Global and network functional connectivity of Nucleus Basalis of Meynert is strengthened in blind individuals

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Declaration of Conflicting Interests

The authors declare no conflict of interests.

Abstract

Vision loss causes dramatic changes in brain function which are thought to facilitate behavioral adaptation. One interesting prospect is that the cholinergic signals are involved in this blindness-induced plasticity. Critically, the nucleus basalis of Meynert is the principal source of the cholinergic signals, however, no studies have yet investigated whether the nucleus basalis of Meynert is altered in blindness. Therefore, here we examined its structure, cerebrovascular response, and the resting-state functional connectivity in blind individuals. We found that the global signal of the nucleus basalis of Meynert as well as its network connectivity with the visual, language, and default mode network is significantly enhanced in early blind individuals. On the other hand, its structure and cerebrovascular response remain unchanged in early blind individuals. Further, we observed that less visual experience predicts stronger global and network connectivity of the nucleus basalis of Meynert. These results suggest that the nucleus basalis of Meynert develops a stronger neuromodulatory influence on the cortex of blind individuals at both global and network levels.

Keywords: nucleus basalis of Meynert, blindness, global connectivity, network connectivity, resting-state, fMRI, plasticity, choline, plasticity

Introduction

The brain retains a profound amount of plasticity which enables us to adapt to environmental demands (Bang and others 2021; Bruel-Jungerman and others 2007). Particularly, loss of vision has been a critical model for investigating brain plasticity. Ample amount of evidence indicates that blind individuals perform better than sighted people at various non-visual tasks including echolocation (Lessard and others 1998; Voss and others 2004), pitch discrimination (Gougoux and others 2004), speech discrimination (Niemeyer and Starlinger 1981), verbal memory (Amedi and others 2003; Hull and Mason 1995) and tactile discrimination (Goldreich and Kanics 2003; Van Boven and others 2000).

One of the influential mechanisms proposed to explain this superior ability of blind individuals is that compensatory alterations occur in the brain which enhance the processing of non-visual input (Fine and Park 2018). Indeed, compelling evidence indicates that the blind individuals' visual cortex becomes recruited for a wide range of non-visual tasks such as Braille reading (Burton and others 2002; Kupers and others 2007; Sadato and others 1996), auditory localization (Norman and Thaler 2019; Voss and others 2006), sensory substitution tasks (Murphy and others 2016; Nau and others 2015; Ptito and others 2005; Striem-Amit and others 2012), verbal memory (Amedi and others 2003), language (Bedny and others 2011; Bedny and others 2015), and mathematics (Amalric and others 2018; Kanjlia and others 2019). When the visual cortex was disrupted by transcranial magnetic stimulation during the task, the performance was impaired in blind individuals (Merabet and others 2009). Beyond the visual cortex, the left superior temporal sulcus, and the fusiform area were shown to be activated to a greater extent during voice

97 discrimination in congenitally blind individuals (Gougoux and others 2009). This line of studies
98 suggests that the cortical functional reorganization occurs in blindness that may modulate
99 various behavioral adaptations.
100
101 In particular, cholinergic signals have been suggested to play a role in blindness-induced
102 compensatory alterations. The nucleus basalis of Meynert (NBM) provides the major source of
103 cholinergic signals to the cortex. The cholinergic input from NBM innervates diffusively the
104 cortex including both primary sensory areas and high-order association areas (Mesulam and
105 others 1983; Mesulam and others 1984). Critically, the cholinergic signals are involved in
106 attention (Everitt and Robbins 1997; Sarter and others 2005) and experience-dependent
107 cortical plasticity (Bakin and Weinberger 1996; Froemke and others 2007; Kilgard and
108 Merzenich 1998). In addition, the cholinergic neurons in NBM are known to rapidly modulate
109 sensory processing (Goard and Dan 2009; Pinto and others 2013). For example, when NBM is
110 stimulated electrically or optogenetically, the cortical coding of visual information in V1 is
111 enhanced (Goard and Dan 2009; Pinto and others 2013) and the performance on a visual task is
112 improved in animals (Pinto and others 2013).
113
114 Relatedly, the input from NBM is thought to play a key role in orchestrating spontaneous
115 activity across the brain (Turchi and others 2018). The resting-state fMRI provides a useful
116 platform to investigate spontaneous brain activity. This spontaneous brain activity is
117 distinguishable into two qualitatively different signals. The first one is a network signal that is a
118 specific correlation between different brain areas. This network signal reflects the functionally

119 connected network architecture (Damoiseaux and others 2006). Further, studies showed that
120 this network signal is constrained by large-scale anatomical connections (Hagmann and others
121 2008; Honey and others 2009). On the other hand, global signal refers to broadly shared signal
122 across the neocortex (Scholvinck and others 2010). This global signal is suggested to reflect
123 large-scale coordination of brain activity (Cole and others 2010). Building on this, a recent study
124 demonstrated that NBM regulates global signal fluctuations (Turchi and others 2018).
125 Specifically, when NBM was inactivated, the global signal components ipsilateral to the
126 injection site were suppressed whereas the specific correlations that define resting-state
127 networks were unaffected. This finding suggests that the input of NBM contributes to the
128 global signals but has little influence on the network signals.
129
130 In the field of blindness-induced plasticity, the amount of choline was observed to be higher in
131 the visual cortex of early blind individuals (Coullon and others 2015; Weaver and others 2013).
132 An interesting question arises from this observation, namely, whether NBM plays a causal role
133 in enhancing the cholinergic signals in the blind's visual cortex. This idea is supported by the
134 fact that NBM sends cholinergic projections to the entire cortex including visual areas
135 (Mesulam and others 1983; Mesulam and others 1984).
136
137 Here, we propose that NBM develops a stronger influence to the neocortex of blind individuals
138 in order to facilitate non-visual processing. Using anatomical MRI and resting-state functional
139 MRI, we provide novel support for this prediction, presenting enhanced global and network
140 connectivity of NBM during rest in early blind individuals. Particularly, the cortical networks

that present increased network connectivity with NBM include visual networks bilaterally (occipital visual cortex, lateral visual cortex, medial visual cortex), language networks of the left hemisphere (inferior frontal gyrus (IFG), posterior superior temporal gyrus (pSTG)), and default mode network (posterior cingulate cortex (PCC)). We further confirmed that these changes in the network and global connectivity of NBM in early blind individuals are not affected by the structural or cerebrovascular changes of NBM. While these alterations of the network connectivity, as well as global connectivity, are significant only within the early blind individuals, the years of visual experience predict both network and global connectivity among early blind, late blind individuals, and sighted controls. These results suggest that NBM may develop stronger cholinergic innervations onto the cortex to support behavioral adaptation in blind individuals.

Materials and Methods

Participants

Forty-nine subjects (23 females, mean age 54.67 ± 2.12) without any history of neurological disorders participated in the study. Seven subjects were congenitally blind, sixteen subjects were late-blind individuals, and twenty-six subjects were sighted controls. One among late blind individuals was later excluded from the entire analysis because the functional MR scan failed to cover NBM. Additional one early blind individual was excluded from the rCVR analysis due to a problem in rCVR computation but included for other analyses. The demographic data of the early and late blind individuals are depicted in Table 1. This study was approved by the Institutional Review Board of Pittsburgh University. All subjects provided written informed consent.

Gender	Age (years)	Onset age (years)	Duration of blindness (years)	Cause
M	58	51	7	Traumatic accident
F	59	53	6	Congenital cataracts, aniridia, pediatric glaucoma
F	53	28	25	diabetic retinopathy
M	62	51	11	Traumatic accident
F	64	0	64	congenital
M	25	0	25	congenital
F	58	0	58	congenital
F	35	31	4	Traumatic accident
M	55	35	20	Trauma accident
M	56	0	56	congenital
M	58	7	51	encephalitis
F	58	46	12	glaucoma
M	18	13	5	pigmentosa
F	60	0	60	retinopathy of prematurity
F	62	0	62	congenital
F	71	59	12	glaucoma
F	60	31	29	glaucoma
M	75	59	16	pigmentosa
F	39	17	22	retinopathy of prematurity
F	30	23	7	tumors
M	63	0	63	retinopathy of prematurity
M	64	54	10	detached retinas

Table 1. Subject demographic and clinical information

MRI data acquisition

MRI data were collected with a 3 T Siemens Allegra MR scanner. Anatomical MR images were obtained using a T1-weighted MPRAGE (176 contiguous 1-mm sagittal slices, voxel size = 1×1×1 mm^3, repetition time (TR) = 1400 ms, echo time (TE) = 2.5 ms, field of view (FOV) = 256×256 mm^2, flip angle = 8°, acquisition matrix = 256×256). Functional images were obtained using a single-shot gradient-echo echo-planar imaging (EPI) sequence (36 contiguous 3-mm axial slices,

175 voxel size = 2×2×3 mm^3, TR = 2000 ms, TE = 25 ms, FOV = 205×205 mm^2, acquisition matrix =
176 64×64) while subjects were at rest with eyes closed. The slices covered the whole brain.
177
178 MRI Voxel-based morphometry (VBM) analysis
179 We conducted VBM analysis to test whether NBM presents any atrophy within the grey and
180 white matter. T1-weighted MRI images were segmented and normalized to MNI space using
181 SPM12 (http://www.fil.ion.ucl.ac.uk/spm/). Then, the images were smoothed using a Gaussian
182 kernel of 6mm FWMH.
183
184 Resting-state fMRI analysis
185 T1-weighted MRI and resting-state fMRI images were preprocessed using CONN's default MNI
186 pipeline in CONN toolbox, version 18.a (www.nitrc.org/projects/conn,RRID:SCR_009550)
187 (Whitfield-Gabrieli and Nieto-Castanon 2012). The default preprocessing steps included the
188 functional realignment and unwarping, slice-timing correction, functional outlier detection,
189 functional segmentation and normalization, structural segmentation and normalization,
190 functional smoothing using a Gaussian kernel of 8mm FWMH. The noise components from
191 cerebral white matter, cerebrospinal fluid, estimated subject-motion parameters, scrubbing,
192 and linear session effects were removed from the functional images for each voxel and each
193 subject using an anatomical component-based noise correction procedure (aCompCor)
194 implemented in CONN's default de-noising pipeline. The functional images were then band-
195 pass filtered to 0.008 Hz – 0.09 Hz.
196

The map of NBM on MNI space was obtained from SPM Anatomy toolbox version 3.0 (Zaborszky and others 2008). In particular, this NBM map was created based on stereotaxic probabilistic maps of the basal forebrain. Magnocellular cell groups in the subcommissural-sublenticular region of the basal forebrain were delineated and then warped to the MNI space (Zaborszky and others 2008). The functional connectivity of the seed NBM was then computed using CONN toolbox. For global correlation analysis, we calculated the average of correlation coefficients between each voxel and the rest voxels of the brain across time series. Then we extracted the global correlation coefficients from the voxels corresponding to seed NBM and averaged them across the seed voxels to identify NBM's brain-wide correlation properties. For ROI-level analysis, we used 30 cortical networks that CONN generated. These include default mode network (bilateral lateral parietal cortex, medial prefrontal cortex, posterior cingulate cortex), dorsal attention network (bilateral frontal eye fields, bilateral intraparietal sulcus), frontoparietal network (bilateral lateral prefrontal cortex, bilateral posterior parietal cortex), language network (bilateral inferior frontal gyrus, bilateral posterior superior temporal gyrus), salience network (anterior cingulate cortex, bilateral anterior insular cortex, bilateral rostral prefrontal cortex, bilateral supramarginal gyrus), sensorimotor network (bilateral lateral sensorimotor cortex, superior sensorimotor cortex), and visual network (bilateral lateral visual cortex, medial visual cortex, occipital visual cortex). We computed the correlation coefficients between the seed NBM and all 30 cortical networks and converted them to z-value using Fisher's r-to-z transformation (Lowe and others 1998). For voxel-level analysis, the correlation coefficients were obtained between the seed NBM and each voxel and were converted to z-value using Fisher's r-to-z transformation.

219

220 rCVR analysis

221 rCVR maps were obtained from the resting-state fMRI images using MriCloud

222 (https://mricloud.org/). Following prior methods (Liu and others 2017), we computed the voxel-

223 wise CVR index (α) using a general linear model between normalized BOLD time series

224 (ΔBOLD/BOLD) and the global signal time series (*GS*). Then we calculated the voxel-wise rCVR

225 by normalizing α by tissue signal intensity averaged across the whole brain (SI). The residuals

226 term (β) was not used for analysis. Below is the summary of these steps.

227 $$\text{rCVR} = \frac{\alpha}{\text{SI}} \text{ where } \alpha \text{ is obtained from } \frac{\Delta\text{BOLD}}{\text{BOLD}} = \alpha \bullet \text{GS} + \beta$$

228

229 We extracted rCVR values from NBM and 30 cortical networks which are in MNI space.

230

231 Statistics

232 For all statistical analyses, we used two-tailed parametric tests with statistical significance set at

233 $P<0.05$. We assessed the assumption of sphericity for all measures ANCOVAs using Mauchly's

234 sphericity tests. When the assumption of sphericity was violated, we reported Huynh-Feldt

235 corrected results. In the following post-hoc tests, we used Bonferroni method to correct the

236 multiple comparisons and reported Bonferroni-corrected P values. For whole-brain voxel-level

237 analysis, a voxel height threshold of $p<0.001$ and a cluster height threshold of p-FDR

238 corrected<0.05 were used.

239

240

241 **Results**

242 We first examined whether the white and grey matter of NBM (**Fig. 1A**) are altered in blindness
243 using VBM analysis. For this, we applied a one-way ANCOVA with a factor group (control, early
244 blind, late blind) to the white and grey matter volumes of NBM as controlling for total
245 intracranial volume and age. The results revealed no significant main effect of group for both
246 white and grey matter (white matter volume: $F(2,43)=1.705$, $P=0.194$, partial $\eta^2=0.073$; grey
247 matter volume: $F(2,43)=1.063$, $P=0.354$, partial $\eta^2=0.047$), suggesting that the anatomical
248 structure of NBM remains intact in blindness.

249

250 Next, we investigated whether NBM presents enhanced global signals in blind individuals. To
251 test for this effect, we computed the global connectivity between NBM and all other cortical
252 voxels. Then, we conducted a one-way ANCOVA with a factor group (control, early blind, late
253 blind) controlling for age. The results showed a significant main effect of group ($F(2,45)=3.530$,
254 $P=0.038$, partial $\eta^2=0.136$; **Fig. 1B**). Further post-hoc tests showed that the early blind group has
255 significantly greater global connectivity compared to sighted controls (control vs. early blind,
256 $P=0.034$, 95% CI=-1.814 – -0.055). However, this increased global connectivity of NBM in the
257 early blind group did not differ from that in the late blind group (early blind vs. late blind,
258 $P=0.248$, 95% CI=-0.268 – 1.605; control vs. late blind, $P=0.957$, 95% CI=-0.923 – 0.391). Further,
259 we examined whether the global connectivity of NBM is associated with the years of visual
260 experience by conducting a partial correlation analysis controlling for age. The results showed
261 that less visual experience predicts a stronger global signal of NBM ($r=-0.453$, $p=0.001$; **Fig. 1C**).

262

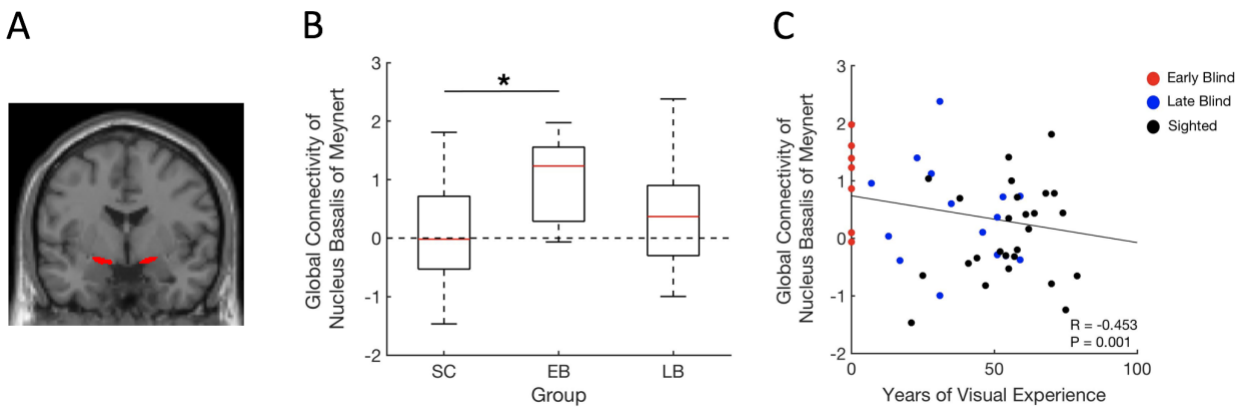


Fig. 1. The Nucleus Basalis of Meynert. (A) Coronal view of the nucleus basalis of Meynert. (B) Global connectivity between the nucleus basalis of Meynert and the entire cortical areas is significantly increased in the early blind group compared to sighted controls. The distributions are represented using box plots. "SC", "EB," and "LB" refer to the sighted controls, early blind and late blind groups. * Bonferroni-corrected $P < 0.05$. (C) Years of visual experience predict global connectivity of nucleus basalis of Meynert. Each point represents one subject. Red, blue and black colors indicate early blind, late blind, and sighted controls. The R and P values in the figure refer to the result of a partial correlation test between years of visual experience and global connectivity of nucleus basalis of Meynert controlling for age. N=48.

Having confirmed the enhanced global signal of NBM in early blind individuals, we further examined whether early blind individuals have increased network connectivity as well. We addressed this question using ROI- and voxel-based analyses. For ROI-based analysis, we created 30 cortical network ROIs (see Materials and Methods) and computed the functional connectivity between NBM and each of the network ROIs. We then conducted a two-way mixed measures ANCOVA with factors group (control, early blind, late blind) and network (30 cortical networks) to the functional connectivity controlling for age. The results revealed a significant main effect of group ($F(2,44)=9.339$, $P<0.001$, partial $\eta^2=0.298$) and significant interaction between group and network ($F(58,1276)=1.754$, Huynh-Feldt correction, $P=0.015$, partial $\eta^2=0.074$) but no main effect of network ($F(29,1276)=0.952$, $P=0.494$, partial $\eta^2=0.021$). Following post-hoc tests showed that the functional connectivity of the early blind individuals is

285 greater than that of the sighted controls and late blind individuals (control vs. early blind:
286 P<0.001, 95% CI=-0.160 – -0.043; control vs. late blind: P=0.760, 95% CI=-0.065 – 0.024; early
287 blind vs. late blind: P=0.008, 95% CI=0.018 – 0.143). Further, a significant interaction between
288 group and network suggests that the functional connectivity changes across the group and that
289 this pattern of change differs across networks.
290
291 Given the significant interaction between group and network, we examined more in detail how
292 the functional connectivity changes across networks by conducting a one-way ANCOVA with a
293 factor group (control, early blind, late blind) to each network as controlling for age. The results
294 revealed a significant main effect of group at visual networks bilaterally (occipital visual cortex:
295 $F(2, 44)=5.491$, P=0.007, partial $\eta^2=0.200$; left lateral visual cortex: $F(2, 44)=8.853$, P=0.001,
296 partial $\eta^2=0.287$; right lateral visual cortex: $F(2, 44)=10.818$, P<0.001, partial $\eta^2=0.330$; medial
297 visual cortex: $F(2, 44)=9.861$, P<0.001, partial $\eta^2=0.310$; **Fig. 2B**), language networks of the left
298 hemisphere (left posterior superior temporal gyrus: $F(2, 44)=10.413$, P<0.001, partial $\eta^2=0.321$;
299 left inferior frontal gyrus: $F(2, 44)=8.105$, P=0.001, partial $\eta^2=0.269$; **Fig. 2B**), and default mode
300 network (posterior cingulate cortex: $F(2, 44)=5.245$, P=0.009, partial $\eta^2=0.193$; **Fig. 2B**).
301 Following post-hoc tests showed that in the occipital visual cortex, the early blind group has
302 higher functional connectivity compared to sighted controls (control vs. early blind: P=0.006,
303 95% CI=-0.441 – -0.060; control vs. late blind: P=0.542, 95% CI=-0.224 – 0.066; early blind vs.
304 late blind: P=0.129, 95% CI=-0.033 – 0.376). In the left lateral visual cortex, the functional
305 connectivity of the early blind group is higher than that of the sighted controls and late blind
306 group (control vs. early blind: P<0.001, 95% CI=-0.465 – -0.117; control vs. late blind: P=0.236,

95% CI=-0.229 – 0.037; early blind vs. late blind: P=0.040, 95% CI=0.007 – 0.383). Similar post-hoc results, that is significantly increased functional connectivity of the early blind group compared to that of the sighted controls and late blind group were observed in the right lateral visual cortex (control vs. early blind: P<0.001, 95% CI=-0.533 – -0.155; control vs. late blind: P=0.083, 95% CI=-0.276 – 0.012; early blind vs. late blind: P=0.039, 95% CI=0.008 – 0.416), medial visual cortex (control vs. early blind: P<0.001, 95% CI=-0.492 – -0.138; control vs. late blind: P=0.389, 95% CI=-0.219 – 0.051; early blind vs. late blind: P=0.013, 95% CI=0.041 – 0.422), left posterior superior temporal gyrus (control vs. early blind: P<0.001, 95% CI=-0.421 – -0.119; control vs. late blind: P=0.098, 95% CI=-0.218 – 0.013; early blind vs. late blind: P=0.041, 95% CI=0.005 – 0.331), left inferior frontal gyrus (control vs. early blind: P=0.002, 95% CI=-0.373 – -0.75; control vs. late blind: P=1.000, 95% CI=-0.097 – 0.131; early blind vs. late blind: P=0.002, 95% CI=0.081 – 0.402), and posterior cingulate cortex (control vs. early blind: P=0.008, 95% CI=-0.359 – -0.044; control vs. late blind: P=1.000, 95% CI=-0.134 – 0.107; early blind vs. late blind: P=0.026, 95% CI=0.018 – 0.357).

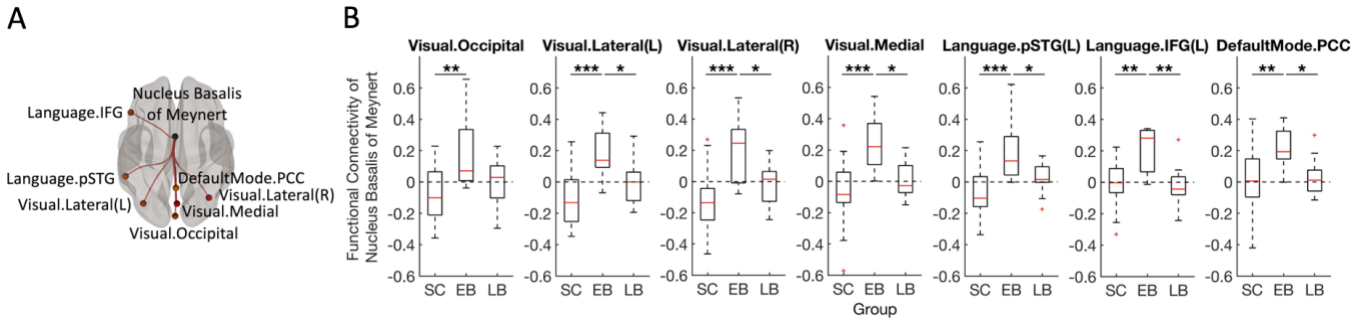


Fig. 2. Early blind individuals have increased functional connectivity between nucleus basalis of Meynert and cortical networks including visual networks, language networks, and default mode network. (A) Schematic depiction of the nucleus basalis of Meynert and seven cortical networks (occipital, lateral, medial visual cortices, left posterior superior temporal gyrus, left inferior frontal gyrus, posterior cingulate cortex) which showed enhanced connectivity with the nucleus basalis of Meynert in the early blind group. (B) Functional connectivity between the nucleus basalis of Meynert and seven cortical networks. The distributions are represented using box

plots and the outliers are plotted as plus signs. “SC”, “EB,” and ”LB” refer to the sighted controls, early blind and late blind groups. * Bonferroni-corrected $P < 0.05$, ** Bonferroni-corrected $P < 0.01$. N=48.

The above analyses were conducted on averaged functional connectivity across large-scale brain networks. For completeness, we also examined the functional network connectivity of NBM at the voxel level. For this, we computed the functional connectivity between NBM and each cortical voxel. We then conducted a one-way ANCOVA with a factor group (control, early blind, late blind) controlling for age. As in the above ROI-level analysis, we observed a significant main effect of group within the visual cortex bilaterally, and the left middle temporal gyrus (**Fig. 3A**). Additionally, the voxel-level analysis found group difference in the bilateral fusiform area, which was previously included in the medial visual network during the ROI-level analysis. Further post-hoc tests (**Fig. 3B**) revealed that this group difference was driven by the early blind group. A comparison between early blind and sighted controls revealed that early blind individuals have greater functional connectivity of NBM at the visual cortex bilaterally, and left superior, middle, inferior temporal gyrus, as well as fusiform area bilaterally. Another comparison between early and late blind groups showed that the early blind group has significantly higher connectivity of NBM at the right visual cortex. On the other hand, late blind and sighted controls did not yield a significant difference at any voxels. These results replicate the ROI-level analysis results although the voxel-level analysis did not observe significant changes within the left inferior frontal gyrus (language network) and the posterior cingulate cortex (default mode network), possibly due to multiple comparisons correction at the voxel level.

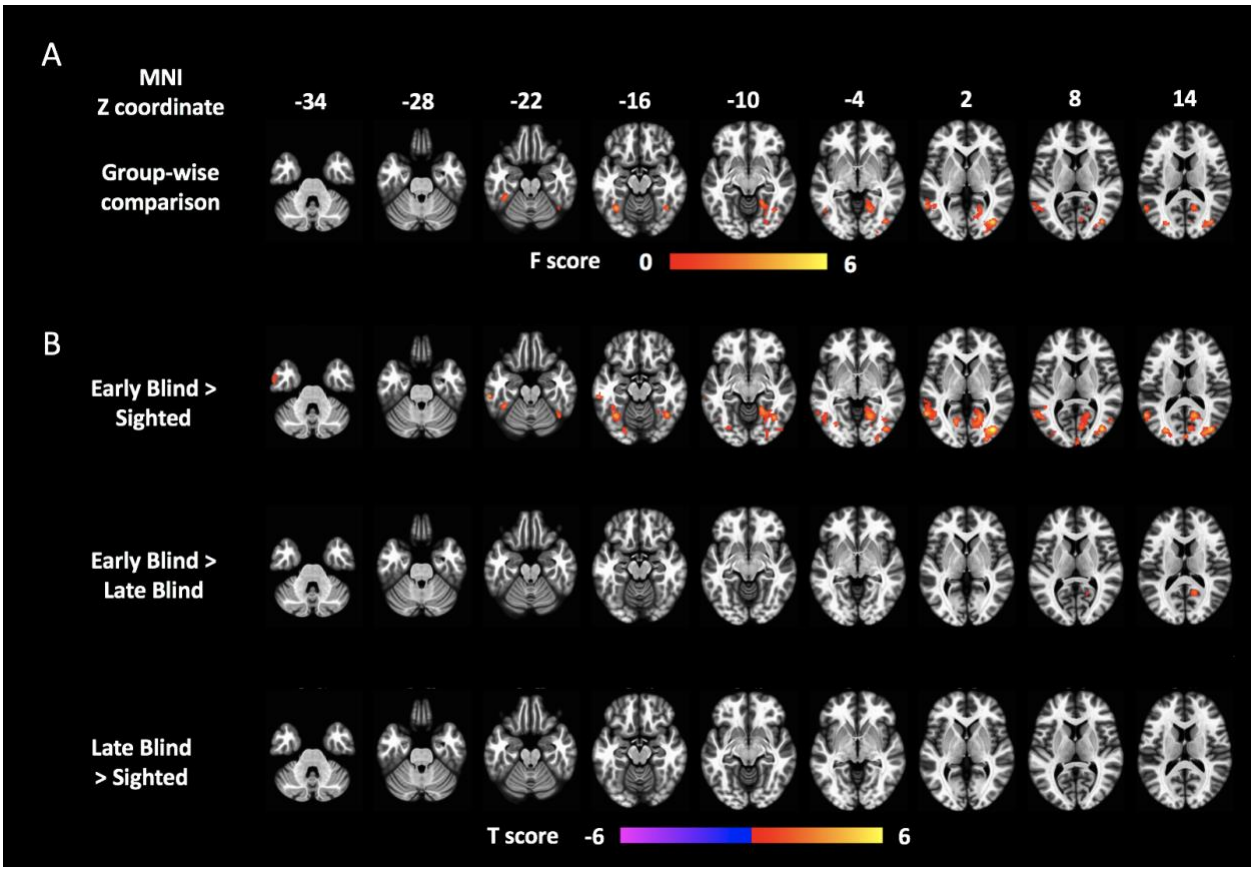


Fig. 3. Group difference of functional connectivity of the nucleus basalis of Meynert. (A) F map for the group-wise difference. Significant group differences are observed in the bilateral visual cortex, left middle temporal gyrus, and bilateral fusiform area. (B) Post-hoc group-wise t-tests between groups. The early blind individuals have increased connectivity of nucleus basalis of Meynert within the bilateral visual cortex, left superior, middle, inferior temporal gyrus, and the bilateral fusiform area compared to sighted controls. Another comparison between early and late blind individuals shows that the early blind group has higher connectivity in the right visual cortex. The late blind and sighted individuals did not show any significant difference. N=48

Since the voxel-level results replicate the findings of the ROI-level analysis, we further explored whether the increase of functional connectivity between NBM and cortical networks is negatively associated with the years of visual experience. For this, we conducted partial correlation analyses controlling for age, using six cortical networks which showed enhanced connectivity with NBM in the early blind group. The results revealed significant correlations

within visual networks (occipital visual network: r=-0.452, p=0.001; left lateral visual network: r=-0.548, p<0.001; right lateral visual network: r=-0.568, p<0.001; medial visual network: r=-0.555, p<0.001), language networks (left posterior superior temporal gyrus: r=-0.527, p<0.001; left inferior frontal gyrus: r=-0.389, p=0.007), and default mode network (posterior cingulate cortex: r=-0.386, p=0.007; **Fig. 4**). The results indicate that less visual experience predicts greater functional connectivity of NBM within these networks.

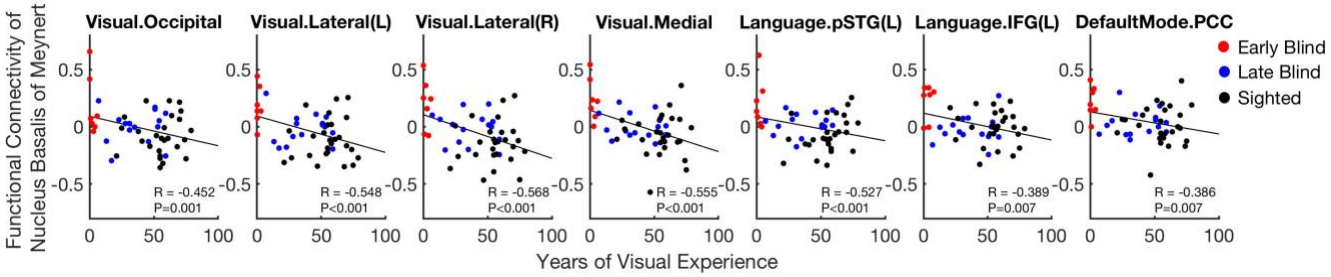
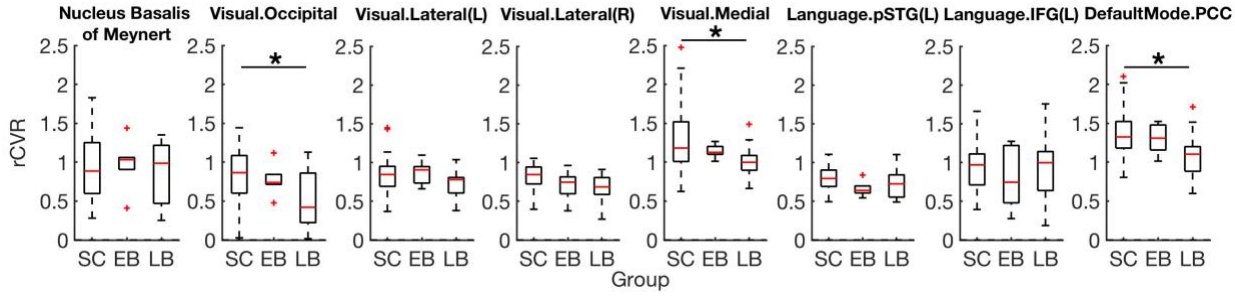


Fig. 4. Years of visual experience predict functional connectivity of nucleus basalis of Meynert. Significant correlations were observed within visual networks (occipital, lateral, medial visual areas), language networks (left posterior superior temporal gyrus, left inferior frontal gyrus), and default mode network (posterior cingulate cortex). Each point represents one subject. Red, blue and black colors indicate early blind, late blind, and sighted controls. For visualization purposes, early-blind data points are plotted apart from each other although their x values are all 0. The R and P values in the figure refer to the results of partial correlation tests between years of visual experience and functional connectivity of nucleus basalis of Meynert controlling for age. N=48.

Finally, we examined whether these global and network signals are affected by cerebrovascular changes using the relative cerebrovascular reactivity (rCVR) map computed from resting-state fMRI (see Materials and Methods). The rCVR measures the cerebral blood vessels' ability to dilate or constrict in response to vasoactive stimuli (Liu and others 2019). Since the BOLD signals in the resting-state fMRI are tightly related to the degree to which cerebral blood

vessels respond to the neurovascular coupling chemical signals (Gauthier and Fan 2019), it is important to examine whether the observed resting-state functional connectivity changes in the early blind group are influenced by cerebrovascular changes. To test whether there are any alterations of rCVR within NBM and the cortical networks that showed significant changes in the early blind group, we applied a one-way ANCOVA with a factor group (control, early blind, late blind) to the rCVR measures controlling for age. We observed no significant main effect of group in NBM ($F(2, 43)=0.257$, $P=0.775$, partial $\eta^2=0.012$), left lateral visual cortex ($F(2, 43)=2.094$, $P=0.136$, partial $\eta^2=0.089$), and left inferior frontal gyrus ($F(2, 43)=0.417$, $P=0.662$, partial $\eta^2=0.019$; **Fig. 5**). However, significant main effect of group was observed in the occipital visual cortex ($F(2, 43)=4.114$, $P=0.023$, partial $\eta^2=0.161$), right lateral visual cortex ($F(2, 43)=3.235$, $P=0.049$, partial $\eta^2=0.131$), medial visual cortex ($F(2, 43)=3.292$, $P=0.047$, partial $\eta^2=0.133$), left posterior superior temporal gyrus ($F(2, 43)=3.279$, $P=0.047$, partial $\eta^2=0.132$) and posterior cingulate cortex ($F(2, 43)=3.725$, $P=0.032$, partial $\eta^2=0.148$; **Fig. 5**). Further post-hoc tests revealed that this significant main effect of group is driven by the reduced rCVR of the late blind individuals, but not by that of the early blind individuals. Specifically, significant differences between sighted controls and late blind group were observed within the occipital visual cortex ($P=0.020$, 95% CI=0.038 – 0.574), medial visual cortex ($P=0.045$, 95% CI=0.005 – 0.576) and the posterior cingulate cortex ($P=0.029$, 95% CI=0.023 – 0.535; **Fig. 5**) but not within the right lateral visual cortex ($P=0.052$, 95% CI=-0.001 – 0.297) and left posterior superior temporal gyrus ($P=0.220$, 95% CI=-0.031 – 0.207). The results suggest that the late blind individuals have impaired rCVR within the occipital, medial visual cortex, and the posterior cingulate cortex. Critically, comparable rCVRs between early blind individuals and sighted

417 controls suggest that the altered global signal and network connectivity of NBM in early blind
418 individuals are not due to cerebrovascular changes, but rather that these changes are primarily
419 driven by the altered neural activity of NBM.
420



421

422 **Fig. 5.** rCVR of the nucleus basalis of Meynert and seven cortical networks that showed
423 significant change in the early blind group. rCVRs of early blind individuals are comparable to
424 those of sighted controls whereas rCVR of late blind individuals are significantly lower than
425 those of sighted controls within the occipital visual cortex, medial visual cortex, and posterior
426 cingulate cortex. The distributions are represented using box plots and the outliers are plotted
427 as plus signs. “SC”, “EB,” and ”LB” refer to the sighted controls, early blind and late blind
428 groups. * Bonferroni-corrected $P < 0.05$. N=47.
429

430

Discussion

The present results provide robust evidence that spontaneous brain activity of NBM is altered in early blind individuals while its anatomical structure and cerebrovascular response are unchanged. Specifically, we observed that both global and network connectivity of NBM is significantly enhanced in early blind individuals. The cortical networks that present increased connectivity with NBM include bilateral visual networks, language networks of the left hemisphere, and the default mode network. Further, the years of visual experience are significantly correlated with both global and network connectivity among early blind, late blind, and sighted individuals. These results provide direct evidence that NBM develops greater neuromodulatory effects on the neocortex of blind individuals, with its strongest effect on early blind individuals.

The cholinergic innervations originating from NBM are known to play a key role in attention (Everitt and Robbins 1997; Sarter and others 2005), experience-dependent plasticity (Bakin and Weinberger 1996; Froemke and others 2007; Kilgard and Merzenich 1998), and sensory processing (Goard and Dan 2009; Pinto and others 2013). At the scale of seconds, the cholinergic neurons of NBM rapidly modulate the visual processing in V1 (Pinto and others 2013) and the release of choline in the cortex is correlated with behavioral performance (Parikh and others 2007). Thus, our results of enhanced global and network connectivity of NBM suggest that blind individuals are under the greater cholinergic influence which underlies the neural processes of attention, plasticity, and sensory processing. This is consistent with the prior observation that the blind individuals have superior capacity at various non-visual tasks

(Amedi and others 2003; Goldreich and Kanics 2003; Gougoux and others 2004; Lessard and others 1998; Niemeyer and Starlinger 1981) and that their visual cortex is recruited for non-visual information processing (Amalric and others 2018; Amedi and others 2003; Murphy and others 2016; Norman and Thaler 2019; Sadato and others 1996). Indeed, prior studies demonstrated that the visual cortex of early blind individuals contains a greater amount of choline (Coullon and others 2015; Weaver and others 2013).

An important question that arises from our study concerns the role of stronger global connectivity of NBM in blind individuals. The brain region that has high connectivity with the rest of the brain suggests that this area is essential for coordinating large-scale brain activity patterns (Cole and others 2010). Thus, our results suggest that NBM exerts greater influence on coordinating the large-scale brain activity in blind individuals. This explanation is in line with a recent observation that NBM modulates the global signals but has minimal effect on the network connectivity (Turchi and others 2018).

Although NBM was reported to play little role in the network connectivity (Turchi and others 2018), we observed that NBM has increased network connectivity in blind individuals at bilateral visual networks, language networks of the left hemisphere, and the default mode network. Different from global connectivity, the network connectivity contains information about functionally connected brain structure (Damoiseaux and others 2006). Thus, our results indicate that NBM is more functionally coupled with the visual, language, and default mode networks in blind individuals. This enhanced functional connectivity may serve to facilitate

475 cholinergic modulation during tasks. Indeed, the brain areas that showed increased activity
476 during the non-visual tasks in blind individuals include the visual cortex (Amalric and others
477 2018; Amedi and others 2003; Bedny and others 2011; Murphy and others 2016; Norman and
478 Thaler 2019; Sadato and others 1996), fusiform area and the left superior temporal sulcus
479 (Gougoux and others 2009). These areas overlap with those that showed increased network
480 connectivity with NBM in the current study. Thus, stronger activation of these cortical areas in
481 blind individuals observed during tasks is likely to be associated with greater cholinergic
482 modulation of NBM.
483
484 The current results raise important questions for future studies. First, it remains unclear
485 whether cholinergic signals in the cortical areas are directly driven by NBM activity in blind
486 individuals. This question can be partly addressed by quantifying the amount of choline from
487 the cortex using magnetic resonance spectroscopy and then examining the correlation between
488 the amount of choline and the functional connectivity between NBM and the corresponding
489 cortical areas. Secondly, it has not been explored yet whether the NBM regulates the neural
490 activity in the cortical networks during non-visual tasks in blind individuals. While our results
491 imply such direct regulation of NBM, we only examined the brain activity during rest, but not
492 during the task. If further studies reveal direct modulation of NBM during tasks in blind
493 individuals, its timescales and impact on the performance are the next important questions for
494 future inquiry.
495

To summarize, our results show that the functional connectivity of NBM becomes strengthened in the absence of visual input at both global and network levels. This alteration appears to arise from neural changes of NBM, but not from structural or neurovascular changes. These findings thus suggest that stronger cholinergic modulation of NBM may serve to facilitate behavioral adaptation in blind individuals.

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