Reduced cholinergic basal forebrain integrity links neonatal complications and adult cognitive deficits after premature birth

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Running Title: Reduced basal forebrain volume in premature-born adults

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Abstract

Background: Premature-born individuals have an increased risk for long-term neurocognitive impairments. In animal models, the development of the cholinergic basal forebrain (cBF) is selectively vulnerable to adverse effects of perinatal stressors, and impaired cBF integrity results in lasting cognitive deficits. We hypothesized that in premature-born individuals cBF integrity is impaired and mediates adult cognitive impairments associated with prematurity.

Methods: We used MRI-based volumetric assessments of a cytoarchitectonically defined cBF region-of-interest to determine differences in cBF integrity between 99 adults who were born very preterm and/or with very low birth weight (VP/VLBW) and 106 term born controls from the same birth cohort. MRI-derived cBF volumes were studied in relation to neonatal clinical complications after delivery as well as intelligence measures (IQ) in adulthood.

Results: In VP/VLBW adults, cBF volumes were significantly reduced compared to term-born adults (-4.5%, $F(1, 202) = 11.82$, $p = 0.001$). Lower cBF volume in VP/VLBW adults was specifically associated with both neonatal complications ($r_{part}(92) = -0.35$, $p < 0.001$) and adult IQ ($r_{part}(88) = 0.33$, $p = 0.001$) even after controlling for global gray matter and white matter volume. In an additional path analytic model, cBF volume significantly mediated the association between neonatal complications and adult cognitive deficits.

Conclusions: Results provide first time evidence in humans that cBF integrity is impaired after premature birth and links neonatal complications with long-term cognitive outcome. Data suggest that cholinergic system abnormalities may play a relevant role for long-term neurocognitive impairments associated with premature delivery.

Keywords:

preterm birth, perinatal stress, substantia innominata, Nucleus basalis Meynert, intelligence, structural MRI

Introduction

Premature birth has a worldwide prevalence of more than 10% and is associated with an increased risk for birth complication and adverse long-term outcomes (1, 2). Adverse outcomes range from growth failure and higher morbidity to impaired neurocognitive development and lower socioeconomic status (3, 4). Risk for adverse outcomes increases substantially for very premature individuals, i.e. born very preterm (VP; gestational age <32 weeks) and/or with very low birth weight (VLBW; <1500g) (4, 5). Neuronal deficits may arise due to a higher propensity for brain injury caused by a higher risk for perinatal stressors such as hypoxic, ischemic, inflammatory, and/or infectious events in combination with developmental disturbances due to immature birth (2, 6).

However, brain mechanisms linking perinatal brain injury and neurodevelopment with long-term neurocognitive outcome after premature birth are still poorly understood. Previous research has shown that perinatal brain injury affects primarily the forebrain's white matter, characterized by immature and vulnerable glia cells and a relatively poor vascularization before 32 weeks of gestation (7). With increasing severity of injury, white matter impairments extend to gray matter, particularly affecting subcortical structures such as thalamus or basal ganglia (8-12). In this regard, the cholinergic basal forebrain (cBF) may be of special interest, since – at least in animal models – proper development of this system of cortically-projecting nuclei has been shown to be highly sensitive to perinatal stressors, such as glucocorticoid exposure (13), hypoxic-ischemic events (14), or neonatal psychosocial stress (15), resulting in structural and functional impairments that persist into adulthood. Moreover, due to the dependency of cortical circuits on cholinergic inputs, the cBF is highly relevant for cognitive functioning and intellectual capabilities (16), possibly linking perinatal brain injury to lasting effects on neurocognitive development. Thus, due to the higher risk for perinatal stressors in premature-born humans, the hypothesis arises that the cBF may be specifically involved in adverse effects of premature birth and associated with impaired neurocognitive development.

While there is currently little data regarding cBF involvement in human premature delivery, this question is of considerable clinical relevance given that cBF deficits may in principle be amenable to targeted interventions for lowering adverse consequences of premature birth.

Recent developments in stereotactic mapping of the cBF nuclei in the human brain have rendered this region accessible to MRI-based volumetric assessments, providing an in-vivo surrogate marker of cBF structural integrity (17-19). Application of these methods to the field of age-related neurodegenerative diseases has provided initial in-vivo evidence for the prominent role of cBF atrophy in the emergence of cognitive deficits in these conditions (20-24). Here, we used these techniques to assess reductions in cBF volumes in VP/VLBW adults as compared to term-born controls, and to further examine the relation of reduced cBF integrity with both neonatal complications and adult cognitive deficits.

Methods and Materials

Participants

Participants were recruited as part of the prospective Bavarian Longitudinal Study (BLS), which investigates a geographically defined whole-population sample of neonatal at-risk infants born in southern Bavaria (25). The current study sample consisted of 99 VP/VLBW individuals and 106 demographically matched term born individuals (control group) from the same obstetric hospitals who participated in a 26 year follow-up assessment including MRI scanning. More detailed information on the BLS and the selection process for the present study is reported in the supplementary material.

MRI assessments were carried out at the Department of Neuroradiology, Klinikum Rechts der Isar, Technische Universität München, Germany (N = 138), and the Department of Radiology, University Hospital Bonn, Germany (N = 67). The study was approved by the local ethics committees of the Klinikum Rechts der Isar and University Hospital Bonn. All study participants gave written informed consent and received travel expenses and a payment for attendance. Gestational age was determined from maternal reports of the last menstrual period and serial ultrasounds during pregnancy (26).

Assessment of neonatal complications

The intensity of neonatal treatment index (INTI) is a measure of neonatal complications (27). Daily assessments of care level, respiratory support, feeding dependency and neurological status (i.e. mobility, muscle tone, and neurological excitability) were carried out. Each of the 6 variables was scored on a 4-point rating scale (0-3). The INTI was computed as the mean score of daily ratings during the first 10 days of life or until a stable clinical state was reached, depending on which occurred first, ranging from 0 (best state) to 18 (worst state).

Cognitive performance assessment

Prior to and independent from the subsequent MRI examination, subjects from the BLS cohort were asked to take part in an assessment of global cognitive functioning at the age of 26 years by trained psychologists who were blinded to group membership. This included a short version of the German version of the Wechsler Adult Intelligence Scale-III (WAIS-III) (28), allowing the computation of a full-scale (F), verbal (V), and performance (P) IQ.

MRI data acquisition

At both sites, MRI data acquisition was initially performed on Philips Achieva 3T TX systems (Achieva, Philips, the Netherlands), using an 8-channel SENSE head coil. Due to a scanner upgrade, data acquisition in Bonn had to switch to Philips Ingenia 3T system with an 8-channel SENSE head coil after N = 17 participants. To account for possible confounds introduced by scanner differences, data analyses included scanner identities as covariates of no interest. Across all scanners, sequence parameters were kept identical. Scanners were checked regularly to provide optimal scanning conditions. Signal-to-noise ratio (SNR) was not significantly different between scanners (one-way ANOVA with factor "scanner-ID" [Bonn 1, Bonn 2, Munich]; P = 0.811). A high-resolution T1-weighted 3D-MPRAGE sequence (TI = 1300 ms, TR = 7.7 ms, TE = 3.9 ms, flip angle = 15°; 180 sagittal slices, FOV = 256 × 256 × 180 mm, reconstruction matrix = 256 × 256; reconstructed voxel size = 1 × 1 × 1 mm3) was acquired.

MRI data processing

MRI data were processed using a fully automated processing pipeline within statistical parametric mapping software (SPM8, Wellcome Trust Center for Neuroimaging) and the VBM8-toolbox (http://dbm.neuro.uni-jena.de/vbm/) implemented in MATLAB R2013a (MathWorks, Natick, MA) as described previously (19, 24). First, MRI scans were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid partitions of 1.5 mm

isotropic voxel-size using the segmentation routine of the VBM8-toolbox. The resulting GM and WM partitions of each subject in native space were then high-dimensionally registered to stereotactic standard space (MNI space). Individual deformation fields resulting from the high-dimensional registration to the reference template were used to warp the GM segments and voxel-values were modulated for volumetric changes introduced by the normalization procedure, such that the total amount of GM volume present before deformation was preserved. All preprocessed GM maps passed a visual inspection for segmentation and registration accuracy.

Individual GM volumes of the cBF were extracted automatically from the warped GM segments by summing up the modulated GM voxel values within a region-of-interest (ROI) defined by a stereotactic atlas of BF cholinergic nuclei in MNI space. This atlas was derived from combined histology and in cranio post-mortem MRI of a 56-year old man without any evidence of cognitive decline or psychiatric illnesses prior to death (18). High-dimensional image registration of the post-mortem MRI data was used to accurately map the histologic delineations of cholinergic forebrain nuclei onto the MNI standard space template. The cBF mask comprises detailed outlines of different cholinergic subdivisions within the basal forebrain, including cell clusters corresponding to the medial septum, diagonal band, nucleus subputaminalis, and nucleus basalis Meynert (18). In the current study we investigated the entire volume of the cBF ROI, including all cholinergic subdivisions, as a proxy for overall BF cholinergic system integrity (Fig. 1).

To study the relative specificity of reduced cBF integrity in VP/VLBW adults and its association with neonatal complications and adult cognitive deficits, we also assessed the respective effects for global GM and WM volume. WM alterations have been described to be the most prevalent form of neuronal damage associated with preterm birth (7) and previous imaging studies found reductions in total WM volume to be a major determinant of IQ in VP/VLBW adolescents (11, 29). To account for differences in head size, cBF as well as

global GM and WM volumes were scaled to the intracranial volume (ICV), calculated as the sum of total segmented volumes of the GM, WM and cerebrospinal fluid partitions.

Statistical analysis

Statistical analyses were carried out using the software package IBM SPSS Statistics version 21. Statistical significance of the differences in ICV-normalized cBF, GM and WM volume between the VP/VLBW and control groups was assessed using analysis of covariance, controlling for MRI acquisition center. In addition to the group-wise volumetric comparisons, partial correlation coefficients of ICV-normalized cBF, GM and WM volume with INTI scores were calculated within the VP/VLBW group, controlling for MRI acquisition center and gender. Partial correlations between cBF volume and INTI score were also assessed when additionally controlling for global GM and WM volume. INTI score was not available for one subject who was excluded from this analysis.

Associations of cBF, GM and WM volume with cognitive test performance were assessed separately for VP/VLBW and controls using partial correlation coefficients controlled for MRI acquisition center and gender. Partial correlations between cBF volume and cognitive test performance were also assessed when additionally controlling for global GM and WM volume. FIQ was used as the primary neuropsychological outcome measure, and secondary correlation analyses assessed domain-specific associations with VIQ and PIQ. Cognitive test performance was not available for five VP/VLBW and three control subjects who were excluded from this analysis.

Mediation analysis

Additional mediation analysis was performed to test whether adult cognitive impairments associated with neonatal complications may be mediated by reduced cBF volume. First, associations between INTI scores and adult IQ scores were established using partial

correlations controlled for gender. In the mediation model, INTI score was entered as the causal variable, FIQ as the outcome variable, cBF volume as the mediator variable, and MRI acquisition center and gender as covariates of no interest. Path coefficients were estimated using (unstandardized) regression coefficients of multiple regression analyses, and statistical significance of the indirect effect was tested using a non-parametric bootstrap approach (with 1000 repetitions) to obtain 95% confidence intervals (30).

Results

Sample characteristics are summarized in Table 1. VP/VLBW and controls differed significantly in gestational age and birth weight. The VP/VLBW group had significantly lower IQ scores compared to the term-born controls.

Cholinergic basal forebrain volumes in VP/VLBW adults and their association with neonatal complications

cBF volumes were reduced in VP/VLBW adults by -4.5% on average compared to controls and this difference was statistically highly significant ($F(1, 202) = 11.82$, $p = 0.001$). Smaller cBF volumes were significantly correlated with a higher INTI score within the VP/VLBW group (Fig. 2; $r_{part}(94) = -0.27$, $p = 0.009$). By contrast, global GM volume did not differ between the two groups ($F(1,202) = 0.39$, $p = 0.53$) and WM volume was only marginally lower in VP/VLBW adults compared to controls (-1.2%, $F(1, 202) = 2.98$, $p = 0.086$). Neither global GM ($r_{part}(94) = 0.07$, $p = 0.50$) nor WM volumes ($r_{part}(94) = -0.16$, $p = 0.13$) were significantly associated with INTI scores, and the correlation between cBF volume and INTI score was increased after additionally controlling for global GM and WM volume ($r_{part}(92) = -0.35$, $p < 0.001$).

Association between cholinergic basal forebrain volumes and general cognitive function in VP/VLBW adults and term-born controls

Among VP/VLBW adults, smaller cBF volumes were significantly associated with a lower FIQ (Fig. 3; $r_{part}(90) = 0.33$, $p = 0.001$), as well as with a lower VIQ ($r_{part}(90) = 0.26$, $p = 0.01$) and PIQ ($r_{part}(90) = 0.31$, $p = 0.003$). Neither global GM nor WM volumes were associated with any IQ measure (all $p \geq 0.35$), and controlling for these global volumes did not markedly alter the association between cBF volume and intelligence scores ($r_{part}(88) = 0.33/0.25/0.33$, for FIQ/VIQ/PIQ, all $p \leq 0.02$). In term-born adults, neither cBF volumes ($r_{part}(99) = -0.17$, p

= 0.10) nor GM or WM volumes (all $p \geq 0.22$) were significantly associated with FIQ, or any other IQ measure.

Mediational effect of cBF volume on the association between neonatal complications and neurocognitive limitations in VP/VLBW adults

Among VP/VLBW adults, the INTI score was significantly associated with a lower FIQ ($r_{part}(90) = -0.21$, $p = 0.04$) and VIQ ($r_{part}(90) = -0.23$, $p = 0.03$), but not with a lower PIQ ($r_{part}(90) = -0.14$, $p = 0.19$). In a mediation analysis (Fig. 4), the effect of the INTI score on adult FIQ performance (total effect: $c = -0.72 \pm 0.33$, $t(90) = -2.18$, $p = 0.03$) was not significant when controlling for cBF volume (direct effect: $c' = -0.46 \pm 0.33$, $t(89) = -1.39$, $p = 0.17$), and the bootstrapped 95% confidence interval revealed the indirect effect (i.e. mediation: total - direct effect) to be significantly different from zero ([-0.70, -0.08]).

Discussion

Structural MRI-based volumetric assessments of a cytoarchitectonically defined cholinergic BF ROI were used to investigate cBF integrity in VP/VLBW adults and to link it with neonatal complications and adult cognition. Volume of the cBF was significantly reduced in VP/VLBW adults in comparison to term-born controls. Among VP/VLBW individuals, reduced cBF volume was associated with both higher neonatal treatment intensity and reduced IQ scores in adulthood. Notably, the association between neonatal complications and adult cognitive problems was found to be mediated by reduced cBF volume. These results provide first evidence in humans that the cBF is involved in long-term adverse effects of premature birth.

Neuropathological studies in premature-born newborns (9) and in-vivo imaging studies in premature-born newborns (10), children (31, 32), adolescents (11, 29, 33), and adults (12, 34-36) reported consistent volume reductions in subcortical brain regions, including brainstem, thalamus, and basal ganglia, but – to the best of our knowledge – volume differences of the cBF have not yet been investigated. By contrast, research in animal models demonstrated a high vulnerability of the developing cBF system against perinatal stressors such as increased glucocorticoids and hypoxic-ischemic events (13-15), which have a high prevalence in premature born infants (2). Our finding of reduced cBF volumes in VP/VLBW adults may reflect an impaired neurodevelopment of this brain system with long-term consequences for cognitive abilities. While we cannot infer developmental trajectories from the examined cross-sectional imaging data acquired in adulthood, a significant association between adult cBF volume reduction and the degree of neonatal complications further supports the idea that development of this brain region is particularly sensitive to perinatal stressors and neonatal treatment. However, more detailed studies in premature newborns and complementary longitudinal studies mapping cBF developmental trajectories are needed to substantiate this finding.

Combining in-vivo cBF volumetry with cognitive performance assessments, we found that reduced cBF volume in VP/VLBW adults was associated with lower intellectual abilities. This is in line with the widely supported role of the cBF system in higher cognitive functions, and may reflect an impaired cholinergic innervation of cognition-relevant cortical circuits (16). Clinicopathologic correlation studies in several neurologic dementing diseases characterized by impaired cBF integrity have demonstrated associations between cBF degeneration and dementia severity (37, 38). Furthermore, reduced cBF integrity has also been associated with more subtle cognitive deficits in conditions with less severe alterations of the cBF system, such as those associated with the normal aging process, developmental disturbances, or mild traumatic brain injury (38-40). Recent neuroimaging studies using MRI-based volumetric assessments of the cBF could replicate associations between reduced cBF integrity and cognitive deficits in neurodegenerative conditions in-vivo (20-24). Our findings in VP/VLBW adults suggest that reduced cBF integrity may be associated with cognitive limitations regardless of the etiology of the deficit.

Importantly, complementary assessments of global GM and WM volume revealed a relatively specific role of reduced cBF integrity for persisting intellectual limitations in VP/VLBW adults. Reductions in cerebral WM volume have been described to be the most prevalent form of neuronal damage associated with premature birth (7) and to be a major determinant of IQ in VP/VLBW adolescents (11, 29). However, in our sample of VP/VLBW adults, total WM volume was only marginally reduced compared to controls and was not related to intellectual impairments. While studies on premature birth-associated cerebral abnormalities in adulthood are still rare, two recent volumetric imaging studies on independent cohorts of VP/VLBW adults (34, 35) reported very similar observations of more subtle between-group differences compared to the data in infants (31, 32) and adolescents (11, 29, 33). Thus, rather than showing global differences, structural abnormalities in VP/VLBW adults were found to be restricted to specific subcortical GM and WM structures and select

cortical limbic and associative regions, indicating that some, but not all, aspects of the adverse effects of premature birth on cerebral structure may normalize during late development. Persistent reductions in cBF volume in VP/VLBW adults are consistent with experimental research in animal models demonstrating a weak potential of the cholinergic forebrain to recover from neonatal lesions (41). While our analyses demonstrate a relatively specific role of the cBF in premature birth-associated cognitive deficits that last into adulthood, one would not expect cBF volume to be the only factor related to variance in intellectual abilities among VP/VLBW adults. This is also reflected in the mild to moderate effect size of the observed associations between cBF volume and IQ scores (r_{part} = 0.26 – 0.33), indicating that impairments in other cortical and/or subcortical regions and their networking through the brain's white matter may affect intellectual abilities in VP/VLBW adults in at least partially independent ways (12, 34, 35). In addition, associations between structural/volumetric brain markers and mental capacities are generally indirect in nature and depend on the degree to which the measured structural abnormalities reflect the functional integrity of the neuronal system under study. In line with these considerations, variance in cBF volume among term-born controls was not found to be linked to differences in intellectual abilities, probably because volumetric differences among normally developed adult subjects are only weak indicators of neuronal functioning. Accordingly, variance in hippocampus volume has been shown to exhibit robust in-vivo associations with memory performance across a wide range of neurologic conditions (24, 42), but not so in healthy adults (43). In addition, the association between cBF volume and cognitive performance may be augmented by comorbid brain lesions in the VP/VLBW group (12, 34-36). Thus, experimental animal research (44) and more recent human in-vivo studies (45) have implicated impaired cBF integrity in diminished neural adaptation to white matter injury, possibly further contributing to worse cognitive outcomes in VP/VLBW adults.

A key finding of our study is that reduced cBF volume in VP/VLBW adults is associated with both neonatal complications and adult cognitive deficits. While our analyses are correlational and do not allow any direct inferences on directionality or causality, additional mediation analysis supports the idea of a causal influence of perinatal stressors on cBF integrity and inter-related cognitive problems. More specifically, factors during the perinatal period may affect development of the cBF; such alterations, in turn, likely influence the brain-behavior developmental trajectory towards worse outcomes; this influence realizes as an iterative process over time, when the child engages in interactions with the environment; interactions which themselves may be altered by the early brain insults. Such a model might open new ways in our thinking about adverse consequences of premature birth complications, both in terms of the prognosis of long-term risk for neurocognitive impairments and the treatment of such complications (4). Due to the lowered capacity of an abnormally developed cortical cholinergic input system to cope with additional disease-related stressors, aberrant development of the cholinergic BF system has been discussed as an important vulnerability factor for adult mental illnesses, such as schizophrenia (46) or late-life cognitive decline and dementia (47). Thus, future studies should carefully examine the possible role of cBF integrity in mediating increased risk profiles for neuropsychiatric disorders in VP/VLBW individuals. Concerning potential treatments, more attention should be paid to interventions which may prevent or reduce the negative impact of perinatal stressors on the cBF. For example, perinatal choline supplementation has been shown to protect cholinergic system integrity in animal models of increased vulnerability to cholinergic deficits, such as modeled by fetal alcohol exposure (48) or genetic predisposition (49). Interestingly, a recent placebo-controlled trial of perinatal dietary choline supplementation in healthy women demonstrated a positive effect on a psychophysiologic outcome marker related to cholinergic system development and schizophrenia risk (46). Early postnatal dietary interventions have already been shown to be able to ameliorate adverse effects of preterm birth on cognitive and brain volumetric outcome

variables in adolescence (50), but specific choline supplementations have not yet been considered. Beyond primary preventive perinatal diet supplementations, pharmacological interventions with pro-cholinergic drugs, such as nicotinergic agonists or acetylcholinesterase inhibitors, might be additional options to ameliorate long-standing cognitive limitations in VP/VLBW adults. Cholinergic drugs are generally well tolerated, have demonstrated nootropic properties in healthy adults (51), and have shown beneficial effects on cognitive disturbances that are believed to be of cholinergic origin in diverse neurologic and psychiatric conditions (52-55).

A limitation of our study is that the volumetric cBF measurement is only an indirect marker of cholinergic system integrity. The correspondence of the analyzed brain region with the cholinergic space of the BF was determined based on stereotactic mapping of the forebrain's cholinergic nuclei using combined histology and post-mortem MRI (18). Notably, we found that the results of our study were robust against slight variations in the definition of the cBF ROI, as they could be fully reproduced using a previously published stereotactic mask of the cBF based on a different post-mortem brain (17) (data not shown). Nevertheless, one cannot exclude that the volumetric measurement may also reflect change in other non-cholinergic neuronal populations. For example, cholinergic neurons in the BF are intermingled, and interact, with cortically-projecting GABAergic neurons, which are increasingly being recognized for their critical role in BF-mediated modulation of cortical activity (56). Moreover, GABAergic neurons have also been reported to be particularly prone to preterm hypoxia (7). Thus, while the observed associations with neonatal complications and adult IQ scores underline the clinical and functional relevance of in-vivo detectable volumetric cBF changes after premature birth, the implications of these alterations for cholinergic neurotransmission and cortical function need to be studied in more detail. Pharmacologic challenge paradigms (57, 58) and functional imaging studies (24, 59) may be suitable analytic approaches to further investigate the nature of reduced cBF volume and its

association with neuro-cognitive function in premature-born adults. Secondly, one should note that the current study focuses on high-risk premature birth, as defined by VP and/or VLBW birth. This approach follows previous framings for defining high-risk prematurity, facilitating comparability with a wide spectrum of human prematurity studies (2, 5). While we demonstrated a link between neonatal medical complications, cBF, and IQ for VP/VLBW persons, we cannot disentangle which medical or prematurity factors (or their interactions) may be most determining for cBF volume and IQ in adulthood. This indeterminacy is partly based on our sample definition following combined VP/VLBW criteria for determining a general high-risk prematurity profile (2, 5), as well as on the employed INTI measure (27), which is a summary score of medical complications after birth without specifying the exact nature of these complications or their underlying causes. Thirdly, the sample of this MRI study was biased towards VP/VLBW adults with relatively lower neonatal complications and higher IQ compared to the entire BLS baseline cohort, given that individuals with more severe impairments were more likely to be excluded in initial screening for MRI or to decline to participate in MRI scanning. Thus, the reported differences compared to term-born controls may be considered conservative estimates of the differences in cBF volume in VP/VLBW adults.

In summary, we studied an in-vivo marker of structural cBF integrity in a large sample of VP/VLBW adults, and provide novel evidence for a previously unappreciated aspect of the neuronal changes underlying lasting cognitive limitations in this population. Further studies using complementary pharmacologic and functional imaging approaches for investigating alterations in the cholinergic system after premature birth and its implications for neuro-cognitive function are warranted.

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Figure legends

Figure 1. Illustration of the cholinergic basal forebrain region of interest

The cholinergic basal forebrain region of interest as defined by a cytoarchitectonic map of cholinergic nuclei in the basal forebrain is superimposed on transparent renderings of the stereotactic standard space template (MNI space).

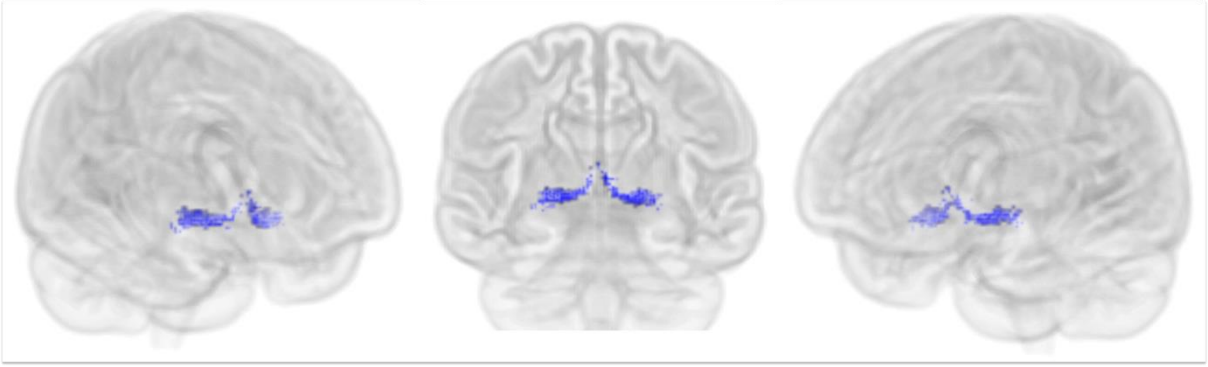
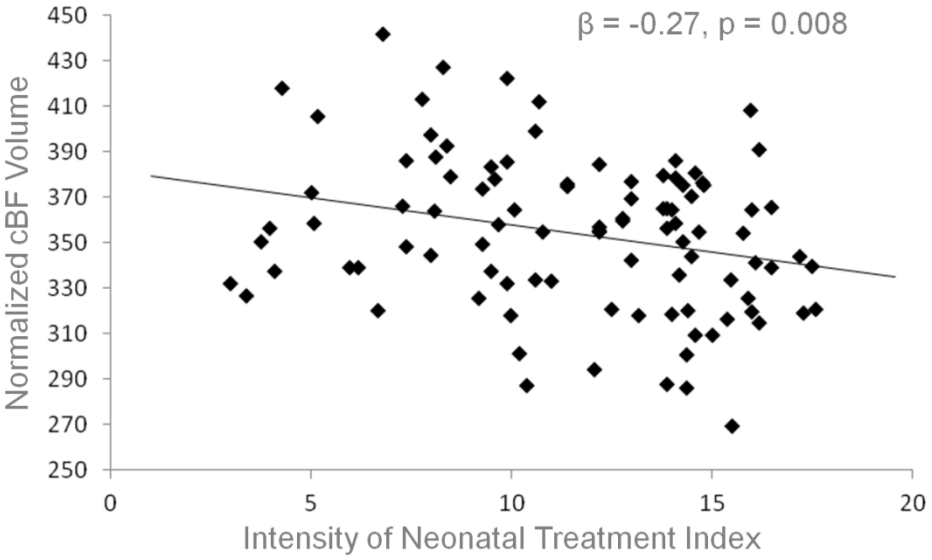
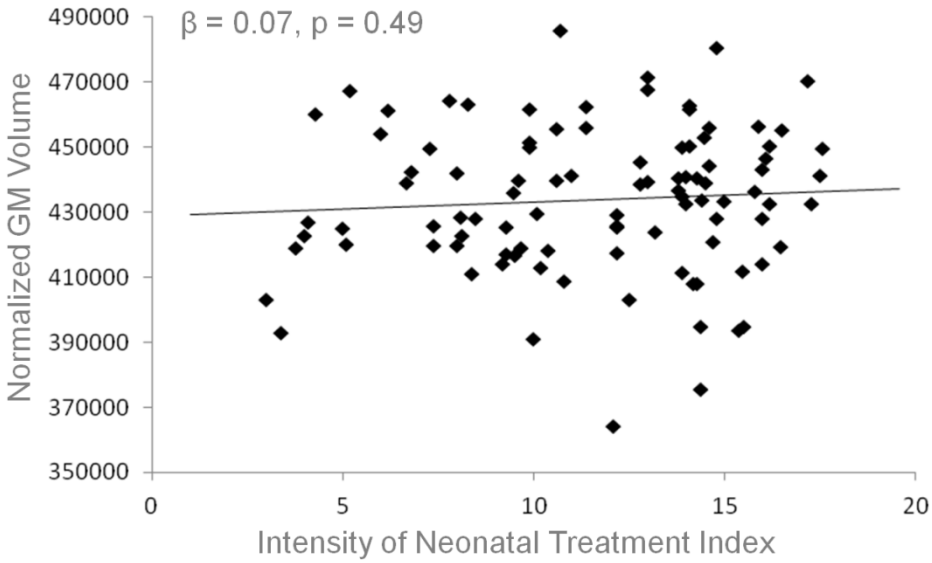


Figure 2. Association of intensity of neonatal treatment with cholinergic basal forebrain and global gray and white matter volume in preterm-born adults

Intensity of neonatal treatment (INTI) scores in preterm-born adults are plotted against cholinergic basal forebrain (cBF, top), global gray matter (GM, middle), and global white matter (WM, bottom) volumes, normalized for intracranial volume (head size). For visualization purposes, all plots include linear regression trend lines with their corresponding standardized regression coefficient and associated p-value. Only cBF volume shows a significant negative association with INTI scores, which is corroborated in covariate-controlled partial correlation analyses (see Results).


β = -0.27, p = 0.008
Normalized cBF Volume
450
430
410
390
370
350
330
310
290
270
250
0
5
10
15
20
Intensity of Neonatal Treatment Index


β = 0.07, p = 0.49
Normalized GM Volume
490000
470000
450000
430000
410000
390000
370000
350000
0
5
10
15
20
Intensity of Neonatal Treatment Index

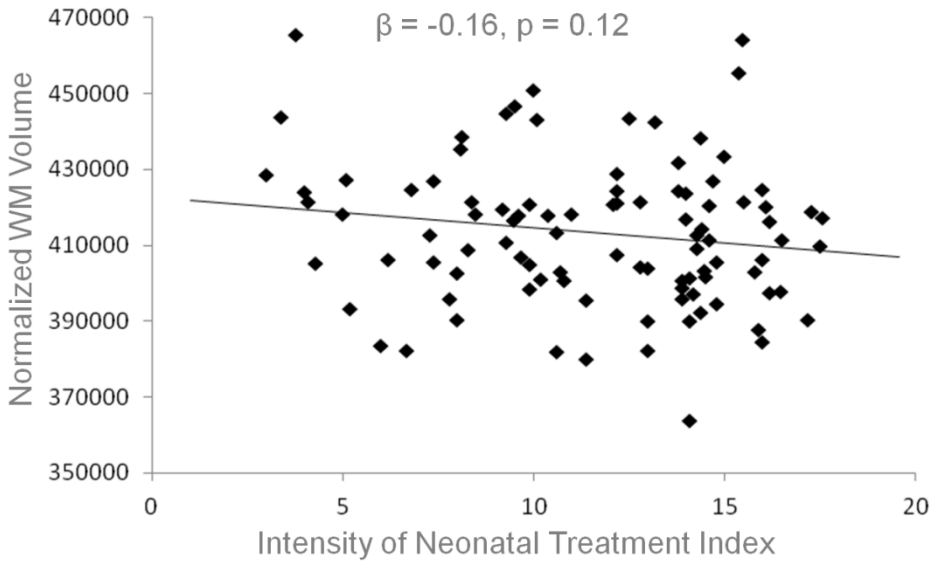

β = -0.16, p = 0.12
Normalized WM Volume
470000
450000
430000
410000
390000
370000
350000
0
5
10
15
20
Intensity of Neonatal Treatment Index

Figure 3. Association of intellectual abilities with cholinergic basal forebrain and global gray and white matter volume in preterm-born adults

Cholinergic basal forebrain (cBF, top), global gray matter (GM, middle), and global white matter (WM, bottom) volumes, normalized for intracranial volume (head size), are plotted against full-scale IQ scores of the Wechsler Adult Intelligence Scale-III in preterm-born adults. For visualization purposes, all plots include linear regression trend lines with their corresponding standardized regression coefficient and associated p-value. Only cBF volume shows a significant positive association with IQ scores, which is corroborated in covariate-controlled partial correlation analyses (see Results).

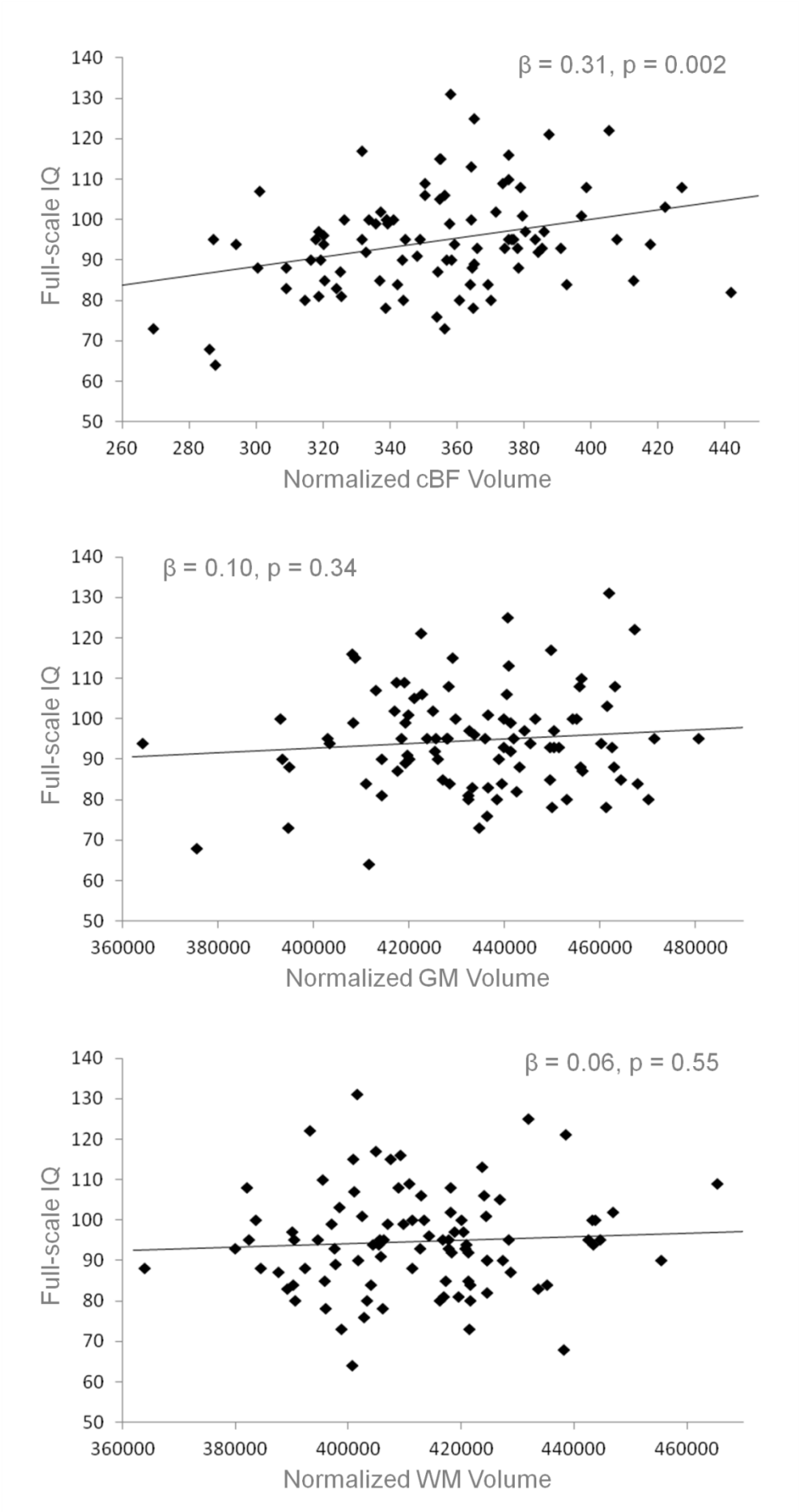
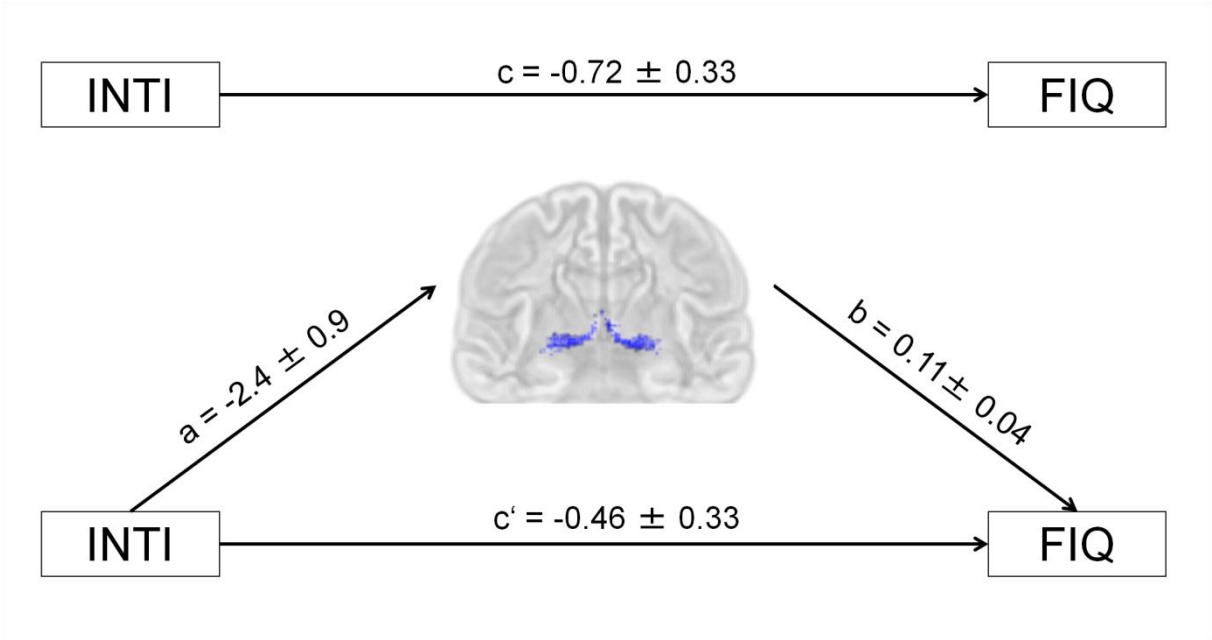

β = 0.31, p = 0.002
Full-scale IQ
Normalized cBF Volume
β = 0.10, p = 0.34
Full-scale IQ
Normalized GM Volume
β = 0.06, p = 0.55
Full-scale IQ
Normalized WM Volume

Figure 4. Cholinergic basal forebrain volume mediates between neonatal complications and neurocognitive limitations in preterm-born adults

Path diagrams illustrating the bivariate model of neonatal complications (INTI scores) affecting neurocognitive limitations (FIQ score) in preterm-born adults, which is being compared to an alternative model of cholinergic basal forebrain volume mediating the effect of neonatal complications on adult neurocognitive limitations. Paths are labeled with the (unstandardized) regression coefficients of the respective effect (± SE). a: effect of causal variable on mediator; b: direct effect of mediator on outcome; c: total effect of causal variable on outcome; c': direct effect of causal variable on outcome. INTI: intensity of neonatal treatment index; FIQ: full-scale intelligence quotient of the Wechsler Adult Intelligence Scale-III.



Tables

Table 1. Sample characteristics of demographic, birth-related, and neuropsychologic variables

	VP/VLBW	Controls
N	99	106
Gender (M/F)	57/42	61/45
Age, yrs (SD)	26.7 (0.6)	26.8 (0.7)
Gestational Age, weeks (SD)	30.4 (2.1)**	39.7 (1.1)
Birth Weight, g (SD)	1325 (314) **	3391 (451)
INTI (SD)	11.6 (3.8)	-
Full-scale IQ (SD)	94.7 (12.4) **	102.7 (11.9)
Verbal IQ (SD)	99.5 (13.8)*	105.9 (14.4)
Performance IQ (SD)	90.2 (13.2)**	98.9 (10.1)

* Significantly different from control group at $p < 0.01$

** Significant different from control group at $p < 0.001$

Statistical significance of group differences was assessed using Fisher's exact test for categorical variables and two-sample t-tests for continuous variables. INTI, intensity of neonatal treatment index (arbitrary units: 0 denotes best, 18 denotes worst state); IQ, intelligence quotient; M/F, male/female; SD, standard deviation