

Structural and functional brain reorganisation due to blindness: the special case of bilateral congenital anophthalmia

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Abstract

Investigating the changes in the brain that result from a loss of sensory input has provided significant insight into the considerable capacity of the brain to reorganise. One of the difficulties in studying sensory-deprived populations is that the time and extent of sensory loss vary significantly. In this review, we consider the changes in the human brain associated with complete absence of visual input resulting from bilateral congenital anophthalmia, in which the eyes fail to develop. We describe the functional reorganisation and associated structural and connectivity changes that occur in the brain of those affected by the condition. By considering animal models of this condition, we investigate the changes that may be occurring on a scale that is not captured by human *in vivo* imaging techniques. Finally, we lay out a model pathway for taking auditory information to the occipital cortex that may be specific to anophthalmia.

1. Background

1.1 Early blindness as a model for understanding cross-modal plasticity

Understanding how the brain develops in the total absence of vision can provide insight into how brain organisation and functional specialisation depend on sensory input. In early blindness, there is significant evidence for a wide range of structural and functional reorganisation in the “visual” system, in particular of auditory processing, some tactile processing, and, in humans, of higher order cognitive functions including language, memory and arithmetic (Bedny, 2017; Merabet and Pascual-Leone, 2010). Studies of such cross-modal plasticity allow us to address questions about the role and extent of visual experience in shaping the brain’s functional organisation (Buchel et al., 1998; Burton, 2003; Gougoux et al., 2004). Importantly, findings in humans from imaging, neuropsychology, and brain stimulation studies indicate that the reorganisation of function to the occipital cortex is not epiphenomenal, rather it contributes to behavioural performance (Amedi et al., 2004; Cohen et al., 1997; Gougoux et al., 2004; Hamilton et al., 2000).

1.2 The role of prenatal experience

While a considerable amount of brain structure and connectivity is predetermined before birth, post-natal experience has an obvious and significant role in shaping the brain. One of the doctrines of brain development and plasticity is ‘use it or lose it’, implying that use of a specific function is required in order for the brain to maintain its processing power. It is also important in the initial development of functional specialisation. For neurons in the brain to become responsive to light, sounds, or touch, it is necessary that they are activated by the specific peripheral stimulation. However, prior to birth, the amount of retinal and hence neural stimulation by light is minimal. Instead, the system can generate endogenous activity through retinal waves that electrically stimulate the immature visual pathway (Katz and

Shatz, 1996). These retinal waves have been shown to propagate through the visual system in neonatal mice prior to eye opening. Thus, even when vision loss occurs very close to birth, as in the case of retinopathy of prematurity, the infant will have experienced endogenous retinal activity during *in utero* development. It is likely that even brief stimulation along the visual pathway is sufficient to initiate mechanisms that support functional specialisation. Coupled with variability in post-natal visual experience including minimal light perception or gradual deterioration of vision, the brain in most early blind populations has had multiple opportunities for input (albeit impoverished) from the visual sense. In contrast, the brain of an individual with bilateral congenital anophthalmia receives no input from the visual sense, either endogenous or from light stimulation. Thus, it provides a rare opportunity to understand how the brain adapts to the total absence of a sense organ.

1.3 The special case of anophthalmia

In this review, we present findings from our own studies of humans who are congenitally blind due to bilateral anophthalmia, a condition in which the eyes are completely absent (see Bridge et al., 2012). This unique model of blindness can help resolve some of the apparent discrepancies in findings from studies of early blindness. Compared with other models of blindness, it is homogeneous with respect to age of sensory deafferentation, visual experience, and severity of visual impairment. We can explore the extent to which the brain's functional architecture relies on activity-dependent mechanisms that occur even before birth. Given the lack of stimulation along the visual pathway, the model offers the earliest opportunity for cross-modal plasticity during development.

Congenital bilateral anophthalmia is rare, occurring in about 1.8 in 100 000 births. Since around 60% of people with bilateral anophthalmia have other systemic disruptions (Bakrania et al., 2007), the number of people who are available to participate in studies of the isolated condition is even more limited. Therefore, to gain an understanding of changes that occur during development, it is important to have animal models that provide detail about brain structure and connectivity and specifically regarding the difference between early postnatal vision loss and anophthalmia.

We will first present our findings from studying brain function in a small group of humans with anophthalmia, which suggest that the functional reorganisation evident at the cortical level is mediated by changes subcortically. We then explore the evidence in support of changes to brain structure and connectivity at cortical and subcortical levels in two animal anophthalmia models, the macaque monkey and the mouse.

2. Anophthalmia

2.1 Humans with anophthalmia

Congenital bilateral anophthalmia occurs often as a result of genetic factors, but with many cases of unknown origin. There were some media reports of clustering of cases of microphthalmia and anophthalmia in England during the late 1980s, with a suggestion that pesticides might be the cause. This conclusion, however, was not supported by epidemiological investigation (Dolk et al., 1998). Interestingly though, this study did find

almost a two-fold increase in prevalence in people living in rural, compared to urban areas, a finding that has yet to be explained. Exposure to certain infections during gestation, such as influenza and Parvovirus B19 has also been associated with anophthalmia (Busby et al., 2005). However, genetic causes appear to account for the largest proportion of cases, particularly in the case of syndromic patients, with a number of genes implicated.

Around 10-20% of people with bilateral anophthalmia have an abnormality related to *SOX2*, which affects eye development, in addition to other types of brain malformations and clinical abnormalities (Bakrania et al., 2007; Williamson and FitzPatrick, 1993). Indeed, a recent study that screened seven genes implicated in anophthalmia or microphthalmia, found *SOX2* point mutations or deletions in 18 patients (Chassaing et al., 2014). The next genes most commonly affected were *OTX2* and *RAX*, both of which had point mutations or deletions in 5 patients. The *RAX* gene is involved in eye and retina development, and has been shown to be essential for normal vertebrate eye development (Mathers et al., 1997). Similarly, *OTX2* is required for both ocular and forebrain development, and mutations can affect the eye and lead to developmental delay (Ragge et al., 2005).

We are extremely fortunate to have had access to, and significant interaction with, a group of young adults with isolated bilateral, congenital anophthalmia (Bridge et al., 2009; Bridge et al., 2012; Coullon et al., 2015a; Coullon et al., 2015b; Watkins et al., 2012; Watkins et al., 2013). One of these individuals has a causal mutation in *OTX2* but the cause of blindness in the others was unknown and they had no family history of micro- or anophthalmia. We used MRI to scan the orbits and cranial nerves of these individuals (Bridge et al., 2012). The globes were absent bilaterally and the optic nerves and chiasm were either undetectable or extremely hypoplastic. Interestingly, the extraocular muscles were present in the socket but were disorganised and reduced in size. Two of the six cases had implants bilaterally into the socket to support prosthetic eyes.

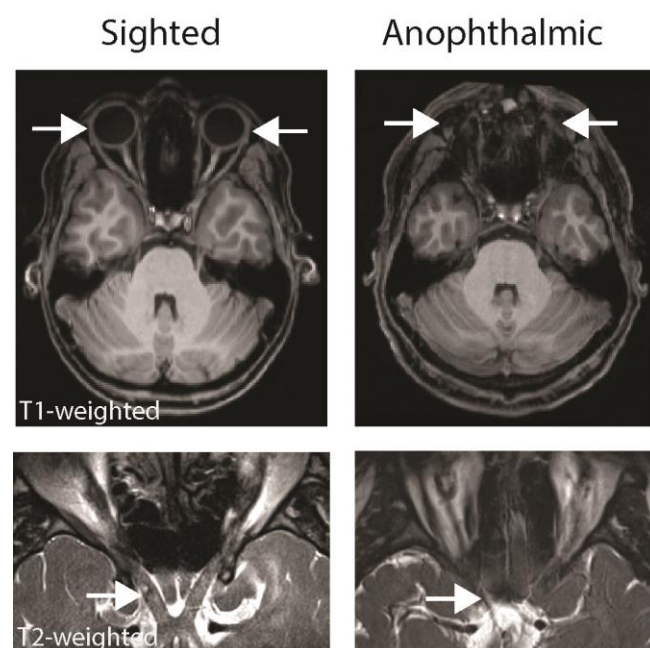


Figure 1: Images in the upper row are T1-weighted structural scans sliced horizontally (axially) through the location of the eyes. The white arrows indicate the location of the

globes in the sighted participant, whereas there is no evidence of eyes in the anophthalmic participant. The lower row shows T2-weighted high-resolution scans sliced horizontally through the optic nerves and chiasm, indicated by the white arrows. The anophthalmic participant shows only a very atrophied optic nerve unilaterally.

2.2 Animal models of anophthalmia

There has been knowledge of a mouse strain that includes individuals with anophthalmia since the 1940s, when it was described in detail by Chase & Chase in a series of manuscripts (Chase, 1942, 1944, 1945). This strain is now known as ZRDCT, and later work found that the responsible mutation in *Rax* resulted in a Methionine -> Leucine substitution in the encoded protein (Tucker et al., 2001). A *Rax* null allele causes widespread central nervous system defects in addition to anophthalmia and leads to neonatal death. While this strain provides an anophthalmic model, the comparison strain that has been used, known as C57, differs beyond the visual system, potentially confounding the results (Masse et al., 2014). To overcome this issue Boire and colleagues backcrossed the two strains, thereby creating a model anophthalmic animal that did not differ globally from the comparison sighted animals.

In the absence of a genetic model, and low natural incidence of congenital blindness, estimated at around 1 per 1000 in a wild colony (Rawlins and Kessler, 1983), the macaque model of anophthalmia involves *in utero* enucleation at different embryonic days. By comparing the effects of enucleation at different developmental stages the effects of any spontaneous retinal stimulation on downstream visual pathways can be determined.

By examining in detail changes in brain structure, function and connectivity across these models and comparing them with the changes in our small sample of humans with anophthalmia, we aim to understand the importance of sensory input to neural development.

3 Cross-modal functional plasticity in human anophthalmia

3.1 Language-evoked activity in the anophthalmic occipital cortex

We used functional MRI to investigate auditory and language processing in our small homogeneous group of people with bilateral congenital anophthalmia. Imaging during an auditory naming task (listening to phrases such as “bees make it” and covertly retrieving a word such as “honey”) revealed task-related activity in the usual left-lateralised cortical network of areas involved in language processing, namely the left inferior frontal gyrus, right and left posterior superior temporal cortex and the supplementary motor cortex on the medial wall (Watkins et al., 2012). However, unlike sighted controls, the anophthalmic group also showed activity in the lateral occipital cortex bilaterally but predominantly on the left (Figure 2A). When we examined the amount of activity across occipital areas, we found that V1 (defined in sighted individuals) was activated to a similar extent as A1, but that activity was considerably greater in areas further along the “visual” processing hierarchy such as in the lateral occipital complex (LOC) and V3A (see Figure 3A). We concluded that unlike in early blindness (Amedi et al., 2003), the hierarchy of processing in

the occipital processing stream (the ventral visual processing stream in sighted individuals) was maintained in anophthalmia. Further support for this conclusion came from measures of how well correlated brain activity was during resting state fMRI between homologous cortical areas in each hemisphere (see Figure 3B). Like A1, V1 showed high interhemispheric correlation in both the anophthalmic and the sighted individuals consistent with the interpretation that these areas are involved in early sensory processing. The interhemispheric correlation reduced along the processing hierarchy, with LOC showing similar levels to the association cortex in inferior frontal gyrus.

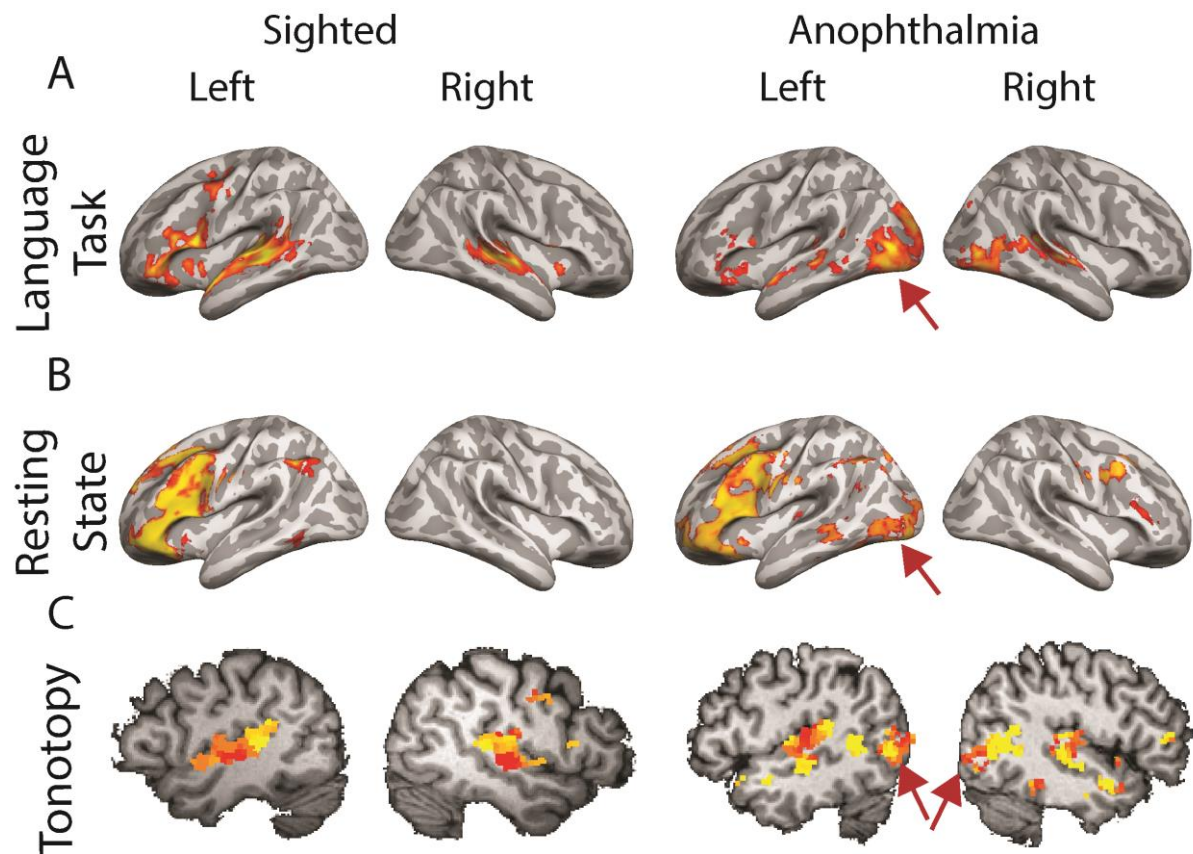


Figure 2. Functional reorganisation in people with anophthalmia. A. The network of cortical areas activated by a language task. In the group with anophthalmia, there was significant activation in occipital regions (red arrow) in addition to the language centres evident in sighted people (Watkins et al., 2012). B. A left lateralised resting state network also included the same occipital region (red arrow) in the anophthalmic group (Watkins et al., 2012). C. Visual motion area V5/hMT+ showed a tonotopic organisation in several of the people with anophthalmia (red arrows); red voxels show a preference for low frequency tones, orange for medium frequencies and yellow for high frequencies (Watkins et al., 2013).

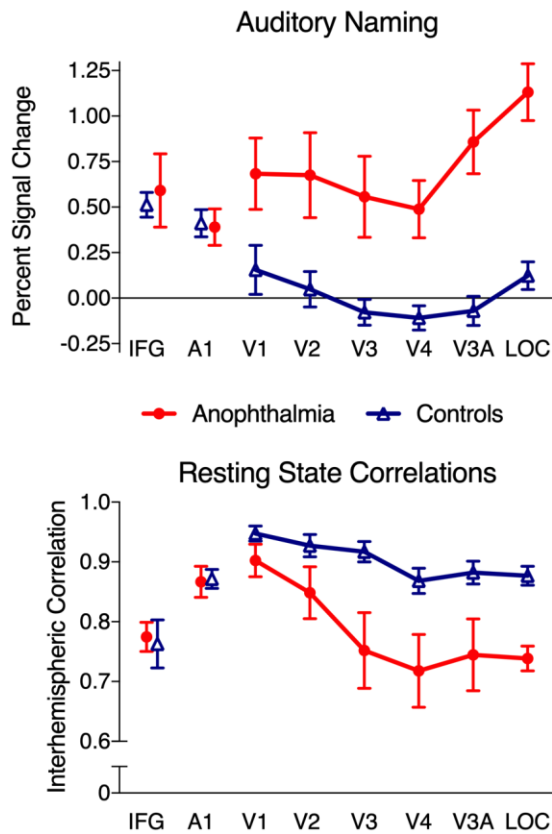


Figure 3. Maintained hierarchy of processing across occipital areas. A. Activity plotted by “visual” area showing highest activity in LOC in the anophthalmic group. B. Correlations between homologous regions in the two hemispheres shows highest correlation for early sensory areas and a decrease along the “visual” processing stream in both groups, more pronounced in the anophthalmic one.

Analysis of resting-state data in populations allows questions about functional organisation to be addressed without interference from tasks and variations in performance that can influence activation patterns. We explored functional connectivity networks in the group of people with anophthalmia and found differences with the sighted group of controls in a left lateralised “language” network (Smith et al., 2009). The same “language” network in anophthalmia showed coordinated functional activity across the left lateral frontal, superior temporal and medial frontal cortex (as in sighted controls) but additionally recruited the left lateral occipital cortex into this network (Figure 2B). Thus, consistent with the task MRI data, the lateral occipital cortex appeared functionally connected with areas typically involved in language processing in the left hemisphere (Watkins et al., 2012).

The findings from the language task and resting-state fMRI raised the notion that functional modules exist early in the developing cortex and are either multisensory or pluripotent (see also Bedny 2017 for a review in other blind populations). In multi-modal areas, deprivation in one sense could lead to inputs from another sense coming to dominate or being unmasked. Modules that show early sensory processing properties for a different sense would reflect reorganisation or rewiring subcortically in the thalamus or earlier in the sensory processing stream. As noted above, the data from early blindness in humans

indicates reorganisation predominantly at the cortical level (Amedi et al., 2003; Klinge et al., 2010).

3.2 Early auditory processing in anophthalmic occipital cortex

To address this question of reorganisation in early sensory processing, we used functional MRI to explore which brain areas showed responses to simple auditory stimuli in people with congenital anophthalmia (Watkins et al., 2013). During passive listening to trains of pure tones of high, medium and low frequencies, the anophthalmic group had reduced activity in the posterior superior temporal gyrus (corresponding to the location of A1) relative to sighted control participants. This finding of reduced response amplitude in auditory cortex to stimulation in the anophthalmic group was confirmed recently using population field mapping (Huber et al., 2019). The activity in V1 did not differ between sighted and anophthalmic groups but another occipital area – corresponding to a probabilistic cytoarchitectonic map of V5/hMT+ in sighted human brains - showed activity in the anophthalmic group bilaterally and was not activated in controls (Figure 2C). The pattern of activity in A1 in both the sighted and the anophthalmic individuals showed the expected frequency preference (tonotopy). In addition, some anophthalmic individuals showed a topographic frequency preference in V5/hMT+ (Figure 2C). This latter finding is consistent with the idea that V5/hMT+ acts as an early sensory processing area in anophthalmic people which may be mediated by direct input from subcortical areas that have reorganised or rewired for auditory processing (e.g. the pulvinar).

3.3 Functional specialisation within the anophthalmic occipital cortex

Taken together, the activity evoked by language processing in lateral occipital cortex and that evoked by pure tones more dorsally in V5/hMT+, indicates preserved functional modularity in occipital cortex in anophthalmia but for auditory input (Figure 4). The finding of a “late” processing area in the visual stream for language supports the hypothesis of a preserved hierarchy of function along the visual processing stream, which is again suggestive that the reorganisation occurs subcortically, potentially “co-opting” inputs from thalamic areas that have had their inputs changed from visual to auditory. Consistent with the idea that the pulvinar might mediate the projection to V5/hMT+ are findings in marmosets with neonatal lesions to V1 that result in maintenance of the projection between the inferior pulvinar and MT (Bridge et al., 2016; Warner et al., 2015). It is possible that prenatal visual deafferentation of V1 (as has occurred in anophthalmia) would similarly result in preservation of this projection and that this could support inputs from other sensory modalities.

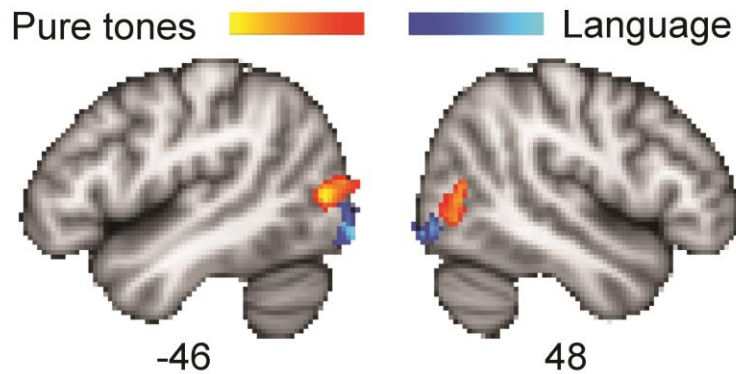


Figure 4. Functional specialisation for audition in the occipital lobe of anophthalmic humans. V5/hMT+ shows selectivity for pure tones, while subjacent lateral occipital cortex is activated by language (from Watkins et al., 2013). Additional modules are likely to exist within the occipital lobe.

3.4 Auditory-evoked activity in anophthalmic subcortical pathways

In the subcortical auditory pathway, there is another candidate region in which rewiring or reorganisation of inputs might occur, namely the tectum, which in humans comprises the superior and inferior colliculi. The superior colliculi are multimodal though predominantly process visual information with other sensory information important for movement control (e.g. eye and gaze movements). They receive direct retinal input and project to the pulvinar (Berman and Wurtz, 2008). The inferior colliculi receive inputs from the cochlear nuclei and in turn send outputs to the medial geniculate nuclei, which then project to cortex. Theoretically, reorganisation subcortically might occur between the colliculi, which in turn would alter the sensory input to the pulvinar and cortex.

To test this hypothesis, we used complex and rich auditory stimuli presented monaurally or binaurally to examine auditory responses in ‘visual’ subcortical structures (Jiang et al., 2013). Activity in the inferior colliculi was significantly reduced in anophthalmia bilaterally in the binaural condition and contralaterally in the monaural condition (Coullon et al., 2015b). However, whereas the sighted people had negligible activity in the superior colliculi for these auditory stimuli, the anophthalmic individuals showed robust auditory-evoked activity (Figure 5). Both sighted and anophthalmic people showed equivalent levels of auditory-evoked activity in the medial geniculate nuclei and in primary auditory cortex. The previously reported reductions in auditory-evoked activity in A1 in anophthalmia occurred during processing of simple pure tone stimuli (Watkins et al., 2012) whereas those used here were rich and complex stimuli comprising both speech and music; the different types of stimuli might explain the different findings in these separate studies. The activation of V1 was negligible in sighted controls during auditory processing but again robustly activated in anophthalmia (Coullon et al., 2015b).

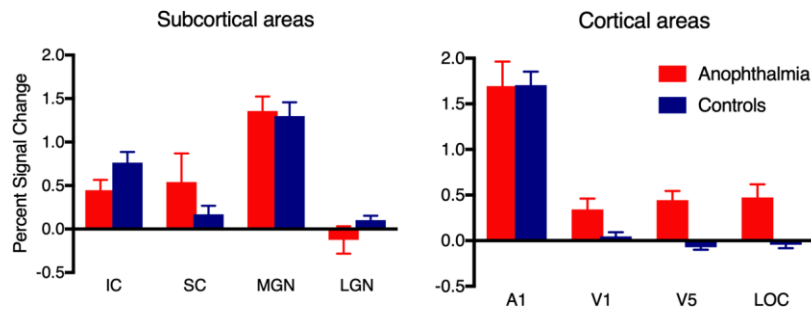


Figure 5. fMRI responses to rich auditory stimulation in anophthalmic and sighted people. Responses in the medial and lateral geniculate are similar in both groups. In contrast the superior colliculus shows significantly greater activity to auditory stimulation in the anophthalmic compared to sighted participants. Only those with anophthalmia showed auditory responses in V1 and extrastriate visual areas. Asterisks indicate regions where the activity was greater in the anophthalmia group than controls.

In none of the studies involving auditory stimuli have we seen activation of the lateral geniculate nucleus (LGN) in the anophthalmic individuals (nor, indeed in the sighted participants; Figure 5). Thus, we have preliminary support for the proposal that cross-modal plasticity has occurred subcortically in anophthalmia, possibly at the level of the colliculi, either by rewiring of inputs between inferior and superior pairs or by unmasking existing auditory inputs to the superior colliculi. From the superior colliculi, existing projections to the pulvinar might support auditory inputs to V5/hMT+, which in turn could project to later areas in the occipital cortex such as the lateral occipital cortex.

To evaluate our proposal that reorganisation of function at the cortical level in humans with anophthalmia is driven by subcortical rewiring, we next turn to evidence from changes in brain structure in the same small group of people with anophthalmia and to both structure and function in the animal models.

4 Structural changes in the brain related to anophthalmia

4.1 *Structural effects of anophthalmia on the human brain*

We have used T1- and proton density-weighted structural MRI to examine the grey matter volume of the cortex and subcortical structures in humans with anophthalmia and diffusion tensor imaging to examine the white matter connectivity (Bridge et al., 2009). The most striking difference in visual brain structures resulting from anophthalmia in humans was the reduction in size of the LGN (Bridge et al., 2009), a finding evident across other types of congenital blindness too (Aguirre et al., 2016; Cecchetti et al., 2016). Detailed proton-density MRIs of the structure (shown in Figure 6A) indicated that it was significantly reduced in size and had lost the characteristic shape that allows for identification in scans from sighted individuals.

Analysis of white matter indicated a significantly lower volume in anophthalmia relative to sighted controls in the region of the chiasm and optic tract. Diffusion metrics indicated globally reduced fractional anisotropy (FA) in the anophthalmic group; FA is a measure of white matter integrity. The reductions in FA were maximal in the corpus callosum and in the occipital white matter bilaterally. Somewhat surprisingly, and in apparent contradiction with the reduction in integrity, the connectivity between the thalamus and the occipital cortex measured by probabilistic tractography of the optic radiations suggested that these tracts were intact and not diminished in terms of volume or strength of connections. Analysis of crossing fibres in these tracts indicated that although the dominant connections are along the direction of the optic radiations, there was a large number of fibres crossing these tracts. This would explain the reduction in FA and is consistent with an interpretation of increased structural reorganisation and changes in connectivity within the occipital lobe. For example, maintenance or strengthening of the pulvinar projection to V5/hMT as hypothesised based on our functional findings. Strengthening of feedforward connections between primary sensory areas in the cortex or feedback connections to occipital areas from association cortex elsewhere would also result in an increase in the crossing-fibre population in the occipital white matter.

Beyond the obvious change in the LGN, the main cortical difference between people with anophthalmia and sighted controls was increased thickness of the cortical ribbon on the banks of the calcarine sulcus, the area that usually corresponds to striate cortex (V1) and the position of the boundary with extrastriate cortex (V2). Increased cortical thickness is another finding that appears to be common to other types of congenital blindness (Aguirre et al., 2016; Park et al., 2009). It could reflect a lack of experience- or activity-dependent pruning, delayed maturation, a failure to specialise, or a change in function. It might also reflect a change in the boundary between V1 and V2 or the existence of an intermediate or hybrid cortex as described previously in monkeys enucleated early in gestation (Rakic 1988; and see below).

We used magnetic resonance spectroscopy to examine the neurochemistry of the cortex around the calcarine sulcus in the anophthalmic individuals (Coullon et al., 2015a). The anophthalmic V1 cortex relative to that in sighted participants, showed elevated levels of choline, glutamate/glutamine, myo-inositol, and creatine. There were no differences between the groups in *N*-acetylaspartate or γ -amino-butyric acid (GABA) in V1 nor in any of the neurochemicals measured for a control region placed medially in M1/S1 cortex. The greater concentration of choline coupled with more grey matter in V1 is consistent with a profile of immature cortex. The greater concentrations of choline and of glutamate/glutamine in V1 also indicate enhanced excitatory circuits within the occipital cortex possibly underpinning increased cross-modal plasticity.

While the region around putative V1 was the only area showing a significant increase in thickness in humans with anophthalmia, increases in grey matter were seen in several regions outside of the occipital lobe, including the intraparietal sulcus (IPS), middle temporal gyrus and superior frontal sulcus. There were reductions in the putamen bilaterally also. Thus, although the changes in gross anatomy are relatively subtle in anophthalmia, they appear far reaching.

To summarise, in human anophthalmia, imaging studies indicate that the LGN is severely affected by the absence of the eyes. The LGN's significant reduction in volume might explain the absence of activation to auditory stimulation that we found in our functional imaging studies (see Figure 5). It is also consistent with the idea that the LGN is not the locus of functional reorganisation due to subcortical rewiring from the neighbouring medial geniculate nucleus (MGN). It is possible, however, that the maintenance of the projection from tissue around the LGN to the occipital cortex is due to fibres from the MGN rather than the vestigial LGN and that these fibres could provide subcortical input to V1 for auditory processing. Other projections to occipital cortex from subcortical, primary sensory or association cortex could explain the increase in the crossing-fibre population within the optic radiations and the reduction in FA. The loss of input from LGN due to light or retinal stimulation could also be responsible for a failure of maturation or specialisation in V1 cortex, which is a potential explanation for the finding that the cortical ribbon is thicker in anophthalmia than in sighted individuals.

4.2 Alterations to the visual pathway in anophthalmic macaque monkeys

Since there is no known model of congenital anophthalmia in the non-human primate, the only approach to determine the neural effects of the condition is to enucleate the foetus *in utero*. Seminal work from the laboratory of Henry Kennedy (Dehay et al., 1996a; Dehay et al., 1996b) used enucleation at different embryonic days to determine that, in general, if the enucleation was carried out early in gestation (prior to embryonic day 81), the structural effects on the visual brain were significantly greater. Indeed, inspection of subcortical structures (reproduced in Figure 6B), shows that following early enucleation, the LGN exhibited a reduction in volume and a loss of distinct magno- and parvocellular layers. Interestingly, late enucleation had little effect on the LGN structure, though it should be noted that the histology was performed days after birth, so there was little opportunity for degeneration.

Neither early nor late enucleation affected the lateral pulvinar volume in the macaque, in spite of the reciprocal connectivity with visual cortex evident in sighted primates (Bridge et al., 2016; Sherman and Guillery, 2002). In contrast, there was a reduction in the volume of the inferior pulvinar in the animals with early enucleation. Since around 60% of this region is involved in saccadic eye movements, a size reduction is perhaps not surprising if the eye musculature is unused because of the absence of the globe (Robinson et al., 1986). Thus, at the subcortical level, both the LGN and at least some pulvinar nuclei appear to require the presence of the eyes during development to form normally.

Interestingly, none of the enucleated animals showed reduction in visual cortex volume relative to total brain volume. However, the volume of the striate cortex both in absolute terms, and relative to visual cortex volume, was significantly reduced. Where classified as striate cortex, the morphological signature, including the stria of Gennari, was comparable in the enucleated and sighted animals. Since the striate cortex is the major target for subcortical projections from the LGN, it is unsurprising that the loss and disorganisation of LGN tissue led to significant disruption at the cortical level.

While the volume of striate cortex was reduced, there was additional 'hybrid cortex' situated within the region normally occupied by V1 in the enucleated animals. This hybrid cortex was distinct from both striate cortex and the adjacent V2 being described as thicker and with a disorganised layer IV. This hybrid cortex was more likely to be present on the operculum, where the central visual field is represented in a sighted animal. In addition to the volume reduction in striate cortex, the gyrification in this region also appeared abnormal (Figure 6C). There was increased folding, particularly in those animals that were enucleated early, although the significance of this abnormal pattern is unclear. Increased gyrification in this region was also described in Rakic's studies of monkeys enucleated early in gestation (Rakic 1986) and in the anophthalmic humans that we studied (see supplementary material in (Bridge et al., 2009)).

Few structural differences between sighted and enucleated animals were evident beyond striate cortex, suggesting the lack of retinal input is less disruptive to extrastriate visual areas than areas earlier in the visual hierarchy.

In a recent study, Magrou and colleagues (Magrou et al., 2018) investigated the connectivity of the structurally defined hybrid cortex present within the area corresponding to striate cortex in sighted animals. They found that local connectivity in this region was considerably more extensive than that typically found in V1. This greater connectivity of the hybrid cortex is consistent with the area behaving as an extrastriate region. Moreover, in accord with the work in human anophthalmia described above, connections between hybrid cortex and LGN were significantly reduced. Additionally, connections between hybrid cortex and inferior pulvinar were significantly increased, indicating an increased role for the pulvinar. Unfortunately, due to the challenges of behaviour testing in the macaque, there is no direct evidence for auditory function within the occipital cortex, and specifically within the structurally defined hybrid cortex in the enucleated animals.

To summarise, in the macaque model of anophthalmia (early enucleation), there are significant effects on the development of the LGN such that its volume is reduced and that its characteristic layer structure is disrupted. The development of the inferior pulvinar is also affected. Cortically, striate cortex is present, but reduced in volume and with abnormal gyrification following enucleation. The reduction in striate cortex is accompanied by an increase in hybrid cortex with a distinct cytoarchitecture in the prenatally enucleated macaque. The hybrid cortex in enucleated macaque resembles extrastriate cortex and has reduced connectivity with the LGN.

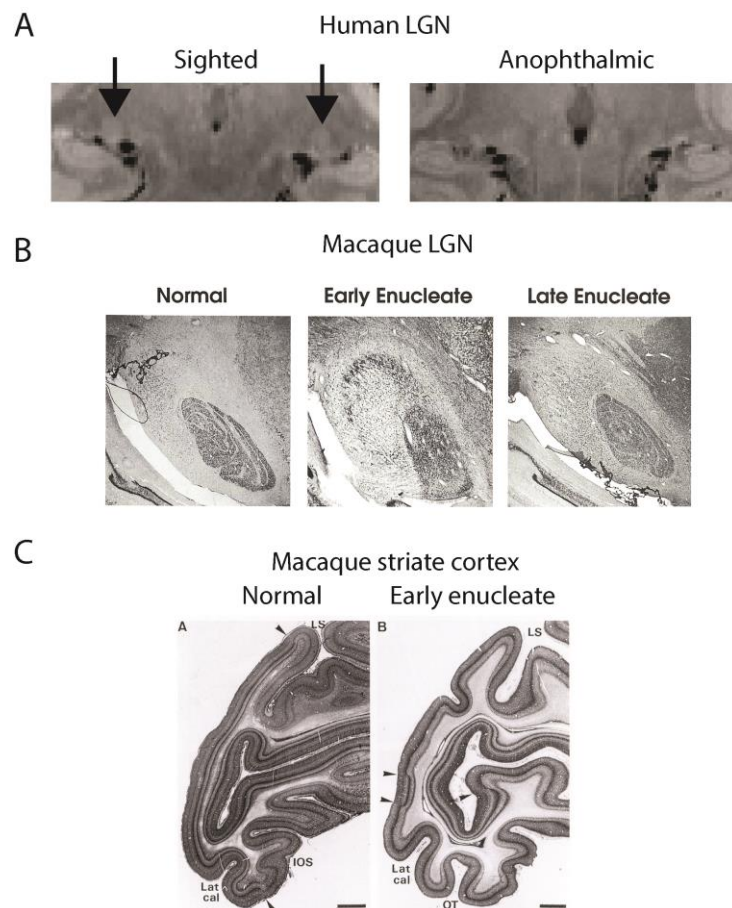


Figure 6. A. The LGN is not evident in the adult human with anophthalmia. B. In the enucleated macaque, the LGN is reduced in size, and shows disordered organisation following early enucleation. C. Abnormal gyrfication in a macaque with early enucleation. Panels B and C reproduced from (Dehay et al., 1996a) with permission.

4.3 Structural and functional differences due to anophthalmia in mice

As discussed earlier, ensuring anophthalmic and sighted strains of mice are matched for global brain structure and volume is important to understand the specific effects of vision loss (Masse et al., 2014). Across a series of studies, Bronchti, Boire and colleagues have systematically investigated differences between anophthalmic, enucleated and sighted mice (Chabot et al., 2007; Masse et al., 2014; Piche et al., 2004).

Findings indicated no global differences in brain structure among the three groups of sighted, enucleated and anophthalmic mice; total neocortical volume and the volume of primary auditory and somatosensory cortex did not differ (Masse et al., 2014). Subcortically too, analysis of the volume of thalamic nuclei indicated that there were no differences among these groups for the ventroposterior somatosensory nuclei or the auditory medial geniculate bodies. In contrast, the visual LGN in both enucleated and anophthalmic animals were significantly smaller compared with sighted ones, and the volume of these nuclei did not differ between the two type of blindness.

At the cortical level, both the volume and surface area of striate cortex were significantly lower in the anophthalmic and enucleated groups compared with sighted, but again there was little difference between them.

The main method for determining functional selectivity for particular brain regions is by using c-Fos activity in combination with immunohistochemistry. With this approach, Chabot and colleagues (Chabot et al., 2007) investigated the differences between wild-type sighted, genetically anophthalmic, and neonatally enucleated mice. Measuring c-Fos activity in response to auditory white noise, they found that anophthalmic mice showed activity in the LGN, V1 and a small amount in V2. In contrast, neonatally enucleated animals showed the greatest amount of auditory-evoked activity in V2, with little in either LGN or V1.

The same group of researchers investigated whether an enriched environment, consisting of a large cage with extensive plastic tubing and food distribution that required exploration, changed activity patterns in anophthalmic mice. C-Fos activity in mice raised in the enriched environment was compared to mice raised in standard mouse cages. They found that c-Fos activity was significantly higher in V1 of the anophthalmic mice raised in the enriched environment compared to standard (Chabot et al., 2007; Piche et al., 2004). Use of non-visual senses therefore appears to increase the degree of reorganisation in the 'visual' cortex, indicating potential use-dependent plasticity.

There have been several tracing studies suggesting altered connectivity within the anophthalmic mouse brain, specifically connections from inferior colliculus to LGN (Chabot et al., 2007; Piche et al., 2004). In contrast, there does not appear to be a direct connection between MGN or inferior colliculus and visual cortex (Chabot et al., 2008). Charbonneau et al. (Charbonneau et al., 2012) performed a comprehensive analysis of both subcortical and cortical projections to V1 in both the sighted and anophthalmic mouse. Interestingly they found that V1 receives extensive input from other sensory cortices (auditory, somatosensory and olfactory) in addition to motor and association regions. This was the case in sighted, enucleated and anophthalmic animals and there were few differences between the groups regarding connectivity. The authors concluded that there was little evidence for novel or enhanced connections to V1 from other cortical sensory systems. Unsurprisingly, the LGN and other visual thalamic nuclei provided the strongest subcortical input to V1 in sighted animals. The important finding, however, that there were no significant differences in subcortical projects to V1 between the sighted and two blind groups of animals. It may be the case, therefore, that in the mouse, the LGN is capable of providing auditory input to V1, even though it is reduced in volume. Alternatively, given the extensive inter-connectivity between sensory cortices in the sighted mouse, cortico-cortical plasticity may be possible with little change in underlying anatomy.

To summarise, both anophthalmic and enucleated mice models show reduced LGN and striate cortex volume relative to sighted mice. Functionally, however, the anophthalmic and enucleated mice differed in terms of reorganisation for auditory processing. Although reduced in volume the LGN and V1 cortex in the anophthalmic mouse was responsive to auditory stimulation, whereas the V2 cortex was maximally responsive in the enucleated mice.

4.4 Similarities and differences in the brains of the human, macaque and mouse models of anophthalmia

The studies of brain structure in anophthalmic humans, macaques and mice consistently show significant effects on the size of the LGN and its cortical target in the occipital lobe, the striate cortex (V1). The volume reduction of the LGN evident in MRI scans of anophthalmic humans is likely due to the loss of lamination evident in the macaque (Dehay et al., 1996a). We suggest, therefore, that, like the anophthalmic macaque, any (non-visual) input to the occipital cortex in humans is unlikely to be projected via the LGN. This could differ from the mouse model, in which auditory-evoked activity was observed in the LGN and in V1 cortex.

The pattern of changes in the pericalcarine cortex in humans, striate cortex in macaque and mouse differed. Without histology in the humans, we are unable to determine the exact volume and boundaries of striate cortex, but, on the banks of the calcarine sulcus, the cortical ribbon was significantly thickened relative to sighted humans. In contrast, in the two animal models, striate cortex volume was reduced. In the macaque, the reduced striate cortex volume was accompanied by additional hybrid cortex that resembled extrastriate cortex and was not strongly connected with the LGN. We suggest, therefore, that the increase in cortical thickness in human pericalcarine cortex may be due to the inclusion of the equivalent of hybrid cortex seen in macaques. The changes in neocortex in these two primate models is possibly related to abnormal patterns of gyrification, which is not a feature of the mouse brain.

The predominant effects of the absence of the eyes were observed in striate cortex in all three models and did not appear to extend to extrastriate areas. This difference could reflect differential effects of retinal waves on striate and extrastriate visual areas. Ackman et al. showed that, at least in mice, retinal waves provide patterned input to the superior colliculus and primary visual cortex, and are the primary source of activity to these areas (Ackman et al., 2012). While data were not shown from LGN, previous work has indicated that interfering with retinal waves monocularly can interfere with the development of LGN structure (Stellwagen and Shatz, 2002). Thus, retinal waves appear to be critical for development of the visual pathway until V1. However, Ackman et al. also showed that in extrastriate areas, there was activity linked to retinal waves but this activity did not drive those visual areas. Rather the retinal waves acted to modulate ongoing activity in extrastriate areas, presumably arising from other neural structures. Thus, if the main pre-natal activity in extrastriate cortex can be generated without retinal waves it is perhaps not surprising that the structure of these areas is less affected by anophthalmia. In contrast, where retinal waves form the driving input to an area, such as LGN and V1, anophthalmia is likely to have a significant effect on development.

5. Linking structure and function: pathways to reorganisation

While there is no question that both anophthalmia and early blindness lead to extensive functional reorganisation in the occipital cortex, it is not clear that the mechanism by which this occurs is necessarily the same in the two cases. Studies in humans with early blindness have investigated alternative models of reorganisation, which include strengthening of

feedforward connections between primary sensory cortical areas, greater feedback to occipital cortex from association cortical areas or changes subcortically in the midbrain or thalamus (Bavelier and Neville, 2002). There is support for a pattern of reversed hierarchy of processing within occipital cortex for verbal memory and language tasks with V1 responding maximally to higher order tasks than to sensory processing for Braille reading (Amedi et al., 2003). In addition, a functional connectivity analysis indicated that reorganisation occurs cortico-cortically, with the maintenance and possible strengthening of cortical inputs from auditory thalamus to both auditory and visual primary cortex as well as strengthening of feedforward functional connections between them (Klinge et al., 2010).

As noted above, our data from functional imaging during language processing in humans with anophthalmia showed no support for a reversed hierarchy of processing with V1 at the top of this hierarchy as seen in studies of early blindness (Amedi et al., 2003; Bedny et al., 2011; Lane et al., 2015; Sadato et al., 1996). This suggests that anophthalmia may differ from other types of blindness in humans in terms of processing in V1. There are two likely explanations for this difference: (i) the input to V1 from auditory and somatosensory thalamus has been strengthened or unmasked due to the very early loss of input from the vestigial LGN (see above); (ii) V1 or striate cortex in anophthalmia is significantly reduced in size as in the anophthalmic animal models and hybrid cortex resembling V2 is present. Our spectroscopy data further suggests that even in adulthood, this cortex remains in an immature state. This would seem to rule V1 out of representing higher order cognitive functions due to feedback from cortical association areas in anophthalmia. It does not rule out strengthening of feedforward connections between primary sensory cortical areas or changes subcortically.

The findings from our study of early auditory processing indicated reduced activity in A1 for pure tones in humans with anophthalmia and there was no obvious activation of the anterior pericalcarine cortex that is known to receive direct input from A1 (Rockland and Ojima, 2003). Structural and functional changes to other sensory cortex have not yet been explored in the animal models of anophthalmia, but based on the human data, we see no evidence of strengthening of cortico-cortical inputs between the early sensory areas.

The activation of area V5/hMT+ as well as V1 during simple processing of pure tone stimuli and the tonotopic pattern observed here in some anophthalmic people was strongly suggestive of reorganisation subcortically. We proposed that the known projection to V5/hMT+ from the pulvinar could support auditory input. To test this, we examined for activation subcortically during complex auditory processing in anophthalmic people. We found evidence of reorganisation of function in the colliculi, with the normally visual superior pairs responding robustly to auditory stimulation and the normally auditory inferior pairs showing reduced activity. The activation levels of the medial geniculate were no different from those in sighted people. The superior colliculi project to the pulvinar. We suggest, therefore, that in the case of anophthalmia, auditory information is projected via the superior colliculus and inferior pulvinar to V5/hMT+ and then to other extrastriate regions. V1 may also still receive input from auditory and somatosensory thalamic nuclei unmasked by the loss of the LGN projection. The proposed model is laid out in Figure 7. There remains a missing piece to this puzzle, which is that we have yet to see robust activation of the pulvinar nucleus in our functional imaging studies of humans with

anophthalmia. Furthermore, the studies in macaque indicated some atrophy of the inferior pulvinar which projects to V5/hMT+. Future studies of humans with anophthalmia will examine both the structure and the function of this nucleus in greater detail.

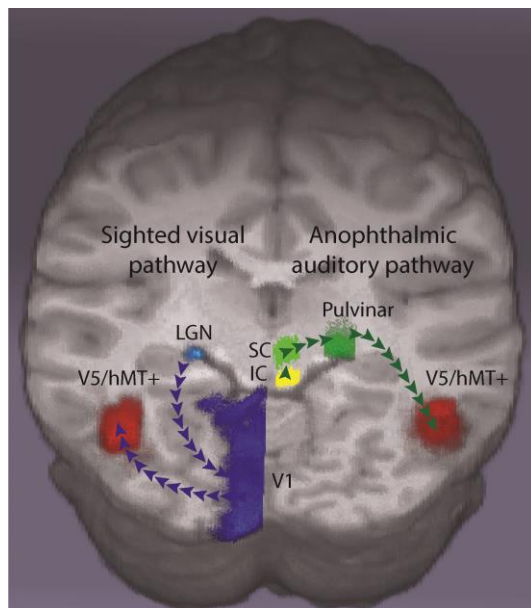


Figure 7. The structures proposed to underlie cross modal plasticity in human anophthalmia. The left hemisphere shows the standard visual hierarchy with visual flow from LGN to V1 to V5/hMT+. The right hemisphere shows the hypothesised projection of auditory information from inferior colliculus (IC) to superior colliculus (SC), via pulvinar to V5/hMT+.

6. Future directions to understand processing

In this review we have considered the extent of neural changes that result from bilateral anophthalmia, across mouse, non-human primate and human. There is evidence that the total absence of input from light, both pre- and post-natally, leads to significant reorganisation, such that areas normally specialised for aspects of visual function take on a specialised auditory function instead. Nonetheless, there remain many questions, not least that it is crucial to demonstrate that the pulvinar responds to auditory stimulation. In addition, with advances in MR technology, it has become possible to measure functional activity in both mice and macaque, so providing an opportunity to monitor the development of cross-modal plasticity through development.

An additional point is that the current review, and indeed the scientific literature on people with anophthalmia, has focussed on auditory processing. It may be that there are additional pathways, and areas of specialisation for other neural functions, such as somatosensation and memory. Reorganisation for these functions is described in other types of congenital blindness but remains to be determined in this specific population.

In conclusion, understanding the connectivity and developmental trajectory of cross-modal plasticity can help untangle the role of experience in specialisation of the brain, but will also become crucial as the development of a prosthetic retina continues to advance.

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