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ORIGINAL ARTICLE

Aberrant Claustrum Microstructure in Humans after Premature Birth

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Abstract

Several observations suggest an impact of prematurity on the claustrum. First, the claustrum's development appears to depend on transient subplate neurons of intra-uterine brain development, which are affected by prematurity. Second, the claustrum is the most densely connected region of the mammalian forebrain relative to its volume; due to its effect on pre-oligodendrocytes, prematurity impacts white matter connections and thereby the development of sources and targets of such connections, potentially including the claustrum. Third, due to its high connection degree, the claustrum contributes to general cognitive functioning (e.g., selective attention and task switching/maintaining); general cognitive functioning, however, is at risk in prematurity. Thus, we hypothesized altered claustrum structure after premature birth, with these alterations being associated with impaired general cognitive performance in premature born persons. Using T1-weighted and diffusion-weighted magnetic resonance imaging in 70 very preterm/very low-birth-weight (VP/VLBW) born adults and 87 term-born adults, we found specifically increased mean diffusivity in the claustrum of VP/VLBW adults, associated both with low birth weight and at-trend with reduced IQ. This result demonstrates altered claustrum

microstructure after premature birth. Data suggest aberrant claustrum development, which is potentially related with aberrant subplate neuron and forebrain connection development of prematurity.

Key words: claustrum, cognitive difficulties, magnetic resonance imaging, mean diffusivity, premature birth, subplate neurons

Introduction

Premature birth is defined as birth before 37 weeks of gestational age (GA), with a worldwide prevalence of about 11% (Chawanpaiboon et al. 2019) and an increased risk for neurodevelopmental impairment and lasting cognitive difficulties (D'Onofrio et al. 2013; Breeman et al. 2015; Twilhaar et al. 2018). Brain aberrations in premature-born individuals have been identified on both microscopic and macroscopic levels (Woodward et al. 2006; Nosarti et al. 2008; Andiman et al. 2010; Ball et al. 2012, 2013; Kinney et al. 2012; Dean et al. 2013; Salmaso et al. 2014; Meng et al. 2016; Hedderich et al. 2019; Volpe 2019). Among microscopic changes, the impact on two large but transient cell populations of intra-uterine brain development stands out, namely on subplate neurons (SPNs), which are relevant for establishing thalamocortical and intra-cortical connectivity (Ghosh et al. 1990; Kostovic and Rakic 1990; Kanold and Shatz 2006; Kanold and Luhmann 2010; Hoerder-Suabedissen and Molnár 2015; Luhmann et al. 2018) and pre-oligodendrocytes (pre-OL), which are pre-cursers of myelin-producing oligodendrocytes of white matter connections (Back and Miller 2014; Back 2017; Volpe 2019). Among macroscopic brain changes, widely distributed impact on white matter structural connectivity (Ball et al. 2012; Meng et al. 2016; Menegaux et al. 2020), gray matter cortical volume and geometry (Nosarti et al. 2008, 2014; Hedderich et al. 2019; Hedderich et al. 2020b), and gray matter subcortical nuclei structure and connectivity has been reported so far (Cole et al. 2015; Scheinost et al. 2017; Aanes et al. 2019; Hedderich et al. 2020a; Schmitz-Koep et al. 2021). Surprisingly, among non-cortical gray matter structures, which have been demonstrated as being affected by prematurity, the claustrum is not yet mentioned. In humans, the claustrum is a thin layer of gray matter subjacent to the insular cortex, located between the external and the extreme capsule (Crick and Koch 2005; Mathur 2014; Smythies et al. 2014). Several reasons support the suggestion that the claustrum is affected by premature birth.

First, to start simple with systematics, while the impact of premature birth on gray matter development is regionally distinct for cortical regions, its impact on subcortical nuclei is noticeably consistent ranging from thalamic nuclei (Ball et al. 2015) to hippocampal subfields (Hedderich et al. 2020a), amygdala (Schmitz-Koep et al. 2021), hypothalamus (personal observation), neuromodulatory nuclei (Grothe et al. 2017), and the striatum (Meng et al. 2016), being adjacent to the claustrum. Therefore, it is at least a matter of systematics to test whether the subcortical claustrum is also affected by prematurity.

Second, more mechanistically, the cellular claustrum development is suggested to depend on SPN development (Watson and Puelles 2017; Bruguier et al. 2020). SPNs such as transient cell population of intra-uterine brain development exhibit peak volumes between weeks 28 and 34 coinciding with premature birth (Ghosh et al. 1990; Kostovic and Rakic 1990; Kanold and Shatz 2006; Kanold and Luhmann 2010; Hoerder-Suabedissen and Molnár 2015; Luhmann et al. 2018). Due to special vulnerability for even transient perinatal hypoxic-ischemic events of premature birth (McQuillen et al. 2003; McClendon et al. 2017),SPNs have been identified as a key pathophysiological pathway for aberrant brain development in prematurity (Volpe 1996; McQuillen and Ferriero 2005; Kinney et al. 2012). Remarkably, distinct populations of SPNs link with claustrum development via their developmental trajectory (Arimatsu et al. 2003; Puelles 2014; Watson and Puelles 2017; Bruguier et al. 2020). Similar to SPNs, claustrum cells arise very early during gestation and cell-tracing studies have identified a subplate part of the claustrum anlage (i.e., first appearing brain parts, which develop to the claustrum) and claustrum proper (i.e., the core of the mature claustrum; in contrast to, for example, the endopiriform nucleus) through gene expression of the SPN-specific gene *Nr4a2* in the developing and mature claustrum (Arimatsu et al. 2003; Hoerder-Suabedissen et al. 2009, 2013; Puelles 2014; Watson and Puelles 2017; Bruguier et al. 2020). Evidence exists that both the claustrum itself and its SPN-dependent development are conserved across mammals, motivating the translation of recent findings from mice to humans (Wang et al. 2010; Puelles 2014; Hoerder-Suabedissen and Molnár 2015; Duque et al. 2016; Bruguier et al. 2020). The link between the claustrum, SPNs, and prematurity suggests a potential relevance for the claustrum in prematurity.

Third, the mature claustrum is the most connected region in the mammalian forebrain in relation to its volume, including reciprocal connections with most cortical areas such as primary, associative, and prefrontal regions (Mathur 2014; Goll et al. 2015; Torgerson et al. 2015; Brown et al. 2017; White et al. 2018; Krimmel et al. 2019). Via its strong impact on pre-OLs, which link with white matter connectivity as pre-cursers of later myelin-producing oligodendrocytes (Back 2017), prematurity impacts myelin-dependent brain connections and thereby the development of sources and targets of such connections (Ball et al. 2015; Volpe 2019). Therefore, due to its high degree of connectivity, the claustrum might be affected by premature birth.

Fourth, the claustrum might be relevant for the increased risk for impaired general cognitive performance in prematurity. Due to its strategic connectivity, the claustrum has been suggested to be involved in basic cognitive functions, particularly both selective attention and task switching/maintaining (Mathur 2014; Goll et al. 2015; Torgerson et al. 2015; Brown et al. 2017; White et al. 2018; Krimmel et al. 2019). For example, in line with theoretical models in mice (Brown et al. 2017), the claustrum amplifies top-down signals of cognitive control from the anterior cingulate to distributed cortical regions and in humans (White et al. 2018), the claustrum is involved in task-onset control across different cognitive task settings (Krimmel et al. 2019). General cognitive functioning, however, is at risk after premature birth with on-average about 12 points lower IQ in adults after very premature birth (i.e., birth before 32 weeks of gestation and/or lower birth weight than 1500g) (Twilhaar et al. 2018; Wolke et al. 2019), suggesting a potential link between altered claustrum and impaired cognitive functioning in prematurity.

Based on these four points, we hypothesized that claustrum structure is impaired after premature birth and such impairment links with deficits in general cognitive functioning. To test these hypotheses, we assessed very premature- and

mature-born adults, namely 70 very preterm/very low-birth-weight (VP/VLBW) born and 87 term-born adults of the population-based Bavarian Longitudinal Study (BLS), with structural and diffusion-weighted magnetic resonance imaging (MRI) (DWI), manual expert MRI annotations of the claustrum, and assessment of general cognitive performance. We assessed claustrum volume by T1-weighted imaging and its microstructure by mean diffusivity (MD) derived from DWI, a measure of cellularity which is sensitive for microstructural aberrations (Le Bihan 2003), and cognitive performance by full-scale IQ.

Materials and Methods

Participants

The participants examined in this study are part of the BLS, a geographically defined, population-based sample of neonatal at-risk children, i.e. born very preterm (VP) <32 weeks GA and/or with very low birth weight (VLBW) <1500 g, and healthy full term (FT) controls who were followed from birth into adulthood (Riegel et al. 1995; Wolke and Meyer 1999). Detailed numbers of included study participants and included imaging procedures passing quality control can be found in Supplementary Figure S1. The final sample numbers were 70 VP/VLBW born adults and 87 term-born adults, who were assessed by structural MRI, diffusion-weighted MRI, and cognitive testing. The study was carried out in accordance with the Declaration of Helsinki and was approved by the local institutional review boards. Written consent was obtained from all participants.

Birth-related Variables

GA was estimated from maternal reports on the first day of the last menstrual period and serial ultrasounds during pregnancy. In cases where the two measures differed by more than two weeks, clinical assessment at birth with the Dubowitz method was applied (Dubowitz et al. 1970). Maternal age, infant birth weight (BW), and intensity of neonatal treatment (INTI) were obtained from obstetric records (Riegel et al. 1995; Gutbrod et al. 2000).

Neurocognitive Assessment

Participants were assessed using an abbreviated version of the German Wechsler Adults Intelligence Scale, Third edition (WAIS-III) (von Aster et al. 2006) and full-scale intelligence quotient (FS-IQ) was calculated.

MRI Data Acquisition

The MRI examinations took place at two sites (Department of Neuroradiology, Technical University Munich (n = 145) and the Department of Radiology, University Hospital of Bonn (n = 67)) on either a Philips Achieva 3T or a Philips Ingenia 3T system using 8-channel SENSE head-coils. Participants were distributed across scanners as follows: Bonn Achieva: 4 VP/VLBW, 9 FT; Bonn Ingenia: 28 VP/VLBW, 14 FT; Munich Achieva: 36 VP/VLBW, 50 FT; Munich Ingenia: 2 VP/VLBW, 14 FT. The image protocol included a high-resolution T1-weighted, 3D-MPRAGE sequence (TI = 1300 ms, TR = 7.7 ms, TE = 3.9 ms, flip angle 15°; field of view: 256 mm × 256 mm) with a reconstructed isotropic voxel size of 1 mm^3 and Diffusion Tensor Imaging. Diffusion weighted images were acquired using a single-shot spin-echo echo-planar imaging sequence, resulting in one non-diffusion weighted image (b = 0 s/mm^2) and 32 diffusion weighted images (b = 1000 s/mm^2, 32 noncollinear gradient directions) covering whole brain with following parameters: echo time (TE) = 47 ms, repetition time (TR) = 20,150 ms, flip angle = 90°, field of view = 224 × 224 mm^2, matrix = 112 × 112, 75 transverse slices, slice thickness = 2 mm, and 0 mm interslice gap, voxel size = 2 × 2 × 2 mm^3.

To account for possible confounds by the scanner-specific differences, all statistical analyses included categorical dummy regressors for scanner identity as covariates of no interest. Across all scanners, sequence parameters were kept identical. Scanners were checked regularly to provide optimal scanning conditions. MRI physicists at the University Hospital Bonn and Klinikum rechts der Isar regularly scanned imaging phantoms, to ensure within-scanner signal stability over time. Signal-to-noise ratio (SNR) was not significantly different between scanners (one-way ANOVA with factor "scanner-ID" [Bonn 1, Bonn 2, Munich 1, Munich 2]; $F(3,182) = 1.84$, $P = 0.11$).

Claustrum Segmentation

The claustrum was segmented manually by an experienced neuroradiologist (D.M.H.) on 3D T1-weighted images after spatially adaptive nonlocal means denoising (Manjon et al. 2010). Tracings were performed on a Wacom Intuos M tablet (Wacom Co., Ltd., Kazo, Saitama, Japan) using ITK-SNAP version 3.4.0 (www.itksnap.org) (Yushkevich et al. 2006) following a predefined manual claustrum segmentation protocol, modified from Davis (2008). Manual segmentations included the dorsal and the ventral claustrum. Examples and detailed information on claustrum segmentations, including the manual segmentation protocol and metrics of intrarater reliability can be found in the Supplemental Information and Figure 1A. In brief, the claustrum was segmented in axial and coronal orientations at optimal image contrast. Intra-rater reliability was assessed in a random subset of 18 individuals by means of modified Hausdorff distance (HD) showing good intra-rater reliability. As a control region, a region-of-interest was placed manually central in the left putamen.

Diffusion Weighted Data Preprocessing and Mean Diffusivity Calculation

Both preprocessing and quality check of diffusion data were previously described in Stampfli et al. (2019) and Menegaux et al. (2020). First, denoising of the raw data was performed using the "dwidenoise function" from the MRtrix3 software package (http://www.mrtrix.org). Diffusion weighted data were then corrected for eddy-current and motion-induced distortions by registration of the diffusion weighted images to the b0 image using the dwipreproc routine from MRtrix3. This function makes use of the eddy tool implemented in FSL (FMRIB, Oxford, UK version 6.0.0). The brain extraction tool (BET) from FSL was then applied to remove non-brain tissue and estimate the inner and outer skull surfaces. Prior to the calculation of MD maps, the diffusion-weighted data were corrected for susceptibility-induced distortions using the bdp correction algorithm implemented in the BrainSuite software package (http://brainsuite.org) (for more details see the method described in Bhushan et al. (2015). In brief, the information from each subject's MPRAGE image is used to estimate a deformation map and un-distort the diffusion data via constrained nonrigid registration. Each unwarped diffusion image was then visually inspected. FA and MD maps were then calculated using the DTIFIT tool implemented in FSL. As a next

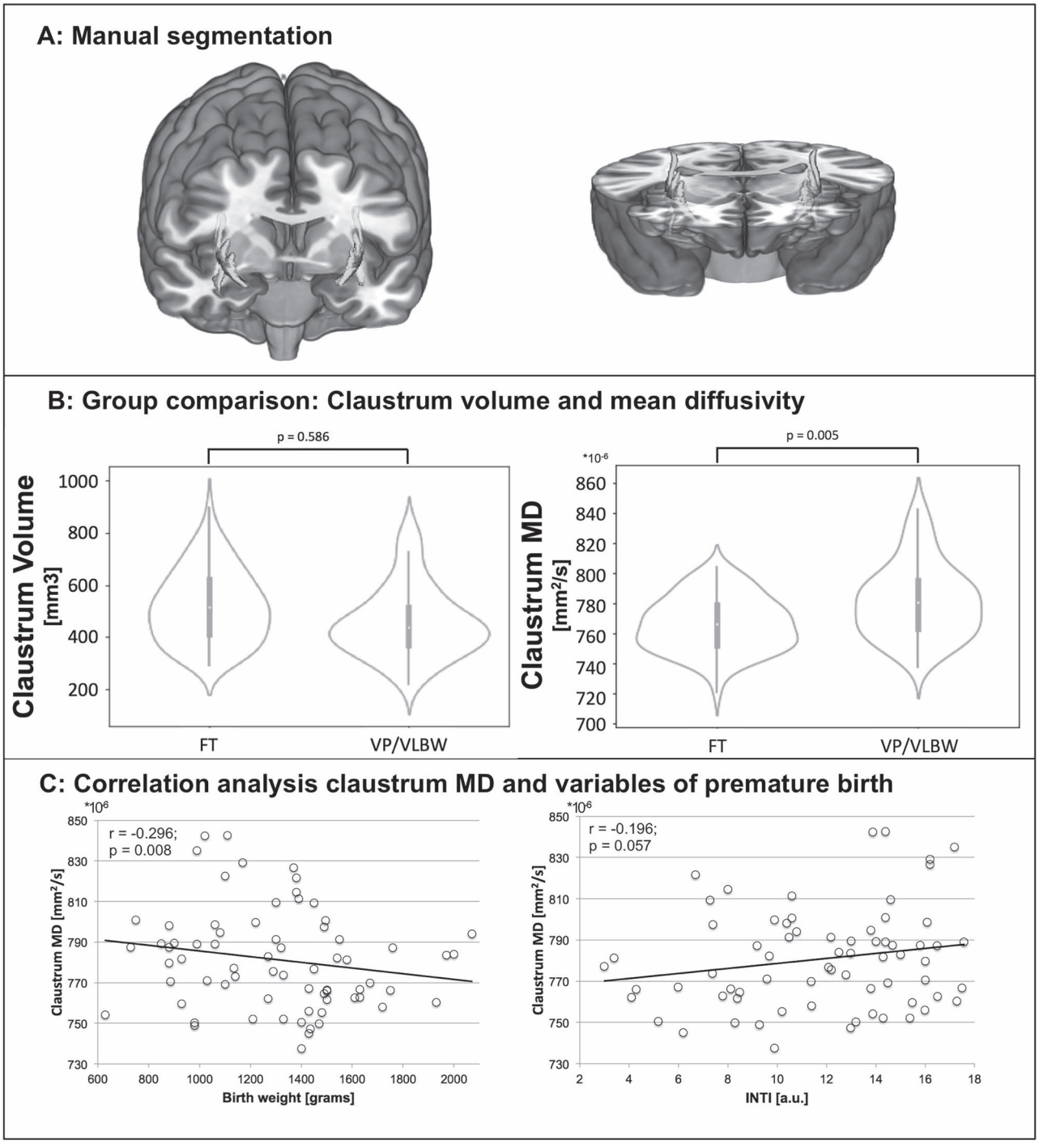

Figure 1. Claustrum anatomy, manual segmentation, group comparison and correlation with perinatal variables

A: Three-dimensional rendering of manual annotation depicts the anatomical localization of the sheet-like claustrum between the basal ganglia and the insular cortex.

B: Left panel: Violin plots of bilateral claustrum volumes derived from manual segmentations and divided by total intracranial volume (TIV) are shown for VP/VLBW and FT individuals. There was no significant group difference for normalized claustrum volumes between VP/VLBW and FT individuals ($P = 0.586$).

Right panel: Violin plots of bilateral claustrum mean diffusivity (MD) are shown for VP/VLBW and FT individuals. Claustrum MD is significantly elevated in VP/VLBW compared to FT individuals ($P = 0.005$).

C: Associations of premature birth variables with claustrum microstructure measured by MD. Claustrum MD is plotted against birth weight (BW) and intensity of neonatal treatment (INTI) in premature-born adults. Plots include linear regression trend lines. Correlation coefficient r and associated P values are given from nonparametric partial correlation correcting for scanner.

Abbreviations: DWI: Diffusion weighted imaging; FT: full term; g: grams; MD: mean diffusivity; T1w; T1-weighted; TIV; total intracranial volume; VP/VLBW: very preterm and/or very low birth weight.

step, the quality assessment of all diffusion datasets was performed based on the following criteria: 1) Calculation of tensor residuals for each diffusion direction and visual inspection of the nine slices with highest residuals in the whole diffusion dataset, 2) Mean intensity plots for each diffusion direction and non-diffusion-weighted images were derived and plotted slice by slice in sagittal, axial and coronal direction. Head motion normally induces peaks, which can easily be spotted on these plots. Based on these criteria and additional visual inspection of DWI data, each participant was classified as having no, slight, or strong artifacts. Only participants with no artifacts were kept thus leading to the exclusion of 19 participants due to motion artifacts, 16 because of insufficient fat suppression (ghosting) artifacts, and one due to corrupted data.

Extraction of Mean Diffusivity within the Claustrum

In order to extract mean MD values within the claustrum, b0 images were coregistered to its individual structural space with FSL FLIRT, using linear registration with a trilinear interpolator and parameters were saved to coregister MD and FA maps to T1-weighted imaging. Segmentation masks of the claustrum were overlaid on MD and FA maps and mean values were extracted.

Statistical Analysis

Statistical analyses were carried out using SPSS (IBM SPSS Statistics, Version 25). General linear models were used to determine whether premature birth status is a significant factor for claustrum volume, MD, FA, and other brain regions MD (the latter two variables concern control analyses; other brain regions concern thalamus, putamen, insular, prefrontal, and somatosensory cortices, defined by "Hammersmith" atlas (Hammers et al. 2003)). Analyses included TIV and scanner as covariates of no interest. Nonparametric partial correlation analyses, corrected for scanner and restricted to the VP/VLBW group, were used to examine the association between claustrum MD and perinatal variables (GA, BW, INTI) and FS-IQ. Statistical significance was set at $P < 0.017$, with this level being corrected for three tests of two hypotheses. The tests concern the two group comparisons for claustrum volume and MD and the correlation analysis of aberrant claustrum structure and FS-IQ in premature born adults.

Results

A detailed description of participants can be found in Table 1. Participants do not differ for age (mean age 26 year) or sex at time of assessment; by design, premature born subjects had lower weight and gestational age at birth, higher intensity of neonatal care at birth, and about 10 points lower IQ at time of assessment.

Claustrum Macro- and Microstructure in Premature-Born Adults

In order to assess whether claustrum volumes differ between VP/VLBW individuals and FT controls, we used a general linear model approach, in which unnormalized claustrum volume was the dependent variable and independent variables were group, TIV, and scanner, with the last two variables correcting for total intra-cranial volume (TIV; in order to be independent of brain size) and scanner differences (in order to be independent of different scanner influences). The mean of left and right claustrum volumes was not different between groups (VP/VLBW: 477.9 ± 12.2 mm^3, FT: 487.3 ± 11.3 mm^3; $P = 0.586$; partial η^2=0.002) (Fig. 1B). This result suggests macroscopically unchanged claustrum after premature birth.

In order to test our hypothesis, namely whether claustrum microstructure differs between VP/VLBW and FT adults, we used a general linear model with bilateral claustrum MD as dependent variable, correcting for scanner and TIV. Claustrum MD values were significantly elevated in VP/VLBW adults (VP/VLBW: 778.4 ± 2.5 × 10^{-6} mm^2/s, FT: 768.6 ± 2.2 × 10^{-6} mm^2/s; $P = 0.005$; partial $\eta^2 = 0.051$) (Fig. 1B). To control this result further for potential confounds of scanner, we restricted the analysis to the largest group of VP/VLBW and FT assessed on only one scanner, which was the Munich Achieva sample with 36 VP/VLBW and 50 FT. For this subsample, we repeated group comparison analysis for claustrum MD by the use of a general linear model, in which bilateral claustrum MD was the dependent variable and independent variables were group and TIV. We found at-trend increased claustrum MD in the prematurity group (VP/VLBW: 773 +/− 3 mm-2/s; FT: 765 +/− 2.8 mm-2/s; $P = 0.054$; partial $\eta^2 = 0.046$), suggesting further that the finding of increased claustrum MD is not confounded by the use of different scanners. These results together indicate microscopically changed claustrum in human prematurity.

In order to ensure that elevated claustrum MD in VP/VLBW adults is not a nonspecific effect of generally altered diffusion measures derived from DWI, we tested for significant group differences of claustrum fractional anisotropy (FA) of water diffusion, using the same general linear model approach as for claustrum MD. We did not find significant claustrum FA group differences (VP/VLBW: 0.400 ± 0.003, FT: 0.403 ± 0.003; $P = 0.467$) (Please also see Figure S9 in Supplemental Material). Furthermore, to ensure that this change in *significance* of group effect on claustrum MD but not FA really reflects distinct group effects on claustrum MD and FA (Nieuwenhuis et al. 2011), we performed an analysis of covariance (ANCOVA) approach directly comparing these effects; we chose claustrum MD as dependent variable, group as independent one, and claustrum FA as co-variate (additionally we included scanner and TIV as nuisance factors). We found a significant main effect of group on claustrum MD ($P = 0.006$; partial $\eta^2 = 0.050$), indicating that, even when accounting for variance of claustrum FA and its interaction with group, the claustrum MD values are increased in premature born adults. This means that claustrum MD is specifically increased in prematurity with respect to other diffusion-derived measures of the claustrum.

In order to ensure that elevated claustrum MD in VP/VLBW adults is not a nonspecific effect of generally altered MD, we tested for significant group differences of MD in other gray matter subcortical and cortical regions such as putamen, insular, prefrontal, and somatosensory cortices, using the same general linear model approach as for claustrum MD. While we found significant group difference for insular MD (VP/VLBW: 904.9 ± 3.2 × 10^{-6} mm^2/s; FT: 880.7 ± 2.8 × 10^{-6} mm^2/s; $P < 0.001$; partial $\eta^2 = 0.174$), there were no significant group differences for prefrontal MD (VP/VLBW: 1000.5 ± 6.0 × 10^{-6} mm^2/s; FT: 992.8 ± 5.3 × 10^{-6} mm^2/s; $P = 0.361$; partial $\eta^2 = 0.007$), somatosensory MD (VP/VLBW: 972.3 ± 6.2 × 10^{-6} mm^2/s; FT: 959.5 ± 5.5 × 10^{-6} mm^2/s; $P = 0.138$; partial $\eta^2 = 0.016$), and left putamen MD (VP/VLBW: 709.1 ± 2.4 × 10^{-6} mm^2/s, FT: 707.8 ± 2.2 × 10^{-6} mm^2/s; $P = 0.700$; partial $\eta^2 = 0.001$). Please also see Figure S10

Table 1 Demographical, clinical, and cognitive data.

	VP/VLBW (n = 70)			FT (n = 87)			
	M	SD	Range	M	SD	Range	P value
Sex (male/female)	43/27			52/35			0.871
Age (years)	26.7	± 0.6	25.8–28.0	26.8	± 0.7	25.6–28.9	0.427
GA (weeks)	30.4	± 2.1	25–36	39.7	± 1.0	37–42	<0.001
BW (g)	1304	± 317	630–2070	3374	± 468	2120–4670	<0.001
Hospitalization (days)	73	± 26	24–141	7	± 3	2–26	<0.001
INTI	11.8	± 3.8	3–18	n.a.	n.a.	n.a.	n.a.
Maternal age (years)	29.3	± 5.0	16–38	29.3	± 5.1	18–42	0.976
Full-scale IQ[a] (a.u.)	93.8	± 12.2	64–125	103.2	± 12.2	77–130	<0.001

Notes: Statistical comparisons: sex, SES with χ^2 statistics; age, GA, BW, Hospitalization, maternal age, and IQ with two sample t-tests. Abbreviations: BW, birth weight; FT, full-term; GA, gestational age; INTI, intensity of neonatal treatment index; IQ intelligence quotient; M, Mean; maternal age, maternal age at birth; n.a., not applicable; VP/VLBW, very preterm and/or very low birthweight.
[a]Data are based on 67 VP/VLBW and 86 FT-born individuals.

in Supplemental Material. Furthermore, to ensure that these changes in *significance* of group effect on MD in claustrum and, for example, prefrontal cortex really reflect changes in group effect on claustrum MD and prefrontal MD (Nieuwenhuis et al. 2011), we performed an ANCOVA approach directly comparing these effects; we chose claustrum MD as dependent variable, group as independent one, and prefrontal cortex MD as covariate (additionally we included scanner and TIV as nuisance factors). We found a significant main effect of group on claustrum MD (P = 0.010; partial η^2 = 0.044), indicating that, even when accounting for variance of prefrontal cortex MD and its interaction with group, the claustrum MD values are increased in premature born adults. This result indicates that increased claustrum MD is not due to general MD changes in structures around the claustrum.

Since previous reports showed sex-specific aberrant brain development after premature birth, we tested whether sex is associated with claustrum MD and whether it interacts with premature birth. Mean claustrum MD values were for female VP/VLBW: 787.3 ± 26.3 × 10^{-6} mm^2/s, male VP/VLBW: 771.2 ± 22.6 × 10^{-6} mm^2/s, female FT: 768.8 ± 19.1 × 10^{-6} mm^2/s, male FT: 760.9 ± 17.1 × 10^{-6} mm^2/s). In this general linear model, both premature birth (F = 9.920; P = 0.002; partial η^2 = 0.063) and sex (F = 4.482; P = 0.036; partial η^2 = 0.029) were significant factors, while there was no interaction effect between premature birth and sex (F = 0.041; P = 0.839; partial η^2 < 0.001). This result indicates that claustrum MD increases in prematurity are not modulated by sex.

In order to further investigate whether prematurity parameters are relevant for MD claustrum increases, we studied the association between claustrum MD and variables of prematurity (i.e., GA, BW, neonatal treatment intensity) using nonparametric partial correlation analysis across subjects of the VP/VLBW group only. For claustrum MD, we found a significant negative correlation with BW (r = −0.296; P = 0.008), a trend-to-significant positive correlation with neonatal treatment intensity (r = 0.196; P = 0.057), and no correlation with GA (r = −0.065, P = 0.303) (Fig. 1C). This result indicates that increases in claustrum MD are related with birth weight in prematurity.

In order to find further evidence for potential SPN-dependence of claustrum MD increases in premature born adults, we investigated further consequences of potentially impaired SPN development in our sample of premature born adults. It is well known that thalamus microstructure is SPN-dependent due to SPN-based control of the development of connections between cortex and the thalamus (Ghosh et al. 1990; Kostovic and Rakic 1990; Hoerder-Suabedissen and Molnár 2015). Therefore, we expected increased thalamus MD in premature born adults. We found significantly increased thalamus MD in premature-born individuals (Thalamus MD FT: 841.1 ± 3.3; Thalamus MD VP/VLBW: 866.0 ± 3.7; F = 23.881; P < 0.001; partial η^2 = 0.137), indicating altered thalamus microstructure in prematurity. This finding is consistent the notion that altered SPN development of premature birth might contribute to both altered thalamus and claustrum microstructure. Or in other words, increased thalamus MD in prematurity supports the suggestion that increased claustrum MD might be related to impaired SPN development.

Claustrum Microstructure Is Associated with Cognitive Performance in Premature-Born Adults

In order to test the functional relevance of aberrant claustrum microstructure in adult VP/VLBW adults, we performed nonparametric partial correlation analysis between claustrum MD and adult FS-IQ, correcting for scanner. We found an at trend-to-significance correlation between claustrum MD and FS-IQ (r = −0.231, P = 0.033) (Fig. 2). In order to exclude that this correlation result is driven by extreme values, we identified outliers >1.5 * interquartile range (IQR) above the third quartile or <1.5 * IQR below the first quartile. We removed two individuals with extremely high claustrum MD and one individual with extremely high IQ. After removing these study participants, we found a stronger association between claustrum MD and full-scale IQ (r = −0.263; P = 0.020). To control for the specificity of claustrum MD in its relation with FS-IQ in premature born adults versus other regions MD relation with FS-IQ, we performed a partial correlation analysis among claustrum MD, prefrontal cortex MD, FS-IQ across premature born adults. Within such approach variance of prefrontal cortex MD is accounted for both further variables i.e., claustrum MD and FS-IQ. We found a negative partial correlation coefficient of r = −0.23 (P = 0.037) for the relation between claustrum MD and FS-IQ, indicating that the at-trend correlation between increased claustrum MD and reduced IQ in premature born adults is specific for claustrum MD versus MD of other brain regions. This result suggests that aberrant claustrum microstructure might be relevant for cognitive difficulties in human prematurity.

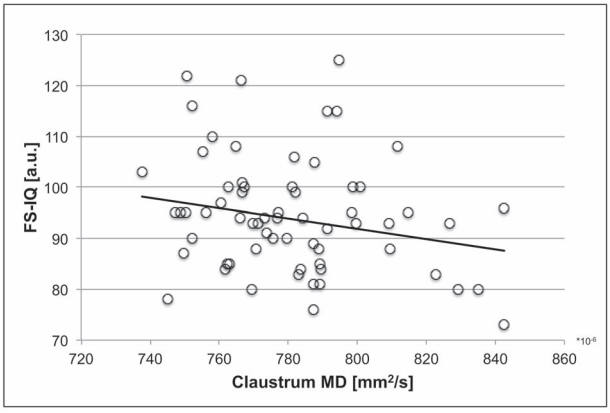

Figure 2. Claustrum microstructure links with general cognitive performance. Scatter plot showing the association between claustrum microstructure measured by mean diffusivity (MD) and FS-IQ measured by Wechsler Adults Intelligence Scale. Correlation coefficient r and associated *P* values are given from nonparametric partial correlation correcting for scanner ($r = -0.231$, $P = 0.033$). Abbreviations: FS-IQ: full-scale intelligence quotient; MD: mean diffusivity; VP/VLBW: very preterm and/or very low birth weight.

Discussion

Using T1-weighted and diffusion-weighted MRI and cognitive assessment, we found birth weight-related, specific increases of claustrum mean diffusivity in young adults after very premature birth, indicating altered claustrum microstructure toward lower cellularity. Furthermore, microstructural claustrum alterations were linked at-trend with lower FS-IQ, suggesting that aberrant claustrum microstructure is relevant for cognitive performance in premature-born adults. To the best of our knowledge, our finding is the first linking human prematurity with the claustrum. Data suggest aberrant claustrum development, which is potentially related with both aberrant SPN and forebrain connection development of prematurity.

Impaired Claustrum Microstructure after Premature Birth

We found increased MD values in the claustrum of VP/VLBW born adults, with increased MD values being correlated with low birth weight. This result suggests aberrant claustrum microstructure due to premature birth in humans. Concerning relative specificity, we found this result to be specific for both MD, since claustrum relative volumes and claustrum FA were not different in VP/VLBW born adults, and the claustrum, since MD in the adjacent putamen or in prefrontal and somatosensory cortices did not differ between VP/VLBW and FT controls. While we found a significant effect of sex toward increased claustrum MD in females, we did not find any interaction between sex and prematurity on claustrum MD, indicating that sex did not modulate the effect of prematurity on claustrum microstructure. We interpret increased claustrum MD after premature birth as an indicator of decreased claustrum cellularity in premature born adults. More specifically, MD has been used as an in-vivo marker of tissue cellularity since it reflects the overall mean squared displacement of protons in the extracellular tissue space (Le Bihan 2003). That means that MD-based "cellularity" reflects all membranes hindering diffusion of water molecules, what includes for example membranes of cell bodies but also membranes of neurites, which penetrate a gray matter structure. While cell body membranes make MD to a marker of cell density for the given gray matter structure, membranes of penetrating neurites might confound the MD signal of this structure. The principle that decreased cell density leads to enhanced mean squared proton diffusion of free water and thus increased MD values was recently validated histologically in a mouse model of traumatic brain injury (Tu et al. 2016). Accordingly, decreasing MD values have been described during normal brain development, indicating increased tissue cellularity in cerebral white and gray matter (Pierpaoli et al. 1996; McKinstry et al. 2002; Ouyang et al. 2019). Moreover, DWI-derived measures such as MD or fractional anisotropy of water diffusion have been used as a marker of white matter and cortical development (Huppi et al. 1998; Miller et al. 2002; Ball et al. 2013; Dean et al. 2013) after premature birth. For example, Dean et al. (2013) found disturbed cortical microstructure, specifically impaired dendritic arborization by means of DWI after premature birth. Consequently, we interpret the significant MD increases in the human claustrum in VP/VLBW born adults as indicators of altered microstructure toward decreased cellularity.

Impaired Claustrum Microstructure Is Associated with General Cognitive Performance in Premature-Born Adults

We found at-trend-to-significance correlation between claustrum MD and FS-IQ in premature-born individuals, suggesting relevance of claustrum microstructure for general cognitive difficulties of premature-born adults. Premature-born individuals are at risk for impaired general cognitive functioning (Twilhaar et al. 2018) and correlations with several aspects of brain structure have been described such as aberrant gyrification, cortical complexity, volume of the corpus callosum, and decreased FA (Nosarti et al. 2004, 2008; Meng et al. 2016; Hedderich et al. 2019; Hedderich et al. 2020b). General cognitive performance is a complex phenomenon and therefore it seems very likely that several brain features contribute to the variance of cognitive performance. Claustrum microstructure might be such a feature, as claustrum connectivity within the forebrain is extraordinary, accompanied by the fact that claustrum function is involved in several basic cognitive processes such as selective attention, task-switching, and cognitive control (Mathur 2014; Goll et al. 2015; Torgerson et al. 2015; White et al. 2018; Krimmel et al. 2019). For example, in mice, the claustrum amplifies top-down signals of cognitive control from the anterior cingulate to distributed cortical regions (White et al. 2018) and in humans, the claustrum is involved in task-onset control across different cognitive task settings (Krimmel et al. 2019).

Prematurity, Claustrum, and Cellular Underpinnings: Is Aberrant Claustrum Microstructure a Window for Impaired SPN Development?

A possible explanation for the observed link between aberrant claustrum microstructure and prematurity might be the dependence of claustrum integrity on SPN development. The claustrum was recently shown by gene expression studies to contain distinct populations of SPNs in mice, which either remain in the adult claustrum and form the so-called subplate part of the claustrum or migrate tangentially toward the subcortical zone of the dorsal pallium in order to control cortical development (Puelles 2014; Puelles et al. 2016; Bruguier et al. 2020).

SPNs are highly vulnerable to perinatal stressors of prematurity such as hypoxic-ischemic events, and therefore, a central mediator of aberrant brain development in prematurity (Volpe 1996; McQuillen and Ferriero 2005; Kinney et al. 2012; McClendon et al. 2017). Both the shared ontogeny of the claustrum and SPNs and the vulnerability of SPN for perinatal stressors of prematurity are consistent with the idea that aberrant adult claustrum microstructure reflects aberrant SPN development after prematurity. This notion is supported by our finding of increased thalamus MD in premature born adults; increased thalamus MD indicates aberrant thalamus microstructure in premature born adults, with such microstructural changes being a potential further consequence of aberrant SPN development in prematurity (Ghosh et al. 1990; Kostovic and Rakic 1990; Hoerder-Suabedissen and Molnár 2015). However, to provide definitive evidence for the idea of SPN dependence of claustrum changes in prematurity, future studies have to demonstrate in humans both, that SPNs of the human claustrum are impaired by prematurity and that this impairment underpins MD increases.

Beyond SPN pathology, alternative nonexclusive explanations for increased claustrum MD in prematurity do exist. First, the claustrum is—relative to its volume—the most connected brain region of the mammalian forebrain, with long, bidirectional, white matter connections to cortical regions, from primary to associative cortices (Druga 2014; Torgerson and Van Horn 2014; Milardi et al. 2015; Torgerson et al. 2015). The development of these connections and thereby their subcortical and cortical sources and targets is strongly dependent on myelin-producing oligodendrocytes (Buser et al. 2012; Back 2017; Volpe 2019). Oligodendrocytes, in turn, develop from pre-oligodendrocytes that are a transient cell population of intrauterine brain development with their peak activity coinciding with typical periods of premature birth (Buser et al. 2012; Back 2017; Volpe 2019). Critically, pre-oligodendrocytes are a main target of adverse perinatal events of prematurity, resulting in widespread and lasting white matter changes of premature born individuals (Ball et al. 2012, 2015; Menegaux et al. 2020). Based on this cascade-like model, the aberrant development of claustrum microstructure might be, therefore, also influenced by the impact of prematurity on pre-oligodendrocytes. Second, related with this, claustrum microstructure might be influenced by aberrant white matter fibers adjacent or invading claustrum borders and impaired neurite development, which is widespread in brains of premature-born individuals (Ball et al. 2013; Meng et al. 2016). Third, more technically but related to the last point, our measure of claustrum MD might be confounded by white matter partial volume effects, which might occur due to the limited spatial resolution of structural MRI and small claustrum dimensions. Taken together, future microscopic studies and neuropathological assessments will be needed to further investigate our suggestion of underpinning causes of claustrum microstructure aberrations in human prematurity.

Strengths and Limitations

Strengths of the current study are that its sample is considerably large and derived from a long-term population study, and that our approach is strongly hypothesis-driven based on very recent translational findings about SPNs and pre-OLs as well as claustrum development and connectivity.

There are several limitations of our study: Our sample is biased to VP/VLBW adults with less severe neonatal complications, less functional impairments, and higher IQ, as individuals with stronger birth complications and/or severe lasting impairments in the initial BLS sample were more likely to be excluded in the initial MRI screening due to exclusion criteria for MRI (for example infantile cerebral palsy). Thus, differences in claustrum microstructure between VP/VLBW and term control adults reported here are conservative estimates of true differences. Furthermore, there are methodological limitations. The tiny structure of the claustrum, which is not amenable to automated segmentation, makes it particularly hard to study using MRI. We have obtained high-quality expert annotations with acceptable intrareader variability, which can be considered the best possible reference standard. Further studies should aim at semi-automated or automated segmentation algorithms for the claustrum in order to facilitate comparable studies in large cohorts. Most importantly, as already mentioned above, although our finding of altered claustrum microstructure is consistent with aberrant SPN subpopulation in prematurity, our approach is not able to provide definitive evidence that claustral MD increases are due to SPN alterations in prematurity. Future translational approach integrating both microscopic and DWI assessments are necessary.

Conclusion

We demonstrate specific, birth weight-related claustrum microstructure alterations in premature-born adults, which link with impaired general cognitive performance. Data suggest aberrant claustrum development, which is potentially related with aberrant SPN and forebrain connection development of prematurity.

Supplementary Material

Supplementary material can be found at *Cerebral Cortex* online.

Data Availability Statement

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to lacking consent from research participants.

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