Disentangling the Prefrontal Network for Rule Selection By Means of a Non-Verbal Variant of the Wisconsin Card Sorting Test

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Abstract: This study disentangles the prefrontal network underlying executive functions involved in the Wisconsin Card Sorting Test (WCST). During the WCST, subjects have to perform two key processes: first, they have to derive the correct sorting rule for each trial by trial-and-error, and, second, they have to detect when this sorting rule is changed by the investigator. Both cognitive processes constitute key components of the executive system, which is subserved by the prefrontal cortex. For the current fMRI experiment, we developed a non-verbal variant of the WCST. Subjects were instructed either to respond according to a given sorting rule or to detect the correct sorting rule, like in the original version of the WCST. Data were obtained from 14 healthy male volunteers and analysed using SPM and a random effects model. All conditions activated a fronto-parietal network, which was generally more active when subjects had to search for the correct sorting rule than when the rule was announced beforehand. Significant differences between these two conditions were seen in the dorsolateral prefrontal cortex (PFC) and the parietal lobe. In addition, the data provided new evidence for the assumption of differentiated roles of the left and right prefrontal cortex. Although the right PFC showed a general involvement in response selection and the execution of goal directed responses, based on given rules, the left PFC was only activated when inductive reasoning and feedback integration was required. *Hum Brain Mapp* 30:1734–1743, 2009. © 2008 Wiley-Liss, Inc.

Key words: fMRI; brain; executive system; prefrontal cortex; visual; spatial; working memory

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INTRODUCTION

The ability to plan and execute goal directed actions is widely assumed to be predominantly a frontal brain function. Cognitive processes involved in goal directed actions include attentional processes, working memory, the ability to follow different streams of actions, and to plan and select appropriate responses. A number of models have been put forward to describe these complex processes, focusing on executive attentional aspects, for example, the model of the “supervisory attentional system” [Shallice,

1982], or working memory [see, e.g., Baddeley, 1992, 2001; Baddeley et al., 2001]. The latter model assumes a central executive system, which has the ability to distinguish between relevant and irrelevant information, to direct attention, or, for example, to plan an appropriate action. Sub-systems within this model are the visual sketchpad and the phonological loop for maintaining information in working memory. Working memory capacity is crucially needed to perform (every-day) tasks or to deal with information, for example, with extracting relevant and rejecting irrelevant information. The central-executive system is supposed to combine and control these sub-systems, and, finally, it is relevant for the execution of an appropriate action, like a response to an external trigger. Anatomically, these functions are assumed to be located in prefrontal and parietal brain areas. Especially, the prefrontal cortex (PFC) has been implicated in the maintenance and manipulation of verbal and non-verbal information, decision-making, preparation and execution of motor actions, and sustained attention and alertness [D'Esposito et al., 1998; Levy and Goldman-Rakic, 2000; Smith and Jonides, 1999; Sturm et al., 1999; Wood and Grafman, 2003].

One "classical" clinically widely applied test for exploring a dysfunction of the executive system is the Wisconsin-Card-Sorting-Test (WCST) [Dehaene and Changeux, 1991]. In this test, the subject has to indicate whether two subsequently presented cards, each showing up to four coloured symbols, are identical with respect to a specific sorting criterion (dimension) that can either be the (i) colour, (ii) symbol, or (iii) number of displayed symbols. At the beginning of a sequence of trials, the subject does not know the correct sorting criterion. Therefore, it has to figure out the correct response by trial and error. After the subject has been able to derive the correct sorting criterion and performed a series of trials with correct responses, the rule is changed by the investigator without any notification. The subject has to detect this change and to derive the new sorting criterion. Therefore, the WCST imposes high demands on working memory and the executive system and cognitive flexibility because all subsequent responses have to be adapted when a negative feedback occurs. For getting the new sorting rule, the subject must therefore memorize previously tested rules and avoid to test them twice, and the subject has to reject some rules by reasoning on the possible feedback [Dehaene and Changeux, 1991].

However, even though neuroimaging data are consistently demonstrating that the performance of the WCST leads to strong activations in the PFC [Konishi et al., 1999a,b, 2005; Lie et al., 2006; Monchi et al., 2001, 2004; Stemme et al., 2005], clinical studies have also shown that there is a lack of sensitivity of the WCST for frontal lesion. These studies have reliably demonstrated that also patients with non-frontal lesions occasionally demonstrate reduced performance in the WCST [Goldstein et al., 2004; Kim et al., 2007; Wildgruber et al., 2000]. Therefore, several modifications of the WCST had been made in the past

[Dehaene and Changeux, 1991; Nelson, 1976; Stuss et al., 2000; van den Broek et al., 1993], which on the other hand influenced the performance differently.

Besides the general lack of sensitivity for frontal lesion, the specificity of left and right prefrontal processes and their relationship to cognitive activation in imaging studies is also still under debate. For example, lesion studies suggest a much clearer behavioural differentiation between patients with left and right frontal lesions than neuroimaging studies do. A common finding in patient data is that patients with right frontal lesions tend to make more perseveration errors and show reduced feedback monitoring, whereas patients with left frontal lesion show a poorer task performance when inductive reasoning and behavioural adaptation are required [see, e.g., Reverberi et al., 2005; Stuss et al., 2000]. In contrast, neuroimaging studies using the WCST demonstrate a more bilateral activation pattern with less clear functional differentiations between left and right PFC [Konishi et al., 2002; Lie et al., 2006; Monchi et al., 2001].

One difficulty in deriving a reliable left-right differentiation of PFC function involved in the executive system may lie in the selection of the stimulus material. Taking the WCST as an example, the simplicity of the original stimulus material makes it most likely that subjects adopt verbal processing strategies, like "three red stars", "two green crosses", etc. Such a verbal rehearsal processes is generally assumed to involve the phonological loop, which is predominantly localized in the left frontal lobe [Baddeley, 2003], close to those areas found to be relevant for inductive reasoning, a key process for solving the WCST. Neuroimaging studies may therefore fail in efficiently disentangling stimulus- and task-driven processes in the left PFC. In accordance with that, functional studies consistently report bilateral frontal activations with more task-dependent variations in the right than left PFC, when, for instance, instructed trials, where the subject was informed about the sorting rule beforehand, are compared to non-instructed trials [Konishi et al., 1999a; Lie et al., 2006]. Although the first condition mainly draws upon working memory, where a previously announced rule has to be maintained, the second condition differently draws upon higher executive processes such as hypothesis generation via feedback integration, updating of working memory, and adaptation of behaviour. Although those processes are likely to involve the left PFC to a higher degree than mere WM processes, the results from neuroimaging studies mainly implicate right PFC.

Here, we are presenting a new variant of the sorting task by using newly developed non-verbal stimulus material. The stimulus material of this WCST study was modified with respect to a couple of parameters. First, the pure geometrical stimuli of the original WCST were replaced by complex and abstract symbols, adopted from the artificial Klingon alphabet [Mecklinger et al., 2000]. Second, the colour of the symbols was changed to shaded and indefinite colours, and third, the dimension "number of symbols"

was replaced by a spatial dimension, where the position on the screen had to be processed. Because of the short presentation and maintenance times, it was expected that the stimuli would be processed more as abstract images than semantically associated objects. Furthermore, the number of repetitions of each stimulus was kept low, so that a naming strategy for coding the stimuli verbally would be inefficient. As in previous studies, instructed and non-instructed trials conditions were included. This causes that the subjects knew beforehand, what are the potential sorting criterions, which is a deviation from the clinical administration of the test. This type of modification was similar to that “64A” version, used by Stuss [2000]. Under both conditions, subjects were asked to decide whether the target stimulus matches the reference stimulus with respect to the requested sorting rule or not.

In general, we expected a replication of the fronto-parietal activation pattern, observed in other WCST functional imaging studies. This includes the prediction that the instructed condition, where only an announced sorting rule has to be applied, will be less demanding than the WCST condition. Because of the attempt to suppress a verbal rehearsal strategy by making the stimulus material not easy to verbalize, it was further hypothesized that when compared with earlier studies, left frontal activations would be reduced, especially during the instructed trials, whereas right frontal activations are expected to be elevated through the processing of object and spatial-related information [Baddeley, 2003]. In contrast to the instructed conditions, the non-instructed WCST-like condition was expected to involve the PFC of both hemispheres as seen in other studies of rule generation [Bunge, 2004; Newman et al., 2003; Ramnani and Owen, 2004; Strange et al., 2001; van den Heuvel et al., 2003]. Therefore, by using non-verbal material, it was hypothesized to disentangle task-related from stimulus-related activations in the prefrontal cortex, which may have being overlapping in earlier studies, using the original WCST stimulus material.

METHODS

Thirteen-right- and one-left-handed male healthy subjects (mean age 24.8 ± 5.1) were investigated, and the study was conducted in accordance with the Declaration of Helsinki. All the subjects gave informed consent according to the institutional guidelines.

Subjects performed four experimental, one baseline, and one rest condition. One of the four experimental conditions was adopted from the WCST, where the subjects had to judge, whether a displayed target card fitted with respect to one dimension of the previously displayed reference card, like colour, symbol, or position on the screen. After three consecutive correct responses in this WCST-like condition, the sorting criterion was changed without notification. This ensured that subjects tried as long as possible in

a given block of trials to work on the correct sorting criterion. Subjects were not informed beforehand about changes in the sorting criterion, and they were unfamiliar with the general concept of the WCST. The other three conditions were “instructed conditions” and served as control condition for the working memory components of the WCST. In these conditions, the respective target dimension was announced before the block of trials (i.e. either “space”, “symbol”, or “colour”). In all the four conditions, subjects were instructed to provide their responses, that is, whether there was a match in the requested dimension or not, via button presses. For “yes” responses, subjects used their index finger; for “no” responses, subjects used their middle finger. The use of the left or right hand for button presses was balanced across the group.

To minimize verbalization of the stimulus material, only abstract symbols, adopted from the artificial Klingon alphabet [Mecklinger et al., 2000], were used. These stimuli were coloured with shaded colour mixtures and displayed at one out of 24 possible positions on the screen. In the baseline condition, subjects were instructed to press one of the response buttons at every second stimulus. During the rest condition, only the fixation cross was displayed and no response was required. During a familiarization session outside the scanner room, subjects were presented with the paradigm and trained with a special training version. This training version consisted of an own set of symbols and contained the instructed and non-instructed conditions, but did not include the un-notified change of the rules under the non-instructed WCST-like condition.

Subjects performed all conditions within a single-session fMRI-experiment. All experimental conditions were arranged as delayed matching tasks, with parametrically varied delays between the reference and the target symbol. All experimental conditions were arranged as blocks of nine trials, with an instruction, displayed before each block. Each condition was repeated four times, resulting in 36 trials per condition. The “WCST” condition thus also contained 36 trials, but these trials were a mixture of “Space”, “Symbol”, or “Colour” sorting trials, and the number of trials per sorting rule depended on the performance of the subject, because the sorting criterion changed only after three correct consecutive responses. All the four experimental conditions had the same temporal structure and stimulus material. Each trial started with a fixation cross (300 ms), followed by the reference symbol, displayed for 500 ms. Thereafter, the fixation cross was displayed again for a parametrically varied delay of 1,500–2,500 ms (varied in steps of 500 ms). The target symbol was then displayed for 1,200 ms during which the response had to be given, whether the target and reference symbol matched with respect to the requested dimension. Feedback was provided by turning the fixation cross for 300 ms into red (wrong) or green (correct), followed by a variable period with no fixation cross (300–900 ms, varied in steps of 300 ms), which marked the end of a trial. Figure 1 illustrates the temporal structure of each trial.

Figure 1.

Experimental design: temporal order of one trial. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The stimuli were presented using a shielded projector inside of the scanner room, projecting onto a screen inside of the MR bore. The subjects' responses were recorded using MR-compatible response devices (LUMItouchT; <http://www.photonixco.com/>). The presentation of the stimuli and the collection of responses were achieved by using presentation (Neurobehavioral System, <http://nbs.neuro-bs.com>).

Data Acquisition

The MR images were acquired using a 1.5 Tesla Siemens MRI system (Siemens Sonata with a Vision long-bore magnet, Erlangen). The imaging procedure started with the acquisition of a high-resolution anatomical image (MPRAGE, TR/TE/FA/FOV 11.08 ms/4.3 ms/15°/230 mm, 200 × 256 matrix, 128 sagittal slices, 1.48 mm thick). For functional imaging, 470 images were acquired; each contained 24 axial slices, oriented in the anterior-posterior commissure plane and covering the whole cerebrum and most of the cerebellum. The parameters of the functional sequence were as follows: GE-EPI, TR/TE/FA/FOV 2,500 ms/66 ms/90°/200 mm, 64 × 64 matrix, ascending slice order, 5 mm thick, 0.5 mm gap between slices.

Pre-Processing and Statistical Analysis

The first three images were rejected in order to reach maximum signal equilibrium in the functional data. The remaining DICOM images were converted into the analyse file format using nICE (www.nordicneurolab.no), whereas all further steps were performed using SPM2 [Friston et al., 1995, 1996, 2000; <http://www.fil.ion.ucl.ac.uk/spm> with all updates until 04/2005] based on MATLAB v6.5.1 (Mathworks). For correcting head-movements, the functional images were realigned to the first image, followed by a slice-timing procedure, using the twelfth slice as temporal reference. The normalization of each subjects dataset into the stereotactical reference space, defined by the

Montreal Neurological Institute, was performed through an adaptation of the optimized voxel-based morphometry method [Ashburner and Friston, 2000; Good et al., 2001; Specht et al., 2005; Wohlschläger et al., 2005]. This procedure includes a coregistration of the T1 image with an averaged image of the functional scans, followed by a segmentation of the T1 image into grey- and white-matter probability maps. Only the grey-matter probability map was used and normalized onto a grey-matter template. The thereby estimated transformation was applied to the functional images, which were re-sampled to a cubic voxel size of 3 mm, and finally smoothed by a Gaussian kernel of 8 mm FWHM.

The fMRI data for each participant were analysed within a fixed-effects statistical model, using the hemodynamic response function as basis functions. Even though the experimental design appeared as block design to the subjects, an event-related design was modelled. This allowed to include for each trial the delay between reference and target symbol as well as the response time for the respective trial as two independent and mean-corrected parameter ("parametrical hybrid design"). Therefore, for each of the four experimental conditions, the design matrix contained beside the stimulus onset also the parametrically varied delays between the reference and target card, and the trial-by-trial response times. Delay-period activity has been repeatedly observed in inferior parietal areas as well as the frontal eye-field [Curtis et al., 2004; Pochon et al., 2001; Pollmann and von Cramon, 2000; Schon et al., 2008]. Therefore, it was expected that the parametrically varied delay will result in delay-dependent activation within this network. The correlation with the response time, however, was expected to be more related to areas for response selection and the executive system. During estimation of the design, the data were high-pass filter (cut-off period 256 s), and estimates were corrected for the intrinsic auto-correlation. For each subject, contrasts were estimated, reflecting the main effects, the additional variations of the BOLD-signal, depending on the delay between reference and target card, and those variations, which were correlated with the trial-by-trial response time. Because of the large behavioural performances differences between subjects during the baseline condition, which was probably caused by an unclear instruction, only the rest condition was considered in the subsequent analyses of the imaging data (Supp. Info. Fig. 1 gives an overview over the design matrix and the specified contrasts).

Because we were mainly interested in the differences between the instructed and non-instructed conditions, we averaged the three instructed conditions by specifying a contrast that displays the averaged across all three instructed conditions ("Working memory" contrast). However, in order to justify this, we explored first the differences between the three instructed conditions with paired *t*-tests. These analyses showed only small differences bilaterally in occipital and superior parietal areas, but not in those areas, described later, especially not in the frontal

lobe. Finally, we also verified that the left-handed subject did not diverge in the overall results.

For exploring the group effects of interest, the main analysis was based on five one-sample *t*-tests. First, we explored the network, activated during the instructed conditions, that is, the “working memory” network, second, the network, which was active during the WCST-like condition, third the difference between these two, fourth, the network, which showed a correlation with the trial-by-trial response times, and fifth, the network that correlated with the trial-by-trial varied delay-time between reference and target symbol. Because we performed a series of *t*-test, we adjusted the threshold to $P < 0.01$ (FDR-corrected [Genovese et al., 2002] and used an extent threshold of at least 20 voxels (corresponding to 540 mm³) per cluster.

For anatomical localization, non-linear transformation of voxel coordinates from Montreal neurological institute to Talairach space were performed, described by Brett (www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml), and, afterwards, the regions were anatomically characterized by the use of the Talairach atlas [Talairach and Tournoux, 1988], the Talairach Daemon (<http://ric.uthscsa.edu/resources>), the Anatomy Toolbox [Eickhoff et al., 2005], and overlays created with MriCron (www.mricron.com).

We supplemented the analysis of frontal activations by region-of-interest (ROI) analyses, using separated ROIs for the dorsolateral and the ventrolateral prefrontal cortex. The ROIs were anatomically defined by using Marina (<http://www.bion.de/>) as well as the Broadmann map, implemented in MriCron, in order to restrict the ROIs to the dorsolateral and ventrolateral part of the prefrontal cortex.

RESULTS

Behavioural Data

In the WCST condition, subjects needed on average 722.6 ± 81.6 ms (mean/sdev) to respond and 1.7 ± 0.8 trials for deriving the right sorting criterion, and 5.6 ± 0.9 rules were detected. In all instructed conditions, subjects were significantly faster [$P < 0.002$ for all pair-wise comparisons in a within group ANOVA ($df = 3,39$)]. Response times between the instructed conditions were also significantly different. Subjects were fastest for spatial judgments (604.9 ± 76.3 ms) and slowest for the symbols (683.7 ± 71.8 ms; colour: 641.2 ± 62.7 ms).

Imaging Data

Compared to the rest condition, the “Working memory”, that is the instructed conditions (Fig. 2a, Table Ia) led to significant activations in a fronto-parietal network as well as in the occipital cortex bilaterally. The most significant activations were seen in the superior and inferior parietal cortex (SPL, IPL), the middle and inferior occipital gyrus (MOG, IOG), the supplementary motor area (SMA), the

Figure 2.

Projection of activations onto lateral and medial views of a standard brain: The upper two rows show the main-effects for the working memory and WCST conditions, the third row shows the differential effect, that is, WCST > working memory condition. All results are displayed with a threshold of $P < 0.01$ (FDR-corrected) and extent threshold of at least 20 voxels per cluster.

anterior and middle cingulate gyrus (ant and mid. CG), the precentral gyrus (PrCG) and dorsal part of the inferior frontal gyrus (IFG), and, finally, also the ventral part of the IFG, extending into the insula. For each region, the activations were almost equally significant in both the hemispheres. In contrast, the dorso- and ventro-lateral PFC, including parts of the middle frontal gyrus and IFG, was activated in the right hemisphere, only.

Comparing the WCST condition to the rest condition, the same network was seen, but with generally elevated significance levels and more extended activations (Fig. 2b, Table Ib). In particular, the activations in the parietal cortex were more inferiorly extended than in the working memory condition, comprising also the angular gyrus (AngG). However, this condition recruited also distinct areas, which were not seen during the working memory. These mostly left-sided areas included the left anterior, dorsolateral, and mid-ventrolateral PFC, comprising parts of the middle and superior frontal gyrus (MFG, SFG), and, subcortically, the caudate nuclei and the thalamus. This was further confirmed by the difference contrast between

TABLE I. The table lists the cluster sizes, t-values, the respective P-values, the MNI coordinates, and the anatomical location of the clusters of activation observed for the three conditions: working memory, WCST conditions, WCST-working memory, thresholded at an FDR-corrected P-value of 0.01 and an extent threshold of at least 20 voxel

Cluster		Voxel			Anatomical location					
p(cor)	Size	p(FDR)	T	Z	x	y	z	Side	Area	Brodmann area
a) Working memory										
0.000	307	0.000	12.60	5.71	33	24	6	R	Ins, IFG	6, 44, 47
0.000	469	0.000	11.23	5.47	-3	3	54	L&R	SMA, MCC	6, 24
0.000	525	0.000	10.27	5.28	-48	-39	48	L	IPL, SPL, SMG	7, 19, 40
0.000	604	0.000	10.07	5.23	45	-45	51	R	IPL, SPL, MOG	7, 19, 40
0.000	75	0.000	9.81	5.18	45	30	18	R	MFG	45, 46
0.000	153	0.000	8.79	4.94	-33	-9	63	L	PrCG	6
0.000	249	0.001	7.13	4.47	-33	-63	-9	L	FG, IOG	19, 37
0.000	103	0.001	6.83	4.38	30	-3	63	R	PrCG	6
0.000	93	0.001	6.67	4.32	-39	3	33	L	PrCG, MFG, IFG	6, 44
0.000	225	0.001	6.39	4.23	36	-69	-9	R	FG, IOG	19, 37
0.005	45	0.001	6.02	4.09	-30	24	3	L	Ins, IFG	47
0.005	44	0.002	5.94	4.06	-6	-75	-21	L	Cerebellum	
b) WCST										
0.000	793	0.000	12.93	5.76	-48	-39	51	L	IPL, SPL, AngG, SMG, MOG	7, 19, 40
0.000	816	0.000	12.21	5.64	6	27	39	R&L	MdFG, SMA, ACC, MCC	6, 8, 24, 32
0.000	187	0.000	11.88	5.58	30	21	0	R	Ins, IFG	47
0.000	1007	0.000	11.57	5.56	42	-57	45	R	IPL, SPL, AngG, MOG	7, 19, 40
0.000	1106	0.000	11.65	5.54	51	9	33	R	MFG, IFG, PrCG	6, 8, 9, 10, 44, 45, 46
0.000	349	0.000	10.74	5.37	36	-60	-27	R	FG, IOG	19, 37
0.000	128	0.000	10.17	5.25	-33	27	-3	L	Ins, IFG	47
0.000	596	0.000	9.52	5.11	-33	-57	-24	L	FG, IOG	19, 37
0.000	506	0.000	7.68	4.64	-30	-9	54	L	PrCG, MFG, IFG	6, 44
0.000	180	0.000	7.40	4.56	-36	48	12	L	MFG	10,46
0.001	70	0.000	6.52	4.27	9	-12	9	R	Caudate nucleus, thalamus	
0.155	24	0.001	5.37	3.83	-9	3	9	L	Caudate nucleus, thalamus	
c) WCST-working memory										
0.000	774	0.000	12.93	5.76	42	-60	45	R	IPL, SPL, AngG, PrCu	7, 19, 40
0.000	551	0.000	12.40	5.67	-45	-57	48	L	IPL, SPL, AngG	7, 19, 40
0.000	1330	0.000	11.66	5.54	30	12	57	R	MFG, IFG, PrCG, SMA, ACC	6, 8, 9,10, 32, 44, 45, 46
0.000	415	0.000	7.73	4.65	-51	18	30	L	IFG, PrCG	6, 44
0.000	187	0.000	7.44	4.57	-30	54	-3	L	MFG	10,46
0.000	83	0.001	7.25	4.51	42	21	-9	R	Ins, IFG	47
0.000	122	0.001	6.51	4.27	-21	-75	-24	L	Cerebellum	
0.001	53	0.001	6.33	4.20	-33	24	-9	L	Ins, IFG	47
0.054	24	0.001	5.97	4.07	-60	-39	-3	L	MTG	21
0.014	33	0.001	5.87	4.03	9	-6	9	R	Thalamus, caudate nucleus	
0.002	47	0.002	5.54	3.90	-36	-57	-33	L	Cerebellum	

Each cluster is defined by its maximal t-values and the respective MNI coordinates. The anatomical descriptions are given by anatomical landmarks as well as Brodmann areas. The order of the descriptions follows the extension of the cluster (voxel size $3 \times 3 \times 3 \text{ mm}^3$), with the location of the global maximum at first, followed by the location of the sub-maxima within that cluster.

SMA, supplementary motor area; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus; MdFG, medial frontal gyrus; PrCG, precentral gyrus; ACC, anterior cingulate cortex; MCG, middle cingulate cortex; SPL, superior parietal lobe; IPL, inferior parietal lobe; AngG, angular gyrus; PrCu, precuneus; IOG, inferior occipital gyrus; MOG, middle occipital gyrus; FG, fusiform gyrus; MTG, middle temporal gyrus; L, left hemisphere; R, right hemisphere; L and R, bilateral activations, stronger on the left; R and L, bilateral activations, stronger on the right.

the WCST and the working memory condition, demonstrating on the one hand, that the activations in the right PFC and bilateral in the parietal lobe were significantly increase. On the other hand, it further confirmed that the areas in the left PFC were exclusively involved in the WCST and not the working memory task (Fig. 2c, Table Ic). In addition, this difference contrast also showed activation in the left middle temporal gyrus.

Neither the correlation of the BOLD signal with the trial-by-trial response time nor the correlation with the trial-by-trial varied delay between reference and target stimulus were significant at the selected thresholds. However, exploring the data with an uncorrected threshold of $P < 0.001$, the results showed the expected dissociation between areas, which correlated with the delay and those, which correlated with the response time. Although the

Figure 3.

Region-of-interest (ROI) analysis for the prefrontal cortex: The figure displays the mean signal change for each region and the error bars indicate 0.95 confidence intervals; * marks significant differences of $P < 0.05$, and “**” of $P < 0.01$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

former one corresponded more with the working memory task, comprising in particular the inferior parietal lobe and the frontal eye field, the latter showed activation in the PFC as well as anterior cingulate gyrus (see Supp. Info. Fig. 2 and Supp. Info. Table I).

Region of Interest Analysis

The ROI analysis revealed (see Fig. 3) that the left dorso-lateral pre-frontal cortex (dlPFC) was exclusively activated under the WCST condition ($P < 0.002$, when compared with the working memory condition), whereas the right dlPFC was activated under both conditions but slightly stronger under the WCST condition, even though, this difference was not significant ($P > 0.05$).

The ventrolateral PFC (vlPFC) was activated under all conditions but the right vlPFC was significantly stronger under the working memory condition ($P < 0.024$), whereas the left vlPFC remains constant under both conditions.

In addition, the right PFC, in particular the right vlPFC were significantly stronger activated during the WM task than the homologue areas of the left hemisphere ($P < 0.05$). There was no significant lateralisation during the WCST condition.

DISCUSSION

The aim of this study was to further disentangle the neural mechanisms in the frontal cortex underlying key executive functions involved in the WCST. To suppress the verbal strategies and hence the left frontal processing only abstract “Klingon” symbols [Mecklinger et al., 2000] were

used, which were coloured in shaded colours. Furthermore, in contrast to the original version of the WCST, the spatial location of the displayed symbol on the screen rather than the number of symbols was used as a target dimension. To disentangle the working memory network involved in the WCST from those activations, exclusively involved in the condition, which draws upon inductive reasoning and behaviour adaptation, the subjects were informed about the sorting criterion in some of the conditions.

The Working Memory Network

The present results replicate earlier studies of working memory in the WCST [see, e.g., Lie et al., 2006]. The detected network is typical for visual working memory conditions, comprising bilaterally parietal, occipital, and frontal areas [see, for a review Naghavi and Nyberg, 2005; Rajah and D’Esposito, 2005; Wager and Smith, 2003]. In addition, the detected network included bilaterally an area anterior to the frontal eye field, in the vicinity of the pre-central sulcus and superior frontal sulcus [Curtis and D’Esposito, 2003], an area which seems to be specialized for spatial working memory as suggested by both the imaging [Courtney et al., 1998; Pollmann and von Cramon, 2000; Sala et al., 2003], and TMS studies [Oliveri et al., 2001].

Although overall the observed working memory network is in good accordance with other studies, one important deviant to our earlier WCST study [Lie et al., 2006] using “classical” stimuli has to be mentioned: In the current study using non-verbal stimuli, we did not observe bilateral prefrontal activations in the instructed conditions. Rather, the frontal activations were more pronounced on the right side, as confirmed by the ROI analysis. We suggest that this right hemisphere preponderance is caused by the use of abstract and shaded-coloured Klingon symbols [Mecklinger et al., 2000] used in the current study to prevent subjects from using verbal strategies. It is well known [see, e.g., the reviews by Smith et al. [Smith and Jonides, 1997, 1998; Smith et al., 1998] that verbal working memory is mainly left lateralized whereas spatial and non-verbal tasks are predominantly right lateralised. Our data thus support the hypothesis that verbalization strategies play an important role in the original WCST, which uses simple symbols, like crosses, circles, triangles, and stars, in simple colours. Consistent with this suggestion, studies using the original WCST always report bilateral prefrontal activations [Konishi et al., 1999a,b; Lie et al., 2006; Monchi et al., 2001, 2004].

The WCST Condition

In addition to the working memory conditions, the WCST condition draws upon evaluation, deductive reasoning, and behaviour adaptation. This will cause both a higher load on the working memory system, but also result in the recruitment of additional processes. Increased

working memory demands are likely to result in an overall increase of the activation in the areas, described earlier. However, more importantly with regard to the purpose of our study are those areas, which were exclusively activated during the WCST, as these areas of differential activations reflect additional cognitive processes, whereas a differential increase of activity in areas activated across all conditions is likely to result from a higher workload [Lie et al., 2006]. In accordance with a model supplied by Bunge [Bunge, 2004], the difference contrast between the WCST and the working memory condition demonstrated several additionally recruited areas, which were clearly separated from those areas, activated in the working memory task. These additional areas were mainly left sided, comprising the left anterior and dorsolateral PFC, left IFG, left thalamus and caudate nucleus, and a small area in the left middle temporal gyrus. By contrast, the activations seen in the parietal and the right frontal lobe are primarily increased and extended into adjacent areas, but predominantly overlapping with those activations, seen under the working memory conditions.

Generally, the prefrontal activations observed nicely overlap with those areas, reported in problem-solving studies [see Bunge, 2004; Newman et al., 2003; van den Heuvel et al., 2003] for further references] and patient studies [Reverberi et al., 2005; Stuss et al., 2000]. In contrast to other studies, finding the strongest effect for rule learning in the anterior PFC [Ramnani and Owen, 2004; Strange et al., 2001], our results comprised the aPFC only partially. The left/right difference in the dlPFC, as revealed by the ROI analysis (see Fig. 3), might provide some further evidence for the view that the right frontal areas, including the cingulate gyrus and SMA, are mainly responsible for goal-directed decisions, based on given or already learned rules. By contrast, the left dlPFC is involved in the generation of new rules and feedback integration under the WCST condition. This aspect is further supported by the activations observed in the caudate nucleus and thalamus. Monchi et al. [Monchi et al., 2001] explored the feedback processing and found a similar relationship between the sub-cortical and PFC areas.

Beside these frontal areas, the WCST contrast also showed a small activation in the left middle temporal cortex. Activation of this area during the WCST is in good agreement with the model by Bunge [Bunge, 2004], in which the anterior and posterior ventrolateral PFC interacts with this area in rule-selection tasks.

Finally, the WCST condition showed bilateral extensions into the inferior parietal cortex and angular gyrus. This might simply reflect a higher working memory load. Alternatively, this may reflect a recruitment of adjacent but functionally distinct areas. Because other studies have shown that higher working memory demand lead to elevated rather than extended activations [Adcock et al., 2000; Bunge et al., 2000], we suggest that the observed effects reflect additional cognitive processes. Activation of inferior parietal cortex, on the left as well as on the right, has been

reported in several other studies, where conceptual processing was required, like, for example, comparative judgments, arithmetics, or problem solving (see, e.g., [Hirsch et al., 2001; Naghavi and Nyberg, 2005; Pinel et al., 2004; Unterrainer et al., 2004]. Especially those studies, which explored the network underlying arithmetical processing [Delazer et al., 2003; Gruber et al., 2001] or the integration of sensory information [Assmus et al., 2003] have shown activation of the left inferior parietal cortex. In contrast, activation of the right inferior parietal cortex has been consistently reported in tasks requiring spatial attention [see, e.g., Fink et al., 2000; Marshall and Fink, 2001]. This interpretation is further supported by the fact that the BOLD signal from this area also correlated with the delay period.

General Discussion

By using material that is less easy to verbalize as the original WCST stimuli, we were able to demonstrate a dissociation between the left and right dorsolateral prefrontal cortex, as the latter one was involved in all task, whereas the left dlPFC was only involved in the WCST task. This clear differentiation was not seen in earlier studies, which used the original material from the WCST [Lie et al., 2006]. Future studies may focus more on this stimulus-dependent effect by direct comparisons. On the other hand, the results reflect also that especially the WCST condition involves both, the left and right dlPFC, which is accordance with the observed weak differentiation between patients with left or right frontal lesion. This general ventral-/dorsal-differentiation is also in line with the meta-analysis by Wager and Smith [2003]. They demonstrated that dorsal frontal cortex is especially involved when executive processes, like information updating, are required, whereas ventral areas are more involved in storage of information. However, also this modification of the WCST does not fully disentangle the networks involved in performing this sorting task. In addition, the strong involvement of parietal areas and even the inclusion of an area within the left middle temporal gyrus under the WCST condition is on the other hand reflecting the repeatedly discussed generally lack of sensitivity of the WCST for frontal lobe lesions [Goldstein et al., 2004; Wildgruber et al., 2000]. To disentangle more the different processes under the WCST condition, the interaction between the detected areas, and their dynamic coupling, future studies may also focus more on the effects during set-shifting behavioural adaptation, as the here presented design does not allow such a comparison due to the low number of rule-shifts that were possible.

CONCLUSION

In summary, this study further disentangles prefrontal cognitive processes involved in the WCST using a novel non-verbal variant of the Wisconsin-Card-Sorting-Test. The results provide further evidence for a differential role of the left and right prefrontal cortex, showing that the right

PFC is especially involved in the implementation of cognitive control and selection of previously learned rules, whereas the left PFC is especially involved during inductive reasoning and hypothesis generation.

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