Cerebral hemodynamics and capillary dysfunction in late-onset major depressive disorder

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ABSTRACT

In major depressive disorder (MDD), perfusion changes in cortico-limbic pathways are interpreted as altered neuronal activity, but they could also signify changes in neurovascular coupling due to altered capillary function. To examine capillary function in late-onset MDD, 22 patients and 22 age- and gender-matched controls underwent perfusion MRI. We measured normalized cerebral blood flow (nCBF), cerebral blood volume (nCBV), and relative transit-time heterogeneity (RTH). Resulting brain oxygenation was estimated in terms of oxygen tension and normalized metabolic rate of oxygen (nCMRO2). Patients revealed signs of capillary dysfunction (elevated RTH) in the anterior prefrontal cortex and ventral anterior cingulate cortex bilaterally and in the insulate cortex compared to controls, bilateral hypometabolism (parallel reductions of nCBV, nCBF, and CMRO2) but preserved capillary function in the cerebellum and brainstem. Our data support that microvascular pathology affects neurovascular coupling in ventral circuits. We speculate that microvascular pathology is important for our understanding of etiology of late-onset MDD as well as inferences about resulting brain activity changes.

1. Introduction

Functional neuroimaging methods have widened our understanding of brain function in major depressive disorder (MDD). Accordingly, studies of the brain’s blood supply and blood oxygenation during rest and functional activation in MDD patients indicate both state- and trait-dependent changes in brain activity within specific brain networks – for overviews see e.g., (Drevets, 2000; Fitzgerald et al., 2008; Gunning and Smith, 2011; Mayberg, 2003; Rigucci et al., 2010; Savitz and Drevets, 2009; Videbech, 2006; Wang et al., 2012).

When brain activity is inferred from functional imaging studies, we assume a one-to-one correspondence between the magnitude of cerebral blood flow (CBF) – or the amplitude of blood oxygenation level dependent (BOLD) signal changes – on one hand, and the underlying brain activity on the other: Neurovascular coupling mechanisms adjust arteriolar diameter, and thereby CBF, according to the metabolic needs of brain tissue, which in turn depends on the level of neuronal activity (Iadecola, 2004). This fundamental assumption was recently shown to represent an oversimplification: While CBF determines net oxygen supply to a given brain region, capillary flow patterns biophysically limit the fraction of the blood’s oxygen, which can be extracted by the tissue (Jespersen and Østergaard, 2012). In the resting brain, capillary flow velocities are highly heterogeneous (Kleinfeld et al., 1998), but during episodes of increased blood flow, i.e., functional hyperemia, capillary flows homogenize (Gutierrez-Jimenez et al., 2016; Jespersen and Østergaard, 2012). This homogenization is crucial for the efficient...
Capillary dysfunction has been implicated in cerebral small vessel disease (SVD) (Ostergaard et al., 2016) and the pathogenesis of Alzheimer’s disease (Eklidson et al., 2017; Nielsen et al., 2017, 2020; Ostergaard et al., 2015, 2013b). Meanwhile, increasing data suggest an association between cerebral SVD and higher risk of depression (Fang et al., 2020; van Agtmaal et al., 2017), dementia, and stroke (Rensma et al., 2018). Manifestations of cerebral SVD may be visualized on magnetic resonance imaging (MRI) of the brain as white matter hyper-intensities (WMHs), small subcortical infarctions, lacunes, microbleeds, perivascular spaces, and global atrophy; for a detailed description of these neuroimaging features - see Wardlaw et al. (2013). The association of microvascular dysfunction, including cerebral SVD, and late-onset depression was recently reviewed by Empana et al. (2021) and comprise alterations in blood-brain barrier permeability, reduced cerebrovascular reactivity, impaired cerebral autoregulation, and disturbed capillary flow patterns, that through complex mechanisms, driven by tissue hypoxia, may result in inflammation, ischemia, and hemorrhage, causing disturbed neuronal functioning in pathways important for affective and cognitive processing. The microvascular dysfunction is initiated by aging, inflammatory stress, and vascular risk factors such as hypertension and diabetes, and although recent publications from large community studies show an association between vascular risk factor burden and incident depression (Adams et al., 2018; Kivimäki et al., 2012), reported associations between depression and vascular risk factors have previously been inconsistent (Valkanova and Ebmeier, 2013) and seem to be age-dependent with lesser association in later life compared to mid-life (Bloch et al., 2021). In addition, depression related to WMHs, a known marker of cerebral SVD, is mostly confined to late-onset debut of depressive symptoms (Salo et al., 2019). Therefore, we wanted to compare the degree of capillary dysfunction in a well-characterized cohort of patients with late-onset MDD to that of an age- and gender-matched control group with similar vascular risk profile (Dalby et al., 2010a). We examined whether brain regions, which showed MDD-specific CBF or BOLD changes in previous neuroimaging studies, revealed signs of capillary dysfunction, and we discuss whether our findings might affect the functional interpretation of those studies or of the aetopathogenesis of MDD.

2. Methods

2.1. Subjects

The study population consisted of 22 patients with late-onset (pre-defined as debut after the age of 50), first-episode MDD and 22 age- and gender-matched controls with no previous history of psychiatric illness. All patients met the DSM-IV criteria (American Psychiatric Association, 2000) for MDD and the ICD-10 criteria (World Health Organization, 1993) for moderate to severe depression within 4 weeks of examination. Controls were recruited through local paper advertisement and matched for age and gender with the patient group. All subjects were assessed with selected parts of the SCAN structured interview (Wing et al., 1998) and were thoroughly interviewed about their medical history. We used the Bech–Rafaelsen Melancholia Scale (Bech, 2002) and Widløcher Depressive Retardation Scale (Widløcher, 1983) to assess severity of depressive symptoms and psychomotor retardation, respectively, in both groups (Dalby et al., 2010a). All controls were additionally screened with the Mini-Mental State Examination ( Folstein et al., 1975) with a minimum score of 29 (results not shown). Age at debut of depressive symptoms in the patient group was determined through detailed patient interview and clinical records; an estimated disease duration at inclusion is calculated in a previous publication (Dalby et al., 2013). Details on vascular risk factors such as hypertension, diabetes, tobacco smoking, and cardiovascular disease were carefully obtained during the clinical interview and by blood samples and electrocardiogram (Dalby et al., 2010a). Records of prescribed medication, including psychotropics, are reported elsewhere (Dalby et al., 2010a). None of the patients were chronic treatment resistant according to the criteria of Souery et al. (1999). None of the controls received any psychotropic medication. Both groups underwent a neurological exam, and all subjects were screened for concurrent medical diseases and alcohol abuse by standard blood tests. Exclusion criteria for both groups included organic brain disease (e.g., former stroke, cerebral vascular malformations, and epilepsy) or other brain injury, lifetime substance dependency (except for tobacco smoking), and contraindications for MRI. The study was approved by the regional ethical committee and was conducted after written informed consent from all participants in line with the Declaration of Helsinki (World Medical Association, 2008).

2.2. Imaging protocol

Cerebral MRI was performed using a 3.0 Tesla GE Signa HDx scanner (GE Medical Systems, Milwaukee, WI, USA) with an 8-channel head coil. The structural MRI included a 3D T1-weighted sequence, an axial T2-weighted, sequence, and an axial T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence, while perfusion-weighted imaging comprised dynamic gradient echo (GRE) (TE/TR=60/1500 ms, FOV=240 mm, 128 × 128 matrix, 5 mm slices with 1.5 mm gap) and spin echo (SE) (TE/TR=30/1500 ms, 60° flip angle, FOV=240 mm, 128 × 128 matrix, 5 mm slices with 1.5 mm gap) echo-planar imaging (EPI) during bolus injection of 0.1 and 0.2 mmol/kg gadobutrol (Gadovist, Bayer Schering Pharma, Berlin, Germany), respectively, followed by injection of 30 ml of physiologic saline at a rate of 5 ml/s. The total MRI protocol acquisition time was approximately 50 min.

2.3. Regions of interest

Several brain circuitries have been implicated in the pathophysiology of MDD. The limbic-cortical dysregulation model (Mayberg, 1997, 2003) describes alterations in a complex functional network of limbic, subcortical, and cortical pathways thought to be involved in MDD symptoms. In recent years, interest has gathered on large-scale, resting-state functional networks of the brain, including the default mode network (DMN) (Buckner et al., 2008; Raichle et al., 2001), which refers to distinct neuronal networks that show correlated, slowly oscillating BOLD signals during wakeful rest. The DMN overlaps with both the cognitive and affective network and includes the medial prefrontal cortex (PFC), ventral anterior cingulate cortex (ACC), posterior cingulate cortex (PCC)/precuneus, and lateral and inferior parietal cortices, all of which shows abnormal resting-state functional connectivity in depressed patients (Dutta et al., 2014; Kaiser et al., 2015; Sheline et al., 2009). These frontal-subcortical circuits are important for executive cognitive functions, mood, emotion, social behavior, and motivation (Salloway et al., 2001), and may therefore be involved in the psychopathologic features of MDD.

We selected regions of interest (ROIs) according to the main cortical and subcortical/limbic structures of both the DMN and the limbic-cortical dysregulation model of depression as illustrated in Figs. 1 and 2. To define the ROIs, we used an anatomical atlas, which is part of the non-linear Montreal Neurological Institute (MINI ICBM152 template (Fonov et al., 2011), and a Brodmann atlas, imported from the MRCro software (Rorden and Brett, 2006) and adapted to the MINI template.

2.4. Image analysis

T1-weighted images were preprocessed using the framework described by Aubert-Broche and colleagues (Aubert-Broche et al., 2013). Briefly, images were denoised (Coupe et al., 2008), bias field corrected (Sled et al., 1998), rigidly (Collins et al., 1994) and non-rigidly (Collins et al., 2013a).
Fig. 1. Illustration of the cortical ROIs, here listed numerically after the roughly corresponding Brodmann areas (Brodmann and Gary, 2006): Premotor frontal cortex and supplementary motor area (confined as BA6), the dorsolateral PFC (BA9 + 46), the anterior/ventromedial PFC (BA10), the orbitofrontal cortex (BA11), the lateral temporal cortex (BA21), the posterior cingulate cortex (BA23 + 31), the ventral ACC (BA24), the subgenual ACC (BA25), the retrosplenial cingulate cortex (BA29 + 30), the dorsal ACC (BA32), the lateral/inferior parietal cortex (BA39 + 40), and insulate cortex. ACC = anterior cingulate cortex; BA = Brodmann areas; PFC = prefrontal cortex.

Fig. 2. Illustration of the subcortical/limbic ROIs, including the caudate nucleus (magenta), putamen (purple), globus pallidus (light blue), subthalamic nucleus (turquoise), thalamus (green), hippocampus (orange), and amygdala (dark orange with green border). In addition, we included the insulate cortex (yellow), brainstem (white) and cerebellum (red). ROIs = regions of interest.
and Evans, 1997) registered to MNI space, and skull-stripped (Fiskildsen
et al., 2012). Brain tissue was classified into gray matter, white matter,
and cerebrospinal fluid using an artificial neural network classifier
(Zijdenbos et al., 1994) and merged with an atlas in MNI space through
the non-rigid transformations to obtain subject-specific ROIs (Collins
et al., 1999). Perfusion images were corrected for possible motion
artifacts using SPM version 8 (http://www.fil.ion.ucl.ac.uk/spm) and
co-registered to the T1-weighted images using a rigid body trans-
formation. Atlas ROIs in MNI space were mapped onto each perfusion
parameter map in perfusion native space using the inverse of the
non-linear and linear transformations.

2.5. Perfusion parameter analysis

Perfusion-weighted images follow the retention of intravenously
injected gadolinium-based contrast agent in the cerebral vasculature
over time. The area under each image voxel’s concentration-time-curve
(CTC) is proportional to local cerebral blood volume (CBV), and
correction (so-called deconvolution) of the CTC for the shape of the CTC
in the supplying artery (the so-called arterial input function) creates an
impulse response curve. The height of this curve is proportional to CBV,
which determines oxygen delivery, while the shape of the curve holds
information on the retention of blood in the voxel’s vasculature – and
therefore be taken as a proxy for capillary density, and SE-based CTH is
assumed to most closely reflect capillary CTH.

A perfusion sequence is most sensitive to contrast agent in capillary-sized vessels (Boxerman et al., 1995). SE-based CBV can therefore be taken as a proxy for capillary density, and SE-based CTH is tested against other ROIs – see discussion in Mouridsen et al. (2014). Gradient echo-based perfusion-weighted imaging is sensitive to contrast agent in all vessels within the voxel, irrespective of size. Thus, GRE-based CBV reflects total blood volume, while GRE-based MTT reflects blood’s transit through small arteries, arteri-
able, capillaries, venules, and small veins. In terms of CTH and RTH, our
knowledge of the relative sensitivities of SE- and GRE-based estimates to
microvascular flow disturbances remains limited. Thus, GRE-based CTH
and RTH estimates have proven more sensitive than GRE-based indices
towards early microvascular flow disturbances in preclinical Alz-
heimers’ disease (Fiskildsen, 2017; Nielsen et al., 2017, 2020). For
completeness, we report both SE- and GRE-based values in this study,
which includes both cerebral, cerebellar, and brainstem ROIs, and report
elevated RTH values as capillary dysfunction, irrespective of sequence.

Our physiological model (Jespersen and Ostergaard, 2012) allows us
to convert estimates of MTT and CTH into predictions of oxygen
extraction fraction (OEF) and the metabolic rate of oxygen (CMRO2),
assuming that oxygen transport in tissue is in a steady state (net oxygen
equation equals oxygen metabolism) at a tissue oxygen tension of 25
mmHg. The model calibration step assumes that OEF in each subject’s
normal-appearing white matter was 30% while CMRO2 was calculated as
OEF×CBF. Finally, CBV, CBF, and CMRO2 measurements were
normalized to whole-brain values (termed nCBF, nCBV, and nCMRO2);
whole-brain data for these parameters showed no significant differences
between groups (data not shown). Our hemodynamic estimates cannot
measure ‘true’ OEF and CMRO2. Instead, elevated OEF or low CMRO2
indicate whether blood supply and/or the capillary distribution of blood
are indicative of low oxygen availability in brain tissue under standard-
ized conditions. If indeed oxygen availability is limited, oxygen tension
(PO2) would be expected to fall, reaching a steady state between oxygen
utilization and (higher) OEF. We estimated PO2 by assuming a normal,
resting CMRO2 of 2.5 mL/100 mL/min (Sette et al., 1989), a capillary
blood volume of CBV=1.6%, and calibrated the rate constant k, which is
necessary to model the bidirectional oxygen transport between blood and
tissue, by requiring that the OEF is 0.3 and PO2=25 mmHg in
whole-brain white matter. If the PO2 required to achieve this oxygen
utilization was low, local hemodynamics were taken to indicate tissue
hypoxia. Since SE data more closely reflect capillary hemodynamics, the
calculations of OEF, CMRO2, and PO2 was applied only to SE data.

2.7. Statistics

Statistical analyses were carried out using Stata release 13 (Stata
Corp LP, College Station, Texas, USA). Differences between patients and
controls in socio-demographic data and clinical variables were evalu-
ated with the Wilcoxon two-sample rank-sum test for continuous vari-
ables and Fisher’s exact test for categorical variables. Perfusion
parameters for each ROI were compared between groups using multiple
linear regression after checking for Gaussian distribution and possible
outliers. A p-value <0.05 was considered statistically significant.

3. Results

Demographic and clinical characteristics of the patients and controls are
presented in Table 1. The two groups were comparable for age,

| Patients (n = 22) | Controls (n = 22) | Statistics |
|-----------------|-----------------|------------|
| Mean | SD | Mean | SD | z | p |
| age (years) | 57.4 | 4.6 | 59.2 | 7.3 | 0.47 | 0.64 |
| gender (female) | 15 | 68.2 | 15 | 68.2 | 1.90 | 0.05 |
| hypertension smoking | 7 | 31.8 | 6 | 27.3 | 0.74 | 0.02* |
| current | 11 | 50.0 | 3 | 13.6 | 0.02* |
| past | 8 | 36.4 | 9 | 40.9 | 1.00 |
| never | 3 | 13.6 | 10 | 45.5 | 0.05 |
| diabetes | 1 | 4.6 | 1 | 4.6 | 1.00 |
| cardiovascular disease | 3 | 13.6 | 0 | 0 | 0.07 |

*p<0.05.
3.1. Perfusion findings

Two patients did not complete the SE perfusion-weighted sequence; one due to patient discomfort and one due to technical problems. Data from one control was excluded as an outlier because of image artifacts. Hence, GRE perfusion data were available for 22 patients and SE data for 20 patients. Both GRE and SE perfusion data were available for 21 controls. Tables 2 and 3 summarizes the significant results from GRE and SE perfusion analyses, respectively. Perfusion results from all ROI analyses are listed in Supplementary Tables 1 and 2, including the total number of subjects for each comparison (some values were omitted due to image artifacts as described above). With the approach stated in Section 2.6, we identified five different patterns in our perfusion data, which we summarize below:

3.1.1. Capillary dysfunction (increased RTH)

Gradient echo perfusion data showed significantly higher RTH in the left and right anterior PFC (BA10) and ventral ACC (BA24) and in the left intermediate frontal area / frontal eye fields (BA8), left dorsal ACC (BA32) and left insulate cortex in patients compared to controls (Table 2). These results remained significant after adjusting for smoking (current, past, or never smoking), cf. Supplementary Table 3. Additionally, SE perfusion data showed significantly higher RTH in the left thalamus and left posterior cingulate (BA23-31) in the patient group (Table 3), but these results did not remain significant after adjusting for smoking (Supplementary Table 3).

3.1.2. Reduced capillary density, CBF, CMRO₂, and P〇₂, with preserved capillary function

We found significantly reduced SE-based nCBV, nCBF, nCMRO₂, OEF and P〇₂ in the subthalamic nucleus and globus pallidus bilaterally in patients versus controls. In addition, SE-based nCBV, nCMRO₂, OEF, and P〇₂ were significantly reduced in the right putamen, and nCMRO₂ and P〇₂ significantly reduced (nCBV only marginally) in the left putamen. The majority of findings stayed significant after adjustment for smoking, although only marginally for nCBF (Supplementary Table 4). Meanwhile, RTH in the same regions was either significantly reduced in the patient group or equal between groups, indicating preserved capillary function. It is generally accepted that capillary density and resting CBF is closely consistent with a shorter-lasting increase in metabolic activity. It is generally accepted that capillary density and resting CBF is closely consistent with a shorter-lasting increase in metabolic activity. This pattern is consistent with long-term adaptation of the cerebellar microvasculature and blood flow to a higher metabolic rate. The brain stem shows hyperemia accompanied by ‘physiological’ homogenization (i.e., reduced SE-based CTH) but no adaptation of capillary density, consistent with a shorter-lasting increase in metabolic activity.

3.1.3. Increased capillary density, CBF, and preserved capillary function

Gradient echo-based nCBF was significantly higher in both the cerebellum and brainstem in patients compared to controls (Table 2), although only with a tendency towards significance after adjusting for smoking (Supplementary Table 3). Meanwhile, SE-based nCBV was significantly elevated in the cerebellum and SE-based CTH was significantly reduced in the brainstem (Table 3) and stayed significant after adjusting for smoking (Supplementary Table 4); also SE-based CTH was significantly reduced in the brainstem after adjustment for smoking. This pattern is consistent with long-term adaptation of the cerebellar microvasculature and blood flow to a higher metabolic rate. The brain stem shows hyperemia accompanied by ‘physiological’ homogenization (i.e., reduced SE-based CTH) but no adaptation of capillary density, consistent with a shorter-lasting increase in metabolic activity.

3.1.4. Isolated perfusion findings

Gradient echo-based nCBF was significantly reduced in the left premotor frontal cortex (PMFC) and supplementary motor area (SMA) (both regions combined in BA6) and nCBV significantly reduced in the left retrosplenial cingulate cortex (BA29+30) in patient group compared to controls.
### Table 3

| Region of interest (ROIs) with significant differences in perfusion parameters between patients and controls for spin echo patients versus controls. **BA** = Brodmann area, **CHT** = capillary transit time heterogeneity; **MTT** = mean transit time; **nCBF** = normalized cerebral blood flow; **nCBV** = normalized cerebral blood volume; **nCMRO2** = normalized metabolic rate of oxygen; **OEF** = oxygen extraction fraction; **PFC** = prefrontal cortex; **P** = oxygen tension; **ROIs** = regions of interest; **RTH** = relative transit time heterogeneity. | \( n \) | \( \mu \) | \( \text{SEM} \) | \( \text{p} \) | \( \text{RTH} \) | \( \text{CBF} \) | \( \text{CBV} \) | \( \text{MTT} \) | \( \text{CTH} \) | \( \text{nCMRO2} \) | \( \text{OEF} \) | \( \text{RTH} \) | \( \text{P} \) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ROIs | | | | | | | | | | | | | |
| Cerebellum | | | | | | | | | | | | | |
| Cerebellum - left | 39 | 0.39 | 0.052 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Cerebellum - right | 40 | 0.31 | 0.049 | 0.71 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Brainstem | | | | | | | | | | | | | |
| Brainstem - left | 39 | 0.48 | 0.067 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Brainstem - right | 40 | 0.48 | 0.067 | 0.71 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Subcortical | | | | | | | | | | | | | |
| Caudate - left | 41 | 0.98 | 0.33 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Caudate - right | 40 | 1.82 | 0.067 | 0.84 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Putamen - left | 41 | 0.98 | 0.33 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Putamen - right | 40 | 1.82 | 0.067 | 0.84 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Thalamus - left | 41 | 0.98 | 0.33 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Thalamus - right | 40 | 1.82 | 0.067 | 0.84 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Subthalamic nucleus - right | 40 | 0.59 | 0.052 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Globus pallidus - left | 40 | 0.59 | 0.052 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Globus pallidus - right | 39 | 0.59 | 0.052 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Cortical | | | | | | | | | | | | | |
| Posterior cingulate (BA31) - left | 41 | 0.59 | 0.052 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Posterior cingulate (BA31) - right | 40 | 0.59 | 0.052 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Retrosplenial cingulate (BA29) - left | 40 | 0.59 | 0.052 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |
| Retrosplenial cingulate (BA29) - right | 40 | 0.59 | 0.052 | 0.69 | 0.06 | 0.05 | -0.16 | 0.105 | 0.80 | 0.041 | -0.44 | 0.041* | 0.53 | 0.002* |

4. **Discussion**

In this study, we extend previous reports on cerebral perfusion changes in MDD by taking capillary function into account when examining patients with late-onset MDD compared to age- and gender-matched controls. The principal findings of our study are (i) microvascular changes consistent with capillary dysfunction in anterior aspects of the DMN and (ii) hypoperfusion with preserved capillary function in major relay stations of the cortico-limbic pathways in depressed patients compared to controls. In addition, we demonstrated (iii) a pattern consistent with hyperactivity of the brainstem and cerebellum in the patient group.

#### 4.1. Capillary dysfunction

Capillary dysfunction affects oxygen extraction efficacy, and neurovascular coupling mechanisms must therefore modify CBF and CBF responses to meet the metabolic needs of brain tissue. We have proposed that slight reductions in oxygen extraction efficacy (i.e., mild capillary dysfunction) may be counteracted by augmented CBF and CBF responses, while more severe capillary dysfunction can only be compensated for by attenuating CBF and CBF responses to limit functional shunting (Ostergaard et al., 2013b). Attenuated CBF- and blood oxygenation changes tend to reduce BOLD signal amplitudes during changes in brain activity, and BOLD responses or fluctuations would therefore be expected to be smaller in MDD patients although brain activity changes are not; see Angleys et al. (2018) for a comprehensive analysis of the effects of CTH changes on the BOLD signal. Sheline et al. (2009) found reduced BOLD changes in the DMN of depressed patients compared to controls during passive observation / active reappraisal of pictures with negative content in ventromedial PFC (BA10), ACC (BA 24/32), lateral parietal cortex (BA39), and lateral temporal cortex (BA21). A recent meta-analysis by Zhou et al. (2020) showed association between subregional DMN activation and rumination, which is strongly correlated to depression. In general, functional imaging studies show hypoactivation during depressed state in structures involved in the cognitive control system in MDD, including the dorsal/pregenual ACC, dorsolateral PFC, insulate cortex, inferior parietal regions, and cerebellum, whereas structures involved in the affective network in MDD, including the ventral ACC, the amygdala, and portions of the OFC and basal ganglia, typically demonstrate increased resting-state metabolism and hyperactivation during depressed state (Fitzgerald et al., 2008; Gunning and Smith, 2011). However, there is some evidence that these patterns of activity may change with increasing age, such as hypoactivation of the ventromedial PFC in late-life depression (Brassen et al., 2008). Our finding of capillary dysfunction in the anterior/ventral aspects of the DMN implies that findings of reduced CBF and CBF responses in this area may be related to either or both reduced neuronal activity and altered neurovascular coupling as a result of capillary dysfunction. Our results thus indicate that caution should be taken when interpreting functional MRI brain activation patterns that rely on blood flow changes in terms of the underlying pathophysiology in conditions with capillary dysfunction.

#### 4.2. Changes in capillary density, CBF, and/or CMRO2, and \( P_{O2} \) with preserved capillary function

In brain regions without capillary dysfunction, CBF and BOLD changes would be expected to reflect the underlying neuronal activity as
previously assumed. Still, our observations of changes in capillary density, possibly to adapt to changes in brain metabolism, may hold clues to changes in brain function that occurred prior to our patients’ first depressive episode. Angioadaptation is understudied in humans but would be expected to take months. Our findings of low nCBV and nCBF in the subthalamic nucleus and globus pallidus and the same tendency in the right putamen (Table 3) could therefore reflect longlasting, reduced activity that abatedted our patients’ first admission. The basal ganglia modulate movement as well as emotional and cognitive behavior through cortical and limbic circuits, including the limbic-cortical-striatal-pallido-thalamic circuitry. Metabolic and CBF abnormalities of these structures have previously been described in MDD – see e.g. reviews by Drevets (2000), Sheline (2003) and Videbech (2000).

Similarly, the cerebellar changes suggest longlasting hyperactivity, consistent with earlier PET studies (Bench et al., 1992; Dolan et al., 1992; Videbech et al., 2002). Besides an extensive motor network, the cerebellum has complex connections with the limbic system, prefrontal cortex, subcortical nuclei, and brain stem nuclei (Rapport et al., 2000), the latter being a main center for monoamine release to the rest of the brain. The functional hyperemia may thus be part of a compensatory response, as proposed by Guo et al. (2013), to accommodate to depressive changes in cognitive and emotional control through dorsal and ventral circuitries.

4.3. Isolated perfusons findings

Evidence of longlasting hypoactivity in terms of reduced capillary density (i.e., reduced nCBV) was observed in the left OFC (BA11) and the adjacent left ventral frontal cortex (BA47) in the patient group, but without significant differences in nCBF and nCMRO₂, suggesting that activity may have increased in relation to the depressive episode. The OFC is involved in emotional expression, memory, and reward-related activity (Salloway et al., 2001). In MDD, metabolic and CBF abnormalities of the OFC have included reports of increased metabolism during the depressed state relative to remission, although these changes seemingly vary according to OFC subregion (Drevets, 2007). Due to our cross-sectional study design and relative broad definition of the OFC (i.e., BA11), our findings in the left OFC are difficult to compare with the existing literature. However, our results imply both state- and trait-related microvascular changes in the left OFC in MDD, and future studies may add more knowledge to this phenomenon.

Conversely, areas where CBF was altered but CBV unaltered, CBF changes are more likely to reflect activity changes that occurred in relation to the current depressive episode, such as for example the increase in brainstem nCBF, which has also been reported in earlier studies (Bench et al., 1992; Dolan et al., 1992; Videbech et al., 2002). However, cross-sectional studies fall short when it comes to interpreting whether reported changes in perfusion parameters occur in close time relation to a clinical depressive episode or antedate a depressive episode by e.g. months. Future longitudinal functional imaging studies taking capillary density and (dys)function into account may shed more light on this state versus trait issue in MDD.

4.4. Cause of capillary dysfunction

The cause of capillary dysfunction is not yet fully established – see Østergaard et al. (2018) for a review of putative sources in stress and depression. Cardio- and cerebrovascular risk factors, such as age, hypertension, diabetes, and smoking variably affect capillary morphology and blood rheology. The putative sources of capillary dysfunction in these factors include pericyte degeneration and pericyte loss, endothelial swelling, change in capillary morphology, thickening of basement membranes, and hyperviscosity – for an overview see Østergaard et al. (2015) and Østergaard (2020). Pericytes have been shown to be involved in the regulation of CBF and CTH (Hall et al., 2014), and their function may be hampered by oxidative stress and neuroinflammatory processes in the neurovascular unit (Najjar et al., 2013). As part of an inflammatory response, pro-inflammatory cytokines are believed to play an important role in the pathophysiology of depression by affecting serotonin (5-HT) metabolism and glucocorticoid secretion, the latter by activation of the hypothalamic-pituitary-adrenal (HPA) axis (Janssen et al., 2010). Taylor et al. incorporated inflammatory processes in a model of late-life depression in which vascular disease and vascular risk factors are important contributors, resulting in impaired cerebral hemodynamics and autoregulation (Taylor et al., 2013). Despite a similar composite vascular risk factor score (Dalby et al., 2015a), our patient group had significantly more current smokers than the controls. Cigarette smoking causes damage to endothelial cells (Chang et al., 2014), possibly through the effect of nicotine on the leukocyte-endothelium interactions via intercellular adhesion systems. Nicotine upregulates the expression of adhesion molecules in the capillary endothelium (Albaugh et al., 2004) and induce leukocyte rolling and adhesion in the cerebral microcirculation (Yong et al., 1997) with resulting inflammation. Although adjusting for smoking did not alter our significant findings of capillary dysfunction in the anterior aspects of the DMN, we cannot preclude that smoking may be a co-factor for capillary dysfunction in late-onset MDD.

White matter hyperintensities of presumed vascular origin (Wardlaw et al., 2013) are a frequent finding on T2-weighted MRI of the aging brain and are regarded as an imaging marker of cerebral SVD (Wardlaw et al., 2015). A comprehensive meta-analysis by Shi et al. (2016) showed that high WMH load is associated with lower CBF globally in most gray and white matter regions, possibly confounded by age and atrophy and with reservations to the fact that areas of WMH was not separated from normal-appearing white matter in the contributing studies. As part of our cohort study, we tested for possible differences in regional WMH load between patients and controls (Dalby et al., 2019) and found an equal distribution of subcortical/deep WMHs in the major lobes between groups. In addition, our previous research has not shown any tract-specific localization of deep WMHs in depressed patients compared with controls (Dalby et al., 2010b). Hence, we have no compelling evidence that capillary dysfunction in localized areas of the frontal subcortical circuitries seen in our depressed patients is a result of regional or tract-specific white matter lesion load, although a common, etiologic pathway is evident.

4.5. Limitations

Implementation of an antidepressant wash-out period was not possible in our study due to ethical considerations. Consequently, all patients in our study were in current antidepressant medical treatment (except for one treatment-naïve patient at inclusion), but not yet remitted. Several studies have addressed changes in CBF and cerebral metabolism following effective pharmacological antidepressant treatment of depression, with normalization of frontal hypometabolism when patients are remitted as the most replicated finding (Fitzgerald et al., 2008; Gunning and Smith, 2011; Mayberg, 2003). However, controversies exist, especially in late-life depression, where age-related changes may be an important co-factor. Mayberg and colleagues showed that the effects of antidepressants (fluoxetine) on regional brain glucose metabolism vary over time and represent a complex combination of both normalization of pretreatment hypometabolism and new adaptive changes in regions without previous metabolic anomalies (Mayberg et al., 2000). In addition, involvement of neuronal circuitries and reversibility of CBF changes may be both state- and trait-dependant (Ishizaki et al., 2008). Unfortunately, the cross-sectional design of our study does not allow us to conclude on the complex interactions between pretreatment abnormalities, attempted compensatory responses, and actual treatment effects. However, the immunoregulatory effects of antidepressants warrant a comment. A wide range of studies have documented that antidepressants, regardless of type, decrease the release of pro-inflammatory cytokines, while increasing the synthesis of...
anti-inflammatory cytokines (Maes, 2001), with possible implications for capillary function. Hence, antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCIs) have been shown to affect capillary permeability, vascular responsiveness, CBF, and cerebral metabolism (Nobler et al., 2002), and may protect or even improve vascular endothelial function (Paranthaman et al., 2012).

Our study design is exploratory with a total of 44 ROIs, which introduces the caveat of multiple comparisons, and only few of the reported significant findings, such as in the basal ganglia, would survive a conservative Bonferroni-correction, which would imply a p-value < 0.001 (the corrected p-values are not shown). However, several of the reported findings are bilateral and consistent across different parameters, which speak against a statistical type 1 error. The statistical power is generally limited by our relatively small sample size.

5. Conclusion

By combining conventional CBF measurements with measurements of capillary flow heterogeneity, we were able to address microvascular function in late-onset MDD. Our data suggest that the activity changes previously reported in certain brain structures, based on CBF measurements, in part reflect altered neurovascular coupling as a result of capillary dysfunction. Accordingly, functional neuroimaging studies may underestimate neuronal activity in areas with reduced CBF and CBF responses in MDD due to changes in neurovascular coupling because of capillary pathology and should thus be interpreted with caution. As capillary dysfunction has been hypothesized to play a role in the development of neurodegeneration, our finding of capillary dysfunction in MDD may thus lead to an improved understanding of the link between MDD and dementia. Future studies should address the extent of capillary dysfunction in MDD and its clinical consequences.

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CRediT authorship contribution statement

Rikke B. Dalby: Data curation, Formal analysis, Conceptualization, Writing – review & editing. Simon F. Eskildsen: Formal analysis, Supervision, Writing – review & editing. Poul Videbech: Conceptualization, Writing – review & editing. Raben Rosenberg: Conceptualization, Writing – review & editing. Leif Østergaard: Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors have no competing or conflicting interests to declare. No pharmaceutical companies, including the supplier of contrast agent, and none of the grant contributors were involved in the design, organization, analysis, or preparation for publication of the study.

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Supplementary materials

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