Title: Top-down Influence on the Visual Cortex of the Blind during Sensory Substitution

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Short title: Sensory Substitution fMRI in the Blind

Abbreviations: Aud/Ins/Lim: auditory/insula/limbic networks; BA: Brodmann area; BOLD: blood-oxygen-level dependent; CSF: cerebrospinal fluid; FC: functional connectivity; fcMRI: functional connectivity magnetic resonance imaging; fMRI: functional magnetic resonance imaging; FDR: false discovery rate; FWE: family-wise error; FWHM: full-width half-maximum; GLM: general linear model; PET: positron emission tomography; ROI: region of interest; SSD: sensory substitution device; SS/M: somatosensory/motor networks; Task-: task-negative networks; Task+: task-positive networks

Abstract

Visual sensory substitution devices provide a non-surgical and flexible approach to vision rehabilitation in the blind. These devices convert images taken by a camera into cross-modal sensory signals that are presented as a surrogate for direct visual input. While previous work has demonstrated that the visual cortex of blind subjects is recruited during sensory substitution, the cognitive basis of this activation remains incompletely understood. To test the hypothesis that top-down input provides a significant contribution to this activation, we performed functional MRI scanning in 11 blind (7 acquired and 4 congenital) and 11 sighted subjects under two conditions: passive listening of image-encoded soundscapes before sensory substitution training and active interpretation of the same auditory sensory substitution signals after a 10-minute training session. We found that the modulation of visual cortex activity due to active interpretation was significantly stronger in the blind over sighted subjects. In addition, congenitally blind subjects showed stronger task-induced modulation in the visual cortex than acquired blind subjects. In a parallel experiment, we scanned 18 blind (11 acquired and 7 congenital) and 18 sighted subjects at rest to investigate alterations in functional connectivity due to visual deprivation. The results demonstrated that visual cortex connectivity of the blind shifted away from sensory networks and toward known areas of top-down input. Taken together, our data support the model of the brain, including the visual system, as a highly flexible task-based and not sensory-based machine.

Keywords: blindness, cross-modal neuroplasticity, functional MRI, sensory substitution, top-down, vision

Highlights

- We used a sensory substitution task to investigate plasticity due to blindness.
- We report increased top-down influence in visual cortex activity of the blind.
- Top-down modulation is greater in congenitally blind compared to acquired blind.
- Blindness increases connectivity between visual and task-positive networks.

1. Introduction

Sensory substitution devices (SSDs) represent one approach to vision rehabilitation for those who have lost sight. These devices use a video camera to take a series of images, convert the images into a cross-modal auditory (Meijer, 1992) or tactile (Bach-y-Rita, 2004) signal, and present this substituting signal in place of direct visual input. SSDs have been shown to impart improved environmental perception in the completely blind (Nau et al., 2013; Nau et al., 2014). The advantages of SSDs include their immediate availability, non-surgical implementation, and relatively accessible cost. Moreover, SSDs have been shown to enable improved function in a wide variety of patients with diverse etiologies in blindness (Nau et al., 2013; Nau et al., 2015; Nau et al., 2014).

In addition to the potential for functional improvements, SSDs provide a unique tool for investigating functional organization of the visual system, as well as neuroplasticity associated with visual deprivation. In sighted subjects, Renier et al. used an SSD task to demonstrate that regions within the occipital lobe that are involved in depth perception do not require visual input, but are more specific to modeling depth regardless of the mode of sensory input (Renier et al., 2005). Early studies of SSDs using positron emission tomography (PET) and functional MRI (fMRI) revealed that the visual cortex was engaged by the interpretation of cross-modal signals in blind subjects (Arno et al., 2001; De Volder et al., 1999; Merabet et al., 2009; Ptito et al., 2005). Collignon et al. further demonstrated a direct relationship between occipital lobe activation and improved SSD use in blind subjects by demonstrating decreased accuracy when subjects had occipital lobe activity disrupted by transcranial magnetic stimulation (Collignon et al., 2007). More recent studies have utilized SSDs to identify regions of the brain that maintain the functions of higher-order visual processing during analogous sensory substitution tasks even

if the subjects have had no prior visual experience (Amedi et al., 2007; Striem-Amit and Amedi, 2014; Striem-Amit et al., 2012a; Striem-Amit et al., 2012b; Watkins et al., 2013).

While previous work has demonstrated the presence of activity within the visual cortex during SSD use following visual deprivation, the cognitive basis of this activation remains unclear. The classical view of visual processing in healthy, sighted subjects posits a bottom-up flow of information from lower-order visual centers to higher-order centers, where visual information is continually integrated along this pathway. However, each of these bottom-up projections also has a reciprocal top-down projection from the higher-order center back to the lower-order center (Gilbert and Li, 2013). These top-down projections carry information about attention and expectation, and can alter perception of the visual environment by modulating the activity of lower-level visual regions in the brain. To date, the role of these projections in cross-modal activity in the visual cortex following visual deprivation remains incompletely understood. In this study, using fMRI and an auditory sensory substitution task in both blind and sighted subjects who had no prior knowledge of the SSD, we aimed to minimize the training time necessary to observe significantly increased visual cortex activity. In this way, we tested the hypothesis that visual cortex activity in the blind is significantly modulated by cognitive processing that is intrinsically present in the brain before there is further reorganization in functional or structural connectivity from long-term sensory substitution training.

To investigate this aim, we compared functional activation in both blind and sighted subjects in response to an auditory sensory substitution task using fMRI in two conditions separated by just minutes: 1. passive listening with no prior knowledge of the SSD, and 2. active interpretation of same auditory sensory substitution stimuli after a brief training session of approximately 10 minutes in the MRI scanner. This type of paradigm, which is necessary to

measure the proportion of activity specific to top-down input, is not usually possible because subjects will have typically undergone training prior to the functional imaging session. We further supplemented this experiment with a separate functional connectivity MRI (fcMRI) study to investigate changes in the intrinsic functional connectivity measured at rest due to visual deprivation.

2. Materials and Methods

All studies were approved by the University of Pittsburgh Institutional Review Board.

2.1. Subject recruitment

Twenty-two blind subjects and twenty age-matched controls were enrolled from the established research registry of the Sensory Substitution Laboratory at the University of Pittsburgh. Blind subjects were confirmed to have no vision beyond light perception bilaterally by the Freiburg Visual Acuity and Contrast Test (FrACT), and all subjects had no known neurological disorders and were right-handed. Subjects were scanned after obtaining informed written consent. See Table 1 for complete demographic information.

2.2. Sensory substitution fMRI experiment

In order to investigate visual cortex activity arising from top-down input during sensory substitution, we scanned each subject twice while presenting them with a set of “soundscapes” generated by The vOICe sensory substitution transformation (Meijer, 1992). These soundscapes represented white horizontal or vertical bars moving across a black background in one of four directions (up, down, left or right). Each stimulus was 10 seconds long consisting of 10 concatenated soundscapes. Over those 10 seconds, the white bar would move across the image from one edge to the opposite edge a single time with constant velocity. The subjects were able

to respond any time during those 10 seconds. Subjects had no prior knowledge of the vOICe device or how to interpret the soundscapes before beginning the experiment. For the first scan, the subjects were presented the soundscapes for passive listening, and were asked to respond with the left arrow on the keypad placed in their right hand whenever they heard a sound to indicate they were able to hear the stimuli. Subjects were instructed to press this button as soon as they heard the soundscapes begin. The subjects then took a short break in the MRI scanner and were provided a brief introduction to The vOICe. This training typically took 10 minutes, during which we described the image-to-landscape transformation and discussed examples. The subjects then repeated the scan, but this time we asked them to interpret the soundscapes as images, determine the direction the bar was moving, and respond on the keypad with that direction. Subjects were instructed to respond as soon as they could discern the direction of motion, bearing in mind that they should listen to at least more than one soundscape in order to determine which direction the bar was moving. By holding the bottom-up input constant, we were able to compare activity between the passive listening (pre-training) and active interpretation (post-training) scans to measure activity specific to top-down input. Structural reorganization of the brain was not expected between the pre- and post-training scans because of the short duration of training employed. E-prime software was used for presenting the stimuli and recording keypad responses given by the subjects while they were in the scanner (Psychology Software Tools, Inc. Sharpsburg, PA, USA), and sounds were played through a pneumatic headphone system (Avotec, Inc., Stuart, FL, USA). Interpretation accuracy and reaction times were recorded by E-prime software and tested for group-wise differences using an ANOVA.

For this experiment, we scanned 18 blind subjects and 13 sighted controls using a 3 Tesla Siemens Allegra MRI scanner. We excluded 8 subjects for large head motion (>3 mm of translation or >3 degrees of rotation in any direction), or for lack of response during the passive listening task to ensure all remaining subjects adequately heard the stimuli. One additional blind subject was excluded due to an indeterminate technical failure. Therefore, final analyses were carried out on 4 congenitally blind subjects, 7 acquired blind subjects, and 11 sighted controls. No significant differences in age were measured between groups with mean age of 50.8 ± 17.5 years in the congenitally blind group, 45.4 ± 16.6 years in the acquired blind group, and 49.9 ± 16.2 in the sighted group (mean $\pm$ standard deviation, $p=0.82$ for ANOVA on age). Demographic information is shown in Table 1.

All blood-oxygenation-level-dependent (BOLD) fMRI data were acquired with a single-shot gradient-echo echo-planar-imaging pulse sequence with the following parameters: 2 s repetition time, 26 ms echo time, 104×104 imaging matrix over a 20.5×20.5 cm² field-of-view, and 28 contiguous 3.24 mm thick axial slices. The slices were arranged to cover most of the cerebrum while ensuring the entire occipital lobe was covered. The acquisition time for each scan was 6 min 14 s, consisting of 15 trials with alternating 14 s of rest and 10 s of task. To assist in image processing we also acquired a T1-weighted anatomical image using a 3D MPRAGE pulse sequence (1.4 s repetition time, 2.5 ms echo time, 1 mm isotropic sampling over a $25.6 \times 25.6 \times 17.6$ cm³ field-of-view, superior-inferior frequency encoding direction). Sighted subjects wore a blindfold for all scans and training.

Data processing was performed with a combination of SPM8 subroutines (<http://www.fil.ion.ucl.ac.uk/spm>), and in-house software implemented in MATLAB (Mathworks, Natick, MA, USA). The T1-weighted image was segmented to obtain a set of tissue

probability maps, which estimated the proportion of each voxel occupied by gray matter, white matter and cerebrospinal fluid (CSF). To preprocess the functional images, we applied slice timing correction and realignment to correct for head motion. The functional images were then co-registered to the T1-weighted image, and normalized to the Montreal Neurological Institute (MNI) ICBM 152 brain template using the T1-weighted image as the source image (Fonov et al., 2011). The images were then masked with a gray matter mask, which was equal to one where the gray matter content was greater than white matter plus CSF content. Images were smoothed with a Gaussian kernel with full-width half-maximum (FWHM) of 8 mm. To calculate an activation map for each subject, data from each voxel were fit with a general linear model (GLM). Explanatory variables in GLM included the task paradigm convolved with a canonical hemodynamic response function, the first temporal derivative of this variable, motion parameters calculated during the realignment, the average time course from the white matter, the average time course from the CSF and a constant. White matter and CSF time courses were included to help control for physiological noise. They were measured from respective masks before applying the gray matter mask, where the white matter or CSF content was >99% and gray matter content was calculated as 0%. A BOLD activation map was then calculated by taking the ratio of coefficient 1 (i.e., the amplitude of the task-related signal change) to the coefficient of the constant term (i.e., the average BOLD signal at rest) and multiplying by 100 to convert to a percentage value.

Statistical analyses were performed at both voxel-wise and region-of-interest (ROI) levels. To display the average activation maps within each group both before and after training, all of the individual activation maps were modeled with a GLM with 6 predictors (one for each combination of group and pre- versus post-training), and average activation maps were tested

with the corresponding t-test. To compare the degree of top-down modulation between groups, we calculated maps of the modulation for each subject by subtracting the pre-training activation map from the post-training activation map. These maps were then tested with an ANOVA to identify regions of the brain where the degree of top-down modulation was significantly different between groups, and post-hoc t-tests were performed to test how top-down modulation compared between groups. T score maps summarizing the results of the voxel-wise testing were thresholded at a family-wise error (FWE) corrected $p < 0.05$ (uncorrected $p < 0.01$, cluster size > 213 voxels). For the ROI analyses, activation was summarized as the average BOLD percent change within the gray matter mask for each individual in four regions of interest: primary visual cortex (Brodmann area 17), secondary visual cortex (Brodmann area 18), tertiary visual cortex (Brodmann area 19), and auditory cortex (Brodmann areas 41 and 42). These ROIs were defined using a publicly available atlas in the MRIcron software package (<http://www.mccauslandcenter.sc.edu/mricro/mricron>). P values of less than 0.05 were considered significant.

2.3. Functional connectivity MRI experiment

In addition to the sensory substitution tasks, we performed fcMRI at rest to assess alterations in intrinsic functional connectivity between visual cortex and other brain regions due to visual deprivation. We collected 8 minutes of BOLD images in the absence of a task with a single-shot gradient-echo echo-planar-imaging pulse sequence and the following acquisition parameters: 2 s repetition time, 25 ms echo time, 64 x 64 imaging matrix over a 20.5 x 20.5 cm² field-of-view, and 36 contiguous 3.24 mm thick axial slices. To improve statistical power, additional subjects were recruited for this portion of the study. In total we scanned 20 sighted controls excluding two for severe head motion, and 22 blind subjects excluding four for motion. As in the SSD task

experiment, sighted subjects were blindfolded during scanning. Demographic information is listed in Table 1.

As described above, images underwent slice timing correction, realignment, normalization to MNI space, and masking with a gray matter mask. Images were smoothed with a Gaussian kernel of FWHM = 5 mm. Nuisance covariables were then regressed out using a GLM. Image data and the covariables were temporally filtered with a pass band from 0.01 to 0.1 Hz. The covariables included the motion parameters from realignment, the average time course from the white matter, the average time course from the CSF, the average time course from the entire brain, and a constant. Then a visual functional connectivity map was constructed for each individual using a seed-to-brain approach by computing the average correlation coefficient between seed voxels in the entire occipital lobe and each voxel in the brain. This large seed ROI allowed detection of alterations in functional connectivity both within the occipital lobe and between the occipital lobe and the rest of the brain. Significant visual functional connectivity within each of blind and sighted groups were tested with a voxel-wise one-sample t-test, and significant differences between blind and sighted groups were tested with a voxel-wise two-sample t-test. FWE corrected $p < 0.05$ was considered significant (uncorrected $p < 0.01$, cluster size > 71 voxels).

For ROI level analysis, we constructed a graph that summarized functional connectivity for each individual. Following image preprocessing, the average time course was calculated from each ROI given by a functionally defined atlas comprising 68 brain regions (Jones et al., 2012), and the functional connectivity graph was generated for each subject by calculating the Pearson correlation matrix on the set of 68 time courses. Therefore the nodes of the graph are given by the regions of the atlas, and the edges of the graph (i.e. the strengths of functional connectivity)

are given by the correlation coefficient of the time courses between each pair of nodes. The nodes are arranged in five modules according to functional similarity and these modules include somatosensory/motor, auditory/insula/limbic, task-positive, task-negative and visual networks. Task-positive networks refer to regions of the brain where activity increases during cognitive tasks, whereas task-negative networks refer to a set of anti-correlated regions of the brain where activity decreases during cognitive tasks (Fox et al., 2005). To focus on the effect of visual deprivation on vision-related connectivity, only edges that involved at least one node from the visual network module were tested. The connectivity strength of each edge in the graph was fit with an ANOVA model and group-wise comparisons were made with the corresponding t-test from this model. P values less than a false discovery rate (FDR) corrected 0.05 were considered significant using ANOVA followed by post-hoc t-tests.

3. Results

3.1. Visual cortex activity is significantly modulated in blind but not sighted subjects after short-term sensory substitution training.

In order to first measure visual cortex activity that was non-specific to the interpretation of SSD signals, we presented the subjects with a set of soundscapes for passive listening. These soundscapes represented white bars moving across a black background in one of four directions (up, down, left and right). During this scan, the subjects had no knowledge of how to interpret the soundscapes as images, and simply responded on a keypad that the sounds were audible. The results of t-tests for the average activation map in each group during this pre-training scan are shown in Figure 1 (FWE corrected $p < 0.05$). As expected, subjects from all sighted, acquired blind and congenitally blind groups exhibited significant positive BOLD responses within the auditory cortex upon passive sound presentation before training. Furthermore, subjects from all

groups exhibited significant positive responses in non-visual higher-order areas including the salience and attention networks, where the salience network involves the anterior cingulate and insular cortices, and the attention network includes superior parietal and superior frontal cortices (van den Heuvel et al., 2009). However, substantial differences in BOLD functional responses in the visual cortex were observed between the sighted and blind groups. In the sighted group, significant negative responses were observed within the visual cortex, with the greatest amplitude in the tertiary visual cortex and the smallest amplitude in the primary visual cortex (Figure 2A). In contrast, the functional BOLD response of the visual cortex of both acquired and congenitally blind subjects was significantly positive. The greatest response was observed within the primary visual cortex, and decreased monotonically from primary to secondary to tertiary visual cortex (Figures 2B and 2C).

Figure 1. Task-induced activation maps overlaid on a T1-weighted template image for sighted, acquired blind, and congenitally blind groups both before and after training. Images are in neurological view (left side of brain on left side of image) and each panel in a row represents a different slice location in the 3D image volume moving from inferior (left) to superior (right) slices. For each group, a t-test is shown for the average activation map for both before and after training using a general linear model with 6 parameters (one for each combination of group and condition).

Figure 2. Summary of region-of-interest analysis of task-based, auditory sensory substitution fMRI experiment in primary (Brodmann Area [BA] 17), secondary (BA 18) and tertiary (BA 19) visual cortices and in auditory cortex (BA 41/42). **(A)** The average BOLD percent change both pre- and post-training within the sighted group (n=11). **(B)** The average BOLD percent change both pre- and post-training within the acquired blind group (n=7). **(C)** The average BOLD percent change both pre- and post-training within the congenitally blind group (n=4). Results are presented as mean $\pm$ standard error. (* $p < 0.05$, paired t-test)

We next took a short break of approximately 10 minutes to introduce the subjects to the vOICE visual-to-auditory transformation in the MRI scanner, and then repeated the fMRI scan using the same set of stimuli. During this second scan, subjects were asked to interpret the soundscapes as images and respond on the keypad with the direction of motion. Using this design, we were able to measure activity specific to SSD interpretation by contrasting the measured activation between the post- and pre-training scans while bottom-up input was held constant. No significant differences between groups were observed in interpretation accuracy (sighted: $62.1 \pm 12.3\%$, acquired blind: $67.9 \pm 12.0\%$, congenitally blind: $31.3 \pm 20.8\%$, mean $\pm$ standard error of the mean; ANOVA $p=0.30$) or in reaction time (sighted: $4.6 \pm 0.65s$, acquired blind: $4.1 \pm 0.73s$, congenitally blind: $3.2 \pm 1.3s$, mean $\pm$ standard error of the mean; ANOVA $p=0.54$). Subjects reported that they understood the SSD coding scheme and the performance across all subjects is significantly greater than chance ($p < 0.001$). The results of t-tests in each group during this post-

training scan are also shown in Figure 1 (FWE corrected $p < 0.05$). Upon active interpretation of the sensory substitution stimuli, the average activation map within the sighted group appeared unchanged compared to the pre-training condition, whereas an increase in the spatial extent and amplitude of positively activated regions was observed within the visual cortex of both acquired and congenitally blind subjects.

To test whether the degree of top-down influence in the visual cortex differed between groups, we calculated maps of the modulation in visual cortex activity (i.e., BOLD percent change in post-training scan minus pre-training scan) in each subject of the sighted and blind groups. We then performed an ANOVA on these maps to detect regions where the degree of top-down modulation was significantly different among all groups, and the results are shown in Figure 3 along with group-wise t-tests from the same model. Task-induced modulation within the visual cortex was significantly greater in acquired blind subjects compared to sighted subjects, while modulation was significantly greater in the congenitally blind subjects compared to both sighted subjects and acquired blind subjects. This statistical testing was also performed at the ROI level, and significant group-wise differences were observed between sighted and blind groups in all three visual ROIs (Figure 4). The amplitude of this task-induced modulation was most pronounced in the tertiary visual cortex and decreased monotonically from tertiary to secondary to primary visual cortex in the blind groups. Furthermore, the degree of task-induced modulation was greatest in the congenitally blind group, intermediate in the acquired blind group and least in the sighted group. Finally, while the top-down modulation appears to be left-lateralized in Figure 3, we summarized change in BOLD % change between pre- and post-training in the left and right occipital cortex and detected no significant differences between

hemispheres both across all subjects, and within each group ($p > 0.25$, paired t-test, data not shown).

Figure 3. Group-wise comparisons of top-down modulation. Images are in neurological view (left side of brain on left side of image) and each panel in a row represents a different slice location in the 3D image volume moving from inferior (left) to superior (right) slices. **(Top row)** F map for significant group-wise differences among all sighted, acquired blind and congenitally blind subjects (FWE corrected $p < 0.05$). **(Bottom 3 rows)** Post-hoc group-wise t-tests between individual groups (FWE corrected $p < 0.05$).

Figure 4. The difference in BOLD percent change between post- and pre-training scans in each of sighted, acquired blind, and congenitally blind groups summarized in each region of interest (mean $\pm$ standard error, * $p < 0.05$, post hoc two-sample t-test following $p < 0.05$ ANOVA). Similar trends were observed in BA 17 ($p = 0.12$, ANOVA) and in BA 41/42 ($p = 0.09$, ANOVA), but the effects did not reach significance in these regions.

3.2. Alterations in intrinsic functional connectivity support increased top-down influence in visual cortex due to visual deprivation.

Lastly, we performed a separate functional connectivity MRI (fcMRI) study to investigate alterations in visual cortex connectivity due to visual deprivation. The results of the fcMRI study are summarized for both voxel-wise (Figure 5) and ROI (Figures 6 and 7) levels. Functional connectivity between visual and somatosensory/motor nodes exhibited lower strength in the blind groups compared to the sighted group. Furthermore, functional connectivity within the visual networks was weaker in the blind groups compared to the sighted group. No significant difference was observed between groups in the visual-to-auditory connections. Conversely, functional connectivity was stronger in several other edges in blind subjects compared to sighted

subjects. The module that contained the most consistent positive (blind>sighted) differences in functional connectivity was the task-positive module, which includes the attention networks and prefrontal cortex, both known regions of top-down input into the visual cortex. Functional connectivity was also stronger in the blind subjects between visual networks and the deep gray nuclei, which serve as relays for top-down projections into the visual cortex. While all deep gray nuclei are clustered in the functional atlas, from the voxel-wise map we can see that this alteration in connectivity is localized the medial nuclei of the thalamus (Fig. 5). The task-negative module also contained some positive differences in visual connectivity in the lateral parietal lobules of the default mode network. No significant differences in functional connectivity were observed between acquired and congenitally blind subjects in either voxel-wise or ROI-based analysis.

Figure 5. Summary of voxel-wise analysis of functional connectivity MRI data. Images are in neurological view (left side of brain on left side of image) and each panel in a row represents a different slice location in the 3D image volume moving from inferior (left) to superior (right)

slices. **(Top row)** F map for significant group-wise differences among all sighted, acquired blind and congenitally blind subjects (FWE corrected $p < 0.05$ **(Bottom rows)** Post-hoc group-wise t-tests between individual groups (FWE corrected $p < 0.05$).

Figure 6. Average graphs of functional connectivity (FC) for each group. The nodes of the FC graph represent 68 brain regions that are given from a publicly available functionally derived brain atlas (Jones et al., 2012). The strength of the FC, or edges between nodes is given by the correlation coefficient of the average time courses from a given pair of nodes. Nodes are arranged within five modules according to functional similarity, which include Somatosensory/Motor (SS/M), Auditory/Insula/Limbic (Aud/Ins/Lim), Task-positive (Task+), Task-negative (Task-) and Visual Networks.

Figure 7. Significant differences between average FC graphs. To focus on the effect of visual deprivation on vision-related functional connectivity, statistical testing was performed only in edges that contained at least one visual node, and the others were masked out in white.

Remaining edges that did not reach statistical significance were masked out in black (FDR corrected $p < 0.05$ considered significant, ANOVA followed by post-hoc t-tests). Most FC differences were observed between sighted and congenitally blind subjects, while no differences were observed between acquired and congenitally blind subjects (graph not shown). Negative differences were observed between visual and somatosensory/motor networks (blue arrows). Positive differences were observed between visual and task-positive networks (bottom red arrow) and the deep gray nuclei (top red arrow). Positive differences were also observed between visual networks and default mode network nodes including the lateral parietal lobules and cingulate cortex (green arrows).

4. Discussion

In this study, we demonstrated that sighted subjects exhibit a negative BOLD response in the visual cortex upon exposure to an auditory stimulus, during both passive listening and active interpretation of the auditory sensory substitution stimuli. This observation, which may reflect decreased neuronal firing, may be attributed to attentional processes and cross-modal interactions (Hairston et al., 2008; Kawashima et al., 1995; Laurienti et al., 2002). In contrast, the BOLD response throughout the visual cortex of blind subjects during the passive listening task was either positive or not significantly different from zero, which was in agreement with previous studies of auditory processing in the visual cortex of blind subjects (Kujala et al., 1995; Weeks et al., 2000). Furthermore, we found that the strength of this response was significantly modulated by active interpretation of the SSD signals in both blind groups but not in the sighted group. Since we held the bottom-up sensory input constant between tasks while minimizing external factors other than the short-term training, we can conclude that a significant portion of visual cortex response to SSD tasks in the blind is due to top-down input. In addition, the degree of this top-down modulation is greatest in congenitally blind subjects, intermediate in acquired blind subjects and least in sighted subjects. These findings were further supported by a separate fcMRI study, which revealed bidirectional alterations in intrinsic functional connectivity associated with visual deprivation. Both of our congenitally and acquired blind subjects exhibited weaker

functional connectivity within visual networks and between visual and somatosensory networks, similar to a previous report in the early blind (Liu et al., 2007). Furthermore, we observed a number of edges with greater strength in the blind subjects compared to the sighted subjects particularly in the congenitally blind group. Many of these edges involve nodes known to have top-down projections into the visual cortex including the attention network and the prefrontal cortex, as well as the thalamus, which serves as a relay for top-down projections.

Studies of the visual system, and specifically in those without sight, have provided important insights into how the brain processes sensory input. Pascual-Leone and Hamilton put forth a metamodal model of the brain in which neural networks in the visual cortex may be best suited to process visual input, but they receive input from all sensory modalities and can unmask these inputs to optimize behavior (Pascual-Leone and Hamilton, 2001). Buchel proposed that visual deprivation would cause a flipping of the normal hierarchy in the visual system in blind subjects, where the primary visual cortex becomes a higher-tier area (Buchel, 2003). Our findings provide an alternate perspective from previous approaches to support a recently proposed model of the brain as a “flexible task-based and not sensory-based” machine (Reich et al., 2012). Work which was synthesized into this model focused on identifying brain regions that conserve their functions in high-level visual processing of the environment when blind subjects are presented with an analogous sensory substitution signal. These regions include the dorsal and ventral visual streams (Striem-Amit et al., 2012b), the visual word form area (Striem-Amit et al., 2012a), medial temporal lobe (Watkins et al., 2013), the lateral occipital complex (Amedi et al., 2007), and extrastriate body-selective area (Striem-Amit and Amedi, 2014). Alternatively, we have approached this model by demonstrating that visual deprivation causes the visual cortex to behave less like a sensory network but more resembling task-positive brain networks. These task-

positive brain networks are highly flexible and their BOLD responses are positively correlated to a wide array of cognitive tasks (Fox et al., 2005). Just as task-positive networks are task-flexible, the visual cortex of blind subjects has demonstrated activity during not only sensory stimuli but during a variety of non-visual, high-level cognitive processing (Kupers and Ptito, 2014; Ricciardi et al., 2014).

Hertz and Amedi recently reported alterations in activity in the auditory and visual cortices in sighted, young adult subjects after a 1-hour training paradigm with The vOICe SSD (Hertz and Amedi, 2014), whereas we do not report any significant differences in our older sighted subjects between pre- and post-training using our 10-minute training paradigm. This apparent discrepancy may be attributed to differences in task difficulty and experimental design. Ptito et al. used positron-emission tomography (PET) and a similar pre- and post-training paradigm via electrotactile stimulation of the tongue to demonstrate that sensory substitution induced activation of the occipital cortex in blind subjects occurred after one week of rigorous training (Ptito et al., 2005). Whereas the study by Ptito et al. concluded that rigorous training was necessary to induce plasticity, our findings do not rely on explicit training-induced structural or functional reorganization of the brain during the experimental session given only 10 minutes of training in the MRI scanner. Instead, we postulate from our fcMRI results that functional brain reorganization has already occurred in the blind subjects prior to pre-training fMRI scans and the short-term sensory substitution training. Therefore, simply altering the way the blind subjects cognitively approach the identical stimuli is sufficient to significantly strengthen the activation of the visual cortex, and that the amplitude of this strengthening is significantly larger in blind subjects than in sighted subjects.

The comparisons of plasticity between congenitally and acquired blind subjects may be of particular interest, as these differences can shed light on the impact of sensitive periods on the development of the visual system and cross-modal connectivity. During the SSD task, significant differences in top-down modulation were observed in all three possible group-wise comparisons (Fig. 3). This finding is in agreement with recent literature that the effects of cross-modal plasticity are evident in those who become blind later in life, but the size of these cross-modal effects are smaller than those observed in congenitally blind adult subjects (Voss, 2013). On the other hand, our analyses did not detect any significant differences in functional connectivity between congenitally and acquired blind subjects, which may be surprising given the results of the SSD task and previous literature on comparing functional connectivity in these two populations. For example, Bedny et al. observed increased functional connectivity between the middle temporal complex and lateral prefrontal regions in congenitally blind compared to late blind subjects (Bedny et al., 2010). Collignon and colleagues first identified a set of regions in the occipital cortex that are selectively activated by spatial processing of auditory stimuli in blind subjects, and then used a psychophysiological interaction analysis to demonstrate that the increase in functional connectivity specific to spatial processing within these regions was significantly greater in congenitally blind subjects compared to late-onset blind subjects (Collignon et al., 2013). Qin et al. also reported differences in functional connectivity between congenitally and late blind subjects mostly outside the occipital lobe, but they did find significantly greater long-range functional connectivity density in the medial occipital gyrus of congenitally blind subjects (Qin et al., 2015). Several factors may have contributed to our observation. First, while we did not detect any statistically significant differences, by inspection of the average functional connectivity graphs we can observe apparent differences particularly in the visual-to-somatosensory/motor edges and perhaps to a lesser extent in visual-to-visual edges,

which do not survive FDR correction (Fig. 6). Second, of the previously cited studies, the approach taken by Bedny et al. was most similar to this study, and they did not detect any significant differences between congenitally and late blind subjects in functional connectivity between the middle temporal complex and any regions within the occipital lobe (Bedny et al., 2010). This observation agrees with our findings in this limited subset of edges where direct comparison is possible. With respect to the remaining studies, it is possible that the analysis used in our work was less sensitive to differences in functional connectivity between these groups compared with other methods that utilized alternate transformations of the functional connectivity data (Qin et al., 2015), or evaluated functional connectivity during the performance of a relevant task (Collignon et al., 2013). Taken together, our study supports the established view that plasticity is evident in late blind subjects but not to the extent observed in congenitally blind subjects, lending further evidence for a sensitive period sometime early in life with long-lasting effects on cross-modal connectivity. The behavioral relevance of these differences in the context of SSD use will be an important subject for future investigation.

We compared the activation between pre- and post-training scans to demonstrate that a significant portion of the visual cortex activity in the blind during SSD interpretation is due to top-down input. However, these data cannot be used to directly determine the cognitive basis of the visual cortex response to the sensory substitution signal. The strengthening of visual cortical activity in the blind may arise from a number of cognitive functions including, but not limited to, attention and/or mental imagery. Another limitation of this study is that our group sizes are limited with wide age ranges due to the difficulty in identifying subjects who fit the inclusion criteria. However, no significant differences in age are observed between these groups, and the increased top-down modulation of the visual cortex in blind subjects is still evident when

controlling for age in the model (data not shown). Furthermore, given that this effect can be detected with such a limited sample speaks to the size of the effect, and makes it more likely that the observed effect may play a biologically-relevant role.

In conclusion, we have shown that the degree of top-down modulation of visual cortex activity during active interpretation of sensory substitution signals is significantly greater in blind subjects compared to sighted subjects after a short duration of 10 minutes of training. Furthermore, the degree of this modulation is greatest in congenitally blind subjects, intermediate in acquired blind subjects and not significantly different from zero in sighted subjects. From this finding we can make two inferences. First, a significant portion of the BOLD response in the visual cortex of blind subjects during sensory substitution use is due to top-down influence on the visual cortex. Second, our data support the model of the brain as a highly flexible task-based machine by demonstrating that a portion of the cortex that normally responds in a stereotyped manner to sensory input becomes more task-flexible in its response following visual deprivation. This second inference is further supported by the functional connectivity study, which shows that the visual networks of the blind exhibit diminished connectivity with both somatosensory and other visual networks, but stronger connectivity with the task-flexible networks in the brain such as the attention network, the prefrontal cortex and the thalamus that are also known areas of top-down input to the visual cortex.

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References

- Amedi, A., Stern, W.M., Camprodon, J.A., Bermpohl, F., Merabet, L., Rotman, S., Hemond, C., Meijer, P., Pascual-Leone, A., 2007. Shape conveyed by visual-to-auditory sensory substitution activates the lateral occipital complex. *Nat Neurosci* 10, 687-689.
- Arno, P., De Volder, A.G., Vanlierde, A., Wanet-Defalque, M.C., Streel, E., Robert, A., Sanabria-Bohorquez, S., Veraart, C., 2001. Occipital activation by pattern recognition in the early blind using auditory substitution for vision. *Neuroimage* 13, 632-645.
- Bach-y-Rita, P., 2004. Tactile sensory substitution studies. *Ann N Y Acad Sci* 1013, 83-91.
- Bedny, M., Konkle, T., Pelphrey, K., Saxe, R., Pascual-Leone, A., 2010. Sensitive period for a multimodal response in human visual motion area MT/MST. *Curr Biol* 20, 1900-1906.
- Buchel, C., 2003. Cortical hierarchy turned on its head. *Nat Neurosci* 6, 657-658.
- Collignon, O., Dormal, G., Albouy, G., Vandewalle, G., Voss, P., Phillips, C., Lepore, F., 2013. Impact of blindness onset on the functional organization and the connectivity of the occipital cortex. *Brain* 136, 2769-2783.
- Collignon, O., Lassonde, M., Lepore, F., Bastien, D., Veraart, C., 2007. Functional cerebral reorganization for auditory spatial processing and auditory substitution of vision in early blind subjects. *Cereb Cortex* 17, 457-465.
- De Volder, A.G., Catalan-Ahumada, M., Robert, A., Bol, A., Labar, D., Coppens, A., Michel, C., Veraart, C., 1999. Changes in occipital cortex activity in early blind humans using a sensory substitution device. *Brain Res* 826, 128-134.
- Fonov, V., Evans, A.C., Botteron, K., Almli, C.R., McKinstry, R.C., Collins, D.L., Brain Development Cooperative, G., 2011. Unbiased average age-appropriate atlases for pediatric studies. *Neuroimage* 54, 313-327.
- Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A* 102, 9673-9678.
- Gilbert, C.D., Li, W., 2013. Top-down influences on visual processing. *Nat Rev Neurosci* 14, 350-363.
- Hairston, W.D., Hodges, D.A., Casanova, R., Hayasaka, S., Kraft, R., Maldjian, J.A., Burdette, J.H., 2008. Closing the mind's eye: deactivation of visual cortex related to auditory task difficulty. *Neuroreport* 19, 151-154.
- Hertz, U., Amedi, A., 2014. Flexibility and Stability in Sensory Processing Revealed Using Visual-to-Auditory Sensory Substitution. *Cereb Cortex*.
- Jones, D.T., Vemuri, P., Murphy, M.C., Gunter, J.L., Senjem, M.L., Machulda, M.M., Przybelski, S.A., Gregg, B.E., Kantarci, K., Knopman, D.S., Boeve, B.F., Petersen, R.C., Jack, C.R., Jr., 2012. Non-stationarity in the "resting brain's" modular architecture. *PLoS One* 7, e39731.
- Kawashima, R., O'Sullivan, B.T., Roland, P.E., 1995. Positron-emission tomography studies of cross-modality inhibition in selective attentional tasks: closing the "mind's eye". *Proc Natl Acad Sci U S A* 92, 5969-5972.
- Kujala, T., Huotilainen, M., Sinkkonen, J., Ahonen, A.I., Alho, K., Hamalainen, M.S., Ilmoniemi, R.J., Kajola, M., Knuutila, J.E., Lavikainen, J., et al., 1995. Visual cortex activation in blind humans during sound discrimination. *Neurosci Lett* 183, 143-146.
- Kupers, R., Ptito, M., 2014. Compensatory plasticity and cross-modal reorganization following early visual deprivation. *Neurosci Biobehav Rev* 41, 36-52.

Laurienti, P.J., Burdette, J.H., Wallace, M.T., Yen, Y.F., Field, A.S., Stein, B.E., 2002. Deactivation of sensory-specific cortex by cross-modal stimuli. *J Cogn Neurosci* 14, 420-429.

Liu, Y., Yu, C., Liang, M., Li, J., Tian, L., Zhou, Y., Qin, W., Li, K., Jiang, T., 2007. Whole brain functional connectivity in the early blind. *Brain* 130, 2085-2096.

Meijer, P.B., 1992. An experimental system for auditory image representations. *IEEE Trans Biomed Eng* 39, 112-121.

Merabet, L.B., Battelli, L., Obretenova, S., Maguire, S., Meijer, P., Pascual-Leone, A., 2009. Functional recruitment of visual cortex for sound encoded object identification in the blind. *Neuroreport* 20, 132-138.

Nau, A., Bach, M., Fisher, C., 2013. Clinical Tests of Ultra-Low Vision Used to Evaluate Rudimentary Visual Perceptions Enabled by the BrainPort Vision Device. *Transl Vis Sci Technol* 2, 1.

Nau, A.C., Pinter, C., Arnoldussen, A., Fisher, C., 2015. Acquisition of Visual Perception in Blind Adults Using the BrainPort Artificial Vision Device. *Am J Occup Ther* 69, 6901290010p6901290011-6901290018.

Nau, A.C., Pinter, C., Fisher, C., Jeong, J.H., Jeong, K., 2014. A standardized obstacle course for assessment of visual function in ultra low vision and artificial vision. *J Vis Exp*, e51205.

Pascual-Leone, A., Hamilton, R., 2001. The metamodal organization of the brain. *Prog Brain Res* 134, 427-445.

Ptito, M., Moesgaard, S.M., Gjedde, A., Kupers, R., 2005. Cross-modal plasticity revealed by electrotactile stimulation of the tongue in the congenitally blind. *Brain* 128, 606-614.

Qin, W., Xuan, Y., Liu, Y., Jiang, T., Yu, C., 2015. Functional Connectivity Density in Congenitally and Late Blind Subjects. *Cereb Cortex* 25, 2507-2516.

Reich, L., Maidenbaum, S., Amedi, A., 2012. The brain as a flexible task machine: implications for visual rehabilitation using noninvasive vs. invasive approaches. *Curr Opin Neurol* 25, 86-95.

Renier, L., Collignon, O., Poirier, C., Tranduy, D., Vanlierde, A., Bol, A., Veraart, C., De Volder, A.G., 2005. Cross-modal activation of visual cortex during depth perception using auditory substitution of vision. *Neuroimage* 26, 573-580.

Ricciardi, E., Bonino, D., Pellegrini, S., Pietrini, P., 2014. Mind the blind brain to understand the sighted one! Is there a supramodal cortical functional architecture? *Neurosci Biobehav Rev* 41, 64-77.

Striem-Amit, E., Amedi, A., 2014. Visual cortex extrastriate body-selective area activation in congenitally blind people "seeing" by using sounds. *Curr Biol* 24, 687-692.

Striem-Amit, E., Cohen, L., Dehaene, S., Amedi, A., 2012a. Reading with sounds: sensory substitution selectively activates the visual word form area in the blind. *Neuron* 76, 640-652.

Striem-Amit, E., Dakwar, O., Reich, L., Amedi, A., 2012b. The large-scale organization of "visual" streams emerges without visual experience. *Cereb Cortex* 22, 1698-1709.

van den Heuvel, M.P., Mandl, R.C., Kahn, R.S., Hulshoff Pol, H.E., 2009. Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Hum Brain Mapp* 30, 3127-3141.

Voss, P., 2013. Sensitive and critical periods in visual sensory deprivation. *Front Psychol* 4, 664.

Watkins, K.E., Shakespeare, T.J., O'Donoghue, M.C., Alexander, I., Ragge, N., Cowey, A., Bridge, H., 2013. Early auditory processing in area V5/MT+ of the congenitally blind brain. *J Neurosci* 33, 18242-18246.

Weeks, R., Horwitz, B., Aziz-Sultan, A., Tian, B., Wessinger, C.M., Cohen, L.G., Hallett, M., Rauschecker, J.P., 2000. A positron emission tomographic study of auditory localization in the congenitally blind. *J Neurosci* 20, 2664-2672.

Table 1. Subject demographic information

Sensory Substitution Task Subjects						
Blind group					Sighted group	
Age	Gender	Cause	Age of onset		Age	Gender
18	M	Retinitis pigmentosa	13		25	F
25	M	Retinopathy of prematurity	Birth		27	M
30	F	Tumors	23		38	F
39	F	Retinopathy of prematurity	17		41	F
55	M	Trauma	35		44	F
56	M	Unknown	Birth		55	F
58	F	Retinopathy of prematurity	Birth		56	M
58	F	Glaucoma	46		58	F
58	M	Encephalitis	7		61	M
60	F	Glaucoma	31		70	F

64	F	Retinopathy of prematurity	Birth		74	M
Additional Task-Free fMRI Subjects						
Blind group				Sighted group		
Age	Gender	Cause	Age of onset		Age	Gender
53	F	Diabetic retinopathy	28		55	F
59	F	Congenital cataracts, aniridia, pediatric glaucoma	53		55	F
60	F	Retinopathy of prematurity	Birth		57	F
62	F	Retinopathy of prematurity	Birth		58	M
62	M	Trauma	51		64	F
63	M	Retinopathy of prematurity	Birth		70	F
64	M	Detached retinas	54		71	M
75	M	Retinitis pigmentosa	59		75	M