesions and finally characterize the most common site of the stroke (for more details, see the methodological sections of the experiments). The second MR-technique employed in this thesis is called perfusion weighted imaging (PWI). PWI is an invasive technique, which requires the endovascular injection of a bolus of paramagnetic tracer (e.g., gadolinium diethyl triamineene pentaacetic acid, Gd-DTPA) to measure brain blood flow. The exogenous agent, being paramagnetic, causes magnetic field disturbances that are measured over time by the MR machine. The disturbances are detected and utilised to generate a signal intensity curve, which has the characteristic to decrease its magnitude when the disturbances caused by the tracer increase (Villringer et al., 1988). For each of the patients I selected in the studies, from these curves I calculated two physiological parameters that characterize perfusion: (i) time-to-peak

(TTP) and (ii) maximum signal reduction (MSR; Figure 1). Those brain areas that are reached by the bolus of contrast agent later in time (TTP) or that are reached by a smaller amount of blood containing the agent (MSR) are indicated as 'hypoperfused' or 'malperfused'. Hypoperfused areas represent those structurally intact brain regions that are receiving enough blood (and hence nutrients and oxygen contained in it) to maintain their structural integrity but not enough to preserve their normal functions. The importance of PWI is that it detects areas of hypoperfusion that cannot be otherwise visualized by structural MRI, since they do not lead to significant changes in proton density as the areas of lesions do (Note: MRI measures a signal from protons, i.e. the nucleus of a hydrogen atom, which is the most common element in brain tissue). For this reason, areas of hypoperfusion need to be studied by means of PWI in order to complement structural investigations and to have a complete characterization of the lesion's influences on human perception and behaviour. It is interesting to note that, above a certain threshold, hypoperfusion may be

followed by a drop down of neural activity and dysfunction of the neural tissue. Indeed, hypoperfusion has been correlated with disappearing of local EEG signals (Hossmann, 1994) as well as areas of malperfusion in localised brain territories have been associated with several cognitive disorders like spatial neglect, visual extinction and language dysfunctions (Karnath et al., 2005b; Hillis et al., 2001, 2002, 2005, 2006).

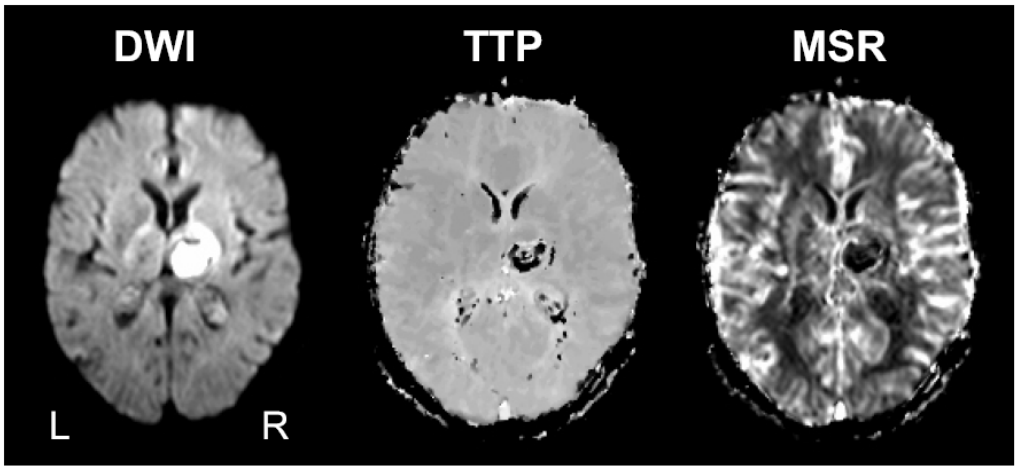

Figure 1: Example of DWI (left), TTP (middle) and MSR (right) maps in a patient with a thalamic lesion. The zone of structurally damaged tissue (thalamus) is clearly visible as an hyperintense area of the DWI map and as a black area of hypoperfusion in the PWI maps.

Although numerous investigations have been conducted so far in stroke patients with spatial and attentional deficits, lively controversies are still present in the scientific environment and new questions need to be addressed before claiming a complete understanding of the issue. For example, very few studies have considered also the metabolic abnormalities that follow the lesions. We designed the experiments presented in this thesis to tackle this particular problem, specifically investigating 'pusher syndrome' and 'visual extinction'. The question we wanted to answer is whether or not there are dysfunctional areas which remain undetected in traditional structural MRI, but that can be detected using alternative MRI approaches. Indeed, in a brain composed of cortical and subcortical networks, lesions in one neural structure can cause dysfunction in other distant but interconnected brain sites.

The last experiment constitutes a different attack on such a problem, with the aim of understanding the role of grey versus white matter tissue in 'spatial neglect'. The work represents a reanalysis of a large sample of 140 stroke

patients (see [Karnath et al., 2004a]) by using group lesion mapping plotted on the atlas of white matter fibres developed by the Juelich group (Buergel et al., 2006). In particular, the experiment faces the lively debate on whether the disruption of cortical structures or subcortical white matter association fibres is responsible for the appraisal of spatial neglect. In order to complement this study, in the present manuscript I also included some preliminary results obtained by using diffusion tensor imaging (DTI) tractography in two patients with spatial neglect.

To conclude, the thesis consists of three experiments, each one of them devoted to one of the following disorders: 'pusher syndrome', 'visual extinction' and 'spatial neglect'. These three parts represent the work conducted for the realization of three papers (see paragraph Contributions) and could be taken separately, although all of them address similar fundamental questions and fit into a coherent theoretical framework for the study of spatial awareness and balance.

2. Overview of the experiments

2.1 Experiment 1 – 'Pusher syndrome'

In a world of perpetual flux, the brain has become outstandingly effective in recognizing and stabilizing the body position in space. For biped species such as the human race, the peculiar need to perceive the correct vertical body orientation in the upright position and react against gravitational and perturbation forces (Massion, 1994) is of particular importance and it is achieved through the convergence of inputs from multiple sources (e.g. vestibular, visual, and somatosensory information; [Horak et al., 1994]). Though, the ability of being aware of our own position in space can be disrupted by peripheral or central damage, which may result in various disorders of posture and of balance control (Horak et al., 1984; Masdeu and Gorelick, 1988; Spinazzola et al., 2003). Among the disorders that affect upright body position, a very intriguing, severe and rather rare one is the 'pusher syndrome' (for review, [Karnath, 2007]). 'Pusher syndrome' is a transient misrepresentation of

body orientation in the coronal (roll) plane, experienced as oriented upright when it is actually tilted nearly 20 degrees to the ipsilesional side (Figure 2). Interestingly, in those patients with ‘pusher syndrome’, the denounced misperception of upright body posture (subjective postural vertical, SPV) is not accompanied by a correspondent disturbed processing of visual and vestibular inputs that determine visual vertical (subjective visual vertical, SVV).

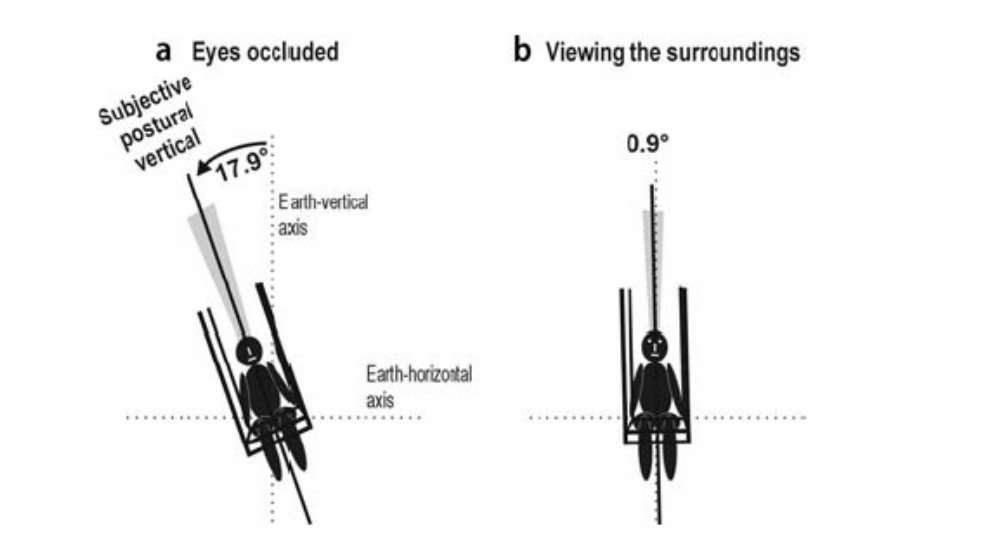

Figure 2: While sitting on a lateral tilt chair, patients with ‘pusher syndrome’ orient their body according to their perceived 'upright' orientation. (a) With eyes occluded, they perceive the upright body posture tilted by nearly 20° ipsilesionally. (b) The same patients, whit eyes open, are able to adjust their position by viewing the laboratory's surroundings (from [Karnath et al., 2000b]).

One possible interpretation of this dissociation is the detachment of the pathway for sensing the orientation of gravity from that one for perceiving the orientation of the visual word. Behaviourally speaking, this means that when patients with 'pusher syndrome' sit on a lateral tilt chair, they align correctly their longitudinal body axis to earth-vertical if they are eye-opened (Figure 2). Otherwise, with occluded eyes, they incorrectly indicate the reached upright body orientation when their body is tilted by nearly 20° towards the side of the brain lesion (Karnath et al., 2000b). This misperception of the SPV leads to postural adjustments consisting in the tendency of the patients to actively push aside from the ipsilesional side with their non-paretic arms and/or legs (Karnath and Broetz, 2003). The evident consequence is that - especially when upright and not supported by a physiotherapist - these patients fall towards their paretic contralesional side (Davies, 1985, 2000; Karnath, 2007).

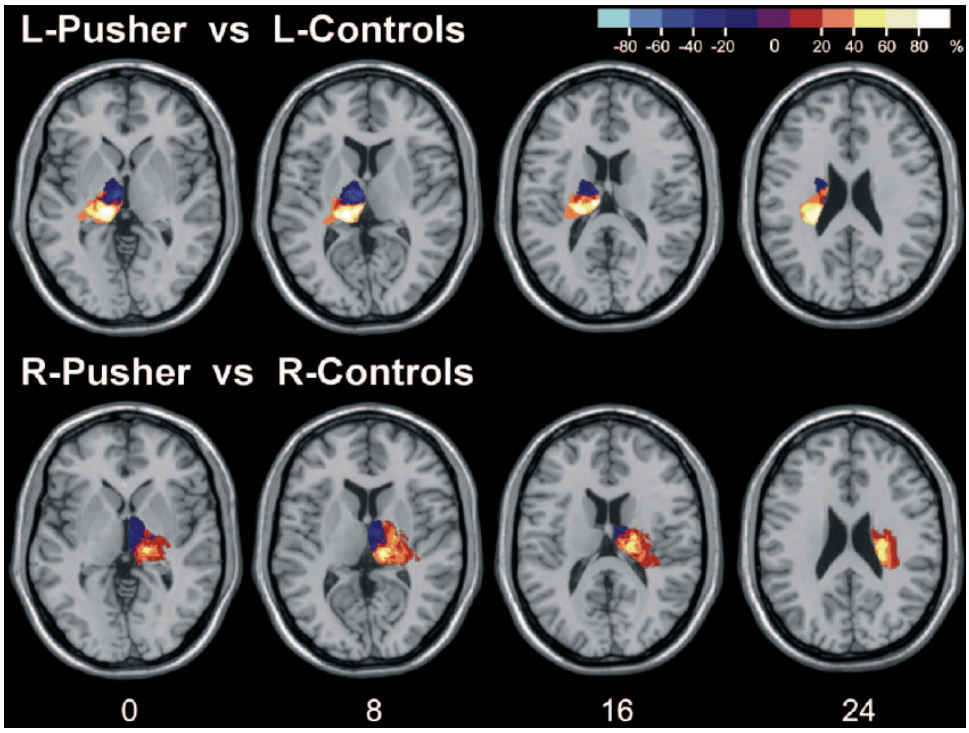

Figure 3: Overlay plots of the left and right thalamic lesions in the groups with (percentage of overlapping in red) and without (percentage of overlapping in blue) 'pusher syndrome' (from [Karnath et al., 2005a]).

Karnath and associates extensively studied the neural basis of this disorder of balance. These authors correlated 'pusher syndrome' with lesions occurring unilaterally in the left or right posterior thalamus (Figure 3 [Karnath et al., 2000a,b; Karnath et al., 2005a]) and - though less frequently - with lesions in the insula and postcentral gyrus in those patients with extra-thalamic strokes (Figure 4, 5 [Johannsen et al., 2006]).

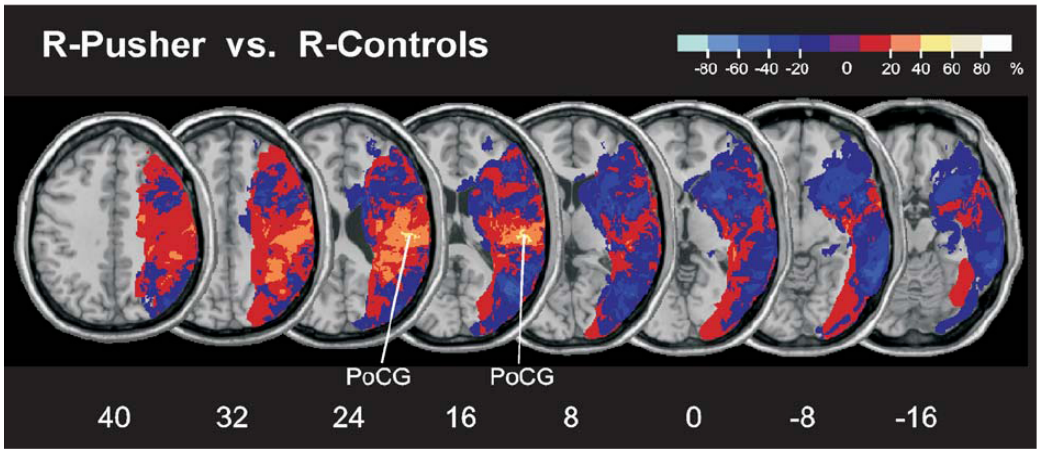

Figure 4: Overlay plots of the extra-thalamic lesions of the right brain damaged group with (percentage of overlapping in red) and without (percentage of overlapping in blue) 'pusher syndrome' (from [Johannsen et al., 2006]). PoCG, postcentral gyrus.

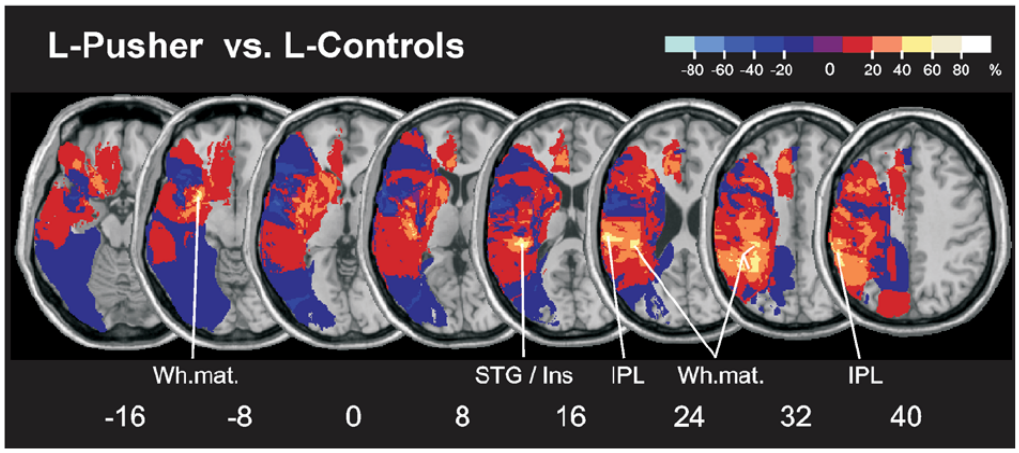

Figure 5: Overlay plots of the extra-thalamic lesions of the left brain damaged group with (percentage of overlapping in red) and without (percentage of overlapping in blue) 'pusher syndrome' (from [Johannsen et al., 2006]). Ins, insula; IPL, inferior parietal lobule; STG, superior temporal gyrus; Wh.mat., white matter.

In order to further deepen our understanding of ‘pusher syndrome’, in this first experiment, we investigated the perfusion deficits in two groups of patients with thalamic and with extra-thalamic strokes showing versus not showing ‘pusher syndrome’. We used diffusion-weighted (DWI) and fluid-attenuated inversion-recovery (FLAIR) imaging to reveal information about irreversibly damaged neural tissue. Moreover, we acquired information on the perfusion deficits by using perfusion-weighted imaging (PWI). Prior positron emission tomography (PET) experiments have shown that thalamic infarctions may be associated with depressed levels of cortical metabolic activity (Baron et al., 1986; Baron et al., 1992; Dieterich et al., 2005). Here, by means of PWI, we investigated whether or not this may also true in the case of ‘pusher syndrome’. Overall, this experiment, allowed us to distinguish the irreversible brain damage from the structurally intact but abnormally perfused tissue in the brains of patients affected by ‘pusher syndrome’. If the results would show that the structural thalamic and extra-thalamic lesions are accompanied by

perfusion deficits in definite brain districts located outside the strokes, then it would be possible to speculate that the neural correlates of 'pusher syndrome' are more widespread than previously thought. Alternatively, if no extra-lesional malperfusion would be found, then it is plausible that those structures identified earlier are the primary substrates of 'pusher syndrome'.

2.2 Experiment 2 – 'Visual extinction'

Visual extinction is a defect of awareness, often concurrent with hemispatial neglect (see experiment 3). Like neglect, visual extinction is asymmetrically associated with right hemisphere damage (Becker and Karnath, 2007). This disorder is a defect of spatial distribution of attention and it is manifest when patients - though able to detect a single stimulus at any spatial location - dismiss contralesional events when presented with a simultaneous ipsilesional stimulus (Driver et al., 1997). It is remarkable to note that patients with extinction have access to sensory information from both sides of space, included the extinguished side, and in fact

they can use this information unconsciously (Driver and Vuilleumier, 2001). In other words, the extinguished left-presented stimuli - although neglected when competing stimuli are presented on the right side - maintain a covert influence on behaviour. For example, extinguished stimuli influence the speed of detecting right-sided stimulations (Di Pellegrino and De Renzi, 1995), they can slow down the account of centrally presented target letters (after the presentation of left-sided letters; [Audet et al., 1991]) and can influence semantic priming of objects (Berti and Rizzolatti, 1992). Numerous hypotheses tried to conceptualise this peculiar phenomenon, which may be described also as a selective impairment of global (bilateral) vs. focal (unilateral) attention. For example, it has been proposed that visual extinction may be due to perceptual rivalry (Denny-Brown et al., 1952), inability of drawing off attention from the stimuli presented on the right side (Posner et al., 1984) or difficulty in distributing attention to multiple targets (Driver and Vuilleumier, 2001). Extinction is not only restricted to the visual domain. Indeed, it can occur

within as well as between different sensory modalities (Deouell and Soroker, 2000; Maravita et al., 2000; Ladavas et al., 2001; Vaishnavi et al., 2001; Rapp and Hendel, 2003). Overall, this may signify that patients with extinction have a high-order distortion in mapping the extrapersonal space as a whole. As mentioned above, visual extinction is often concurrent with spatial neglect. However, these two disorders have evident differences and may be decoupled. There is still much debate as far as the lesion locations of extinction are concerned (as it is the case for spatial neglect). Subcortically, visual extinction has been reported after damage of the basal ganglia, thalamus, internal capsule, and of the periventricular white matter (Vallar et al., 1994). In the cortex, a number of lesion (Critchley, 1949; Heilman and Valenstein, 1972; Vallar et al., 1994) and transcranial magnetic stimulation (TMS) (Pascual-Leone et al., 1994; Hilgetag et al., 2001; Dambeck et al., 2006; Battelli et al., in press) studies on stroke patients and healthy subjects respectively, have suggested the involvement of the posterior parietal lobe. This localization has been

questioned by the work of Karnath and associates (2003). By applying group lesion analysis techniques (Rorden and Karnath, 2004), these authors narrowed down the neural correlates of visual extinction to the region at the intersection of the right ventral inferior parietal lobule, caudal superior and middle temporal cortex, and the lateral occipital cortex – broadly defined as the temporoparietal junction (TPJ; Figure 6). Supporting these last findings Meister and colleagues found – contrarily to prior TMS studies – that healthy subjects that underwent temporary inactivation of the right TPJ demonstrated extinction-like performances in a detection task of visual stimuli presented unilaterally versus bilaterally (Figure 7; [Meister et al., 2006]). Otherwise, the application of TMS in a more rostral location over the STG did not evoke any of such extinction effects.

In the experiment presented in this thesis, we aimed at giving some evidence that could help solving the controversy on the neural correlates of visual extinction. Particularly, we investigated extinction after subcortical damages to understand whether or not it may be

explained by dysfunction of structurally intact cortical structures. In order to do so, we compared thirteen patients with right basal ganglia lesions showing versus not showing visual extinction. We used diffusion-weighted (DWI) and fluid-attenuated inversion-recovery (FLAIR) imaging to reveal information about irreversibly damaged neural tissue and perfusion-weighted imaging (PWI) to measure the perfusion of brain areas and to detect the functioning of the structurally intact cortical tissue.

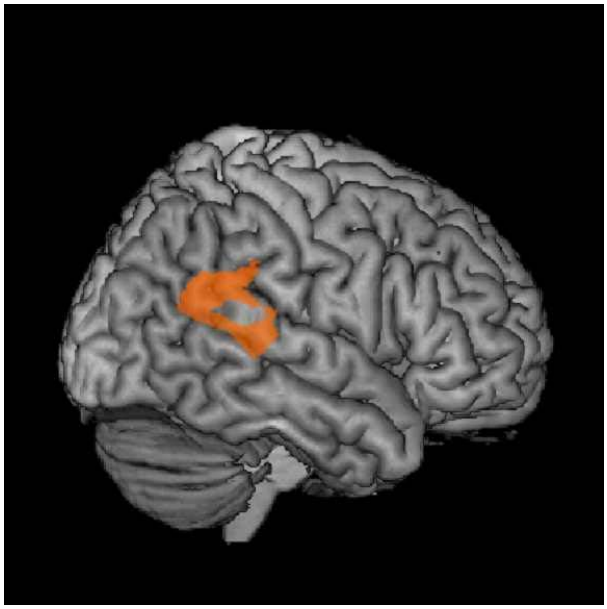

Figure 6: Representation of the surface view of the center of overlap (orange) in patients with visual extinction (adapted from [Karnath et al., 2003]).

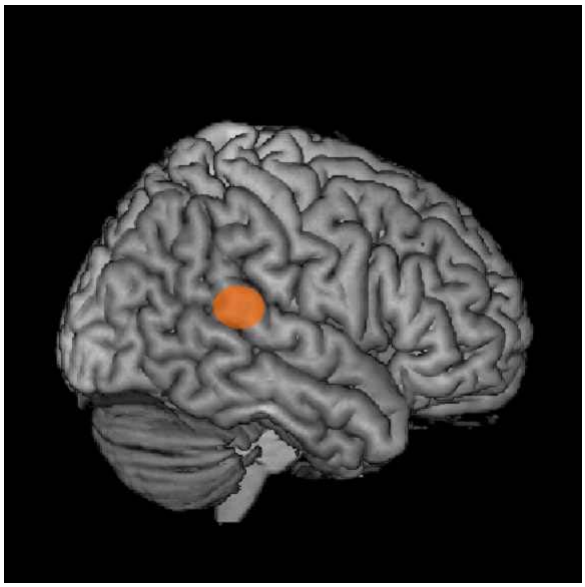

Figure 7: Representation of the brain region (TPJ area) stimulated with TMS by Meister et al. (2006).

Again, as in the first experiment of the thesis, this procedure allowed us to distinguish the irreversible brain damage from the structurally intact but abnormally perfused tissue. In other words, the information obtained allowed us to investigate whether or not the subcortical structural lesions of the basal ganglia are accompanied by perfusion deficits in distinct cortical brain areas, beyond the basal ganglia. If it is the case, we would be able to show the involvement of structurally intact but malperfused cortical tissue in visual extinction even in the case of subcortical strokes, establishing a direct

relationship between subcortical strokes and cortical dysfunction.

2.3 Experiment 3 – 'Hemispatial neglect'

The third problem we sought to investigate is that of hemispatial neglect. Spatial neglect is a rather frequent and severe disorder of spatial awareness, subsequent to unilateral right-hemisphere brain damage. Differently form patients with 'pusher syndrome' (experiment 1) that exhibit a misperception of the orientation in the coronal (roll) plane, stroke patients with hemispatial neglect have a disorder that impacts on the perception of the transverse (yaw) plane (Figure 8). Specifically, neglect patients have an impulsive bias to deviate eyes and head ipsilesionally on the yaw plane (Fruhmann-Berger and Karnath, 2005), leading to the inability to account for stimuli presented on the contralateral (left) side of the lesion and to orient towards them. Clinically speaking, hemispatial neglect provokes a deep debilitation of daily life. From the point of view of basic research, investigating the anatomical substrate of neglect is also

critical to understand the brain networks that lie beneath (i) spatial awareness and cognition and (ii) non-spatial functions, such as the aptitude to maintain vigilant attention.

Lesion studies performed on stroke patients with neglect have identified three major cortical areas that may be credited as the neural correlates of the disorder (Figure 9): the right inferior parietal lobule (IPL), the temporo-parietal junction (TPJ) (Heilman et al., 1983; Vallar and Perani, 1986; Vallar, 2001; Mort et al., 2003) and less commonly the right dorsolateral frontal cortex (Vallar and Perani, 1986; Husain and Kennard, 1996; Committeri et al., 2007). While the earliest findings have been demonstrated by means of computerized tomography (CT), the development of more sophisticated MRI modalities and lesion mapping tools has driven an increasing interest towards the identification of the neural substrates of spatial neglect. The increasing number of researches on this topic has sometimes produced results that are in apparent contrast one to each other, thus provoking an exceptionally lively debate. For example, in

recent years, some studies have proposed that lesions of the right superior temporal cortex (STG) and adjacent insula, and not of the inferior parietal lobule as commonly thought, have a decisive influence on the disorder (Karnath et al., 2001, 2004a; Buxbaum et al., 2004; Corbetta et al., 2005; Committeri et al., 2007).

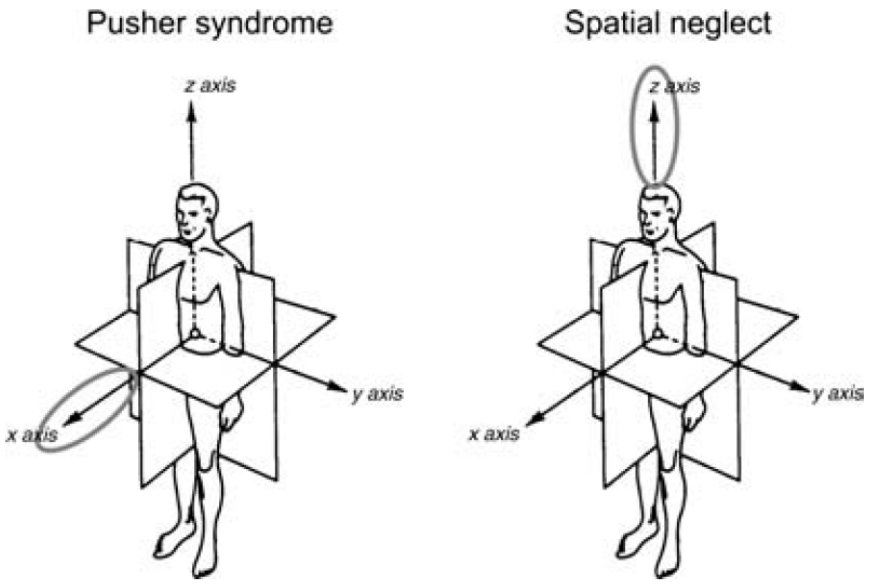

Figure 8: Representation of the disorder of spatial awareness in 'pusher syndrome' and spatial neglect (from [Karnath, 2007]). While 'pusher syndrome' is characterized by a misperception of body orientation in the coronal (roll) plane, spatial neglect is a disturbance of the transverse (yaw) plane (see text).

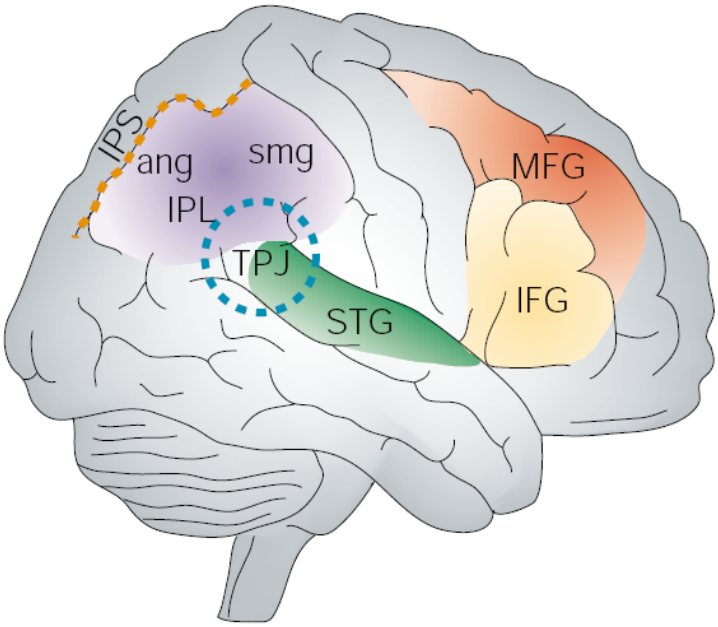

Figure 9: Schematic representation of the sites of damage in patients suffering from spatial neglect. The regions affected encompass the temporal-parietal junction (TPJ), the inferior parietal lobe (IPL), the superior temporal gyrus (STG), the intraparietal sulcus (ISP) and, more rostrally, the inferior frontal gyrus (IFG) and the middle frontal gyrus (MFG) (from [Husain and Rorden, 2003]).

Interestingly enough, neglect has been associated also with subcortical lesions of the putamen, pulvinar and caudate nucleus (Karnath et al., 2002). How subcortical damage may be related to the above mentioned cortical findings has been investigated by means of perfusion studies, which revealed that subcortical strokes may determine cortical hypoperfusion in the STG, IPL and

TPJ in those patients with spatial neglect (Hillis et al., 2002; Hillis et al., 2005; Karnath et al., 2005b). For those wishing to scientifically study neglect's anatomical basis, the data collected throughout the years suggest that neglect is a deficit associated with disruption of a spread system dedicated to spatial perception, more than to damage of single cortical or subcortical modules. Moreover, in order to try to accommodate the findings on spatial neglect within a single framework, it is interesting to note that most of the lesioned and/or hypoperfused areas mentioned above are located within the right cortical middle cerebral artery territories. This means that these neural districts receive a common blood supply, which would explain why dysfunctions (lesion or hypoperfusion) have been found alternatively in these areas. Above, I have mentioned that neglect may be associated with the disruption of a widespread network of spatial perception, which includes various cortical as well as subcortical neural structures. As a matter of fact, the discussion on neglect has been recently shifted from the cortical toward a more subcortical-based perspective.

Already back in the 1970s, Mesulam suggested that spatial neglect might result from disruption of white matter association tracts between posterior parietal, frontal and cingulate cortices, as well as the reticular formation (Mesulam and Geschwind, 1978; Mesulam 1981; 1985). Today, this hypothesis has been interrogated again by means of the new MR-based diffusion tensor imaging (DTI) techniques, which allow virtual brain dissections *in vivo* and permit the examining of the long association neural pathways that connect different lobes and lobules of the brain. The hypothesis behind is that spatial neglect, such as other neuropsychological disorders, would best be interpreted as a 'disconnection syndrome' and would result from the disruption of specific association pathways (Catani, 2006; Bartolomeo et al., 2007; He et al., 2007). Some authors recently exhibited that the disconnection of the superior longitudinal fasciculus (SLF, Figure 10) which connects the parietal and frontal cortices (Thiebaut de Schotten et al., 2005; Bartolomeo et al., 2007) and the inferior occipitofrontal fasciculus (IOF, Figure 10;

[Urbanski et al., 2008], Figure 11a) which connects ventrolateral frontal cortex with posterior temporal, inferior parietal and occipital cortices, may lead to spatial neglect. To me, the correlation between the disruption of the IOF and spatial neglect shall be more extensively verified.

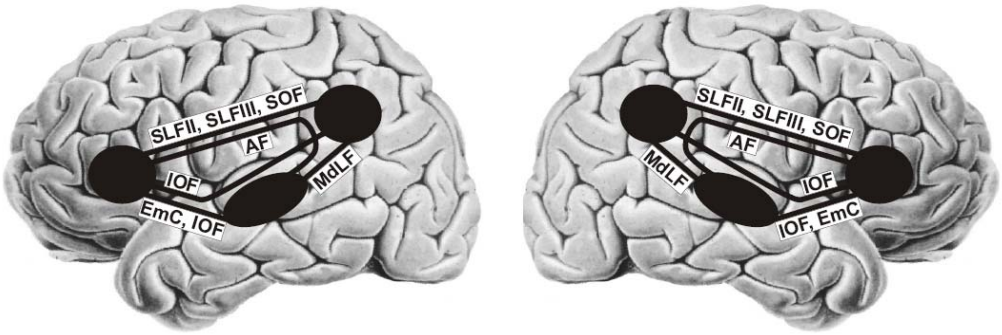

Figure 10: Schematic representation of the perisylvian network of white matter fibers that connect: the inferior parietal lobe with the forsolateral frontal cortices (SLF and SOF, see text), the latter areas with the temporal cortices and the insula (AF, IOF, EmC) and the superior temporal cortex with the inferior parietal lobule (MdLF). SLF, superior longitudinal fasciculus; SOF, superior occipitofrontal fasciculus; AF, arcuate fasciculus; IOF, inferior occipitofrontal fasciculus; EmC, extreme capsule; MdLF, middle longitudinal fasciculus (from [Karnath, 2009]).

As a matter of fact, I could detect the IOF in a neglect patient with a small lesion (Figure 11b and see Discussion). He and colleagues (2007) recently found that damage of the SLF and of the arcuate fasciculus (AF, it consists of fibres that arch around the caudal end of the sylvian fissure, and project to the lateral prefrontal cortex, see also Figure 10) were associated with severe (but not mild) spatial neglect. Of extreme interest is that these authors also investigated the functional connectivity based on coherent fluctuations of fMRI between distant brain regions. Interestingly, they found that the disruption of the white matter association tracts was correlated with interruption of the functional cortical connectivity within the dorsal and ventral attention networks. The overall picture that the findings depicts is that spatial neglect, beyond cortical strokes, may be associated with the disruption of subcortical tracts underneath the sylvian fissure and with cortical dysfunction subsequent to these damages.

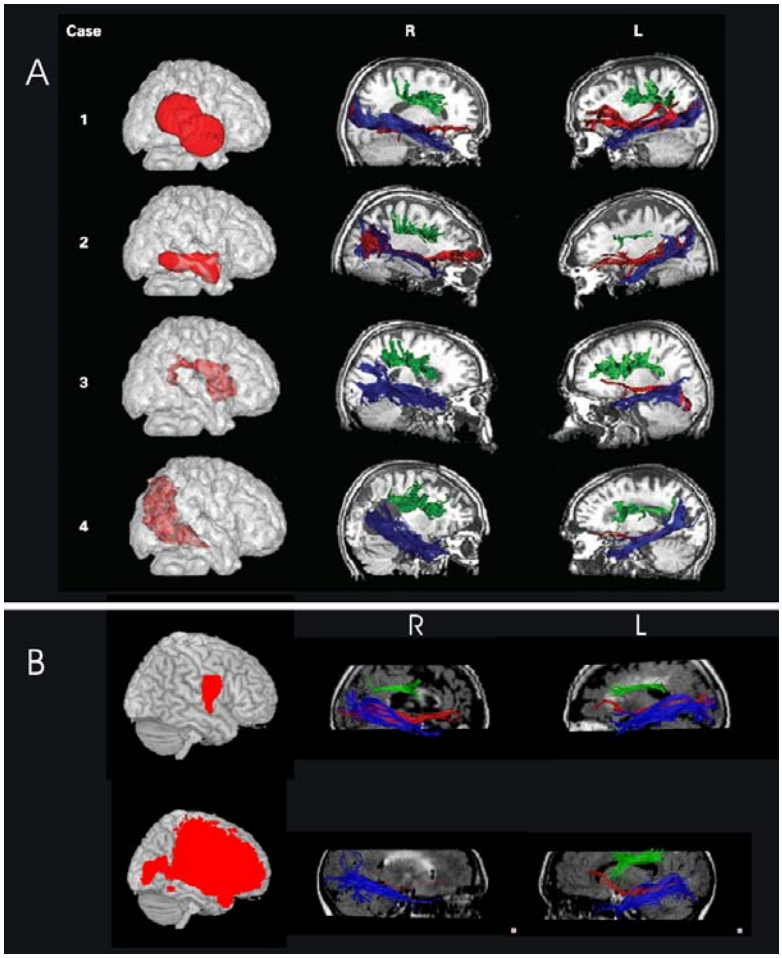

Figure 11: A) Three dimensional reconstruction of the lesions (left) and DTI-tractography of the long association fibre tracts in four patients with (3, 4) and without (1, 2) spatial neglect. In those patients with spatial neglect, the right inferior fronto-occipital fasciculus (in red) is missing. The other tracts depicted are the superior longitudinal fasciculus (in green) and the inferior longitudinal fasciculus (in blue) (from [Urbanski et al., 2008]). B) Reconstruction of the lesions and tractography of two patients with spatial neglect. In one patient the right inferior fronto-occipital fasciculus (in red) is present, in the other is absent together with the superior longitudinal fasciculus (Ticini, preliminary results).

In order to deepen this aspect and to understand the role of cortical and subcortical structures in spatial neglect, we designed the experiment presented in this thesis (Karnath, Rorden, Ticini, 2009). Our analysis was conducted on a large 7-years sample of 140 right-hemispheric stroke patients (see [Karnath et al., 2004a]) and aimed at quantifying how much of the lesioned areas in neglect patients overlap with the perisylvian white matter tracts. Unfortunately we did not have actual data of these patients that could show the subcortical connectivity. Thus, we relied for our investigation on a new white matter atlas developed by the Jülich group, based on the analysis of the brain cytoarchitecture in a sample of ten different human post-mortem brains (Amunts and Zilles, 2001; Zilles et al., 2002).

3. Methods and Results

3.1 Methods experiment 1

Note: the methods presented in this section are reproduced from the correspondent paper:

Ticini L. F., Klose U., NaegeleT. and Karnath H.O. (2009) Perfusion imaging to investigate the neural substrates involved in controlling upright body position. ***PLoS ONE*** 4(5):e5737. doi:10.1371/journal.pone. 0005737.

Subjects

Nineteen patients with first-ever stroke centering either on the thalamus (n=11) or sparing the thalamus (n=8) consecutively admitted to the Centre of Neurology in Tübingen were included. Since stenoses are known to produce false-positive depictions of perfusion deficits, especially in time-to-peak perfusion images (Yamada et al., 2002), we excluded those patients with a haemodynamically relevant extracranial stenosis in the

internal carotid arteries, i.e. ≥ 70%, demonstrated by Doppler sonography. The number of potential participants further had to be limited with respect to proper kidney functions due to the use of contrast agent. The patients were divided into two groups with and without pusher syndrome (Table 1) according to standardised testing for pusher syndrome (see below). All patients gave their informed consent to participate in the study which has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Clinical investigation

Pusher syndrome was diagnosed using the standardised Scale for Contraversive Pushing (SCP) (Karnath et al., 2000a; Karnath and Broetz, 2007) at the same day of MR acquisition. The SCP assesses 1) symmetry of spontaneous posture, 2) the use of the non-paretic arm or leg to increase pushing force by abduction and extension of extremities, and 3) resistance to passive correction of posture. These variables are determined both when

patients were sitting (feet with ground contact) and standing.

		Thalamic brain lesion		Extra-thalamic brain lesion	
		Pusher syndrome	No pusher syndrome	Pusher syndrome	No pusher syndrome
Number		5	6	4	4
Sex		3f, 2m	2f, 4m	4m	2f, 2m
Age (yr)	Mean (SD)	67.8 (6.1)	56.5 (9.6)	64.5 (16.6)	64.7 (13.8)
Etiology		0 Infarct 5 Hemorrhage	4 Infarct 2 Hemorrhage	4 Infarct	4 Infarct
Lesion volume (% of RH)	Mean (SD)	4.8 (2.5)	2.2 (3.5)	15.9 (4.0)	8.3 (3.1)
Lesion side		2 RBD / 3 LBD	5 RBD / 1 LBD	4 RBD	4 RBD
Paresis of contralesional side	% present	100	66.6	100	100
Arm	Median (range)	2 (0-3.5)	4 (1-5)	1.1 (0-2.5)	3.5 (3-4)
Leg	Median (range)	3 (2.5-4)	5 (2-5)	2.6 (2-3)	3.5 (3-4)
Visual field deficit	% present	0	16.6	0	0
Spatial neglect (total	LBD	0/2*	0/1	0/0	0/0
number/max.)	RBD	2/2	0/5	2/4	2/4
Aphasia (total number/max.)	LBD	3/3	0/1	0/0	0/0
	RBD	0/2	0/5	0/4	0/4
SCP posture					
Sitting	Median (range)	1 (0-1)	0	1 (0.75-1)	0
Standing	Median (range)	1	0 (0-0.25)	1 (0.75-1)	0
SCP extension					
Sitting	Median (range)	1 (0.5-1)	0	0.5 (0.5-1)	0
Standing	Median (range)	1	0	0.75 (0.5-1)	0
SCP resistance					
Sitting	Median (range)	1	0	1	0
Standing	Median (range)	1	0	1	0

Table 1: Demographic and clinical data of the patients with and without pusher syndrome. f, female; m, male; *, one patient could not formally be tested for spatial neglect; RH, right hemisphere; LBD, left brain damage; RBD, right brain damage; SCP, Scale for Contraversive Pushing (Karnath and Broetz, 2007).

In patients with pusher behavior, all three criteria had to be present and the patients had to show at least a total score of 1 (max. = 2, sitting plus standing) with respect to their spontaneous posture, at least a score of 1 (max. = 2, sitting plus standing) concerning the use of the non-paretic arm and/or leg to increase pushing force by

abduction and extension, and had to show resistance to passive correction of posture. Details of the SCP assessment are presented in Table 1. The degree of paresis of the upper and lower limbs was scored with the usual clinical ordinal scale, where ‘0’ stands for no trace of movement and ‘5’ for normal movement. Spatial neglect was diagnosed when the patient exhibited the typical clinical behaviour, such as spontaneous eye and head orientation towards the right (Fruhmann-Berger and Karnath, 2005). In addition, all patients were further assessed with the following three clinical tests: the ‘Letter cancellation’ task, the ‘Bells test’, and a copying task (Johannsen and Karnath, 2004). Neglect patients had to fulfill the criterion for spatial neglect in at least two of these tests. Full details about the test procedure and criteria are described elsewhere right (Fruhmann-Berger and Karnath, 2005). Aphasia was assessed conducting a bedside examination that evaluated spontaneous speech, auditory and reading comprehension, picture naming, reading, and oral repetition. Visual field defects were assessed using standardized neurological examination.

MR imaging and analysis

For the depiction of structurally lesioned brain tissue we used diffusion-weighted imaging (DWI) and T2-weighted fluid-attenuated inversion-recovery (FLAIR) imaging. DWI is very sensitive to infarct especially very early after stroke onset where it proves to be superior compared to conventional MR and CT imaging (Mullins et al., 2002). FLAIR imaging represents a T2-weighted imaging protocol in which the signal from the cerebrospinal fluid is suppressed. FLAIR images provide high sensitivity for acute and subacute infarcts (Brant-Zawadzki et al., 1996; Noguchi et al., 1997; Ricci et al., 1999). For lesion delineation, we used DWI imaging within the first 48 h post-stroke and FLAIR sequences when imaging was conducted 48 h or later after stroke onset infarcts (Brant-Zawadzki et al., 1996; Noguchi et al., 1997; Ricci et al., 1999; Schaefer et al., 2002). The mean time between stroke and imaging and clinical investigation for the thalamic stroke patients was 9.6 (SD 6.1, range 4-18) days in the group with pusher syndrome and 7.2 (SD 7.9, range 2-23) days in the group without

the disorder (t = 0.56, p = 0.591, two-tailed). For the patients with extra-thalamic lesions the mean time was 3.5 (SD 4.7, range 0-10) days in the group of pusher patients and 3.0 (SD 4.1, range 0-9) days in the group without pusher snydrome (t = 0.16, p = 0.878, two-tailed). Scans were obtained on a 1.5-T echoplanar imaging (EPI) capable system (Magnetom Sonata, Siemens, Erlangen, Germany). The FLAIR sequence was acquired with 72 axial slices (thickness 1 mm, interslice gap 1 mm), a field of view (FOV) of a 192 x 256 mm², matrix 192 x 256 pixels, repetition time (TR) of 9310 ms and an echo time (TE) of 122 ms. DWI was performed with a single-shot EPI spin echo sequence (TR 3200 ms; TE 87 ms; FOV 230 x 230 mm²; matrix 128 x 128 pixels; slice thickness 5 mm; gap 1mm; b-values of 0, 500 and 1000 s/mm²). The boundary of the lesion was delineated directly on the individual MRI image for every single transverse slice using MRIcron software (Rorden, Karnath and Bonilha, 2007) (http://www.mricro.com/mricron). In order to illustrate the common region of structurally lesioned brain tissue

per group, both the scan and lesion shape were then transferred into stereotaxic space using the spatial normalization algorithm provided by SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). For determination of the transformation parameters, cost-function masking was employed (Brett et al., 2001). In patients with thalamic strokes, left and right lesions had been found to affect homologues structures (Karnath, Ferber and Dichgans, 2000a,b). In the present analysis, we thus switched the left-sided thalamic lesions and relative perfusion maps to the right side in order to obtain a larger data basis for the subtraction analysis (Rorden and Karnath, 2004). Hypoperfused brain tissue was visualized using perfusion-weighted imaging (PWI; Belliveau, et al., 1990). Fifty repetitions of perfusion-weighted EPI sequences (TR 1440 ms; TE 47 ms; FOV 230 x 230 mm²; matrix 128 x 128; 12 axial slices; slice thickness 5 mm; gap 1 mm) were obtained with 20 ml gadolinium diethyl triamineene pentaacetic acid (Gd-DTPA) bolus power injected at a rate of 3-5 ml/s. The amount of bolus used depended on the body-weight of

the subject. Time-to-peak (TTP) maps were calculated to characterize malperfusion. TTP represents the time at which the largest signal drop occurs in the signal intensity curve with respect to the first image. It is generated directly from the signal intensity curve and does not rely on deconvoluting algorithms or the choice of adequate input functions (Calamante, Gadian, and Connelly, 2002; Thijs et al., 2004). In order to identify common regions of perfusion abnormality, the PWI volumes were spatially realigned and then transferred into stereotaxic space using the spatial normalization algorithm provided by SPM2. The normalized TTP maps were spatially smoothed with a Gaussian filter of 2 mm. For SPM normalization, we used a template featuring symmetrical left-right hemispheres (cf. Aubert-Broche et al., 2003). Subsequently voxel-wise inter-hemispheric comparisons were performed for each individual before extracting perfusion deficit volumes. This method takes regional biases for perfusion parameters into account, as each region is compared voxel-by-voxel to its mirrored region, thereby comparing homologous regions and

avoiding a region-specific bias (Karnath et al., 2005b). For the normalized TTP maps, we subtracted from each voxel of the affected hemisphere its mirrored voxel in the unaffected hemisphere. For the determination of volumes with perfusion abnormalities we defined the threshold for TTP delays ≥ 3.0 s. The TTP delay threshold was based on previous observations that TTP delays > 2.5 s in Wernicke's area were associated with language dysfunction (Hillis et al., 2001), and that the general functional impairment of stroke patients correlated best with the volume of PWI abnormality for TTP delays ≥ 4 s (Neumann-Haefelin et al., 1999). The area of mismatch between DWI/FLAIR and PWI abnormalities, i.e. the zones of structurally intact but dysfunctional neural tissue, was determined by subtracting for each subject the normalized DWI/FLAIR map from the normalized TTP delay map. Finally, we compared perfusion abnormalities in the patient groups with and without pusher syndrome. For this purpose, the superimposed mismatch images of the groups without pusher syndrome were subtracted from the overlap mismatch images of the groups with

pusher syndrome [details concerning the subtraction technique are given in Rorden, and Karnath, 2004)].

Addendum

In this first experiment we analyzed a further marker of abnormal perfusion, namely the maximal signal reduction (MSR). While TTP is a parameter that depicts the time of arrival of the blood in the brain tissue, MSR measures the amount of blood flow reaching the brain and is closely related to relative cerebral blood flow (rCBF) in stroke patients (Klose et al., 1999; Liu et al., 2002). In this study we determined a threshold for these two parameters: TTP delays $\geq$ 3 s and MSR fractions $\leq$ 40 %. Previous studies have suggested that these values represent the thresholds that delineate a behaviorally relevant malperfusion of brain tissues. For example, TTP delays > 2.5 s in Wernicke's area were associated with language dysfunction (Hillis et al., 2001); moreover, the functional impairment of stroke patients correlated best with PWI abnormality of TTP delays $\geq$ 4 s (Neumann-Haefelin et al., 1999). As far as MSR is concerned, no specific

correlations between inter-hemispheric and behavioral deficits have been reported so far. However, MSR is closely related to relative cerebral blood flow (rCBF) in stroke patients (Klose et al., 1999; Liu et al., 2002). Thus, the threshold for the MSR parameter that we used (≤ 40 %) has been based on the observation that for rCBF the averaged values of 37% and 43% (relative to unaffected contralesional tissue) characterize best the penumbra, i.e. the non-functional but salvageable tissue, surrounding the infarct (Neumann-Haefelin et al., 2000; Schlaug et al., 1999). These MSR-based fraction values are also in agreement with CBF thresholds derived from positron emission tomography studies. The mean CBF in normal brain tissue is approximately 50 ml/100g/min, while functional impairment occurs at flow thresholds of around 20 ml/100 g/min, i.e. when the CBF is reduced to about 40% (for a review see [Baron, 2001]).

3.2 Results experiment 1

The experimental aim was to compare the brain hypoperfusion produced by thalamic and extra-thalamic

lesions in patients with 'pusher syndrome'. We were principally interested in looking for the patterns of malperfusion produced by these lesions. To achieve this, we divided the two groups according to the site of lesion (thalamic and extra-thalamic strokes, Table 1) and constructed the respective overlay plots of the normalized DWI/FLAIR (Figure 12 and 16) and PWI data (Figure 13-15, 17a). Furthermore, in order to better depict those areas specifically malperfused in only the extra-thalamic stroke patients with 'pusher syndrome', we subtracted the perfusion maps of an appropriate control group of stroke patients without 'pusher syndrome' from the perfusion maps of the pusher groups (Figure 17b). Below, I present the results for the two groups separately.

'Pusher syndrome' after thalamic lesions

The investigation of the perfusion maps obtained from the patients with lesions centering on the thalamus (Figure 12) revealed the following. For both the groups of patients with (Figure 13) and without (Figure 14) 'pusher syndrome', significant overlap of perfusion

deficits in numerous regions were depicted when the lowest thresholds were applied to the TTP and MSR parameters (TTP delay ≥ 0 s and MSR fraction ≤ 100 %). As the thresholds increased (TTP delay ≥ 1.5 s and MSR fraction ≤ 70 % as well as TTP delays ≥ 3 s and MSR fraction ≤ 40 %), the areas of malperfusion became smaller and smaller. Only few voxels in both the groups of patients with and without 'pusher syndrome' and for both TTP and MSR parameters survived the level of thresholds depicting dysfunctional neural tissue (dark blue color in figure 13 and 14 indicates malperfusion in n=1 subject). The area of higher overlap of hypoperfusion was found within the thalamic lesions. Figure 15 illustrates the overlay plots of the perfusion abnormalities beyond the structural damage (i.e., the hypoperfusion within the lesion is not shown). Obviously, the figure indicates the absence of a common pattern of hypoperfusion associated with 'pusher syndrome' outside the area of the lesion. This result suggests that those patients with 'pusher syndrome' following thalamic lesions did not show a systematic

involvement of dysfunctional brain areas in addition to those structurally damaged. The comparison of structurally damaged areas with those malperfused, rather suggested large portions of overlap between the two.

Beyond the posterior thalamus, 'pusher syndrome' is associated with lesions affecting the postcentral gyrus and the insula (Johannsen et al., 2006). To further test if lesions centering on the thalamus (Figure 12) induce malperfusion in the structurally intact postcentral gyrus and/or insula in pusher patients, we used the anatomical parcellation of the MNI single-subject brain by Tzourio-Mazoyer et al. (2002) implemented in MRIcron (Rorden et al., 2007). In each subject, we determined the percentage of perfusion abnormalities for TTP delays ≥ 3 s and MSR fractions ≤ 40 % within the two regions of interest (ROIs): the postcentral gyrus and the insula. For both parameters TTP and MSR we found only few perfusion abnormalities within each single ROI. The statistical comparison between pusher patients and controls revealed no significant differences (TTP delay volumes postcentral gyrus: $U=10.5$, $p=0.29$; insula:

U=12, p=0.52; / MSR fraction volumes postcentral gyrus: U=12.5, p=0.36; insula: U=13.5, p=0.73, two-sided).

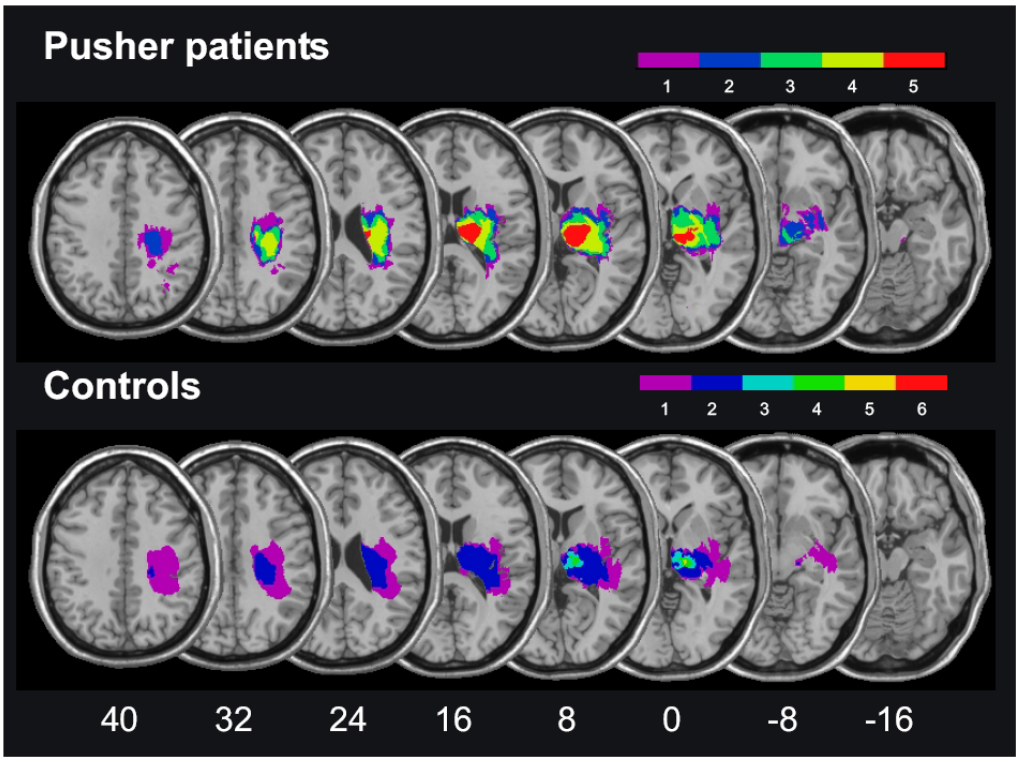

Figure 12: Overlay plots of the normalised structural lesions (based on normalized DWI or FLAIR images) for the groups of patient with and without 'pusher syndrome'. The number of overlapping areas is illustrated by different colours, coding increasing frequencies from violet (n=1) to red (n=5) for pusher patients and from violet (n=1) to red (n=6) for the patient group without 'pusher syndrome' (controls). MNI z-coordinates of the transverse sections are given.

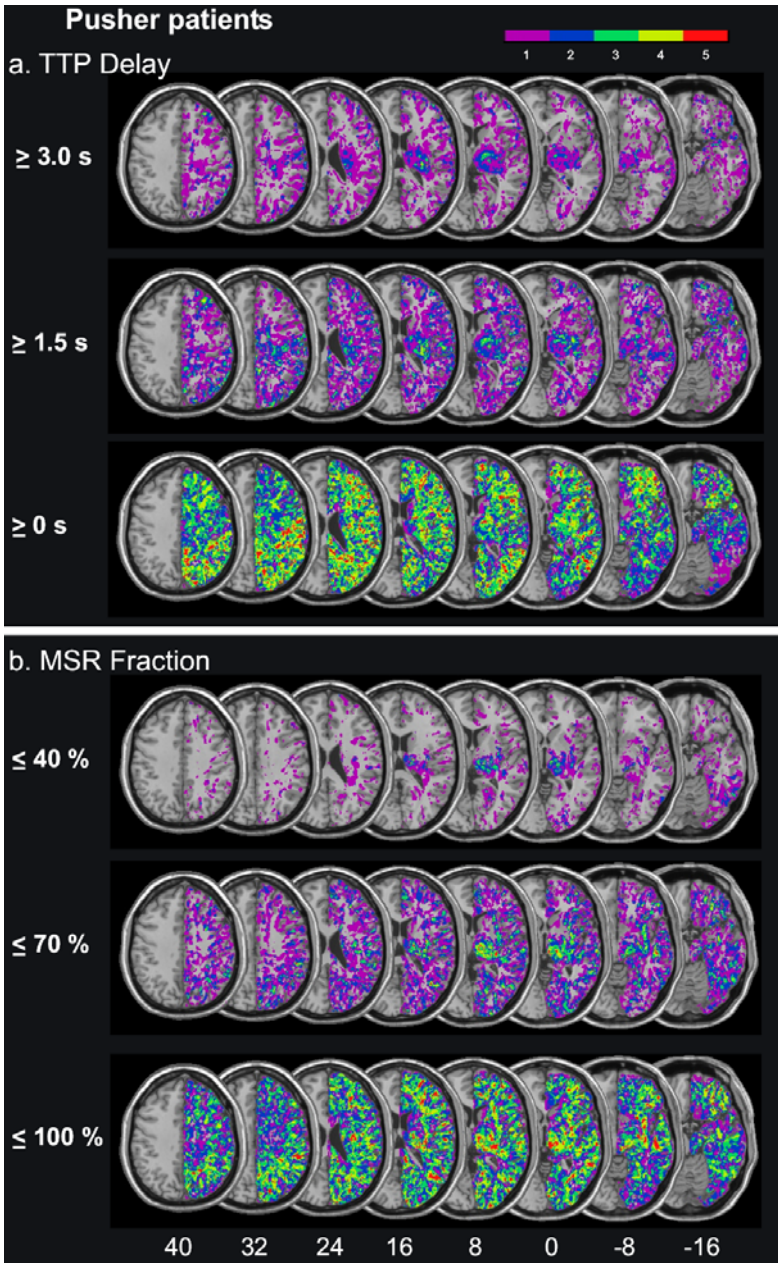

Figure 13: Overlay plots of (a) the normalised TTP delay maps and (b) the normalised MSR fraction maps of the patient group with 'pusher syndrome' depicted for different thresholds. The number of overlapping areas are illustrated by different colours, coding increasing frequencies from violet (n=1) to red (n=5). MNI z-coordinates of the transverse sections are given.

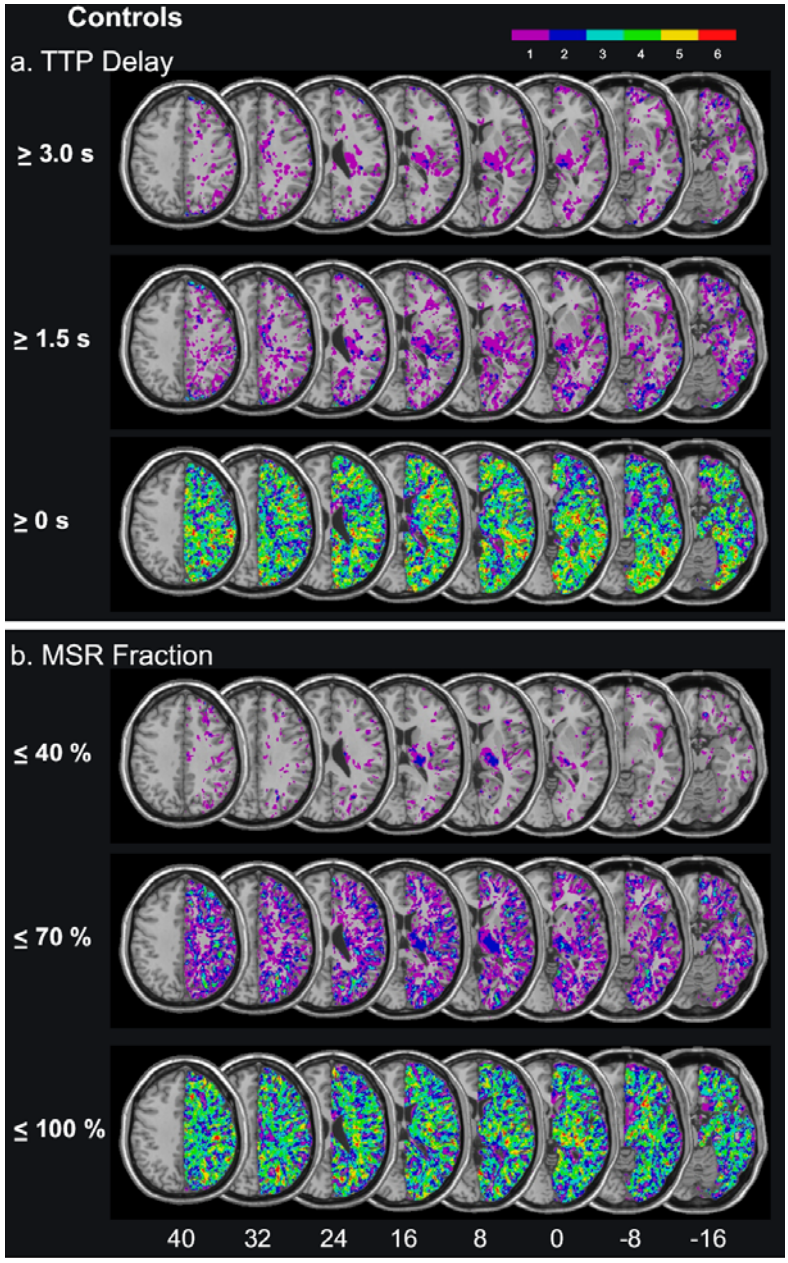

Figure 14: Overlay plots of (a) the normalised TTP delay maps and (b) the normalised MSR fraction maps of the patient group without 'pusher syndrome' (controls) depicted for different thresholds. The number of overlapping areas are illustrated by different colours, coding increasing frequencies from violet (n=1) to red (n=6). MNI z-coordinates of the transverse sections are given.

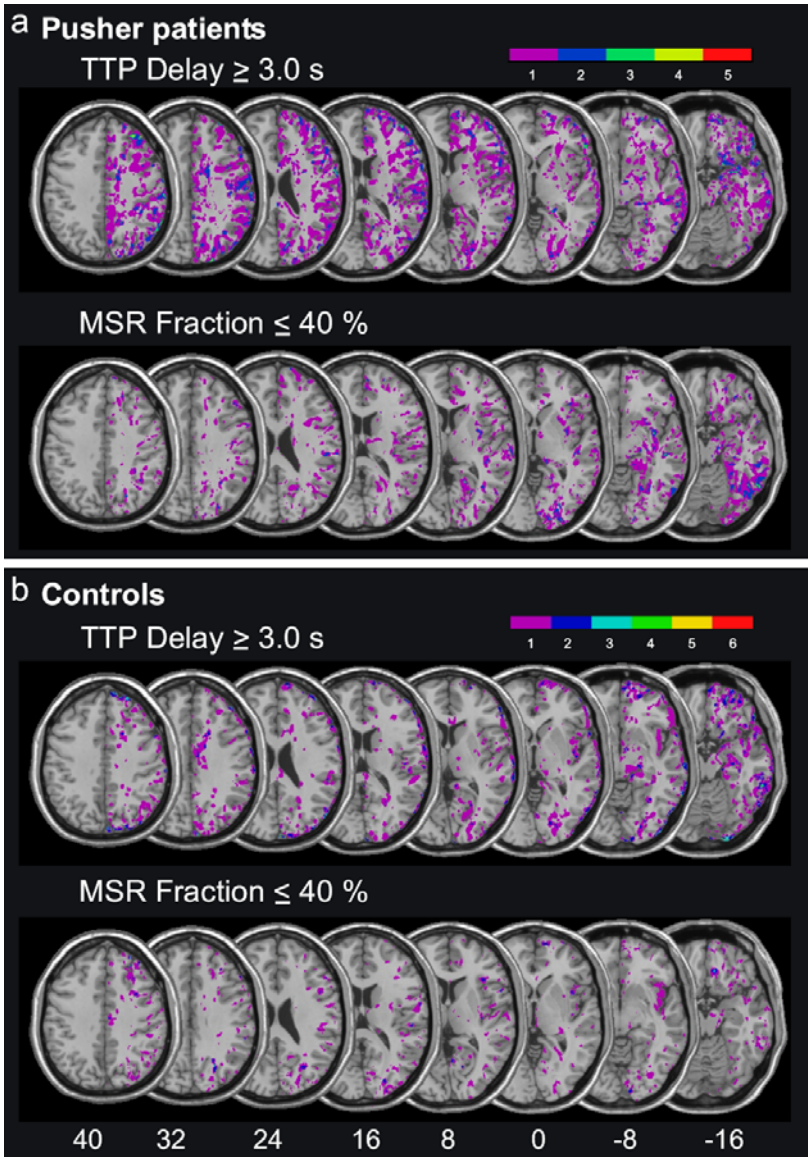

Figure 15: Overlay plots of the patient groups (a) with and (b) without 'pusher syndrome', showing the common regions of structurally intact but dysfunctional brain tissue. The first rows depict the mismatch between TTP abnormalities and DWI/FLAIR; the second rows the mismatch between MSR abnormalities and DWI/FLAIR. The number of overlapping areas are illustrated by different colours, coding increasing frequencies from violet (n=1) to red (n=5) for pusher patients and from violet (n=1) to red (n=6) for

the patient group without pusher syndrome (controls). MNI z-coordinates of the transverse sections are given.

'Pusher syndrome' after extra-thalamic lesions

The overlay plots of the normalized DWI/FLAIR data for the group of pusher patients with extra-thalamic strokes revealed lesions affecting the insula, frontal and rolandic operculum, inferior frontal gyrus, pre- and postcentral gyri, as well as part of the corticospinal tract, inferior occipitofrontal and uncinate fasciculi (Figure 16). The labeling of these areas was conducted with the help of the anatomical parcellation of the MNI single-subject brain by Tzourio-Mazoyer et al. (2002) implemented in MRIcron software (Rorden et al., 2007; http://www.mricro.com/mricron) and the Jülich probabilistic cytoarchitectonic atlas for the white matter fiber tracts (Buergel et al., 2006). The lesions of the control group without 'pusher syndrome' centered on the insula, rolandic operculum, superior temporal gyrus as well as part of the corticospinal tract (Figure 16).

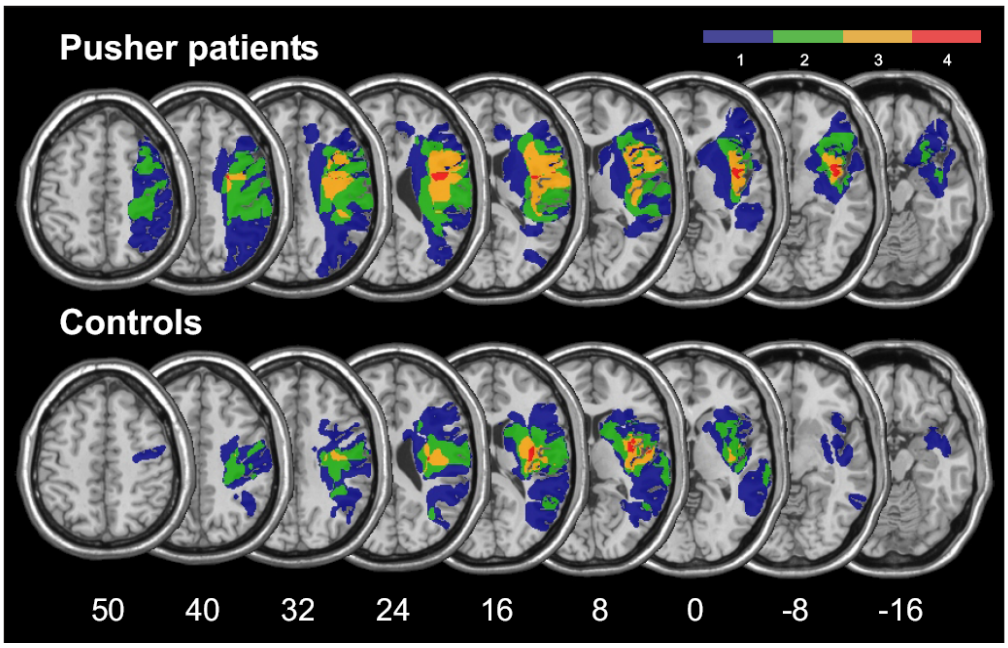

Figure 16: Overlay plots of the normalised structural lesions (based on normalized DWI or FLAIR images) for the groups of patient with and without 'pusher syndrome'. The number of overlapping areas is illustrated by different colours, coding increasing frequencies from blue (n=1) to red (n=4). MNI z-coordinates of the transverse sections are given.

The aim of the second part of the experiment was to identify those areas that were structurally intact but hypoperfused in 'pusher syndrome' subsequent to extra-thalamic strokes. Figure 17a shows the superimposed overlap image PWI/DWI mismatch images of the individuals in the group of patients with 'pusher syndrome' and in the group without the disorder.

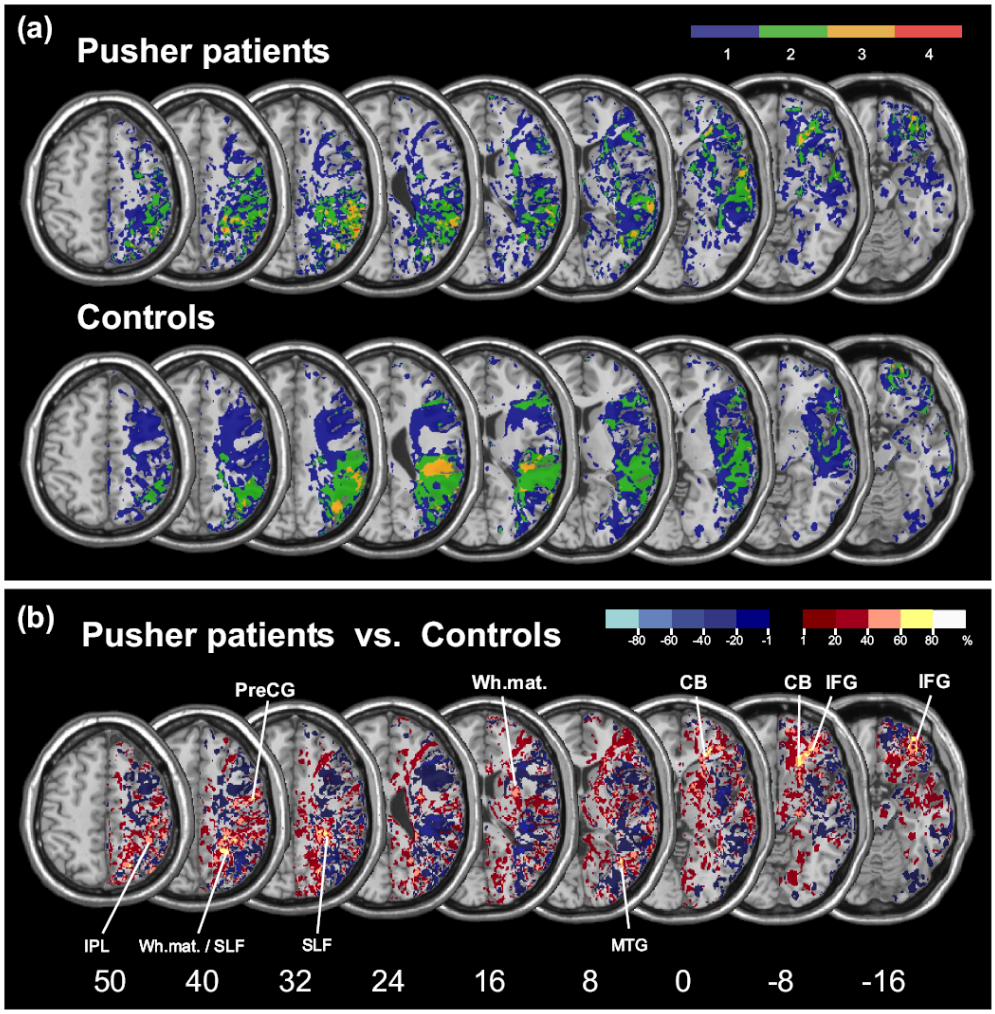

Figure 17: (A) Overlay plots the normalised TTP delay maps showing the common regions of mismatch between DWI/FLAIR and PWI abnormalities, i.e. of structurally intact but abnormally perfused tissue, for the group of pusher patients and the group of patients without 'pusher syndrome'. The number of overlapping areas with abnormal perfusion is illustrated by different colours, coding increasing frequencies from blue (n = 1) to red (n = 4). (B) Overlay plot of the subtracted superimposed mismatch images of the pusher group minus the mismatch images of the group without 'pusher syndrome'. The percentage of overlapping areas of structurally intact

but abnormally perfused tissue in the pusher group after subtraction is illustrated by five different colours, coding increasing frequencies from dark red (difference = 1-20%) to white (difference = 81-100%). Each colour represents 20% increments. The different colours from dark blue (difference = -1% to -20%) to light blue (difference = -81% to -100%) indicate regions abnormally perfused more frequently in patients without 'pusher syndrome' than in the pusher group. Regions where there is an identical percentage of abnormal perfusion in both groups (= 0%) are not depicted in the figure. MNI z-coordinates of the transverse sections are given. IFG, inferior frontal cortex; PreCG, precentral gyrus; SLF, superior longitudinal fasciculum; MTG, middle temporal cortex; CB, callosal body; Wh.mat., white matter; IPL, inferior parietal lobule.

The direct contrast of the common areas of malperfusion in the patients with 'pusher syndrome' versus the area in the patients without the disorder is presented in figure 17b. In the patients with 'pusher syndrome', we found the maximum of perfusion deficits in the structurally intact inferior frontal gyrus from MNI coordinates (x, 35; y, 38; z, -16) over (x, 33; y, 40; z, -8) to (x, 39; y, 41; z, 0), the middle temporal gyrus (x, 40; y, -64; z, 8), precentral gyrus (x, 51; y, -5; z, 40), inferior parietal lobule (x, 40; y, -42; z, 50), and parietal white matter at

coordinates (x, 24; y, -52; z, 40). Further, small parts of the callosal body from coordinates (x, 22; y, 29; z, -8) to (x, 27; y, 36; z, 0), of the temporal white matter (x, 30; y, 1; z, 16), and of the superior longitudinal fasciculus from (x, 31; y, -37; z, 32) to (x, 26; y, -40; z, 40) were affected.

3.3 Methods experiment 2

Note: the methods presented in this section are reproduced from the correspondent paper:

Ticini L. F., de Haan B., Klose U., NaegeleT. and Karnath H.O. (in press) The role of the temporo-parietal cortex in subcortical visual extinction. J Cogn Neurosci.

Subjects

Thirteen patients with first-ever subcortical stroke centering on the basal ganglia consecutively admitted to the Centre of Neurology in Tübingen were included in the study. Since stenoses are known to produce false-positive depictions of perfusion deficits, especially in

time-to-peak perfusion images (Yamada et al., 2002), we excluded those patients with a haemodynamically relevant extracranial stenosis in the internal carotid arteries, i.e. ≥ 70%, demonstrated by Doppler sonography. Due to the use of contrast agent, the number of potential participants had to be further limited with respect to their kidney functions. Following standardized clinical testing (see below), the patients were divided into two groups. One group suffered from visual extinction (with or without additional auditory and/or tactile extinction) as well as from spatial neglect (n=8), while the other group had spatial neglect only (n=5). All patients gave their informed consent to participate in the study which has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Clinical and demographic data of all patients are given in Table 2.

Clinical investigation

The patients were clinically tested for visual, auditory, and tactile extinction. For each modality, 10 unilateral

stimuli on either side and 10 bilateral stimuli were presented in a pseudo-random order.

Patient	Gender	Age	Extinction						Spatial neglect				
			Visual§		Tactile§		Auditory§		Letter Cancellation (hits)		Bells test (hits)		Copying (% omitted)
			L	R	L	R	L	R	L	R	L	R	
E1	F	48	0	10	10	10	10	10	8	29	0	14	25
E2†	M	66	0	10	0	10	0	10	4	24	2	4	50
E3	F	46	5	20	8	10	8	10	20	27	8	15	0
E4	F	78	0	10	0	10	0	10	-	-	2	11	62.5
E5	M	65	0	10	0	10	0	10	10	26	1	13	37.5
E6	M	53	0	10	0	10	0	10	0	14	0	6	87.5
E7	F	68	0	10	0	10	0	10	1	27	0	15	37.5
E8	F	68	0*	10	-	-	0	10	0	3	0	4	87.5
N1†	M	71	10	10	10	10	10	10	-#	-#	-#	-#	87.5
N2	F	46	10	10	10	10	10	10	24	30	-	-	25
N3	M	75	9	10	8	10	8	10	0	23	0	10	-
N4	F	39	9	10	0	10	10	10	18	28	2	14	12.5
N5	M	52	8	10	8	10	8	10	0	13	1	15	0

Table 2: Demographic and clinical data of the patients with and without visual extinction following lesions centring on the right basal ganglia. E, patients with visual extinction and spatial neglect; N, patients with spatial neglect only; †, patients with hemorrhagic strokes (all other subjects suffered from ischemia); F, female; M, male; L, left; R, right; §, absolute numbers of the stimuli detected on the lesional (right) and contralesional (left) sides under bilateral simultaneous stimulation (patients with inability to detect unilateral stimulations were excluded because of primary sensory defects; all the patients presented in the table reported 100% of the unilateral stimuli on each side correclty) ; *, patient with additional left inferior quadrantanopia (tested for visual extinction in the preserved visual field); -, not testable; #, in this patient the Albert's test (Albert, 1973) could be applied revealing 0 hits on the left and 13 on the right.

Visual extinction was tested by the usual clinical confrontation technique. Movements of the examiner's left and/or right index finger were presented in the patient's left and/or right visual half field. One of the thirteen investigated patients had additional left inferior quadrantanopia. In this subject, visual stimuli were presented in the intact visual field. Auditory and tactile stimulation were conducted with the patient's eyes closed. The auditory modality was tested by rustling with a small piece of paper near the patient's left and/or right ear. Tactile extinction was investigated by applying short fingertips on the dorsal surface of the patient's left and/or right hand while the patient's arms lay in front of them. If the patient was not able to report this gentle unilateral tactile stimulation at the contralateral hand due to left-sided sensory loss, the examination was repeated with softly twitching at the left and/or right shoulder. Patients were classified as showing extinction when they reported at least 90% of the unilateral stimuli on each side correctly, but failed to perceive the left stimulus during bilateral stimulation in >50% of the trials. Spatial neglect

was diagnosed when the patients showed the typical clinical behavior, such as spontaneous eye and head orientation towards the ipsilesional side (Fruhmann-Berger and Karnath, 2005), orienting towards the ipsilesional side when addressed from the front or the left, and ignoring contralesionally located people or objects. In addition, all patients were further assessed with the following clinical tests: the 'Letter cancellation' task (Weintraub and Mesulam, 1985), the 'Bells test' (Gauthier et al., 1989), and a copying task (Johannsen and Karnath, 2004). Not all subjects could be investigated with all three tests due to different clinical constraints. Nevertheless, neglect patients had to fulfill the criterion for spatial neglect in at least two of the three tests. Full details about the test procedure and criteria are described elsewhere (Fruhmann-Berger and Karnath, 2005).

MR imaging and analysis

For the depiction of structurally lesioned brain tissue we used diffusion-weighted imaging (DWI) imaging within

the first 48 h post-stroke and T2-weighted fluid-attenuated inversion-recovery (FLAIR) sequences when imaging was conducted 48 h or later after stroke onset (Brant-Zawadzki et al., 1996; Noguchi et al., 1997; Ricci et al., 1999; Schaefer et al., 2002). The mean time between stroke and imaging as well as the clinical investigation was 4.9 days (SD 3.8) in the group with visual extinction and spatial neglect and 4.5 days (SD 5.4) in the group with spatial neglect only ($t=0.14$, $p=0.891$, two-tailed). Scans were obtained on a 1.5-T MR system (Magnetom Sonata, Siemens, Erlangen, Germany). The FLAIR sequence was acquired with 72 axial slices (thickness 1 mm, interslice gap 1 mm), a field of view (FOV) of a 192 x 256 mm², matrix 192 x 256 pixels, repetition time (TR) of 9310 ms and an echo time (TE) of 122 ms. DWI was performed with a single-shot EPI spin echo sequence (TR 3200 ms; TE 87 ms; FOV 230 x 230 mm²; matrix 128 x 128 pixels; slice thickness 5 mm; gap 1mm; b-values of 0, 500 and 1000 s/mm²). The boundary of the lesion was delineated directly on the individual MRI image for every single transverse slice

using MRIcron software (http://www.mricro.com/mricron; Rorden et al., 2007). In order to illustrate the common region of structurally lesioned brain tissue per group, both the scan and lesion shape were then transferred into stereotaxic space using the spatial normalization algorithm provided by SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). For determination of the transformation parameters, cost-function masking was employed (Brett et al., 2001). Dysfunctional brain tissue due to abnormal perfusion was visualized using PWI (Belliveau et al., 1990), performed in the same scanning session as the structural scans. Fifty repetitions of perfusion-weighted EPI sequences (TR 1440 ms; TE 47 ms; FOV 230 x 230 mm²; matrix 128 x 128; 12 axial slices; slice thickness 5 mm; gap 1 mm) were obtained with gadolinium diethyl triamineene pentaacetic acid (Gd-DTPA) bolus power injected at a rate of 3-5 ml/s. The amount of bolus used depended on the body-weight of the subject. Time-to-peak (TTP) maps were calculated to detect possible brain areas of dysfunction. TTP represents the time at which the largest signal drop

occurs in the signal intensity curve with respect to the first image. TTP maps are generated directly from the signal intensity curves and do not rely on deconvoluting algorithms or the choice of adequate input functions (Calamante et al., 2002; Thijs et al., 2004). In order to identify common regions of perfusion abnormality in the two groups of patients, the PWI volumes were spatially realigned and then transferred into stereotaxic space using the spatial normalization algorithm provided by SPM2. The normalized TTP maps were spatially smoothed with a Gaussian filter of 2 mm. For SPM normalization, we used a template featuring symmetrical left-right hemispheres (cf. Aubert-Broche et al., 2003). Subsequently voxelwise interhemispheric comparisons were performed for each individual before extracting perfusion deficit volumes. This method takes regional biases for perfusion parameters into account, as each region is compared voxel-by-voxel to its mirrored region in the unaffected hemisphere, thereby comparing homologous regions and avoiding a region-specific bias (cf. Karnath et al., 2005b). For each voxel of the affected

right hemisphere the TTP value of its mirrored voxel in the unaffected left hemisphere was subtracted, resulting in the 'TTP delay'. For the determination of volumes with perfusion abnormalities we defined the threshold for TTP delays ≥ 3.0 s. The resulting maps are denoted 'TTP delay maps' in the following. The TTP delay threshold was based on previous observations that TTP delays > 2.5 s in Wernicke's area were associated with language dysfunction (Hillis et al., 2001), and that the general functional impairment of stroke patients correlated best with the volume of PWI abnormality for TTP delays ≥ 4 s (Neumann-Haefelin et al., 1999). The area of mismatch between DWI/FLAIR and PWI abnormalities, i.e. the zones of structurally intact but dysfunctional neural tissue, was determined by subtracting for each subject the normalized DWI/FLAIR map from the normalized TTP delay map. Finally, we compared perfusion abnormalities in the patient groups with and without visual extinction. For this purpose, the superimposed mismatch images of the group without visual extinction were subtracted from the overlap mismatch images of the group with visual

extinction. Details concerning the subtraction technique were given elsewhere (Rorden and Karnath, 2004).

3.4 Results experiment 2

In both the groups of neglect patients, with or without visual extinction, the subcortical lesions were centered on the right basal ganglia, as depicted by the overlay plots of the spatially normalized DWI/FLAIR data (Figure 18). The comparison of lesion volumes between the patients with visual extinction (mean: 17% of right hemisphere volume; SD 10.2) and controls (9.9%; SD 8.7) revealed a numerical, non-significant difference ($U=12$, $Z=-1.17$, $p=0.242$). The perfusion abnormalities associated with these lesions are represented for each group in figure 19. To highlight only those brain regions typically hypoperfused in patients with visual extinction, we subtracted the overlay mismatch images of the group without visual extinction from the overlap mismatch images of the extinction group (Figure 20). We found that patients with extinction were characterized by a maximum of abnormal perfusion in the right TPJ. By

using the anatomical parcellation of the MNI single-subject brain by Tzourio-Mazoyer et al. (2002) implemented in MRIcron software (Rorden et al., 2007), we found that the hypoperfused area was clustering around MNI coordinates (x, 58; y, -38; z, 24 and x, 62; y, -26; z, 11) in the posterior part of the STG, the posterior part of the MTG (around x, 50; y, -61; z, 4 and x, 62; y, -48; z, 0), the angular gyrus (around x, 46; y, -44; z, 32 and x, 48; y, -45; z, 30 and x, 38; y, -57; z, 38), and the supramarginal gyrus (x, 60; y, -37; z, 26). Small clusters within the inferior frontal cortex were also observed.

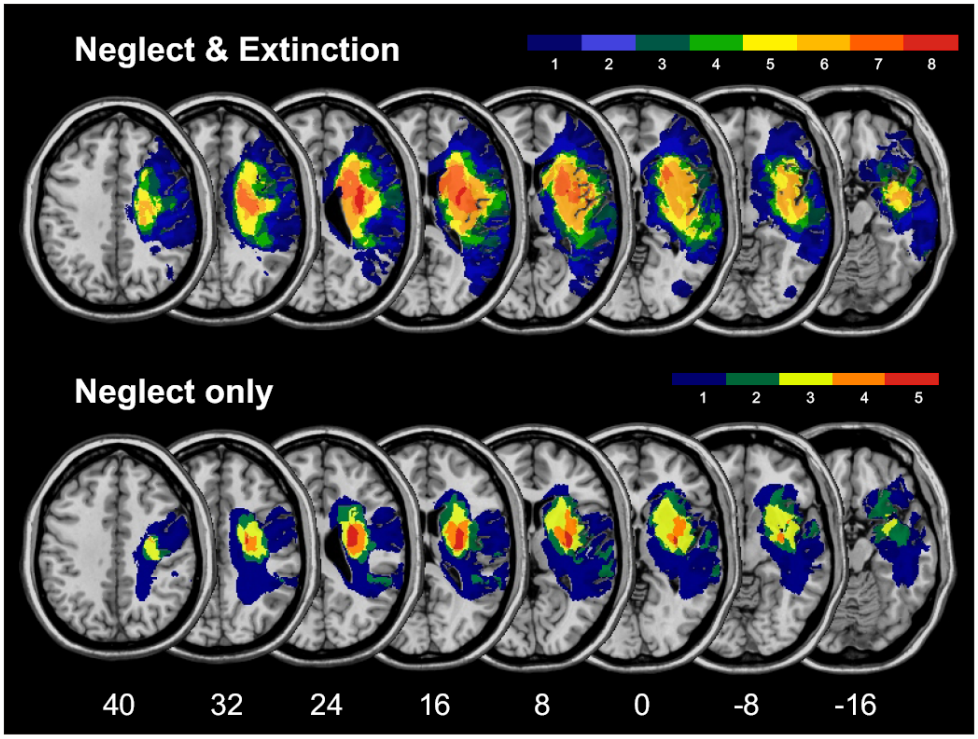

Figure 18: Overlay plots of the normalized structural lesions (based on normalized DWI or FLAIR images) for the groups of patient with and without visual extinction after subcortical lesions centring on the basal ganglia. The number of overlapping areas is illustrated by different colours, coding increasing frequencies from dark blue (n=1) to red (n=max). MNI z-coordinates of the transverse sections are given.

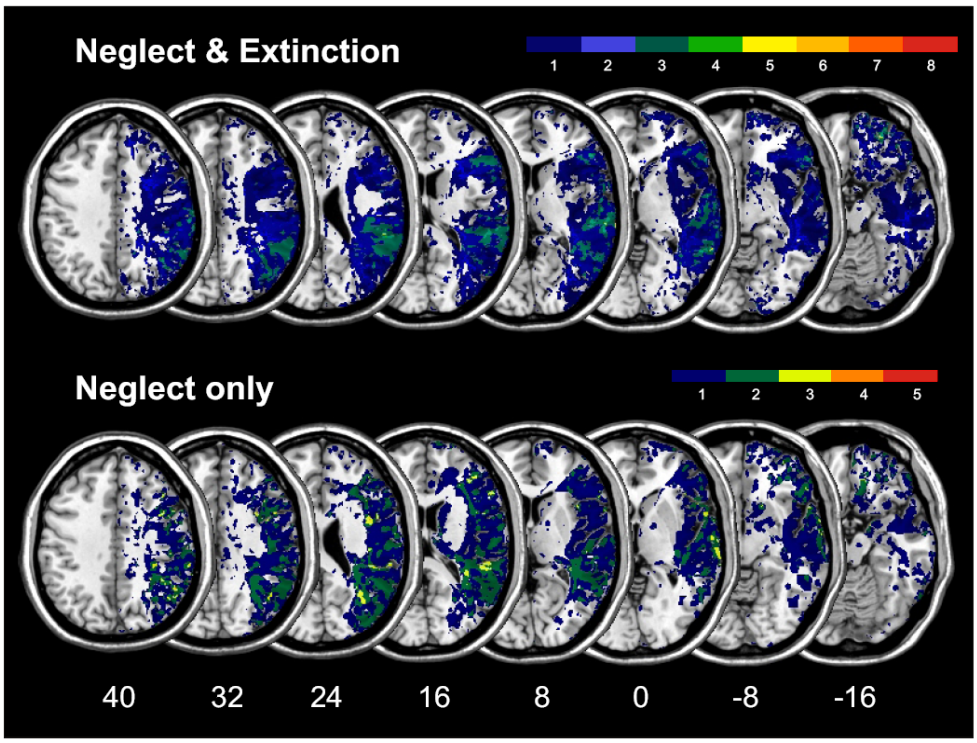

Figure 19: Overlay plots of the normalised TTP delay maps showing the common regions of mismatch between DWI/FLAIR and PWI abnormalities, i.e. of structurally intact but abnormally perfused tissue, for the groups of patients showing vs. not showing visual extinction. The number of overlapping areas with abnormal perfusion is illustrated by different colors, coding icreasing frequencies from dark blue (n=1) to red (n=max.). MNI z-coordinates of the transverse sections are given.

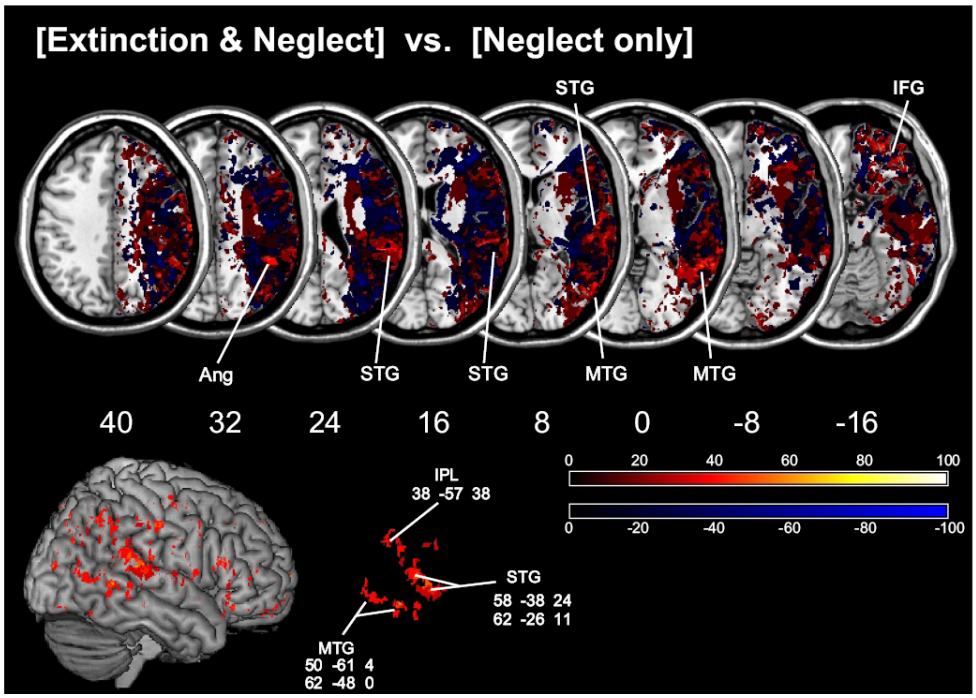

Figure 20: (A) Overlay plot of the subtracted superimposed mismatch images of the visual extinction group with additional neglect minus the mismatch images of the group with pure neglect (i.e. with neglect but no extinction). The percentage of overlapping areas of structurally intact but abnormally perfused tissue in the extinction group after subtraction is illustrated by five different colours, coding increasing frequencies from dark red (difference = 1-20%) to white (difference = 81-100%). Each colour represents 20% increments. The different colours from dark blue (difference = -1% to -20%) to light blue (difference = -81% to -100%) indicate regions abnormally perfused more frequently in patients with only neglect than in extinction patients (with additional neglect). Regions where there is an identical percentage of abnormal perfusion in both groups (= 0%) are not depicted in the figure. MNI z-coordinates of the

transverse sections are given. Ang, angular gyrus; STG, superior temporal gyrus; MTG, middle temporal gyrus; IFG, inferior frontal gyrus. (B) Surface view of the centre of overlap (orange). It represents cortical regions that are damaged > 30% more frequently in extinction patients (with additional neglect) than in patients with only neglect (who had no extinction). MNI z-coordinates of the locations marked are given. IPL, ventral part of the inferior parietal lobe (including inferior parietal lobule, angular gyrus and supramarginal gyrus); MTG, caudal part of the middle temporal gyrus; STG, caudal part of the superior temporal gyrus.

3.5 Methods experiment 3

Note: the methods presented in this section are reproduced from the correspondent paper:

Karnath H.O., Rorden C. and Ticini L. F. (2009) Damage to white matter fiber tracts in acute spatial neglect. ***Cerebral Cortex***. 19:2331-7.

Subjects and Methods

Data were drawn from 140 consecutively admitted stroke patients with circumscribed right hemisphere lesions

from a well-defined recruitment area belonging to the University of Tuebingen, sampled over a period of 7 years (Karnath et al. 2004a). The full details regarding patient characteristics, test criteria, etc., are reported in our previous work (Karnath et al. 2004a). In short, following standardized testing for spatial neglect by using the ‘‘Letter cancellation’’ task (Weintraub and Mesulam 1985), the ‘‘Bells test’’ (Gauthier et al. 1989), the ‘‘Baking tray task’’ (Tham and Tegner 1996), and a copying task (Johannsen and Karnath 2004), the subjects were divided into a group of 78 patients with spatial neglect and 62 control patients who did not show the disorder. Magnetic resonance imaging (MRI) or computerized tomography (Spiral-CT) was carried out in each subject. Lesion mapping was aided by diffusion-weighted imaging for MRI occurring within the first 48 h poststroke and T2-weighted fluid-attenuated inversion recovery sequences when MRI was conducted 48 h or later after the stroke. The mean time between lesion and the MRI was 5.0 days (standard deviation [SD] 5.4). In those subjects who underwent the CT imaging protocol,

the mean time since lesion and the CT was 6.7 days (SD 8.4). All lesions were drawn manually (while being blind for the diagnosis of spatial neglect) on axial slices of a T1-weighted template MRI scan from the MNI (http://www.bic.mni.mcgill.ca/cgi/icbm_view) using the MRIcro software package (Rorden and Brett, 2000) with a 1 x 1 mm in-plane resolution. This template is approximately oriented to match Talairach space (Talairach and Tournoux, 1988) and is distributed with MRIcro. Lesions were mapped onto the slices that correspond to z-coordinates – 40, –32, –24, –16, –8, 0, 8, 16, 24, 32, 40, and 50 mm in Talairach space by using the identical or the closest matching axial slices of each individual. The full details regarding imaging protocols etc. are reported in our previous work (Karnath et al. 2004a).

Analysis of White Matter Lesion Anatomy

For the present investigation, we used the new nonparametric mapping software that is distributed as part of the MRIcron toolset (Rorden et al. 2007).

Specifically, we performed the nonparametric Liebermeister test to identify voxels that when injured predicted the presence of spatial neglect. The resulting statistical map was adjusted for multiple comparisons by using a Bonferroni-corrected $P < 0.05$ threshold. Therefore, our initial analysis was based on this statistical map that illustrated all the voxels that predicted the presence of spatial neglect. In a first analysis, we determined the percentage of these voxels that comprised white matter compared with gray matter tissue. We segmented the individual T1-weighted template MRI scan from the MNI (http://www.bic.mni.mcgill.ca/cgi/icbm_view) by using Statistical parametric mapping routines (SPM5; http://www.fil.ion.ucl.ac.uk/spm/). The percentage of overlap of white versus gray matter tissue with the statistical lesion map was determined by using MRIcron software (Rorden et al. 2007). In order to identify the specific fiber tracts that predict spatial neglect, we segmented the thresholded Liebermeister statistical map using the white matter fiber tracts from the human

probabilistic cytoarchitectonic atlas (Buergel et al. 2006). This atlas is in the same space as the MNI reference brain, with each atlas map illustrating the relative frequency with which a certain fiber tract of 10 normal human brains was present (e.g., a 30% value reflects that the fiber tract was present in that voxel for 3 out of 10 brains). The number of overlapping voxels between the statistical lesion map and the anatomical fiber tract map in the right hemisphere was determined by using the SPM anatomy toolbox of the Juelich atlas (Eickhoff et al. 2005).

Addendum I:

To identify those brain areas affected by the lesion map, we used the anatomical parcellation of the MNI single-subject brain by Tzourio-Mazoyer et al. (2002) implemented in MRIcron (Rorden et al., 2007).

Addendum II, DTI reconstruction:

The diffusion tensor imaging (DTI, 6 directions, b=800) data of two neglect patients (E8 and N2) from the second

experiment of the thesis (see Table 2 and Methods) were studied. Fibre tracking of the superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF) and the inferior fronto-occipital fasciculus (IOF) was performed with TrackVis 0.4.2 (http://trackvis.org), using a two-regions of interest (ROIs) approach (Catani et al., 2002). The reconstructed tracts are displayed in 3D in Figure 11b.

3.6 Results experiment 3

The experimental aim of this experiment was to deepen the role of cortical and subcortical structures in a large 7-years sample of 140 right-hemispheric stroke patients (see [Karnath et al., 2004a]). Our analysis began with a statistical voxelwise lesion-behaviour mapping (VLBM) approach in order to identify those voxels that would predict the brain sites more frequently lesioned in patients with spatial neglect versus those patients without the disorder. The result in figure 21 depicts the territory composed by those voxels that were significantly more affected in patients with spatial neglect versus controls.

We found statistically significant voxels (which persisted after the statistical threshold Bonferroni-corrected alpha level of $P < 0.05$) within the right superior temporal gyrus (STG) at MNI coordinates (x 66; y –16; z 8) and the right periventricular white matter at coordinates (x 28; y –7; z 24). Other brain structures were affected: large parts of the right insula, the planum temporale, and operculum (including the opercular portion of the inferior frontal gyrus).

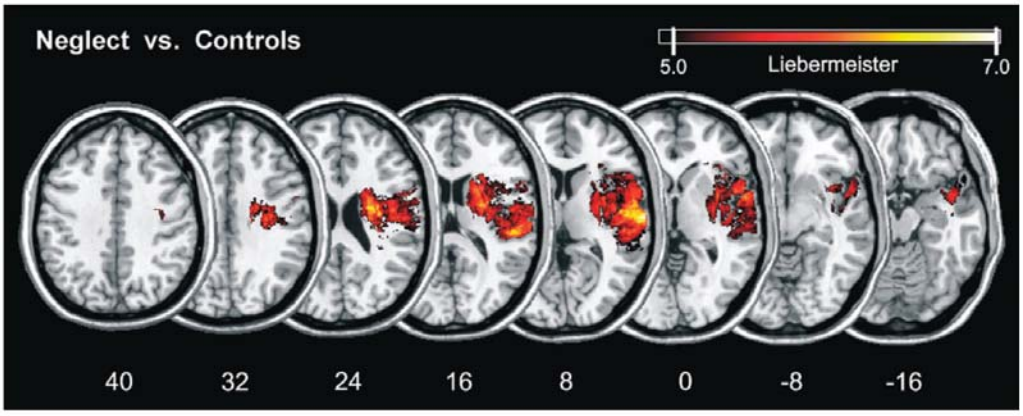

Figure 21: Statistical map of the 140 subjects with (78) and without (62) spatial neglect by using the Liebermeister approach (Rorden et al., 2007). The voxels depicted in the picture are those that persisted after the statistical threshold (see text) and represent the areas statistically more frequently damaged in patients with spatial neglect. MNI z-coordinates of the transverse sections are given.

In the cortex, the lesion also covered parts of the pre- and postcentral gyri and the inferior parietal lobule. Subcortically, the putamen, the head of caudate nucleus as well as the external and extreme capsule were involved. Overall, the lesioned area obtained by using the Liebermeister approach (Rorden et al., 2007) spread over a larger region when compared the area of significant difference produced by Karnath and colleagues (2004a) using the more conservative chi-square test on the same group of patients. To note, there were not voxels more affected in patients without spatial neglect versus those patients with spatial neglect. This result obtained from the statistical map, however, could not allow discerning whether the lesion was affecting more white or grey matter tissues. To specify the percentage of their involvement, we used the MNI brain segmented in its white and grey matter (see Methods) and we found that 36.9% of the white matter and 63.1% of the grey matter components were lying beneath the area of the statistical lesion map of figure 21. This outcome obviously suggests that, in the group of patients showing spatial neglect, the

grey matter tissue is more affected than the white matter tissue. At this point, our interest was to quantify the overlapping of the statistical lesion map with each one of the white matter fibre tracts that could be of relevance for spatial neglect. We thus overlaid the lesion map (Figure 21) onto the probabilistic atlas of the white matter fibre tracts from the Juelich group (Figure 22). At first, we took into account the maximum of overlapping between the lesion map and the probabilistic anatomical atlas. In other worlds, we considered the whole volume of the probabilistic fibre bundles, including those voxels of lowest probability. The result revealed that patients with spatial neglect (vs. controls) had lesions affecting 22.4% of the superior longitudinal fasciculus (SLF), 26.3% of the inferior occipitofrontal fasciculus (IOF), 39.6% of the superior occipitofrontal fasciculus (SOF) as well as 7.5% of the uncinate fasciculus (UF), 18.4% of the corticospinal tract (CT), and 46.9% of the acustic radiation (AR). Figure 23 graphically displays these data. We did not find any overlap between the statistical map and the optic radiation (OR), corpus callosum (CC),

fornix and cingulum. In order to increase the reliability of these first findings, we again quantified the percentage of the fibres affected, this time applying a threshold of 30%. This method restricted the analysis only on those voxels of the white matter located within the 30% isocontours of the atlas [i.e. those voxels present in at least 3 (30%) of the 10 postmortem brains].

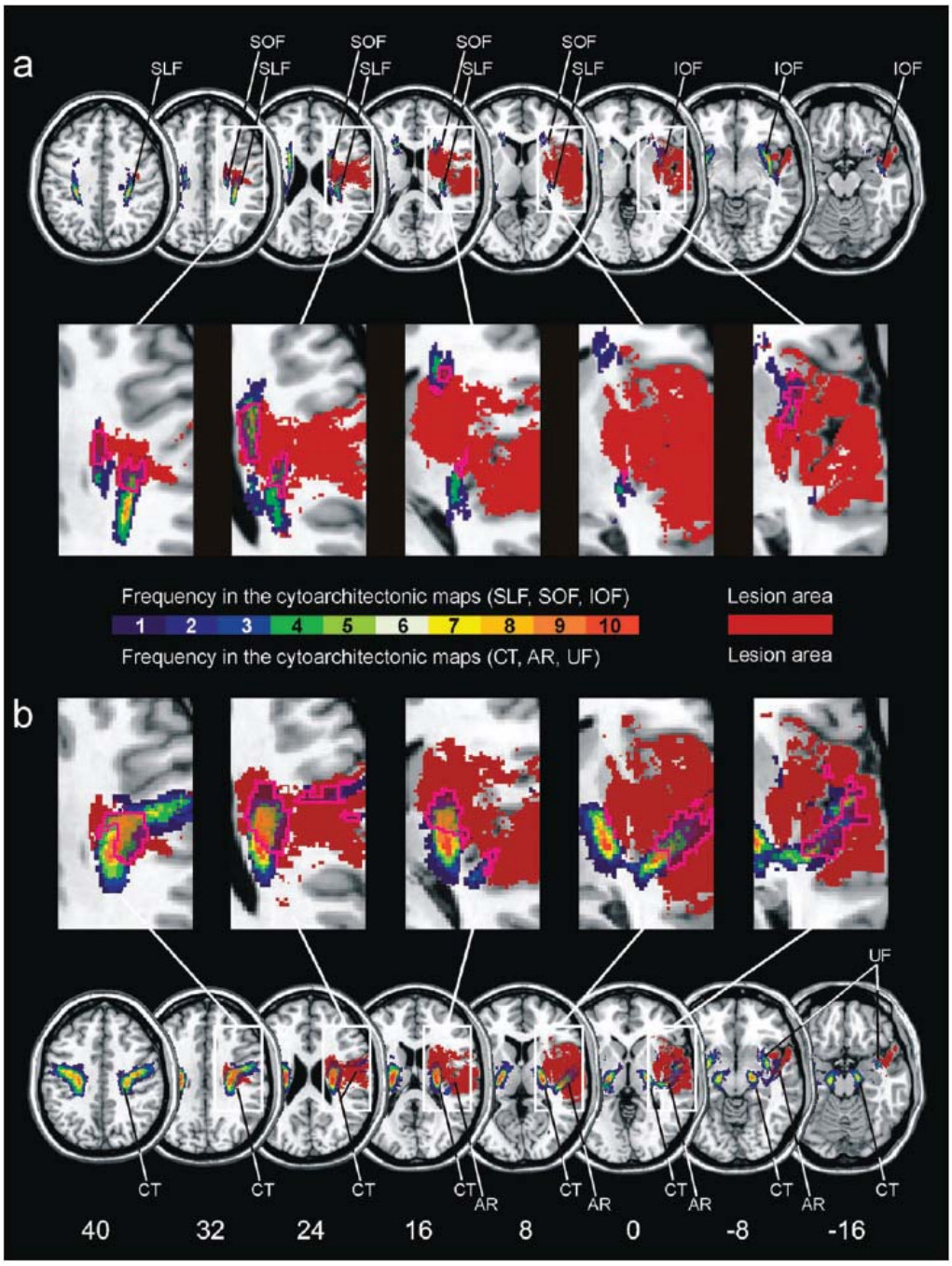

Figure 22: Overlay plot of the statistical lesion map shown in figure 21 (in homogeneous brown colour) over the cytoarchitectonic maps of the white matter tracts from the Juelich atlas, for both the hemispheres. In the centre of the figure the frequency of overlapping

of the white matter tracts (from 1 to 10) is shown. It represents the frequency of appearance of each fibre bundle (from 1 to 10 post-mortem brains) in each voxel. The area of overlapping between the Juelich atlas and the statistical lesion map is outlined in pink. a) The overlap of the lesioned area over the superior longitudinal (SLF), inferior occipitofrontal (IOF) and superior occipitofrontal (SOF) fasciculi. b) The overlap of the lesioned area over the corticospinal tract (CT), acoustic radiation (AR) and uncinate fasciculus (UF). MNI z-coordinates of the locations marked are given.

We found that 7.0% of the SLF, 8.2% of the IOF, and 12.7% of the SOF as well as 0.6% of the UF, 9.5% of the CT, and 15.0% of the AR were affected by the lesion. Moreover, when considering the volume of the lesion that overlapped with the fibre bundles of the Juelich atlas, we found an overlapping of only 10.9% (or 3.4% when the 30% isocontour was applied) of the lesion volume over the fiber tracts. In other words, we found that between 89.1% and 96.6% of the lesioned area affected brain structures other than the perisylvian white association fibres.

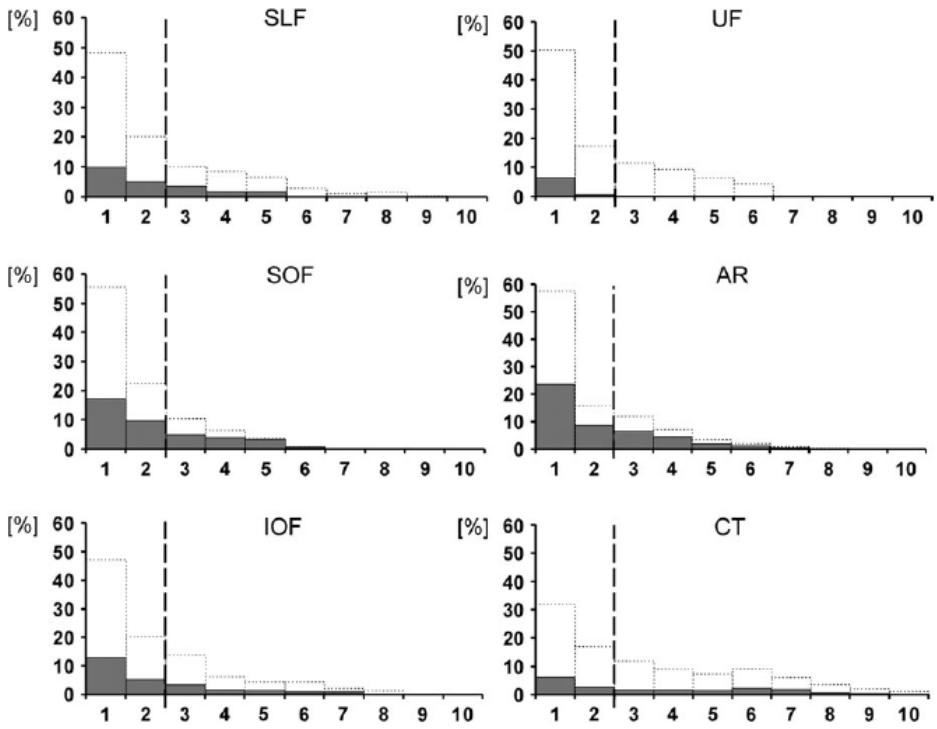

Figure 23: Graphical representation that quantifies the overlapping of the statistical lesion map with each one of the white matter fibre tracts that could be of relevance for spatial neglect (from Figure 22). The frequency (from 1 to 10) in the x-axis represents the colour coding for the Juelich atlas, as in figure 22 (see text). The dashed bars correspond to the percentage for which each frequency (from 1 to 10) is represented in the whole volume of each fibre tract, from the right hemisphere only (the sum of the percentages of the dashed bars is 100%). The grey bars indicate the percentage of voxels lesioned for each frequency. The dashed line represents the 30% isocontouts for the Juelich atlas (see text). SLF; superior longitudinal fasciculus; IOF; inferior occipitofrontal fasciculus; SOF, superior occipitofrontal fasciculus; CT, corticospinal tract; AR, acoustic radiation; UF, uncinate fasciculus.

A second analysis (logistic regression) conducted by Prof. Rorden (Karnath, Rorden, Ticini, 2009) revealed that the disruption of the perisylvian white matter tract IOF (together with the lesion volume) is the best predictor of spatial neglect. Interestingly enough, Urbanski and colleagues (2008) suggested a similar result by reconstructing the white matter fibres in two patients with spatial neglect ([Urbanski et al., 2008]; Figure 11a). The fact that these authors grounded their report only on two patients with spatial neglect, suggested me to prove their result. I conducted the same analysis on two patients with spatial neglect (see Addendum II of the Methods) and I could reproduce their findings only in a patient with a big lesion. Indeed this patient had disruption of the IOF as well as of the SLF. In the patient with spatial neglect and a smaller lesion, the right IOF was preserved together with the other fascicles (Figure 11b). To my view, the real contribution of IOF's disconnection in spatial neglect has still to be confirmed (see also Discussion).

4. Discussion

4.1 Experiment 1

Experiment 1 demonstrates that the damage of specific areas of the cortex (Figure 16) and of the thalamus (Figure 12) in patients with 'pusher syndrome' is not accompanied by large regions of common perfusion deficits beyond the structural damage (Figure 15, 17). This result is partly surprising and partly not. Below, I will explain why and I will face the problem of 'pusher syndrome' after thalamic and extra-thalamic strokes separately. As far as the thalamic damage is concerned, it is known that lesions to this subcortical structure may be associated with a widespread hemispheric metabolic depression (Baron, 2001; Baron et al., 1986; Baron et al., 1992) and may result in various neuropsychological deficits, such as neglect or aphasia (Carrera and Bogousslavsky, 2006), or balance disorders (Masdeu and Gorelick, 1988). This is not surprising, if considering that the thalamus is tightly connected to many other cortical and subcortical structures because of its fundamental role

in integrating and transmitting afferent information from the brain stem, the cerebellum, and the basal ganglia to the cortex. Therefore, to a certain degree, it would be plausible to expect that, also in patients with 'pusher syndrome' the subcortical damage would end up in a widespread malperfusion. At a first glance, the surprise lies in the fact that our results, depicted by normalized TTP and MSR maps, show the opposite (Figure 15). However, looking only at the final conclusions may be misleading. Indeed, the outcomes of this experiment should be interpreted in the following way: there is indeed a widespread pattern of malperfusion in the hemisphere affected by the thalamic lesion, as it is shown in figure 13 and 14 for different thresholds. However, there is not a *specific* cortical area of malperfusion after (i) applying a threshold that delineate tissue dysfunction, (ii) voxel-wise inter-hemispheric comparisons to avoid local bias of the signal and (iii) the group analysis that directly contrasted patients with stroke showing vs. not showing the disorder (see Methods). In other words, our investigation was a group study that aimed at

determining those areas of malperufsion that were dysfunctional in only the group of patients with ‘pusher syndrome’. Evidently, the outcome showed that cortical dysfunction is not an indispensable prerequisite if thalamic stroke patients exhibit ‘pusher syndrome’. It has been hypothesized that ‘pusher syndrome’ is indicative of the existence of two anatomically separated neural pathway, one for sensing the orientation of gravity and controlling upright body posture and one for perceiving the orientation of the visual world (see Overview). This idea is grounded on behavioral evidences: indeed, in both healthy subjects as well as stroke patients, a dissociation between the ability to perceive (i) the orientation of the visual world with respect to the own body (subjective visual vertical, SVV) and (ii) the own body position with respect to graviception (subjective postural vertical or SPV, affected in ‘pusher sundrome’) has been frequently reported (Bisdorff et al., 1996; Anastasopoulos et al., 1997; Mittelstaedt and Mittelstaedt, 1997; Mittelstaedt, 1998; Karnath et al., 2000b; Jarchow et al., 2003; Kaptein and Van Gisbergen, 2004, 2005). Interestingly,

SVV is mediated by the visual-vestibular system and is preserved in pusher patients. Anatomically speaking, damage to the posterior thalamus has been also shown to determine visual-vestibular deficiencies, beyond 'pusher syndrome'. This structure is indeed a relay station for the ascending visual-vestibular input mediated by visual, vestibular and proprioceptive sense organs in the head and neck to the multisensory vestibular areas in cortex (Brandt et al., 1994). Its role in this context has been established by vestibular stimulation in animal experiments (Buttner and Henn, 1976) as well as by human functional imaging studies using vestibular, somatosensory, and optokinetic stimulation (for a review [Karnath and Dieterich, 2006]). Moreover, acute or subacute unilateral posterolateral thalamic strokes in patients with visual-vestibular defects have been observed to cause deactivation of the multisensory vestibular temporo-parietal cortex in the ipsilateral hemisphere (Brandt et al., 1994). Pondering on the differences between these and our findings, the evident difference is the lack of an involvement of the vestibular

cortex in thalamic strokes patients with ‘pusher syndrome’. From this perspective, our evidences showed that upright body posture (SPV) and visual-vestibular (SVV) processing may differentiate behaviorally as well as anatomically. The second step was to discern the cortical substrate typical for ‘pusher syndrome’ in patients with strokes sparing the thalamus (Figure 16). Previously, it has been reported that small areas within the posterior insula, superior temporal gyrus, postcentral gyrus, and inferior parietal lobule are affected in pusher patients ([Johannsen et al., 2006]; Figure 4 and 5). Our results showed that, after extra-thalamic strokes, additional areas are abnormally perfused beyond these structural damages, namely small regions in the structurally intact inferior frontal gyrus (IFG), middle temporal gyrus, precentral gyrus, inferior parietal lobule (IPL), and parietal white matter. Further, small parts of the callosal body (CB), of the temporal white matter, and of the superior longitudinal fasciculus (SLF) were more frequently involved (Figure 17). Are these brain structures distinct from those involved in visual-

vestibular perception? Our study did not allow answering this question. Indeed, the human neural substrates for vestibular processing had been identified in the posterior insula, the inferior parietal lobule, and the superior temporal gyrus (for review [Karnath and Dieterich, 2006]). Obviously, overall it seems that 'pusher syndrome' follows the disruption of a network that involves subcortical as well as cortical neural structures that partially overlap with those involved in visual-vestibular processing. It has been speculated that incoming signals from somatic graviceptors of the trunk via the renal, the phrenic, or the vagus nerves mediate upright body posture (Mittelstaedt, 1964, 1998). The fact that in patients with 'pusher syndrome', the lesions have been found to affect unilaterally the posterior thalamus, particularly the left or the right ventroposterior and the lateral posterior nuclei (Karnath et al., 2000a, 2005a), may suggest that the thalamus is an essential step in processing of SPV. Indeed, from these nuclei, axonal fibers project to numerous other structures. These connections have been traced invasively in non-human

primates (Jones, 1985; Padberg and Krubitzer, 2006; Cappe et al., 2007) and *in vivo* studies in humans (Behrens et al., 2003; Johansen-Berg et al., 2005). In particular, the thalamocortical axons arising from the ventroposterolateral and the ventroposteromedial nuclei project to primary somatosensory cortex in the postcentral gyrus (Brodmann areas 3a, 3b, 1, and 2) and to the secondary somatosensory cortex in the parietal operculcum and to the insula (Jones, 1985; Engelborghs et al., 1998). This anatomical connectivity is evident also in functional studies in which stimulation of the vagus nerve influenced neural activity in the thalamus, the insular cortex and the postcentral gyrus, among other brain regions (Bohning et al., 2001; Chae et al., 2003; Narayanan et al., 2002). To conclude, our results suggested that (i) in pusher patients with thalamic strokes there is no need of additional malperfusion beyond the lesion in order to provoke the disorder; (ii) in pusher patients with extra-thalamic strokes some cortical areas, but not the thalamus, are malperfused, beyond the structural damage. Overall, we suggest that the normal

functioning of a network composed of both extra-thalamic as well as posterior thalamic structures is integral to perceiving gravity and controlling upright body orientation in humans.

4.2 Experiment 2

Experiment 2 reports that the integrity of the right temporoparietal junction (TPJ) is central to detect visual stimuli under conditions of rivalry (Figure 20). As a matter of fact, we found that this area is dysfunctional (though structurally intact) in those patients suffering from visual extinction, even in the case of subcortical strokes centered on the right basal ganglia (Figure 18). This is quite an important finding, due to the fact that it gives a straightforward interpretation of visual extinction after subcortical strokes as a disorder that requires, in order to be apparent, a concomitant cortical dysfunction. As I emphasized in the overview of this experiment, visual extinction has been associated subcortically with damage of, among others, the basal ganglia (Vallar et al., 1994) and cortically with virtual (Pascual-Leone et al.,

1994; Hilgetag et al., 2001; Dambeck et al., 2006; Battelli et al., in press) or real (Critchley, 1949; Heilman and Valenstein, 1972; Vallar et al., 1994) lesions of the posterior parietal lobe as well as virtual (Meister et al., 2006) or real (Karnath et al., 2003) lesions of the right TPJ. Here, we showed that extinction after subcortical lesions is dependent on cortical dysfunction of the right TPJ together with caudal parts of the right superior and middle temporal gyri as well as parts of the supramarginal and angular gyri (Figure 20). A similar result was obtained previously by Karnath and associates (2005), when investigating five patients with spatial neglect vs. five controls without the syndrome. However, due to the fact that spatial neglect and visual extinction are often coupled, the experiment of Karnath and associates depicted the dysfunctional area present in patients with both the disorders. Here, we went further in the investigation and decoupled patients with spatial neglect according to presence/absence of visual extinction. We obtained a group of eight patients with concurrent spatial neglect and visual extinction and a

group of five patients with only spatial neglect (Table 2). Although our results are fitting very well with those observations showing the involvement of the right TPJ in visual extinction ([Karnath et al., 2003; Meister et al., 2006]; Figure 6 and 7 respectively), they are not corresponding to the findings of Hillis et al. (2006). Indeed, Hillis revealed that visual extinction is associated with dysfunction, i.e. with structural or perfusion abnormalities, of the right occipital visual association cortex (right Brodmann area [BA] 19). However, against this conclusion, transcranial magnetic stimulation (TMS) over the parietal lobe but not over the occipital or temporal lobes elicited extinction-like phenomena in normal subjects (Pascual-Leone et al. 1994). The issue of the role of the visual cortex could not be clarified in our experiment (though, we did not find malperfusion in visual areas) and our findings support more the view that extinction is not necessarily associated with dysfunction of BA 19. It is also interesting to note that Hillis and associates (2006) correlated visual extinction neither with a dysfunctional TPJ nor with any of the deep brain areas

reported in previous studies (Vallar et al., 1994; Manes et al., 1999). These authors suggested that the divergence is caused by the time between imaging and stroke onset, which for their patients was less than 24 hours. The assumption behind the reasoning of Hillis and colleagues is that patients with small strokes may have initially showed extinction and neglect but recovered in the first few days after stroke, while patients with larger lesions may have failed to recover, thus giving a preference towards an association of visual extinction with larger lesions covering also the TPJ. Again, we could not discern whether this is a valid reason or not. Further studies are needed to answer this specific question. It is of extreme interest to note that the unsuccessful perception of contralesional events in patients with extinction may affect more than only the visual modality (Stone et al., 1993; Karnath et al., 2003; Hillis et al., 2006). Not surprisingly, the patients we studied had also other forms of extinction, concurrent with the visual one. Five out of eight patients (62.5%) of our extinction group had concurrent visual and tactile extinction and six out of

eight (75%) had concurrent visual and auditory extinction. These numbers are consistent with the percentages found by Karnath and colleagues (2003). How these data are reflected on the pattern of cortical malperfusion? As I mentioned above, the caudal parts of the right superior and middle temporal gyri as well as parts of the supramarginal and angular gyri were found dysfunctional along with the TPJ. At least part of this pattern of malperfusion could be associated with tactile extinction, since it has been shown to correlate with dysfunction of the right inferior parietal lobule, including angular and supramarginal gyri (BA 39/40; [Hillis et al. 2006]). Moreover, part of the malperfusion within the auditory cortex in the STG could be correlated with auditory extinction. Overall, our data indicate an involvement of area TPJ in processing stimuli from different modalities. These results are supported by prior neuroimaging studies that showed the involvement of TPJ in processing salient multimodal visual, tactile and auditory events (Downar et al., 2000; Corbetta and Shulman, 2002). Interestingly enough, grounding their

assumptions on fMRI experiments on healthy subjects, Corbetta and colleagues embedded the TPJ in a model of spatial attention, anatomically and functionally based on diverse cortical areas distributed along a frontoparietal network. In particular, these authors suggested that TPJ area plays a role in a ventral frontoparietal network of attentional processing, which includes also the right ventral frontal cortex (Corbetta and Shulman, 2002; Corbetta et al., 2008). The model calls for an additional system located more dorsally and involving dorsal parietal and frontal cortices. This dorsal system would be dedicated to the top-down control mechanism that generates endogenous signals about possible eventualities and sends out signals that bias the processing of the correct stimulus features and locations in sensory cortex. Otherwise, the ventral network would be devoted to a bottom-up control mechanism for the detection of behaviourally relevant stimuli. These two systems would interact and the latter would behave as a ‘circuit breaker’ for the dorsal system, directing attention to salient events (Corbetta and Shulman, 2002). Within this context, it is

worth mentioning that the right TPJ area is more activated in stimulus-driven fashion, for example when subjects expect a stimulus in one location but it unexpectedly appears at another in the Posner spatial cueing paradigm (Arrington et al., 2000; Corbetta et al., 2000; Macaluso et al., 2002), when distracters shares critical features with the target stimulus (Serences et al., 2005), when a target is presented among other stimuli (Downar et al., 2000, 2001; Serences et al., 2005), or in active execution of voluntary visual exploration (Hopfinger et al., 2000; Nobre et al., 2002; Himmelbach et al., 2006). Coming back to stroke patients' studies, another way to explain the phenomenon of visual extinction would be by giving to the right hemisphere a specialization for global attention, which would be disrupted in right side stroke patients. Following this interpretation, extinction would be an impairment of bilateral (global) vs. unilateral (focal) attentional distribution. As a matter of fact, a selective impairment of the global mode has been reported in patients with right side lesions (Robertson et al. 1988; Marshall and

Halligan, 1995). Supporting these data, a recent fMRI experiment on normal subjects showed the activation of the right inferior parietal lobule (IPL) by bilateral (global) attentional distribution. The authors proposed that unilateral lesions of the right IPL cannot be compensated by the contralesional hemisphere and thus give rise to selective impairment of global attention and visual extinction (Cicek et al., 2007). There are some last comments still to consider, which however are not strictly related to the TPJ functions mentioned so far. Beyond its contribution to the ventral attentional network, area TPJ has been proposed to play a role in non-spatial encoding of salient stimuli (Marois et al., 2000; Adler et al., 2001). Further, neuroimaging studies have found activation of the TPJ (predominantly in the right hemisphere) in several aspects of multisensory integration, visuo-vestibular processing, perception and imagery of body parts, and the processing of biological motion (for reviews [Blanke and Arzy, 2005; Karnath and Dieterich, 2006]). Moreover, localised lesions (Blanke et al., 2004) and electrical cortical stimulation

(Blanke et al., 2002) of this area may evoke out-of-body experiences. Overall, these findings suggest that the TPJ area has functions that go beyond the spatial and attentional systems and that are worth to investigate in detail in future scientific works. To conclude, we compared spatial neglect patients with subcortical right basal ganglia lesions showing versus not showing left-sided visual extinction. We observed that in those patients with extinction the subcortical damage did not determine the disorder *per se* but required additional concurrent cortical dysfunction clustered around the right TPJ. Importantly, our results related tightly prior subcortical stroke findings to cortical reports. The outcome supports the idea that the TPJ area plays a decisive role for the visuo-spatial attentional network. Our result matches with those studies that observed visual extinction after (real/virtual) lesions centring on the cortical area TPJ (Karnath et al., 2003; Meister et al., 2006) or subcortical lesions of the basal ganglia (Vallar et al., 1994).

4.3 Experiment 3

In this experiment we aimed at understanding the role of grey versus white matter tissue in spatial neglect, in a reanalysis of a large 7-years sample of 140 right-hemispheric stroke patients (see also [Karnath et al., 2004a]). For our analysis, we used the non-parametric Liebermeister test to highlight the areas of lesion that characterized neglect syndrome (Rorden et al., 2004) and subsequently plotted them over the Juelich cytoarchitectonic atlas of white matter fibres (Buergel et al. 2006). We quantified the brain structures affected by the statistical map representing the lesion area in neglect patients and we found that 96.6% of this territory was outside the perisylvian white matter fiber tracts of the right hemisphere, which are supposed to be disrupted in spatial neglect. Indeed, the lesion was covering the superior temporal, inferior parietal, inferior frontal, and insular cortices, as well as the putamen and caudate nucleus. Our findings obviously suggest that cortical and subcortical lesions other than the perisylvian white matter tracts would best predict spatial neglect. This is not

surprising since, as we have seen above (see Overview), spatial neglect has been mostly associated with grey matter lesions (Figure 9) of the temporoparietal junction (Mort et al., 2003; Vallar, 2001), rostral parts of the superior temporal gyrus (Committeri et al., 2007; Karnath, 2001; Karnath et al., 2004a), the ventrolateral prefrontal cortex (Husain and Kennard, 1997), the medial temporal lobe (Mort et al., 2003), as well as subcortically the basal ganglia and the thalamus (Karnath et al., 2002). However, Doricchi and Tomaiuolo (2003) found that white matter injuries were predicting spatial neglect in a population of 'chronic' neglect patients (mean interval post-stroke of 121 days), and subsequently He and colleagues (2007) reported that damage of the SLF and of the AF were associated with severe (but not mild) spatial neglect. Overall, this might mean that patients with persistent and severe symptoms of spatial neglect would in fact show disruption of the subcortical white matter fibres. Interestingly, an increasing number of scientific works refer to the disruption of white matter association fibres as a predictor of spatial neglect. The idea behind is

that this disorder may represent a ‘disconnection syndrome’; in plain English, a syndrome that follows disruption of functional and anatomical connectivity between brain areas (Mesulam and Geschwind 1978; Watson et al., 1974, 1978; Catani, 2006; Bartolomeo et al., 2007; He et al., 2007). Recent investigations indeed found that the disconnection of the superior longitudinal fasciculus (SLF; [Thiebaut de Schotten et al., 2005; Bartolomeo et al., 2007]) and the inferior fronto-occipital fasciculus (IOF; [Urbanski et al., 2008]) may lead to spatial neglect. Also a subsequent analysis on the data presented in this thesis (conducted by Prof. Rorden) agreed in viewing IOF’s disruption as predictive of spatial neglect (Karnath, Rorden, Ticini, 2009). The IOF (Figure 10) runs medially in the temporal lobe and inferiorly in the frontal lobe and its fibres are compacted in the anterior floor of the external capsula. It connects rostrally the ventrolateral frontal cortex with posterior temporal, inferior parietal and occipital cortices ([Nieuwenhuys et al. 1988, Catani et al. 2002]; Figure 24). However, the parietal fibres of the inferior fronto-

occipital fasciculus are questioned (M. Catani, personal communication).

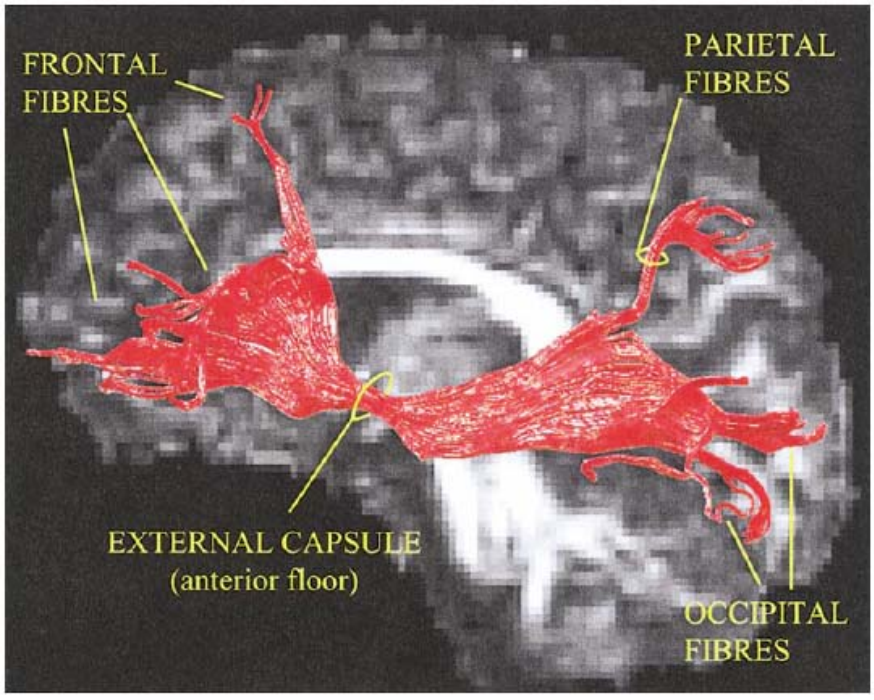

Figure 24: Lateral left view of the inferior fronto-occipital fasciculus (IOF, from [Catani et al., 2002]).

They may represent artefacts or false-positives in the DTI reconstruction. The IOF has two frontal projections, one lateral and the other anterior that can be seen respectively in yellow and red in figure 25. The parietal projections of the ventrolateral frontal fibres (in yellow, Figure 25) are false-positive. In other words, the tracking algorithm that

allows reconstructing the fiber tracts is biased by the close location of different fibre packed within the external capsula. This of course shapes the DTI representation, visualizing connections between the verntrolateral frontal fibers of the IOF and the posterior parietal regions. In their DTI-based study, Urbanski and colleagues (2008) claimed that the IOF was disrupted in two patients with spatial neglect ([Urbanski et al., 2008]; Figure 11a). The analysis I conducted on two other patients with spatial neglect (see Addendum II in the Methods) could reproduce these findings only in one patient with a very big lesion, in which both the IOF as well as of the SLF were disrupted. Otherwise, in the other neglect patient with a smaller lesion, the right IOF was preserved together with other fascicles (Figure 11b). The rostro-caudal tract associated with neglect is the superior longitudinal fasciculus (SLF; [Doricchi and Tomaiuolo, 2003; Thiebaut de Schotten et al., 2005]). The fibres of the SLF are integral part of the perisylvian network (see Overview), which overall seems to be involved in determining, when damaged, spatial neglect.

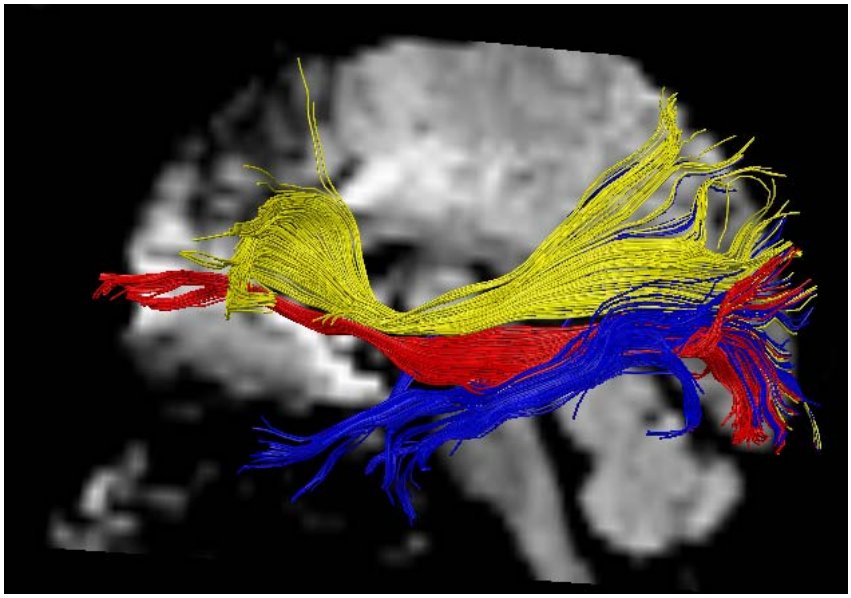

Figure 25: Lateral left view of the inferior fronto-occipital fasciculus (IOF, in red), inferior longitudinal fasciculus (ILF, in blu) and, in yellow, ventrolateral frontal fibres of the IOF that project to parietal regions instead of projecting to the occipital cortex. The parietal projections of the ventrolateral frontal fibres are false-positive.

This fascicle was not found much involved by the structural damage in our experiment (7%), and in my preliminary results, depicted in figure 11b, the SLF was disrupted only in that patient with a widespread cortical and subcortical lesion. Contrarily to the investigations mentioned above, where DTI was used, in the present thesis, we relied on the cytoarchitectonic atlas of ten

post-mortem brains developed by the Juelich group (Buergel et al., 2006). Although this atlas allowed us plotting and studying a group of 140 patients with and without spatial neglect, there is a deficiency to stress. For the intrinsic nature of the procedure used by the Juelich group, which consisted in the mapping of the tracts on coronal brain sections, some rostro-caudal perisylvian fibres could not be defined properly. In particular the arcuate fasciculus (AF, Figure 10) could not be distinguished from the SLF (K. Amunts, personal communication) and clearly the fibres of the SLF could not be traced in their more rostral and caudal sectors. The atlas thus depicts only the very central part of the SLF fascicle. This of course may have biased our results that, however, report an involvement – though not significant (except for the IOF) – of perisylvian white matter fibres in spatial neglect. In favour of the use of the Juelich atlas is the fact that the microscopic procedure certainly allows mapping anatomical structures in a more detailed way than diffusion tensor tractography (Buergel et al., 2006). To conclude, we shall stress that the investigators have to

be cautious when questioning about the sites of lesions in spatial neglect. It seems that being thoughtful is essential because of the numerous conflicting evidences. Moreover, we shall consider that spatial awareness indeed require a distributed network of cortical and subcortical structures and, thus, a lesion in one of these components might determine spatial neglect also through indirectly affecting other components of the system. The evidences reported in our study are more in favour of a cortical contribution in spatial neglect; however they don’t exclude subcortical white matter involvement suggesting that a point of view that includes both perspectives is perhaps the soberest approach to look at this common deficit of spatial awareness.

5. Conclusion

This doctoral thesis describes part of my work performed in the Section of Neuropsychology, University Clinic of Tübingen. The manuscript has been organised in three parts that represent three experiments and corresponding papers (see Contributions) devoted to the investigation of disorders of spatial awareness in human beings, specifically: 'pusher syndrome', 'visual extinction' and 'spatial neglect'. Although the experiments focus on different deficits, and thus could be taken separately, they address similar central questions for the study of spatial awareness and balance. I extensively dealt with each one of them in the related chapters. The study of the disorders of spatial awareness in stroke patients is not only of particular concern from a clinical point of view, but has also the potential to help further our understanding of the neural basis of spatial and attentional processing. With the final aim to deepen the characterization of the neural sites that contribute to spatial awareness, I studied the subcortical as well as the cortical dysfunctions (lesion

and/or hypoperfusion) of groups of stroke patients. Interestingly, the results suggested that there are specific networks involved in spatial awareness and balance, which may be disrupted in stroke patients. As far as 'pusher syndrome' is concerned, we confirmed that this disorder of space perception in the coronal plane is associated mainly with dysfunction of the thalamus and its cortical projections in the postcentral gyrus, insula and proximal areas. In future studies, it would be of scientific interest to employ diffusion tensor imaging technique (DTI) in order to identify those white matter bundles disrupted in 'pusher syndrome' and to describe the reorganization of subcortical and cortical structures and relative functions during the process of recovery (e.g. by means of fractional anisotropy in DTI studies or by fMRI). In the second experiment, I reported that 'visual extinction' is associated with cortical malperfusion and dysfunction of the temporo-parietal junction, even in the case of subcortical strokes centred on the basal ganglia. This finding, by showing a direct relationship between subcortical strokes and cortical dysfunction, fills the gap

between those studies that found extinction only associated with subcortical strokes and those that recognized the syndrome after cortical damages. This information might be useful also for clinical purposes, suggesting that reperfusion methodologies may grant an earlier recovery of extinction, at least in acute patients. Furthermore, in the last experiment we studied 'spatial neglect' in a group of 140 stroke patients, whose lesions were plotted over the probabilistic cytoarchitectonic atlas from the Juelich group. Specifically, this investigation aimed at clarifying whether neglect is more associated with damage of cortical structures or more with the disruption of subcortical white matter tracts. Although there are some methodological limitations, highlighted in the text, our work contributed to the lively discussion on the neural correlates of spatial neglect. Our results depicted that neglect is mainly determined by cortical strokes. However, parts of the perisylvian white matter association tracts were also found lesioned. Thus, the result suggested that we shall not exclude that subcortical white matter perisylvian lesions may impair the network

of spatial awareness. As far as the methodology is concerned, in the investigations I used structural magnetic resonance imaging (MRI) to study the irreversible damage, and perfusion weighted imaging (PWI) to describe those areas of dysfunction in structurally intact tissue. One limitation of studying stroke patients by means of PWI, which I also encountered during my work, is the fact that the employment of an extra-vascular bolus for the blood tracking is always problematic. Indeed, the patients have to correspond to certain pre-requisites (e.g. good kidney's clearance) and suitable patients are often not keen to be investigated by means of invasive methods. In order to solve this issue, it would be of extreme interest to utilize a recently developed MRI perfusion technique, called arterial spin labelling (ASL). ASL measures cerebral perfusion relying on the use of magnetically labelled water protons as an endogenous tracer, thus it does not require the injection of contrast agents. This technique of course may also be applied to explore the neural basis of cognitive deficits, giving the possibility to investigate a

larger number of patients with cognitive disorders. Another aspect that emerged from my work is that, in order to have a comprehensive view of the brain processes is of key importance to face our investigations by using different methodologies (e.g. fMRI, DTI, PWI, MEG). This multimodal approach would eventually allow disentangling and understanding the numerous implications of brain lesions and would also have further implications in proving the helpful use of imaging as biomarker of the patients' chances to respond to different forms of treatments (e.g. reperfusion, rehabilitation).

To conclude, I focused my work on the study of stroke patients in view of the fact that they give us direct neuropsychological evidences of the functions of single brain modules. Certainly, further steps have to be undertaken to interpret thoroughly the neural correlates of spatial awareness. Nevertheless, I hope that the research conducted for this thesis will contribute to deepen the knowledge of the networks of spatial perception in normal and pathological conditions.

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Luca Ticini - Tübingen, March 2009

Contributions

An aim of the section of Neuropsychology at the University Clinic of Tübingen, headed by Prof. Hans-Otto Karnath, is to collect a large database of stroke patients exhibiting neuropsychological deficits. In general terms, this *modus operandi* has the purpose to study groups of patients distinguished as far as their neuropsychological disorders and lesion locations are concerned. At last, this may contribute to ascertain the precise functions of single brain areas. Within this context, patients with 'spatial neglect', 'visual extinction' and 'pusher syndrome' have been collected by the members of the Section of Neuropsychology during the years before and during my permanence at the University Clinic of Tübingen. As far as 'pusher syndrome' is concerned, the section typically relied on the help of the group of physiotherapists headed by Dr. Doris Broetz, who clinically investigated the patients. Moreover, the work of the section of Neuropsychology is assisted by the Department of Neuroradiology at the University of Tübingen. In particular, Prof. Thomas Nägele and his co-

workers permitted the use of an invasive perfusion imaging protocol introduced in the standard magnetic resonance imaging (MRI) acquisitions. Thanks to the physicians and their associates, the patients could also receive appropriate care during these delicate processes. Last but not least, the Section of Experimental MR of the CNS, in the person of Dr. Uwe Klose, together with Regine Zopf, has developed a protocol that allows the study of perfusion MRI in groups of patients. The analysis of the perfusion data that I selected for the experiments of the thesis has been based on this procedure. Overall, my work has been possible thanks to the valuable help of all these persons, without them the collection and study of the patients included in the thesis would have largely not been possible.

The results of the experiments 1, 2 and 3 presented in the following pages have been organized respectively in three papers:

1. Ticini L. F., Klose U., NaegeleT. and Karnath H.O. (2009) Perfusion imaging to investigate the neural substrates involved in controlling upright body

position. *PLoS ONE* 4(5): e5737. doi:10.1371/journal.pone.0005737.

2. Ticini L. F., de Haan B., Klose U., NaegeleT. and Karnath H.O. (in press) The role of the temporo-parietal cortex in subcortical visual extinction. *J Cogn Neurosci*.
3. Karnath H.O., Rorden C. and Ticini L. F. (2009) Damage to white matter fiber tracts in acute spatial neglect. *Cerebral Cortex* 19:2331-7

More in particular, I list below the specific contributions in each experiment.

Experiment 1

The first part of the experiment 1 is based on the idea of Prof. Karnath to study the cortical perfusion deficits in patients suffering from 'pusher syndrome' after thalamic damage. Together, we further developed this design in the second part of the experiment, to study the perfusion deficits in 'pusher syndrome' after extra-thalamic lesions. In this experiment, I used the data of patients collected by Leif Johannsen, Monika Fruhmann Berger and Regine

Zopf. The clinical investigation of the patients was conducted by Leif Johannsen, Monika Fruhmann-Berger, Doris Broetz and Regine Zopf. The perfusion and anatomical data were collected by Prof. Thomas Nägele and his co-workers (Department of Neuroradiology). My work consisted in the selection of those pre-investigated patients suitable according to the sites/kind of lesions and their neuropsychological disorders; the complete analysis of the lesion data and perfusion MRI data; the elaboration of the final results; the writing of the paper (see above), with the precious assistance, contribution and experience of Prof. Karnath.

Experiment 2

The experiment 2 has been conceived by me and supported by Prof. Karnath. This experiment represents an interesting extension of the reports that the Section of Neuropsychology has previously published on visual extinction (Karnath et al., 2003) and spatial neglect (Karnath et al., 2005b). In this experiment I used the data of patients collected by Leif Johannsen, Monika Fruhmann Berger and Regine Zopf. The clinical

investigation was conducted by Leif Johannsen, Monika Fruhmann Berger and Regine Zopf. The perfusion and anatomical data (included DTI) were collected by Prof. Thomas Nägele and his co-workers (Department of Neuroradiology). My work consisted in the selection of the pre-investigated patients suitable according to the sites/kind of lesions and their neuropsychological disorders; the complete analysis of the lesion data and perfusion MRI data; the elaboration of the final results; the writing of the paper (see above), with the precious assistance of Prof. Karnath. Moreover, I conducted a first analysis of the data of two of the patients included in this study by means of diffusion tensor imaging tractography. The preliminary results are shown in figure 11b and commented in the text.

Experiment 3

Our synergic sharing of ideas is also visible in experiment 3. The originality of this paper consists in the use of the histological maps of the human white matter fiber tracts provided by the Juelich probabilistic cytoarchitectonic atlas in order to investigate the white

matter connectivity in a large sample of 140 stroke patients with and without spatial neglect. We already used with success the Juelich atlas in two papers not included in the present thesis. These are the following:

- Papageorgiou E., Ticini L.F., Hardiess G., Schaeffel F., Wiethoelter H., Mallot H., Bahlo S., Wilhelm B., Vonthein R., Schiefer U. and Karnath H.O. (2008) The pupillary light reflex pathway. Cytoarchitectonic probabilistic maps in hemianopic patients. *Neurology*, 70 (12): 956-63
- Staudt M., Ticini L.F., Grodd W., Krägeloh-Mann I., Karnath H.O. (2008) Functional topography of early periventricular brain lesions in relation to cytoarchitectonic probabilistic maps. *Brain and language*, 106 (3):177-83

In this experiment, the clinical investigation of the 140 patients was conducted by Susanne Ferber, Marc Himmelbach, Leif Johannsen, Matthias Niemeier and Ulrike Zimmer. The anatomical data were collected by Dr. Wilhelm Küker and co-workers at the Department of

Neuroradiology. In the third experiment, my contribution was: the development of an appropriate quantitative analysis that allowed the study presented in the paper, i.e. the quantification of white versus grey matter brain tissue affected by the lesions and the identification of the percentage of white matter fibers affected by the lesions in patients with spatial neglect; the execution of the analyses; the elaboration of the final results together with Prof. Karnath; the preliminary analysis of DTI data.