Long-Term Neurobiological Consequences of Early Postnatal hCMV-Infection in Former Preterms: A Functional MRI Study

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Abstract: Early postnatal infection with human cytomegalovirus (hCMV) may contribute to an adverse cognitive outcome in early preterm-born children (PT). We here set out to explore whether long-term neurobiological consequences of such an infection are detectable using fMRI in children and adolescents who were born very preterm and who either did (PT_{hCMV+}) or did not (PT_{hCMV-}) suffer from an early postnatal hCMV-infection, when compared with typically developing healthy control (HC) subjects. Overall, data from 71 children and adolescents could be included, 34 PT (of which 15 were PT_{hCMV+} and 19 were PT_{hCMV-}) and 37 HC. Using a recently established “dual use” fMRI task, we investigated language and visuospatial functions. There were significant activation differences in the left hippocampus (PT > HC and PT_{hCMV+} > HC), and in the right anterior cingulate cortex (PT_{hCMV-} > PT_{hCMV+}) when performing the language task. Surprisingly, only a small region in the occipital cortex showed a significant activation difference (HC > PT_{hCMV-}) when performing the visuospatial task. Targeted analyses revealed differences in gray matter volume, but not density, in several brain regions. Our results suggest that long-term neurobiological consequences of an early postnatal hCMV infection are detectable even in older children and adolescents formerly born very preterm, compatible with a higher effort when performing a cognitive task. This suggests that measures to prevent such an infection are warranted. Furthermore, an interrelation of brain structure and function was detected that may constitute a severe confound when using fMRI to compare structurally differing groups. *Hum Brain Mapp* 35:2594–2606, 2014. © 2013 Wiley Periodicals, Inc.

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INTRODUCTION

Prematurity, i.e., birth before 37 weeks of gestation, represents a considerable risk factor for later developmental disabilities [Allen, 2008]. Progress in neonatal intensive care has led to increased rates of survival particularly in early preterm born (< 32 weeks of gestation) and very low birth weight children (< 1500 g) [Allen et al., 1993; Euser et al., 2008; Philip, 2005]. However, these children still have a high rate of neonatal complications, which again are strongly associated with neurodevelopmental disability later in life [Behrman and Butler, 2007; Eichenwald and Stark, 2008; Fanaroff et al., 2007]. The classical neurological disability in former preterms is cerebral palsy, the incidence of which has decreased in the last years [Krägeloh-Mann and Horber, 2007; Platt et al., 2007]. Simultaneously, less obvious cognitive impairment across several domains becomes an increasingly important object of investigation [Aylward, 2003]. For example, problems with visuo-spatial stimuli, nonverbal reasoning, language comprehension, and difficulties in the simultaneous processing of complex stimuli have repeatedly been described in preterm-born children [Barre et al., 2011; Foulder-Hughes and Cooke, 2003; Hård et al., 2000; Hellgren et al., 2007; Johnson, 2007; Ment et al., 2003; Narberhaus et al., 2009; Stjernqvist and Svenningsen, 1999; Taylor et al., 2002; Wolke and Meyer, 1999]. These cognitive impairments are of particular importance because they severely influence the child's health-related quality of Life and are a burden for parents and caregivers as well as society as a whole [Mathiasen et al., 2009; Petrou and Kahn, 2012; Petrou et al., 2011; Volpe, 2009; Zwicker and Harris, 2008]. This underlines the importance of investigating the reasons underlying adverse cognitive outcomes.

Human cytomegalovirus (hCMV) is a common virus, detectable in about 50% of the U.S. population [Bate et al., 2010; Gandhi and Khanna, 2004; Staras et al., 2006]. Transmission occurs through bodily fluids and the following primary infection is usually mild or asymptomatic; thereafter, hCMV persists in leucocytes [Crough and Khanna, 2009]. Particularly, in times of stress, it may reactivate, which may or may not be accompanied by clinical symptoms [Prösch et al., 2000]. Furthermore, hCMV is among the most common congenital infections [Bate et al., 2010; Dollard et al., 2007; Grosse et al., 2008], making it an important cause for neurological and sensory impairment in children [Dollard et al., 2007; Fowler et al., 1992; Pass et al., 2006; Stagno et al., 1981]. Preterm-born children have long-since been known to be at high risk for showing signs of hCMV infection [Johnson et al.,

1986; Paryani et al., 1985; Yeager et al., 1983]. However and most importantly, these early studies did not differentiate between congenital and postnatally acquired infections, and only in recent years, postnatal, vertical transmission via hCMV-containing breast milk has come into focus [Vochem et al., 1998]. For example, Hamprecht et al. [2001] showed that hCMV reactivates in nearly all seropositive mothers giving birth prematurely and is consecutively detectable in their breast milk; the mother-to-infant transmission rate was 37%, and almost half of these babies were symptomatic [Hamprecht et al., 2001]. Since then, preventive measures to deactivate hCMV in breast milk have been suggested [Goelz et al., 2009; Hamprecht et al., 2004; Maschmann et al., 2006], but the justification of this effort has been questioned [Bryant et al., 2002]. With some studies showing no long-term neurological or neurocognitive effects in infants and younger children [Kurath et al., 2010; Vollmer et al., 2004] and others detecting such effects in older children [Bevot et al., 2011; Goelz et al., in press], postnatally transmitted hCMV via breast milk feeding of preterm-born babies has remained a matter of controversy for years [Luck and Sharland, 2009]. Particularly in the light of the possibility to prevent transmission, further studies investigating the neurobiological basis of these long-term consequences seem warranted.

Functional magnetic resonance imaging (fMRI) is a noninvasive tool that, while difficult to apply, allows to investigate brain activation already in children [Byars et al., 2002; O'Shaughnessy et al., 2008; Wilke et al., 2003a, 2005]. It was previously used to assess differences in activation patterns between former preterms (PT) and healthy controls (HC), including cognitive domains in which PT have been shown to be particularly impaired and that are well-amenable to being studied using fMRI (such as visuo-spatial abilities and language processing) [Ment et al., 2006; Nosarti et al., 2006; Peterson et al., 2002; Santhouse et al., 2002]. Furthermore, multiple brain regions have been shown to display reduced gray matter (GM) volume in PT, when compared with HC [Allin et al., 2004; Nosarti et al., 2011; Taylor et al., 2011], and structural abnormalities have been related to functional activation differences in this group [Gimenez et al., 2005; Lawrence et al., 2010]. It is important to remember that accounting for structural differences by spatial normalization is a necessary part of the data preprocessing procedure when performing group studies, to achieve spatial overlap between corresponding brain regions. Recently, we reported that apparent functional differences between groups of patients with large brain lesions disappeared after correcting for local GM-volume differences [Dinomais

et al., 2011]. In the light of the above, this prompted us to explore whether such a structure–function relationship may also play a role in this setting, and may be a confound for fMRI group comparisons between PT and HC.

The aim of our study was to investigate the long-term neurobiological consequences of preterm-born children and adolescents with early postnatal hCMV infection, using fMRI. As we hypothesized that group differences would be apparent both in the language and the visuo-spatial domain, a “dual-use” task [Ebner et al., 2011] was used. We investigated group differences between PT with (PT_{hCMV+}) and without (PT_{hCMV-}) early postnatal hCMV-infection and HC. We were also interested in exploring the interrelation of structural and functional group differences.

SUBJECTS AND METHODS

Participants

We included former preterms (PT) with (PT_{hCMV+}) and without (PT_{hCMV-}) early postnatal hCMV-infection as well as healthy control subjects (HC). PT were participants of a prospective follow-up study aimed at exploring effects of early, vertically transmitted postnatal hCMV infection [Bevot et al., 2011; Hamprecht et al., 2001; Neuberger et al., 2006; Vollmer et al., 2004]. They were identified using hospital records and were contacted by mail. All but three weighed less than 1,500 g at birth, qualifying them as very low birth weight babies, and all but one (who was born in the 32nd week) were born before the 32nd week of gestation, qualifying them as very preterm born babies. They were born between July 1995 and March 2000 and were treated in the neonatal intensive care unit at University Children’s Hospital, Tübingen, Germany. All PT were hCMV-negative postnatally, excluding congenital hCMV infection. PT_{hCMV+} experienced seroconversion following feeding with hCMV+ breastmilk, while PT_{hCMV-} had no exposition, or exposition without seroconversion in the first 3 months of life [Hamprecht et al., 2001; Neuberger et al., 2006]. HC were recruited from the community by advertisements in the press and public announcements. Ethical approval for the study was obtained from the ethics committee of the University Hospital Tübingen, and written informed consent was obtained for all participants. Verbal assent was also obtained from all participants, who were compensated for time and travel expenses.

Participant Selection

All participants were screened by the study coordinator during a telephone interview regarding in- and exclusion criteria. General MR contraindications applied for all participants. Additionally, specific exclusion criteria for HC were a history of neurological or psychiatric disorders, hearing deficits, cognitive impairment, history of neonatal infection (including neonatal hepatosplenomegaly), or pre-

mature birth (< 37 weeks of gestation). At this stage, 18 participants were excluded (8 PT, 10 HC). After scanning, data from 12 participants (7 PT, 5 HC) was excluded due to either imaging artifacts or excessive motion (see below).

Neurological and Neuropsychological Assessment

Based on the well-known deficits following preterm birth in the language as well as in the visuo-spatial domain, neuropsychological testing included the HAWIK-IV, the German version of the Wechsler Intelligence Scale for Children [Petermann and Petermann, 2007], as well as the DTVP-A [Developmental Test of Visual Perception—Adults and Adolescents, Reynolds et al., 2002]. These tests are standardized instruments for assessing IQ (including Fullscale-IQ [FIQ] as well as verbal comprehension and perceptual reasoning) and visuo-spatial abilities (VMII, the DTVP-A’s visuo-motor integration index). Due to the substantial impact of the maternal education level (MEL) on neurocognitive outcome in PT, MEL was assessed in all participants, in years of education, as a proxy for socioeconomic status [Krägeloh-Mann and Lidzba, 2012; Voss et al., 2012]. In all subjects, handedness was assessed using the Edinburgh Handedness Inventory [Oldfield, 1971], the results of which correlate well with more extensive test batteries [also in children; Wilke et al., 2008]. In the PT groups, gross motor functions were categorized using The Gross Motor Function Classification System, ranging from 0 (no gross motor function impairments) to V (severe impairment) [Palisano et al., 1997; Russell et al., 1989]. Any occurrence of a score of I–V was considered pathological. For assessing hand function, The Bimanual Fine Motor Function [Beckung and Hagenberg, 2002] was used, ranging from 0 (no impairment) to V (severe impairment in hand function of both hands). Again, any occurrence of a score of I–V was considered pathological. Neurological impairment was a contraindication in HC.

Image Acquisition

Participants were scanned on a 1.5-T MR scanner (Avanto, Siemens Medizintechnik, Erlangen, Germany), using a 12-channel head coil. To minimize head motion during the scan, special care was taken to ensure comfortable subject placement and a foam cushion was used. The acquisition included a T1-weighted 3D-data set (TR = 1,300 ms, TE = 2.92 ms, 176 contiguous sagittal slices with an in-plane matrix of 256 × 256, yielding a voxel size of 1 × 1 × 1 mm³) and a T2-weighted echo-planar imaging (EPI) sequence (TR = 3,000 ms, TE = 40 ms, 40 contiguous axial slices with an in-plane matrix of 64 × 64, yielding a voxel size of 3 × 3 × 3 mm³). A gradient-echo B0-field-map was also acquired (TR = 546 ms, TE = 5.19/9.95 ms, with the same slice prescription as the EPI sequence). All anatomical images were read by an experienced pediatric neuroradiologist for incidental findings as well as for signs

of early brain injury [such as enlarged ventricles or white matter (WM) hyperintensities indicative of periventricular leucomalacia; Volpe, 2009].

Task

To assess both the language and the visuo-spatial domain, a “dual-use” fMRI task was used [vowel identification task, VIT; Ebner et al., 2011; Wilke et al., 2006]. In the active condition (VIT_{AC}), images of common objects were visually presented to participants and they needed to decide whether the vowel /i/ was present in the name of the object (for example, a ship [German: Schiff] requires a button press, while a horse [German: Pferd] does not). In the control condition (VIT_{CC}), two unnamable, complex visual patterns (mathematically generated fractals) were presented and participants needed to decide whether the smaller one fits into the larger one “like a piece of a puzzle.” Visual stimuli were presented on a screen using a beamer located outside the scanner room. To allow for performance monitoring, participants’ responses were recorded using a single MR-compatible push button (Current Design, Philadelphia, PA). Task adherence was assumed if performance during VIT_{CC} was above chance-level [as VIT_{AC} is more ambiguous because of possible synonyms; Ebner et al., 2011; Wilke et al., 2006]. Overall, 110 image volumes were acquired in 5:30 min. Participants were familiarized with the task during an extensive offline training session [Byars et al., 2002].

Image Data Processing

Image preprocessing and statistical analysis was performed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK), running in Matlab (Mathworks, Natick, MA). The first 10 scans of each run were discarded to allow for equilibration of the longitudinal magnetization. The remaining 100 scans (five blocks each of the VIT_{AC} and VIT_{CC} condition) were realigned to the mean volume and unwarped using the individually acquired fieldmap, simultaneously removing both EPI-distortions and motion*B0 interaction effects [Andersson et al., 2001]. Motion during the task was assessed as total displacement (resulting from translation and rotation) at the average cortical distance [Wilke, 2012], and subjects with total displacement exceeding voxel size (3mm) were excluded. Functional images were coregistered to the anatomical image, and global signal drifts were removed [Macey et al., 2004]. Finally, they were smoothed with a 9 mm full width at half maximum Gaussian filter.

Anatomical images were segmented into GM, WM, and cerebrospinal fluid (CSF), using a priorless extension [Gaser et al., 2007] of SPM8’s unified segmentation approach [Ashburner and Friston, 2005], with spatial normalization initially based on custom-made tissue priors [Wilke et al., 2008]. Thereafter, a diffeomorphic anatomical

registration using exponentiated Lie algebra (DARTEL) algorithm [Ashburner, 2007] was used for more precise inter-subject alignment into a final, study-specific space. It should be noted that the normalization to a study-specific template, particularly in children [Wilke et al., 2002, b,2008] precludes reporting stereotaxic coordinates in standard space.

Functional MRI Statistics

Functional images were statistically analyzed on the first level using the General Linear Model [Friston et al., 1995], contrasting both VIT_{AC} > VIT_{CC} (assessing the language domain) and VIT_{CC} > VIT_{AC} (assessing the visuo-spatial domain). Individual parameter maps were then transformed to group space applying the spatial transformation parameters derived as part of the DARTEL-procedure. For group analyses, a three-group ANOVA model was used, including sex, age, EHI, FIQ, VMII, and MEL as covariates of no interest. For the main effects over all and within groups, significance was assumed at $p \leq 0.01$, FWE-corrected for multiple comparisons. The main effects over each group were calculated and combined to serve as masks to spatially restrict ensuing between-group comparisons to these regions of interest only (ROI-VIT_{AC} and ROI-VIT_{CC}). To assess between-group effects, significance was assumed at $P \leq 0.001$, uncorrected. For all analyses, small clusters not exceeding 10 voxels were discarded, which was above the cluster size expected to arise by chance given the estimated smoothness of our analyses. PT (PT_{hCMV+} and PT_{hCMV-}) were compared with HC as well as against each other. Additionally, PT_{hCMV+} and PT_{hCMV-} were separately compared with HC.

To assess the influence of structure on function, the sum of voxel values in each subject’s modulated as well as nonmodulated GM tissue map was calculated within the regions of interest defined from the group activation patterns (ROI-VIT_{AC} and ROI-VIT_{CC}). Modulated GM reflects true tissue volume [Good et al., 2001], while unmodulated GM reflects “tissue density” [Ashburner and Friston, 2000]. This difference is important here as functional images are usually normalized without taking normalization-induced volume changes into account. Therefore, differences between “volume” and “density” reflect the impact of spatial normalization, which may be different between the groups.

Statistical Analyses

Statistical analyses of demographic variables were performed in SPSS 20 (IBM, Armonk, NY). Owing to sample size considerations, these analyses were performed with nonparametric statistics. For comparison of demographic variables, Fisher’s exact Test and Mann-Whitney U-Test were used for categorical and continuous variables, respectively, with significance assumed at $P \leq 0.05$. Results are presented as medians and standard error of the mean. For

TABLE I. Demographic details of the preterm-born children and adolescents

	hCMV+	hCMV−	P
Sex (m:f)	10:5	12:7	n.s. ^a
EHI	0.7 (0.1)	0.83 (0.1)	n.s. ^b
Left-handedness ^F	3	4	n.s. ^a
Age at assessment (years)	14.3 (0.4)	15.0 (0.3)	n.s. ^b
FIQ	93 (4.0)	99 (4.7)	n.s. ^b
VIQ	93 (4.7)	103 (4.5)	n.s. ^b
VMII	92 (2.8)	101 (3.6)	n.s. ^b
MEL	12 (0.8)	12.0 (0.7)	n.s. ^b
Gestational age (weeks)	29.7 (0.5)	27.0 (0.5)	n.s. ^b
Birth weight (g)	1,265 (101)	970 (64)	n.s. ^b
Percentile	60 (8.3)	30 (5.9)	n.s. ^b
Intracerebral hemorrhage (any) ^F	3	4	n.s. ^a
Bronchopulmonary dysplasia ^F	1	5	n.s. ^a
Retinopathy of prematurity ^F	4	7	n.s. ^a
GMFCS/BFMF ^F	0/0	1/1 ^{SS}	n.s. ^a
MR abnormalities ^F	4	4	n.s. ^a
Performance (%)	100 (0.4)	100 (0.8)	n.s. ^b

Results are given as medians (SEM); EHI: Edinburgh Handedness inventory score; FIQ: Full-scale IQ; VIQ: verbal IQ; VMII: Visuo-motor integration index; MEL: maternal education level; GMFCS: gross motor function classification system; BFMF: bimanual fine motor function; n.s.: not significant; ^FFrequencies; ^{SS}same subject; performance was defined as the percentage of correct button presses in the VIT_{CC}.

^aFishers exact Test.

^bMann-Whitney U-Test.

comparison of GM volume and density, a two-group ANOVA was calculated, again including sex, age, EHI, FIQ, VMII, and MEL as covariates of no interest, with significance assumed at $P \leq 0.05$.

RESULTS

Characteristics of the Study Population

Overall, data from 71 children and adolescents could be included in this study: 15 PT_{hCMV+} (5f), median age 14.3 (.42), range 11.5–16.1 years; 19 PT_{hCMV−} (7f), median age 15.0 (.26), range 12.1–16.1 years; and 37 HC (20f), median age 12.2 (.42), range, 7.9–17.8 years.

Demographic details of the PT (PT_{hCMV+} and PT_{hCMV−}) are summarized in Table I. There were neither differences in the sex and handedness distribution nor in age. Also, when performing a median split on the preterm group according to birth weight (above or below the median of 1,135 g), or gestational age (above or below the median of 28.4 weeks), there were no significant differences in the frequencies of hCMV+ or hCMV− participants. Furthermore, both groups were comparable with regard to several neonatal indicators and risk factors, including complications such as intracerebral hemorrhage, bronchopulmonary dysplasia, and retinopathy of prematurity. There

were also no differences in their current motor functions. On clinically reviewing their MR imaging data, minor changes, compatible with premature birth, were seen in several PT subjects, but again, no group differences were observed.

Epidemiological details of PT and HC can be found in Table II. There were neither significant differences in sex composition or handedness nor in the proportion of left-handedness, but HC were significantly younger. As expected, HC had significantly higher IQs (both full-scale, FIQ and verbal, VIQ) and higher visuo-spatial ability scores. Furthermore, MEL was also higher in the HC. Consequently, age, FIQ, VMII, and MEL were included in statistical group comparisons as covariates of no interest, in addition to sex and handedness scores.

Task Adherence

All included subjects completed the task successfully, and their performance was above chance level in each case. They showed a median of 100 (0.4)% (range 89.5%–100%) correct button presses in the VIT_{CC} which indicates constant task adherence as well as a substantial ceiling effect. One girl showed a consistently inverse answering pattern as she had misunderstood the instructions; as this was verified directly after the scan, task adherence could be assumed and her images were included. There were no differences between the groups (see Tables I and II).

Functional MRI Results

Over the whole group, activation in VIT_{AC} was observed primarily in left inferior frontal and temporal regions (see Fig. 1, upper panels). Furthermore, activation could be seen in the left basal ganglia and in right posterior-

TABLE II. Demographic details of the preterm-born (PT) and healthy control (HC) children and adolescents

	PT	HC	P
Sex (m:f)	22:12	17:20	n.s. ^a
EHI	0.8 (0.1)	0.8 (0.1)	n.s. ^b
Left-handedness ^F	7	2	n.s. ^a
Age at assessment (years)	15.0 (0.2)	12.2 (0.4)	< 0.001 ^b
FIQ	96 (3.2)	109 (1.4)	< 0.001 ^b
VIQ	103 (3.3)	111 (1.7)	0.007 ^b
VMII	93 (2.4)	106 (1.9)	0.027 ^b
MEL	12 (0.5)	16.0 (0.5)	0.002 ^b
Performance (%)	100 (0.5)	100 (0.5)	n.s. ^b

Results are given as medians (SEM); EHI: Edinburgh Handedness inventory score; FIQ: Full-scale IQ; VIQ: verbal IQ; VMII: Visuo-motor integration index; MEL: maternal education level; n.s.: not significant; ^FFrequencies; performance was defined as the percentage of correct button presses in the VIT_{CC}.

^aFishers exact Test.

^bMann-Whitney U-Test.

Figure 1.

Group activation map for the main effects over all groups; upper panel: active condition (VIT_{AC}); lower panel: control condition (VIT_{CC}). Results are rendered on the custom-made GM template. Results are shown on the custom-made GM template at $P \leq 0.01$, FWE-corrected for multiple comparisons. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 2.

Between-group comparisons in ROI- VIT_{AC} : very similar activation differences in the left hippocampus when comparing $PT > HC$ (top) and $PT_{hCMV+} > HC$ (bottom). Results are shown on the custom-made GM template at $P \leq 0.001$, uncorrected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

temporal as well as cerebellar regions, and in medial pre-SMA (supplementary motor area), left hippocampus, and anterior cingulate cortex.

When comparing PT and HC, there were significant activation differences in the ROI- VIT_{AC} in the left hippocampus ($PT > HC$). A very similar pattern was observed when comparing PT_{hCMV+} and HC ($PT_{hCMV+} > HC$; see Fig. 2), while no differences were seen when comparing PT_{hCMV-} and HC (data not shown). When comparing PT_{hCMV+} and PT_{hCMV-} , there were significant differences in activation in the ROI- VIT_{AC} in right anterior cingulate cortex ($PT_{hCMV+} > PT_{hCMV-}$; see Fig. 3).

Over the whole group, activation in VIT_{CC} was observed primarily in bilateral posterior parietal regions, with right-hemispheric dominance (see Fig. 1, lower panels). Further activation could be seen in occipital, superior frontal, and cerebellar regions.

Comparing PT_{hCMV-} and HC, there were significant activation differences in the ROI- VIT_{CC} in right occipital cortex ($HC > PT_{hCMV-}$; see Fig. 4). None of the other group comparisons showed significant differences.

Impact of Structure on Function

Within the regions activated in the main effects over all groups, there were no significant differences between GM

Figure 3.

Between-group comparisons in ROI-VIT_{AC}: activation differences in right anterior cingulate cortex ($PT_{hCMV+} > PT_{hCMV-}$). Results are shown on the custom-made GM template at $P \leq 0.001$, uncorrected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

density or GM volume in ROI-VIT_{AC} between PT and HC (data not shown). This indicates that there is no significant structural difference in these brain regions in either native or normalized space. In contrast to this, there were no significant differences in GM density in ROI-VIT_{CC} but there were significant differences in GM volume, with HC showing significantly higher GM volumes (see Fig. 5; $P \leq 0.031$). This indicates that there is a significant difference in tissue volume in native space which is undone by spatial normalization.

In the light of these results, we conducted post hoc comparisons on the functional group differences as shown in Figures 2–4. There were no significant GM volume differences for the results shown in Figures 2 and 3, but there were significant volume differences in the region shown in Figure 4 (see Fig. 6; $P \leq 0.022$), with HC

Figure 4.

Between-group comparisons in ROI-VIT_{CC}: activation differences in right occipital cortex ($HC > PT_{hCMV-}$). Results are shown on the custom-made GM template at $P \leq 0.001$, uncorrected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 5.

Sum of voxel values in modulated and nonmodulated GM, calculated within ROI-VIT_{CC}. Note difference in tissue volume (i.e., in native space; left), but not in tissue density (i.e., in normalized space; right). Results are in arbitrary units. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

showing higher GM volumes than PT_{hCMV-} . This indicates that there is a significant difference in tissue volume in native space which is undone by spatial normalization.

DISCUSSION

This study was aimed at investigating brain activation as a function of performing a combined language/visuo-spatial task to detect the possible long-term neurobiological consequences of an early postnatal hCMV infection in PT. Our main findings were distinct activation differences in key brain regions as a function of both prematurity and postnatal hCMV infection. Furthermore, there were group differences in GM volumes within the activated brain regions which were masked by spatial normalization, potentially confounding group comparisons. These results shall now be discussed in more detail.

Figure 6.

Sum of voxel values in modulated and nonmodulated GM, calculated within the occipital activation difference (cf., Fig. 4). Note difference in tissue volume (i.e., in native space; left), but not in tissue density (i.e., in normalized space; right). Results are in arbitrary units. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Activation Patterns: VIT_{AC}

During the active condition of the vowel identification task (VIT_{AC}), we found the expected activation pattern in classical inferior-frontal language regions [Ebner et al., 2011; Everts et al., 2009; Wilke et al., 2006] (see Fig. 1) as well as in a number of other regions including the anterior cingulate and the left hippocampus. Within these regions, PT as a whole showed significantly stronger activation than HC in the left hippocampus, known to be associated with verbal working memory [Binder et al., 1997; Stark and Squire, 2000]. It is particularly interesting to note that this stronger activation can be seen only in the PT_{hCMV+} subgroup when compared with the HC (see Fig. 2), but not in the PT_{hCMV-} subgroup. This suggests that the group activation difference between HC and PT is driven by those PT with an early postnatal hCMV infection. Previous studies have also observed such stronger activation in PT and have interpreted them as indicative of a higher cognitive demand, or as a compensatory mechanism in the light of hippocampal impairment [Gimenez et al., 2004; Lawrence et al., 2010; Peterson et al., 2002]. It was suggested that this reflects working memory impairment in PT [Gimenez et al., 2004, 2005]. Post hoc analyses of our neuropsychological data also showed impaired working memory functions in our PT, as detected by the appropriate subtest of the HAWIK (data not shown). Our results suggest that this activation difference is not a function of prematurity per se, but may rather be more specific, more pronounced, and/or more prevalent in those PT with an early postnatal hCMV infection. This would be in line with results from *in vitro* studies, showing a propensity of CMV to the hippocampus [Cheeran et al., 2009; Kosugi et al., 2005; Li et al., 2009].

When directly contrasting the two groups of PT, there was a stronger activation of the PT_{hCMV+} subgroup in the anterior cingulate. Previous studies have linked such activation with task difficulty, memory demands, and cognitive load [Narberhaus et al., 2009; Nosarti et al., 2006; Paus et al., 1998]. As in the case of the hippocampal activation, this group difference is well in line with the interpretation of the task requiring more cognitive effort in the group of the PT_{hCMV+}. The fact that this difference cannot be observed in the behavioral measures (task performance) is not surprising considering the ceiling effect in this rather simple task; hence, this stronger activation could be considered to reflect successful compensation, although further analyses of more difficult tasks would be required to determine the extent to which this compensation can be observed.

Activation Patterns: VIT_{CC}

During the control condition (VIT_{CC}), all groups showed activation in the expected and previously described brain regions involved in visuo-spatial processing [Ebner et al., 2011]. Within these regions, differences between groups

could only be found in a small occipital cluster, where HC showed higher activation than PT_{hCMV-} (see Fig. 4). As this is a brain region involved in low-level visual processing [Milner and Goodale, 2006; Somers et al., 1999], it could point toward a group difference in visual acuity; however, all subjects had normal or corrected-to-normal vision. With more complex visual perception problems being more prominent in PT [Hellgren et al., 2007; Narberhaus et al., 2009], a group difference not detected by the cursory assessment of visual acuity implemented here cannot wholly be ruled out. However, this is less likely due to the normal neurological exam in almost all our PT [O'Reilly et al., 2010].

There were no activation differences between the groups in parietal brain regions typically associated with visuo-spatial processing [Milner and Goodale, 2006]. This was surprising in the light of the widely described deficits observable in PT in this domain [Hellgren et al., 2007; Narberhaus et al., 2009] which is also detectable in our cohort (reflected in the differences in the visuo-motor integration index [VMII]; see Table II). As we opted to include this index into our statistical model, this may have mitigated some group differences. Furthermore, the task may again have been too easy to put a high-enough strain on visuo-spatial brain regions for differences to become measurable. Such a ceiling effect was observable in the behavioral measures, where PT and HC were all very good at solving the task, and not significantly different. However, there may be an alternative explanation for this lack of activation differences, as discussed in the next paragraph.

Structure–Function Relationship

The impact of prematurity on brain structure has now been demonstrated in several studies [Kesler et al., 2008; Nosarti et al., 2002; Peterson et al., 2000], and it has been suggested that the observable differences are a complex mixture of direct injury, resulting developmental aberrations, and compensatory mechanisms [Volpe, 2009]. And while functional MRI is the method of choice to non-invasively investigate brain function in children [O'Shaughnessy et al., 2008; Wilke et al., 2003a], there may be a potential confound when investigating groups with a co-occurring difference in brain structure.

When comparing brains in a voxelwise fashion, spatial normalization is usually used to achieve spatial overlap between corresponding brain regions. We have previously shown that a severe bias is introduced when using inappropriate reference brains [Wilke et al., 2002], which was avoided here by using custom-generated pediatric reference data and appropriate data processing streams [Wilke et al., 2008]. However, when using functional MRI to compare groups with grossly different brain structure, we recently reported that brain lesions present in one but not in the other group lead to apparent differences in functional measures [Dinomais et al., 2011]. In the light of

these findings, we now aimed at assessing whether more subtle structural group differences may also influence functional group comparisons.

To this effect, we investigated GM volume within the brain regions activated on the group level. We could find neither group differences (in volume) in the areas activated during VIT_{AC} nor in the regions showing group differences (in function) within these regions (hippocampus and anterior cingulate; cf., Figs. 2 and 3). However, there were differences in GM volumes in the brain regions activated by VIT_{CC}, with HC showing significantly higher GM volumes than PT (see Fig. 5). This volume difference is well in line with previous studies investigating such populations, where they were considered to be related with the visuo-spatial impairments seen in PT [Hellgren et al., 2007; Narberhaus et al., 2009]. Furthermore, significantly higher GM volume in HC was found in the occipital cluster where activation differences were found (HC > PT_{hCMV-}, see Fig. 6). As the significant differences in GM volume disappeared when GM density was investigated instead [which (as the functional images) is not corrected for the volume changes occurring during spatial normalization; Good et al., 2001], these volume changes in native space are masked by spatial normalization following transformation to a standard space. This is significant insofar as these volume changes are now routinely “undone” by modulation with the Jacobian determinant of the transformation matrix in structural [Good et al., 2001], but not in functional MRI studies.

These results allow drawing several conclusions: first, they suggest that the observable group activation differences in VIT_{AC} are not confounded by underlying structural brain differences. This is important for their interpretation as they can now safely be interpreted “functionally,” in the light of compensation, or higher cognitive demand, as laid out above. Second, we failed to detect group activation differences in parietal brain regions where they could have been expected due to the observable neuropsychological deficits and previous studies [Hellgren et al., 2007; Narberhaus et al., 2009] while, crucially, we do detect significant GM volume differences in these very brain regions (HC > PT). Third, and conversely to the last scenario, we did detect unexpected group activation differences in occipital cortex while again simultaneously observing significant volume differences in this very cluster (HC > PT_{hCMV-}). We have previously shown the impact of overt brain lesions on apparent functional differences [Dinomais et al., 2011], and it is obvious that “where there is no brain, there is no activation,” and that therefore large brain lesions may impair the voxelwise comparison of two groups. However, the latter two findings reported above may now be interpreted to represent evidence for false negative, or false positive, findings in functional group activation differences due to subtle structural group differences, significantly expanding our previous work.

This of course has important repercussion: while previous studies (as this one) investigated structure and func-

tion [Gimenez et al., 2005; Lawrence et al., 2010; Peterson et al., 2002] there is as yet no comprehensive approach that accounts for the tissue volume change occurring during spatial normalization in functional group studies. As mentioned above, the modulation with the Jacobian determinant [Good et al., 2001] is used in structural studies but is not as easily applicable in functional studies as the BOLD signal is a direct property of the tissue, and its scaling is not as easily justified. Although usually, anatomical data will be acquired alongside functional data to be used for data processing such as spatial normalization, as done here, at current, there is not even an approach that allows to routinely investigate this interplay in fMRI studies. Furthermore, the exact nature of the interplay between structure and function is not clear and needs to be investigated more closely with regard to its impact on group comparisons. However, as functional group comparisons are commonly done between groups also demonstrating structural differences [such as schizophrenia, bipolar disorder, or attention-deficit/hyperactivity disorder; O'Reilly et al., 2010; Somers et al., 1999; Strakowski et al., 2011; Usher et al., 2010] the need for further research investigating the extent of the issue, as well as looking for solutions, seems evident.

Strengths and Limitations

We were able to recruit a large cohort of children and adolescents formerly born very preterm, as well as typically developing healthy controls. As all PT were born and followed-up in our center, we were able to extensively characterize their neonatal period, including their initial postnatal hCMV status (all negative) and the exact point in time when seroconversion occurred in the PT_{hCMV+}. This is important to rule out congenital hCMV infection and a bias introduced by more neonatal complications in one group. As Table I shows, there were no differences between the PT_{hCMV-} and the PT_{hCMV+} groups in several neonatal indicators, allowing to interpret our group results as being associated with hCMV-infection.

The PT had IQ scores within the normal range, but their results were significantly lower than those of the HC in the general as well as the specific visuo-spatial abilities, which is in line with previous studies [Bhutta et al., 2002; Johnson, 2007; Northam et al., 2011]. MEL is also known to influence outcome after preterm birth [Krägeloh-Mann and Lidzba, 2012; Voss et al., 2012] and is furthermore linked with prematurity itself [Brooks-Gunn et al., 1996; Kesler et al., 2008; Lieberman et al., 1987; Ment et al., 2003] and with hCMV infection [Bate et al., 2010; Bevot et al., 2011], making it hard to distinguish these effects. We also found higher MEL in our HC when compared with the PT in this study. Finally, the age range of our HC was wider, and they were slightly younger than our PT. In the interest of increasing power, we still opted to include all HC, but to account for these group differences [which are reflective of the difficulties also

observed by others when following up preterms; Callanan et al., 2001], these factors (age, FIQ, VMII, and MEL) were included as covariates in all statistical analyses. Although it must be expected that some of the differences between groups will be therefore be assigned to these cofactors, we aimed at investigating the core group deficit not explained by either of these factors. Our results must therefore be considered to be conservative, under rather than overestimating the group differences. Furthermore, a response bias cannot be ruled out when recruiting preterms as only those with a better outcome may have responded to our letters; hence, this cannot be considered an epidemiological study representative in all respects to the preterm population as a whole. However, post hoc analyses provided no evidence for a response bias: of 94 subjects approached, 49 subjects responded, while recruitment failed for 45 subjects (21 were approached and declined, and 24 were lost to follow-up). Responders were not significantly different from the full cohort in sex distribution (35m/14f vs. 68m/26f), gestational age (28.6 [0.3] vs. 28.5 [0.2] weeks), birth weight (1185 [49.5] vs. 1092 [33.8] g), or rate of postnatal hCMV infection (26/23 vs. 50/44). We therefore believe that this is not a severe bias.

With regard to data processing, it should be noted that using a customized template precludes the use of standard space coordinates with respect to the underlying structure, and also precludes direct comparisons with other studies. However, due to the strong impact of using nonappropriate reference data [Wilke et al., 2002, 2003b, 2008] we opted to construct our own reference data for this specific, large population. Finally, it must be acknowledged that functional group differences were only considered within the brain regions activated by the group effect (constituting the a priori hypothesis), such that no statement can be made about other brain regions.

SUMMARY AND CONCLUSIONS

With this study, including rather large and well-characterized cohorts of PT with or without an early postnatal hCMV infection [Hamprecht et al., 2001], we can demonstrate that fMRI detects neurobiological long-term effects of such an infection. Importantly, the well-described increased hippocampal activation of PT when compared with HC [Gimenez et al., 2004, 2005; Lawrence et al., 2010; Peterson et al., 2002] could be shown to be driven mainly by the PT_{hCMV+} subgroup in our sample, suggesting that more compensatory activation is required in those former preterms having undergone the infection in the neonatal intensive care unit. In line with this, they showed a higher activation in the anterior cingulate, which can again be interpreted to reflect executive control processes [Adleman et al., 2002]. Although the impact of this early infection is still under discussion [Bryant et al., 2002], our results are in line with recent studies demonstrating that the detrimental effect of this infection can be observed clinically [Bevot et al., 2011, Goelz et al., in press]

and provide the first evidence that such neurobiological consequences can be detected even in children and adolescents with an overall normal IQ. Future studies will have to investigate in how far this infection contributes to the overall morbidity of prematurity, but our results suggest that it has tangible effects that can be detected using current neuroimaging approaches, in line with the previous suggestion of their high sensitivity [Narberhaus et al., 2009]. Hence, they support the notion that efforts to protect preterm-born children from a postnatal hCMV-infection via breast-milk are warranted.

Furthermore, combined functional and volumetric analyses suggest that there is an impact of MR data processing on the resulting group analyses in the presence of subtle structural group differences. Both the lack of expected and the presence of unexpected functional group differences were accompanied by structural differences in these very same regions, suggesting an interrelation of structure and function that may be both mitigated or exaggerated by spatial normalization. There is at current no unifying framework allowing to investigate or compensate for this potential bias, but the field of imaging neuroscience may face a potentially serious confound if our findings should be confirmed.

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