Cytomegalovirus (CMV) infection is usually inapparent in healthy adults but persists for life. Neural progenitor/stem cells are main CMV targets, and dentate gyrus (DG) a major neurogenic niche. Smaller DG volume has been repeatedly reported in severe mental illness (SMI). Considering the suggested immune system, blood–brain barrier and DG disturbances in SMI, we hypothesized that CMV exposure is associated with smaller DG volume in patients, but not healthy controls (HC). Due to the differential male and female immune response to CMV, we hypothesized sex-dependent associations. 381 adult patients with SMI (schizophrenia spectrum or bipolar spectrum disorders) and 396 HC were included. MRI scans were obtained with 1.5T Siemens MAGNETOM Sonata scanner or 3T General Electric Signa HDxt scanner, and processed with FreeSurfer v6.0. CMV immunoglobulin G antibody concentrations were measured by solid phase immunoassay. We investigated main and interaction effects of CMV status (antibody positivity/CMV+ vs. negativity/CMV-) and sex on DG in patients and HC. Among patients, there was a significant CMV-by-sex interaction on DG (p = 0.009); CMV+ male patients had significantly smaller DG volume than CMV− male patients (p = 0.001, 39 mm³ volume difference) whereas no CMV-DG association was found in female patients. Post-hoc analysis among male patients showed that the CMV-DG association was present in both hemispheres and in both patients with schizophrenia spectrum and bipolar spectrum disorders, and further, that higher CMV antibody titers were associated with smaller DG. No CMV-DG association was found in HC. The results indicate a DG vulnerability to CMV infection in men with SMI.
The dentate gyrus (DG) is the preprocessor of the hippocampus receiving incoming information from the entorhinal cortex and preparing it for further cortex amnesia 3 (CA3) processing (Jonas and Lisman, 2014). Smaller DG volume has been repeatedly reported in schizophrenia (Haukvik et al., 2018, 2015; Mathew et al., 2014). In a sample of patients with schizophrenia and HC, DG volume was positively associated with visual learning and speed of processing (Nakahara et al., 2018). Smaller DG volume has also been reported in bipolar disorder (Haukvik et al., 2018, 2015; Mathew et al., 2014). In a sample of patients with schizophrenia and HC, DG volume was positively associated with visual learning and speed of processing (Nakahara et al., 2020). Of note, the DG is one of the two major neurogenic niches in the adult mammalian brain with NSPCs giving rise to new neurons (Drew et al., 2013; Eriksson et al., 1998; Moreno-Jimenez et al., 2019). There is evidence that human CMV targets DG prenatally (Teissier et al., 2014), but also postnatally in immunocompromised adults (Yoon et al., 2017).

It has been shown that blood–brain barrier (BBB) deficiency facilitates CMV invasion into the brain while inflammation accelerates CMV reactivation rates (Dupont and Reeves, 2016; Kawasaki et al., 2017). Both BBB hyperpermeability and inflammatory environment have been suggested to be implicated in schizophrenia and bipolar disorder (Goldsmith et al., 2016; Hope et al., 2009; Najjar et al., 2017; Patel and Frey, 2015). Further, immune-system abnormalities (Muller and Schwarz, 2010; Rosenblat and McIntyre, 2017; van Kesteren et al., 2017) and the immature DG (iDG) endophenotype (Walton et al., 2012), where the DG has abundant immature granule cells, have also been suggested in both disorders. Taken together, the suggested BBB-deficiency, inflammatory environment, immune system disturbances and iDG in severe mental illness (SMI) support the notion that the DG of patients with SMI may be particularly susceptible to CMV infection. Houenou et al. investigated the whole hippocampal volume and showed, partially in line with this hypothesis, that CMV seropositivity and serointensity were associated with smaller right hippocampus in patients with schizophrenia and bipolar disorder, but not in healthy individuals (Houenou et al., 2014).

Sex-dependent CMV effects on human immune system have been reported with the female immune system better controlling latent CMV infection (Di Benedetto et al., 2015; van der Heiden et al., 2016). This finding suggests that a putative detrimental effect of latent CMV infection on DG may be stronger in males. However, to our knowledge, there are no studies investigating latent CMV effects on immune system of patients with SMI, and further, congenital CMV infection seems to be more detrimental among females (Picone et al., 2005; Watt et al., 2016).

We hypothesized that previous CMV exposure and subsequent latency, as reflected by CMV immunoglobulin G (IgG) seropositivity, is associated with smaller DG volume among patients with SMI (schizophrenia spectrum or bipolar spectrum disorders), but not among healthy controls (HC). We further hypothesized that putative CMV-DG associations may be sex-dependent. To the best of our knowledge, this is the first study investigating associations between CMV IgG status and DG volume.

2. Material and methods

2.1. Participants

The Thematically Organized Psychosis (TOP) study is a thematic research effort on psychotic disorders and is the main study protocol at the Norwegian Centre for Mental Disorders Research (NORMENT, Oslo, Norway: www.med.uio.no/norment/english). Patients were recruited from outpatient and inpatient psychiatric units in Oslo, Norway, while HC were recruited from the same catchment area using the national population register. In the TOP study, the following exclusion criteria were applied for both patients and HC: intelligent quotient (IQ) < 70; age outside the range of 18–65 years; previous moderate or severe head injury; a neurological disorder or medical conditions thought to interfere with brain function. Medical doctors and psychologists assessed the patients with the Structured Clinical Interview for DSM-IV axis I disorder (SCID-I) module A-E (First et al., 1996), and the HC with the Primary Care Evaluation of Mental Disorders (Prime-MD) (Spitzer et al., 1994). HC with previous or current psychiatric disorders including substance use disorder (including alcohol use disorder) or with close relatives with SMI were excluded.

For the current study, participants were drawn from the TOP study cohort (2005 to 2014) if they had both CMV and MRI data (n = 798). We subsequently performed quality control for the whole hippocampus and subfield volumes as described in the supplementary material resulting in the exclusion of 21 subjects. The final sample (after the exclusion of the 21 subjects) consisted of 381 patients with SMI (patients with schizophrenia spectrum disorders, i.e. schizophrenia (n = 114), schizophreniform disorder (n = 16), schizoaffective disorder (n = 34)), delusional disorder (n = 9), brief psychotic disorder (n = 5) and psychotic disorder not otherwise specified (n = 45), and patients with bipolar spectrum disorders, i.e. bipolar I disorder (n = 93), bipolar II disorder (n = 59) and bipolar disorder not otherwise specified (n = 6), and 396 HC. Breakdown by patient/control status, CMV IgG status, sex and scanner for the final sample (n = 777) and the excluded subjects (n = 21) is demonstrated in Suppl. Table 1 and 2, respectively.

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study was approved by the local ethics committee and the Norwegian Data Inspectorate. All participants gave written informed consent.

2.2. Measures and medication

Level of education has been largely used as an indicator of socioeconomic status capturing the transition from parental to individual socioeconomic status (Galobardes et al., 2006). We therefore used years of education as socioeconomic status indicator. SMI can impact patients’ education level, and in patient analyses, we also used a categorical maternal education variable (1. primary school; 2. upper secondary school; 3. college/university). We assessed alcohol use with the alcohol use disorder identification test (AUDIT) (Bohn et al., 1995) and drug use with the drug use disorder identification test (DUDIT) (Berman et al., 2005). Further, we assessed the speed of processing with the Digit Symbol from the Welcher Adult Intelligence Scale III (WAIS-III) (Kaufman and Lichtenberger, 1999) and the visual learning with the Rey-Osterrieth Complex Figure Test (ROCFT long term memory) (Meyers and Meyers, 1995). We evaluated the patients with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), the Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Inventory of Depressive Symptoms, clinician rated (IDS-C) (Rush et al., 1996). We calculated the duration of illness (DOI) defined as the time passed since the first psychotic episode for schizophrenia spectrum and the first affective episode for bipolar spectrum. We assessed the current use (yes/no) of antipsychotic, antiepileptic, lithium and antidepressive medications, and for patients on antipsychotics we calculated the current chlorpromazine equivalent doses (CPZ) in mg/day (Andreasen et al., 2010).

2.3. Serology assessment

Blood samples were drawn from all participants. Serology assessments were performed at the Stanley Neurovirology Laboratory (Johns Hopkins University School of Medicine, Baltimore, MD, USA). CMV IgG antibody concentrations were measured by solid-phase immunoenassay and expressed as continuous (serointensity) and dichotomous measures.
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volumes during the same scan session with the following parameters: time (TI)
Echo time (TE)
Magnetom Sonata scanner
with an 8-channel head coil. T1-weighted sequences, Siemens 1.5T
head coil, and 337 scans with 3T General Electric Signa HDxt scanner
described ( Dickerson et al., 2014, 2003 ). In the present study, we
positivity was based on standards run with each sample as previously
described ( Dickerson et al., 2003 ). The cut-off for CMV antibody
concentrations were measured by solid-phase immunoassays and expressed as dichotomous measures (seropositivity/seronegativity) as previously described ( Dickerson et al., 2003 ).

2.4. MRI

We obtained 440 T1-weighted MRI (magnetic resonance imaging) scans using 1.5T Siemens MAGNETOM Sonata scanner with a standard
head coil, and 337 scans with 3T General Electric Signa HDxt scanner with an 8-channel head coil. T1-weighted sequences, Siemens 1.5T
Magnetom Sonata scanner: A sagittal magnetization prepared rapid gradient echo (MPRAGE) sequence was used to acquire two T1-weighted volumes during the same scan session with the following parameters: Echo time (TE) = 3.93 ms, repetition time (TR) = 2730 ms, inversion time (TI) = 1000 ms, flip angle = 7°; Field of view (FOV) = 24 cm, voxel size = 1.33 × 0.94 × 1 mm, number of partitions = 160. The two vol-
umes obtained were averaged during post-processing to increase signal-to-noise ratio (SNR). T1-weighted sequences, General Electric 3T Sigma
HDxt scanner: A 3D fast spoiled gradient echo (FSPGR) sequence was used to acquire T1-weighted volumes using the following parameters: Echo time (TE) = MinFull, repetition time (RT) = 7.8 ms, inversion time (TI) = 450 ms, Field of view = 256x256 mm, voxel size = 1x1x1.2 mm, flip angle = 12°, 170 sagittal slices.

MRI scans were processed using the FreeSurfer v6.0. Quality in-
spection and editing was performed by trained research assistants
following standard FreeSurfer procedures ( McCarthy et al., 2015 ). Hippocampal subfield volumes were obtained with the hippocampal
subfields module of the development version of Freesurfer v6.0. This module generates an automated segmentation of the hippocampal for-
mation subfields using a combination of ex vivo and in vivo MRI data, and in comparison to previous in vivo atlases better matches values from histological studies ( Iglesias et al., 2015; Saygin et al., 2017 ) (Fig. 1). We calculated the DG volume as the sum of the labels for the left and right
granule cell layer of dentate gyrus (GC-DG) and the left and right CA4. We performed quality control for the whole hippocampus and subfield
volumes and the procedure is described in the supplementary material.

2.5. Statistics

The following bivariate and multivariate analyses were conducted separately for patients and HC. In the bivariate analysis, we assessed group differences between CMV IgG seropositive (CMV+ ) and CMV IgG seronegative (CMV− ) participants in sex, age, education years, estimated total intracranial volume (ICV), tobacco use, handedness, AUDIT and DUDIT scores, as well as the correlations between each of these variables and DG volume (Table 1 ). In the patient group, we further assessed group differences between CMV+ and CMV− patients in DOI, PANSS total score, YMRs score, IDS-C score and medication use including CPZ (Table 1 ), as well as their correlations with DG volume (Table 1 ). In our multivariate models (analysis of covariance; ANCOVA), we investigated main and interaction effects of CMV IgG status (CMV + vs. CMV− ) and sex on DG volume, whilst controlling for scanner and variables that differentiated CMV+ and CMV− participants or were correlated with DG in the bivariate analysis. The level of significance for main and inter-
action effects was set at 0.05. We followed up statistically significant CMV IgG status-by-sex interactions with simple main effect analyses. Specifically, we interpreted the simple main effect of CMV IgG status on DG in men, and the simple main effect of CMV IgG status on DG in women, and we thereby accepted statistical significance for the simple main effects at the Bonferroni-adjusted alpha level of 0.025 (0.05/2).

In the whole sample, data on speed of processing and visual learning
was available for 454 participants (286 patients and 168 HC) and 428 participants (264 patients and 164 HC), respectively. We ran two hier-
archical multiple regressions to predict speed of processing and visual
learning from DG volume inserting the following covariates: ICV, scanner, sex, age and patient/control status. In the final multiple
regression models, we retained the covariates that led to statistically
significant increase in R 2 in the hierarchical regressions.
We conducted all the analyses with IBM SPSS Statistics 25

3. Results

3.1. Patient analysis

CMV+ and CMV− patients did not significantly differ in sex, age,
education years, ICV, tobacco use, handedness (right-handedness vs. left-handedness/ambidexterity), AUDIT score, DUDIT score, DOI,
médication variables, PANSS total score, YMRs score or IDS-C score
assessed with t-tests for quantitative variables and chi-square tests for
categorical variables (Table 1 ). For 101 patients, we had data on
maternal education level; CMV+ and CMV− patients did not differ in
maternal education level assessed with chi-square test ( p = 0.552).
Women had smaller DG volume than men assessed with point-biserial

Fig. 1. Coronal slice of the left hippocampus. We calculated the dentate gyrus as the sum of the labels for the granule cell layer of dentate gyrus (GC-DG) (light blue) and the cornu ammonis 4 (CA4) (brown).
correlation, \( r_{pb} \) (37 9) = -0.265, \( p < 0.001 \), ICV was positively correlated with DG volume assessed with Spearman’s correlation, \( r(S79) = 0.609 \), \( p < 0.001 \), whereas no correlations were found between the other analyzed variables and DG volume (Table 1).

In the multivariate model (ANCOVA), we investigated main and interaction effects of CMV IgG status and sex on DG volume whilst controlling for ICV and scanner. There was a significant CMV IgG status-by-sex interaction on DG volume, \( F(1,375) = 6.933, p = 0.009 \), a positive association between ICV and DG volume (\( p < 0.001 \)), whereas CMV IgG status (\( p = 0.075 \)), sex (\( p = 0.239 \)) and scanner (\( p = 0.149 \)) were not associated with DG volume. Simple main effect analysis showed that among men, there was a statistically significant effect of CMV IgG status on DG, \( F(1,375) = 10.471, p = 0.001 \), partial eta-squared = 0.027, whereas among women, there was no such effect, \( F(1,375) = 0.340, p = 0.560 \). Specifically, adjusted mean DG volume in CMV + men (1078 mm\(^3\)) was smaller than in CMV- men (1117 mm\(^3\)), a statistically significant difference of 39 mm\(^3\) (95% CI, 15 to 62), \( p = 0.001 \), whereas adjusted mean DG volumes in CMV + and CMV- women were 1113 mm\(^3\) and 1106 mm\(^3\), respectively, a non-statistically significant difference of 7 mm\(^3\), \( p = 0.560 \) (Table 2 & Fig. 2A).

3.2. Healthy control analysis

CMV + and CMV- HC differed significantly in age, \( t(394) = -2.585, p = 0.010 \), with CMV + HC being older than CMV- HC, but not in sex, education years, ICV, tobacco use, handedness (right-handedness vs. left-handedness/ambidexterity), AUDIT score or DUDIT score. Among men on antipsychotics, we had data on current CPZ for 115 CMV- and 157 CMV + patients (Table 1). In the multivariate model (ANCOVA), we investigated main and interaction effects of CMV IgG status and sex on DG volume whilst controlling for ICV and scanner. There was no significant CMV IgG status-by-sex interaction, \( F(1,390) = 2.766, p = 0.097 \), ICV was positively associated with DG volume (\( p < 0.001 \)), whereas CMV IgG status (\( p = 0.606 \)), sex (\( p = 0.139 \)) and scanner (\( p = 0.108 \)) were not associated with DG volume. As CMV + and CMV- HC differed significantly in age, age was inserted as a covariate in the multivariate model but this did not change the main results showing no main or interaction effects of CMV IgG status and sex on DG volume whilst controlling for ICV and scanner. Applying a false
Table 2
The results of the two-way analysis of covariance (ANCOVA) among patients with severe mental illness. There was a significant CMV-by-sex interaction on dentate gyrus (DG) (*p = 0.009) which we followed up with simple main effect analysis in men and women. CMV+ men had significantly smaller DG volume compared to CMV- men (*p = 0.001), whilst controlling for scanner and estimated total intracranial volume (ICV). No such association was found in women (*p = 0.560). ICV was positively associated with DG volume (*p < 0.001). Significant associations (*p < 0.05) are shown in bold.

| ANCOVA with simple main effect analysis on DG volume | F  | P value | Partial eta squared | Estimated DG volumes (mm$^3$) |
|-----------------------------------------------------|----|---------|---------------------|-------------------------------|
| CMV status (CMV$^+$/CMV$^-$)                        | 3.20 | 0.075  | 0.008               | CMV$^-$ CMV$^+$               |
| Sex                                                 | 1.39 | 0.239  | 0.004               |                               |
| CMV-by-sex interaction                              | 6.93 | 0.009  | 0.018               |                               |
| Scanner                                             | 2.10 | 0.149  | 0.006               |                               |
| Estimated total intracranial volume                 | 185.10 | <0.001 | 0.330               |                               |

* For the simple main effects, statistical significance was accepted at the Bonferroni-adjusted alpha level of 0.025.

3.3.3. CMV antibody titers
The CMV antibody concentration range was 0.1 to 34.3, with a mean (standard deviation) of 3.4 (4.2), and a median (interquartile range) of 3 (4.3). In the bivariate analysis, we searched for correlations between all variables and CMV antibody titers as well as DG. Both ICV and scanner were significantly correlated with DG, and were thereby included in the multivariate model, whereas no other variables were correlated with DG or CMV antibody titers (Suppl. Table 4). We ran a multiple regression to predict DG volume from CMV antibody titers, ICV and scanner. Antibody titers were inversely associated with DG volume (standardized regression coefficient ($\beta$) = -0.146, *p = 0.009), ICV was positively associated with DG volume ($\beta$ = 0.671, *p < 0.001), whereas scanner was not associated with DG volume (*p = 0.075) (Suppl. Table 5). The unstandardized regression coefficient for the antibody titers was –3.74, showing that an increase in CMV IgG concentration of 1 unit was associated with a decrease in the DG volume of 3.74 mm$^3$.

3.3.3. CMV antibody titers
The CMV antibody concentration range was 0.1 to 34.3, with a mean (standard deviation) of 3.4 (4.2), and a median (interquartile range) of 3 (4.3). In the bivariate analysis, we searched for correlations between all variables and CMV antibody titers as well as DG. Both ICV and scanner were significantly correlated with DG, and were thereby included in the multivariate model, whereas no other variables were correlated with DG or CMV antibody titers (Suppl. Table 4). We ran a multiple regression to predict DG volume from CMV antibody titers, ICV and scanner. Antibody titers were inversely associated with DG volume (standardized regression coefficient ($\beta$) = -0.146, *p = 0.009), ICV was positively associated with DG volume ($\beta$ = 0.671, *p < 0.001), whereas scanner was not associated with DG volume (*p = 0.075) (Suppl. Table 5). The unstandardized regression coefficient for the antibody titers was –3.74, showing that an increase in CMV IgG concentration of 1 unit was associated with a decrease in the DG volume of 3.74 mm$^3$.

Table 3
Cytomegalovirus (CMV) immunoglobulin G (IgG) simple main effects on the whole hippocampus and 11 hippocampal subfields by hemisphere among men with severe mental illness whilst controlling for estimated Total Intracranial Volume and scanner. Nominally significant associations are shown in bold.

| Volumes in mm$^3$ | Left hemisphere | Right hemisphere | P value |
|-------------------|-----------------|------------------|---------|
| N                 | CMV$^-$         | CMV$^+$          | P value |
| Whole hippocampus | 3583            | 3497             | 0.021   | 3637            | 3560             | 0.034 |
| CA1               | 668             | 652              | 0.066   | 698             | 684              | 0.134 |
| Molecular layer   | 579             | 562              | 0.011   | 590             | 574              | 0.015 |
| Hippocampal tail  | 580             | 569              | 0.292   | 596             | 582              | 0.154 |
| Subiculum         | 449             | 443              | 0.297   | 442             | 432              | 0.076 |
| DG                | 549             | 529              | 0.003*  | 568             | 549              | 0.003* |
| Presubiculum      | 323             | 317              | 0.193   | 297             | 294              | 0.412 |
| CA2/CA3           | 215             | 206              | 0.023   | 230             | 224              | 0.087 |
| Hippocampal fissure | 151           | 152              | 0.966   | 154             | 156              | 0.533 |
| Fimbria           | 87              | 89               | 0.406   | 84              | 89               | 0.028 |
| HATA              | 65              | 64               | 0.296   | 68              | 67               | 0.456 |
| Parasubiculum     | 69              | 67               | 0.418   | 65              | 65               | 0.653 |

CA: cornu ammonis, DG: dentate gyrus, HATA: hippocampal-amygdaloid transition region
Survives false discovery rate (FDR) correction for multiple testing

Fig. 2. A) Dentate gyrus (DG) volumes in mm$^3$ in cytomegalovirus (CMV) immunoglobulin G (IgG) seronegative (CMV-) and seropositive (CMV+) men and women with severe mental illness (SMI). CMV+ men had significantly smaller DG volume than CMV- men (*p = 0.001). B) DG volumes in CMV- and CMV+ male and female healthy controls (HC).

*p = 0.001.
3.3.4. HSV1 and HSV2
For 196 and 134 men with SMI, we had data on HSV1 (110 HSV1-/86 HSV1+) and HSV2 (117 HSV2-/17 HSV2+) IgG status, respectively. We ran an ANCOVA investigating the main effect of HSV1 IgG status on DG volume whilst controlling for ICV and scanner. There was no HSV1 IgG status effect (p = 0.165) on DG volume. We finally ran an ANCOVA investigating the main effect of HSV2 IgG status on DG volume, controlling for ICV and scanner. As in the HSV1 analysis, there was no HSV2 IgG status effect on DG (p = 0.765).

3.4. Post-hoc analysis among all men

We aimed to determine whether DG volume differed between CMV- men with SMI and CMV+ healthy men, and between CMV- men with SMI and CMV- healthy men. Among CMV- men (n = 226), patients were younger than HC, (t(224) = 2.752, p = 0.006, and reported fewer education years than HC, (t(224) = 5.636, p < 0.001. Age and education years were not associated with DG, r(224) = 0.050, p = 0.458 and r(224) = 0.033, p = 0.619, respectively. Both age and education years were included in the multivariate model among CMV- men. Among CMV- men (n = 190), patients and HC did not differ in age, (t(188) = -0.633, p = 0.528, while reported patients fewer education years than HC, (t(188) = 5.794, p < 0.001. Age and education years were not associated with DG, r(188) = 0.093, p = 0.201 and r(188) = 0.111, p = 0.126, respectively. The education years variable was included in the multivariate model among CMV- men. We ran two multivariate models (ANCOVAs), one among CMV+ men searching for diagnosis status effects (SMI/HC) on DG, whilst controlling for scanner, ICV, age and education years, and one among CMV- men searching for diagnosis status effects (SMI/HC) on DG, whilst controlling for scanner, ICV and education years. Among both CMV+ and CMV- men, patients had smaller DG volume compared to HC; mean difference = 61 mm³, p < 0.001, partial eta-squared = 0.092, and mean difference = 36 mm³, p = 0.010, partial eta-squared = 0.036, for CMV+ and CMV- men, respectively.

3.5. Cognitive measures analysis

The details of the hierarchical regressions are presented in Suppl. Tables 6 and 8. In the final multiple regression on visual learning, DG was significantly positively associated with visual learning (p = 0.001) (Suppl. Table 7). In the final multiple regression on speed of processing, DG was not associated with speed of processing (p = 0.168) (Suppl. Table 9).

4. Discussion

In the present study, among patients with SMI, we found a significant CMV IgG status-by-sex interaction (p = 0.009) on DG, with CMV+ male patients having significantly smaller DG volume than CMV- male patients (p = 0.001), whereas no DG volume difference was found between CMV+ and CMV- female patients (p = 0.560) (Fig. 2). No main or interaction effects of CMV IgG status and sex on DG were found in HC. Post-hoc analysis among men with SMI showed that a) the inverse association between CMV antibody positivity and DG volume was similarly present in right (p = 0.003) and left hemisphere (p = 0.003) (Table 3), b) there were no significant associations between CMV antibody positivity and other than DG right or left hippocampal subfields or the right or left whole hippocampal volume indicative of a DG specificity (Table 3), c) the CMV-DG association was present in both patients with schizophrenia spectrum (p = 0.014) and bipolar spectrum disorders (p = 0.05), d) there was a significant inverse association between CMV IgG antibody titers and DG volume (p = 0.009), and e) there was no association between HSV1 or HSV2 antibody positivity and DG volume. HSV1 and HSV2 are Herpesviridae of the subfamily Alph herpesvirinae (Davison, 2010) and the absence of associations with DG is suggestive of a CMV specificity. Finally, we found that among both CMV+ and CMV- male participants, patients had significantly smaller DG volume than HC. The effect size of the diagnosis status effect on DG volume was larger in CMV+ men, where 9% of the variation in DG volume was explained by the diagnosis status (vs. 4% in CMV- men), reflecting the CMV effect on DG in male patients shown in the main analysis.

The inverse association between CMV exposure and DG volume might be due to the suggested CMV tropism for progenitor/immature cells (Dupont and Reeves, 2016; Luo et al., 2008; Teissier et al., 2014) that are abundant in human DG (Moreno-Jiménez et al., 2019). Granule cells are the principal cells of the DG and it is unequivocal that new granule cells are generated in mammalian DG throughout life (Drew et al., 2013). This adult hippocampal neurogenesis in human DG is a phenomenon first reported by Eriksson et al. (Eriksson et al., 1998) and has been supported by some but not all subsequent studies. In the most recent study, Moreno-Jimenez et al. showed that adult hippocampal neurogenesis is abundant in the DG of neurologically healthy humans, where thousands of immature neurons could be identified (Moreno-Jimenez et al., 2019). The immature cells were mainly located in the hilar border of the granule cell layer (Moreno-Jiménez et al., 2019). In the present study, we calculated DG as the sum of the granule cell layer of the DG and the CA4 layer. The latter is the hilar region of the DG and is located within the DG borders (Iglesias et al., 2015). The presence of a CMV-DG association restricted to SMI may be due to the suggested iDG endophenotype in DG (Hajighara et al., 2013; Walton et al., 2012). The iDG is characterized by the presence of abundant DG granule cells which remain in an immature state, not reaching adequate maturation and integration (Hajighara et al., 2013). The greater presence of immature cells may be due to a compensatory mechanism as a result of primary mature cell loss or due to maturation arrest (Walton et al., 2012). The observed CMV-DG association in SMI may also be related to a BBB hyperpermeability (Najjar et al., 2017; Patel and Frey, 2015). There are indications of BBB disruption across the major psychiatric disorders (Greene et al., 2020), mainly in schizophrenia (Najjar et al., 2017; Pollak et al., 2018) but also in bipolar disorder (Kaminsky et al., 2020; Patel and Frey, 2015). The etiopathology of the BBB hyperpermeability in SMI is not well understood but may include genetic factors, chronic neuroinflammation and environmental factors such as infections and injuries (Najjar et al., 2017; Pollak et al., 2018). Such a BBB dysfunction in SMI could result in a greater CNS accessibility of CMV and/or host immune response components. The greater susceptibility of the DG of patients with SMI could also be due to inflammatory environment (Goldsmith et al., 2016; Hope et al., 2009) enhancing CMV replication rates (Dupont and Reeves, 2016) as well as immune disturbances in SMI (Muller and Schwarz, 2010; Rosenblat and McIntyre, 2017; van Kesteren et al., 2017). By contrast, the DG of HC does not appear susceptible to the CMV infection, possibly due to normal DG maturation, BBB integrity, a non-inflammatory environment and an adequate host immune response.

Importantly, the present results show a CMV-DG association in men but not women with SMI. This may be due to the observed sex-dependent host immune response to latent CMV. Among both middle-aged and elderly adults, the CMV impact on the immune system has been found to be more pronounced in men whereas women appear to better be able to control latent CMV (Di Benedetto et al., 2015; van der Heiden et al., 2016). It has been suggested that women are generally immune-privileged, possibly due to the enhancing impact of estradiol on immune response, whereas testosterone has been reported to be mainly immunosuppressive (Furman et al., 2014; Giebing-Kroll et al., 2015). We are tempted to speculate that the brain volume changes in CMV+ male patients is the result of a progressive atrophic process related to chronic CMV latent infection, a more acute CMV detrimental effect on brain tissue during the primary infection and/or the subsequent CMV reactivations. Due to the cross-sectional design of the study, we cannot deduce from the present results when the primary CMV infection occurred. Congenital CMV infection is lethal in approximately 1/10 of cases (Kawasaki et al., 2017). Although often catastrophic, the incidence of the congenital CMV infection is 0.2–2% in different
populations (Kawasaki et al., 2017), with a recent Norwegian study showing such a vertical transmission in the lower limit of the range (0.22%) (Barrill et al., 2018). By contrast, the exposure to CMV after birth is very common, and CMV IgG seropositivity constantly increases both during childhood/adolescence (Voigt et al., 2016) and in adulthood (Lachmann et al., 2018). In the present study, more than half of the participants were CMV + meaning that only an insignificant portion was infected before birth. Our results cannot thereby be explained by a non-lethal ‘mild’ congenital CMV infection. Of note, not only antibody positivity but also higher antibody titers, possibly reflecting more frequent reactivations during the latent infection period (Dickerson et al., 2014; Iglesias-Escudero et al., 2016), were associated with smaller DG volume in men with SMI. Taken together, our results are suggestive of a DG vulnerability to CMV latent infection especially with higher frequency of reactivations.

The potential underlying mechanisms by which CMV exposure is associated with smaller DG volume might include brain invasion of CMV and/or host immune response components. In the context of a congenital CMV infection, CMV can disrupt the BBB facilitating the viral invasion into the brain parenchyma (Kawasaki et al., 2017). CMV-infected cells have been found in numerous brain regions including the limbic system and the DG, with progenitor cells showing the greatest susceptibility (Teissier et al., 2014). CMV-infected neurons have also been reported in the DG of immunocompromised adults (Yoon et al., 2017). Such an invasion of pathogens into the brain is typically followed by abundant inflammatory response and immune cell migration (Dahm et al., 2016). We speculate that even in SMI and due to the suggested BBB hyperpermeability (Najjar et al., 2017; Patel and Frey, 2015), a postnatal CMV exposure leads to viral invasion into the brain and infection of neuronal cells followed by host inflammatory response and subsequent atrophic process shown as smaller volume in MRI. Another plausible explanation is that CMV does not invade into the CNS of patients with SMI, but is associated with the DG volume decrease through autoimmune cross-reactivity. A substantial overlap of CMV proteins and human proteins involved in neuronal migration, a phenomenon still present in the DG of adults, has been recently reported, suggesting that the host immune response to the CMV infection may cross-react with brain proteins resulting in neuropathological aberrations (Luchese et al., 2020).

In a recent study, Nakahara et al. reported smaller hippocampal GC-DG, CA4, molecular layer, tail and CA1 volumes in patients with schizophrenia compared to HC, with the largest volume difference found for the GC-DG (Nakahara et al., 2020). In the whole sample of patients and HC, GC-DG volume was positively associated with visual learning and speed of processing (Nakahara et al., 2020). In the present study, in the whole sample of patients and HC, DG was positively associated with visual learning but not with speed of processing. Cognitive impairment including visual learning deficits are key symptoms in schizophrenia (Green, 2006), and can also be present in patients with bipolar disorder (Titsipa and Fountoulakis, 2015). In a recent study of a sample overlapping with ours, visual learning showed substantial deficiencies in most patients with SMI (intermediate and impaired cognitive groups) compared with patients of the intact cognitive group and HC (Vaskinn et al., 2020).

The DG volume difference between CMV + and CMV- men with SMI was 39 mm³ with 3% of the variation in DG volume attributed to CMV status. The effect size was small to medium (Lakens, 2013), and of note, comparable to the effect of other environmental factors as well as psychosis on DG. The putative impact of environmental factors on DG is largely unexplored, and to the best of our knowledge, there are no previous studies investigating associations between exposure to CMV or other pathogens and DG volume. Interestingly, traumatic experiences have been associated with smaller DG volumes. In particular, childhood maltreatment was associated with smaller DG volume (Teicher et al., 2012). The variation in DG volume attributed to childhood maltreatment was 4% and 1% for left and right DG, respectively (Teicher et al., 2012). Further, patients with posttraumatic stress disorder have also been reported to have smaller DG than HC, with a similar DG volume difference as between CMV + and CMV- male patients in the present study (Hayes et al., 2017). Finally, in recent studies applying the same atlas as in the present study (Iglesias et al., 2015), the DG volume in patients with first-episode psychosis (Baglivo et al., 2018) or schizophrenia (Nakahara et al., 2020) compared to HC was similarly smaller as between CMV + and CMV- men with SMI in the present study.

The present study has certain limitations. First, despite the fact that we have identified and taken account of putative confounders including education level, tobacco, alcohol and drug use, DOI and medication use, we cannot rule out that other unknown factors may influence the CMV-DG association. Further, we do not know when during the lifespan the CMV exposure took place. This is a study with cross-sectional design and long-term course is not known. Another limitation of the study is the use of two different scanners. Although not statistically significant for the smaller 3T subgroup, CMV + male patients had smaller DG volume than CMV- male patients in both 1.5T and 3T subgroups, and further there was no CMV IgG status-by-scanner interaction (supplementary material). Further, when segmenting 1 mm scans, volumes of internal hippocampal subfields should be interpreted with caution, and the present results should be replicated in independent samples ideally with higher resolution data.

5. Conclusions

To conclude, we have shown that CMV previous exposure and current latency, as reflected by CMV IgG antibody positivity, is associated with smaller DG volume restricted to men with SMI which is suggestive of a DG vulnerability to CMV infection in this patient group. As smaller DG volumes have been repeatedly reported in SMI, our results indicate that CMV may be an important environmental factor that contributes to the smaller DG volume in men with SMI.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.05.009.

References

Andreasen, N.C., Presler, M., Nopoulos, P., Miller, D., Ho, B.-C., 2010. Antipsychotic dose equivalents and dose-years: a standardized method for comparing exposure to different drugs. Biol Psychiatry 67 (3), 255–262.
Baglivo, V., Cao, B., Mwangi, B., Bellani, M., Perlini, G., Lasalvia, A., Dusi, N., Bonetto, C., Cristofalo, D., Alessandrinì, F., Zoccatelli, G., Cicerì, E., Dario, L., Enrico, C., Francesca, P., Mazzi, F., Paolo, S., Balestrieri, M., Soares, J.C., Ruggerì, M., Brambilla, P., Group, G.U., 2018. Hippocampal Subfield Volumes in Patients With First-Episode Psychosis. Schizophr Bull 44, 552-559.
Spitzer, R.L., Williams, J.B., Kroenke, K., Linzer, M., deGruy, F.V., 3rd, Hahn, S.R., Brody, D., Johnson, J.G., 1994. Utility of a new procedure for diagnosing mental disorders in primary care. The PRIME-MD 1000 study. JAMA 272, 1749–1756.

Teicher, M.H., Anderson, C.M., Polcari, A., 2012. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. Proc Natl Acad Sci U S A 109, E563–572.

Teissier, N., Fallet-Bianco, C., Delezioe, A.-L., Laquerriere, A., Marcorelles, P., Khung-Savatovsky, S., Nardelli, J., Cipriani, S., Cabra, Z., Piccone, O., Golden, J.A., Van Den Abbeele, T., Gresens, P., Adle-Biassette, H., 2014. Cytomegalovirus-induced brain malformations in fetuses. J Neuropathol Exp Neurol 73 (2), 143–158.

Tsitsipa, E., Fountoulakis, K.N., 2015. The neurocognitive functioning in bipolar disorder: a systematic review of data. Ann Gen Psychiatry 14, 42.

van der Heiden, M., van Zelm, M.C., Bartol, S.J.W., de Rond, L.G.H., Berbers, G.A.M., Boots, A.M.H., Buism, A.M., 2016. Differential effects of Cytomegalovirus carriage on the immune phenotype of middle-aged males and females. Sci Rep 6, 26892.

van Kesteren, C.F., Gremmels, H., de Witte, L.D., Hol, E.M., Van Gool, A.R., Falkai, P.G., Kahn, R.S., Sommer, I.E., 2017. Immune involvement in the pathogenesis of schizophrenia: a meta-analysis on postmortem brain studies. Transl Psychiatry 7, e1075.

Vaskinn, A., Haatveit, B., Melle, I., Andreassen, O.A., Ueland, T., Sundet, K., 2020. Cognitive Heterogeneity across Schizophrenia and Bipolar Disorder: A Cluster Analysis of Intellectual Trajectories. J Int Neuropsychol Soc 26 (9), 860–872.

Voigt, S., Schaffrath Rosario, A., Mankertz, A., 2016. Cytomegalovirus Seroprevalence Among Children and Adolescents in Germany: Data From the German Health Interview and Examination Survey for Children and Adolescents (IGGOS), 2003-2006. Open Forum Infect Dis 3, ofv193.

Walton, N.M., Zhou, Y., Kogan, J.H., Shin, R., Webster, M., Gross, A.K., Heusser, C.L., Chen, Q., Miyake, S., Tajinda, K., Tamura, K., Miyakawa, T., Matsumoto, M., 2012. Detection of an immature dentate gyrus feature in human schizophrenia/bipolar patients. Transl Psychiatry 2, e135.

Watt, A.P., Loughlin, L., Coyle, P.V., 2016. Congenital CMV disease – A female bias in Northern Ireland? J Clin Virol 77, 99–100.

Wills, M.R., Poole, E., Lau, B., Krishna, B., Sinclair, J.H., 2015. The immunology of human cytomegalovirus latency: could latent infection be cleared by novel immunotherapeutic strategies? Cell Mol Immunol 12 (2), 128–138.

Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry 133, 429–435.

Zuhair, M., Smit, G.S.A., Wallis, G., Jabbar, F., Smith, C., Devleesschauwer, B., Griffiths, P., 2019. Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review and meta-analysis. Rev Med Virol 29 (3), e2034. https://doi.org/10.1002/rmv.v29.310.1002/rmv.v2034.