Do the Congenitally Blind Have a Stria of Gennari? First Intracortical Insights In Vivo

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The primary visual cortex V1, when dissected, is characterized by an easily identifiable anatomical landmark: the stria of Gennari or Gennari stripe. However, the origin and function of the Gennari stripe is so far unknown. In order to shed some light on this question, we acquired 7-T magnetic resonance imaging (MRI) brain scans of congenitally blind (CB) people, who have never had visual experience. If the stria of Gennari requires visual input to develop or to maintain its homeostasis, such subjects should lack this structure. If it is reliably detectable in the CB, it must form and persist independently of visual sensation. This question has never previously been explored in living subjects. For the first time, the use of 7-T high-resolution MRI enables such investigations because of the excellent signal-to-noise ratio at this magnetic field strength. For comparison, we scanned sighted subjects using the same experimental parameters. We detected the stria of Gennari reliably in both sighted and blind subjects, showing that this anatomical feature is not a developmental result of visual input, and it does not degenerate in the absence of visual input.

Keywords: blind, plasticity, primary visual cortex, stria of Gennari, 7-T MRI

Introduction

The striate cortex in the occipital calcarine sulcus is one of the most intensely studied parts of the brain in a wide range of species, including humans. It was among the first areas for which a structure-function relationship was successfully established, as the main cortical center for simple visual processing (Schäfer 1888; Bolton 1900); nowadays, the terms “striate cortex” and “primary visual cortex” (V1) are used in a virtually synonymous manner. One striking feature of the primary visual cortex is an approximately 300-μm-thick horizontal band of myelinated fibers within layer IV (Funkhouser 1915), which is visible to the naked eye in cadaver brain sections. It is most commonly known as the stria (or stripe, band, or line) of Gennari (1782), sometimes also called the stripe of Vicq d’Azyr (1786; Simpson and Crompton 2008), and it is a special manifestation of the outer band of Baillarger (1840), which can be found almost throughout the whole isocortex on a microscopic level. Brainstein discussed the formation of heavily myelinated cortical layers, arguing that the majority of the horizontal axonal fibers within layer IV, which form the stria of Gennari, are collaterals deriving from pyramidal cells in layer III (Brainstein 1962). Nevertheless, its functional significance remains unknown, and, for being such an obvious structure, the literature is surprisingly silent about it. Likewise, the specific circumstances of its development have not been elucidated in detail. As with any given cortical area, the formation of the primary visual cortex requires an orchestrated interaction between genetically determined, intrinsic predispositions to express specific features and extrinsic triggers provided by synaptic stimulation during development (Kahn and Krubitzer 2002). The differentiation of a pluripotent embryonic neuron is heavily dependent on the spatial position within the cortex and available connections. This general principle has been elaborated in the “radial unit model” (Rakic 1988), which postulates a predetermined columnar organization of the cerebral cortex as the result of migratory processes. The functional significance of a given column is determined by its synaptic connections and not completely predefined, even though there is a strong bias due to locally available thalamocortical and corticocortical projections. In animal models, deafferentation (depriving a brain area of its natural input or target) (Pons et al. 1991) and reaffecteration (guiding sprouting neurons to an unphysiological target) (Sur et al. 1988; Roe et al. 1990) resulted in adaptive processes involving both structural and functional reorganization within the brain. In the case of the visual system, Wiesel and Hubel (1963) were the first who reported that closing one eye early in development affected the activation of cells in the visual cortex of kittens. In later studies, it was shown that prenatal (Dehay et al. 1989) and perinatal (Rakic et al. 1991) enucleation in macaques leads to changes in the occipital lobe, resulting in a much smaller, yet histologically identifiable Brodmann area 17 (primary visual cortex V1), as well as the emergence of a new area “X” (Rakic et al. 1991). Similar experiments on ferrets (Kahn and Krubitzer 2002) demonstrated a functional reorganization of the remaining area 17, responding to either auditory or somatosensory stimuli, or both. A lowered metabolic activity in the primary visual cortex after bilateral visual occlusion was reported in monkeys (Kennedy et al. 1976). Despite all such reports on changes in the primary visual cortex of sightless animals, already in early studies on enucleated cats (Shook et al. 1985) and monkeys (Dehay et al. 1989), a normal laminar appearance of the primary visual cortex was detected.

In similar vein, functional magnetic resonance imaging (fMRI) studies conducted with blind human subjects showed a recruitment of primary and secondary visual areas in processing of speech (Röder et al. 2002), music (Ross et al. 2003), or Braille reading (Burton et al. 2002). The stria of Gennari was not investigated in any of these studies, since no methodology was then available, but one can speculate about its fate after deafferentation: persistence would imply a dominance of local, “hard-wired” processes, whereas degeneration or hypoplasia should result if fiber expression depends on projections from the lateral geniculate body (LGN). Alternatively, input from other structures—replacing lost LGN projections and occupying free binding sites—might also lead to development of the stria; however, it is unlikely that a structure as unique as the stria of Gennari would receive...
adequate stimulation from any other structure than its predetermined input. Some early histological studies on human brains assessed variations of the primary visual cortex in anophthalmia, a condition in which patients are born with no eyes and have therefore never experienced visual input. However, reports are conflicting as to whether a stria of Gennari was present (Cosmetatos 1931; Recordon and Griffiths 1938) or not (Duckworth and Cooper 1966; Haberland and Perou 1969; Brunquell et al. 1984).

In the present study, we conducted high-resolution structural MRI at 7-T in birth-blind subjects and sighted controls (SCs). Coronal sections were oriented perpendicular to the main course of the calcarine fissure with the aim to display the stria of Gennari, if present. Previous studies have proven the possibility of localizing the stria at 1.5 T (Clark et al. 1992, in vivo), 3 T (Barbier et al. 2002, in vivo), and 7 T (Hinds et al. 2008, ex vivo; Hinds et al. 2009). By this means, we hoped to resolve the question whether the development of the stria of Gennari depends on retinal input, and therefore should be seen as a structure exclusively dedicated to vision, or if it is preserved in individuals with no visual experience and might thus have a much broader function.

Materials and Methods

Subjects
The study was approved by the ethics committee of the University of Leipzig, and informed consent was obtained from every subject. Thirteen congenitally blind (CB) subjects (7 females, 21–70 years, 46 years mean) were included in the study. Reasons for blindness were retinoblastoma, retrolental fibroplasias, retinitis pigmentosa, lack of the optic nerves, microphthalmia, and the complete lack of eyeballs. For comparison, 15 SCs (8 females, 20–31 years, 25 years mean) were scanned using the same scan protocol.

MRI Acquisition
All experiments were performed with a 7-T whole-body MR scanner (MAGNETOM 7T, Siemens Healthcare Sector) using a 24-channel phased array head coil (Nova Medical Inc.). For positioning of the high-resolution sequences, a 3D whole-brain data set was acquired using a magnetization-prepared rapid gradient-echo sequence (repetition time [TR] = 3 s, echo time [TE] = 2.6 ms, flip angle = 6°; bandwidth = 260 Hz/Px, and isotropic voxels of 0.8 mm³). Subsequently, 30 slices were scanned, approximately orthogonal to the calcarine sulcus, using a turbo spin-echo (TSE) sequence (TE = 27 ms, nominal refocusing flip angle = 180°, bandwidth = 80 Hz/Px, isotropic voxels of 0.5 mm³, turbo factor = 2). Because the blind subjects were naive to MRI, scan time was kept as short as possible. TR varied between 2.1 and 5.7 s depending on the subject-specific specific absorption rate (SAR) limit. When possible, a second set or third set of images with the same voxel size was acquired using either a different TSE protocol (TR = 3.1–5.1 s, TE = 40 ms, bandwidth = 40 Hz/Px, other parameters equal) or a spoiled gradient-echo sequence (TR = 1.5–2.0 s, TE = 23 ms, flip angle = 60°–70°, bandwidth = 60 Hz/Px). These images were not included in the quantitative comparison. Instead, they were used as confirmation that the structure identified as the stria of Gennari is located at the same position in all the acquisitions and is, hence, not a sequence-related artifact. A single scan took about 10 min. depending on the specific TR, which corresponded to the tolerance duration of the MR naive blind subjects. The tolerance duration was not related to the high field strength of 7 T but simply to the necessity of lying still in the scanner bore. Therefore, longer overall experimental times of around 30–45 min had to be split into 10-min periods. The high SNR provided by the field strength of 7 T made averaging of acquisitions unnecessary. Instead, all the MR images presented resulted from a single scan.

Complete coverage of the occipital lobe was impossible within one scan with the TSE protocol consisting of thirty 0.5-mm-thick slices. However, even when a second or third scan was tolerated by the subjects, the remaining posterior or anterior part of V1 was not scanned. Instead, the first scan was repeated with different scanning parameters to check for possible sequence-related artifacts.

Analysis
The images were first visually examined to select the slices in which the stria of Gennari could most easily be identified. Using those slices, image intensity profiles were measured along cortical normals between the white matter boundary and the cortical surface, at positions where the slice orientation was approximately perpendicular to the calcarine sulcus. Figure 1 shows a coronal section of the calcarine sulcus with examples of typical profile positions. From the estimated profiles, the "intensity dip" representing the stria of Gennari was taken as the central point of the profile. In each direction along the profile (toward white matter and toward cerebrospinal fluid), 3 additional points were taken into account. Thus each profile consisted of 7 points in total, with the Gennari stripe in the middle. The profiles were drawn manually, and their positions were chosen randomly on the selected slices. The profiles were estimated on magnified sections of unsmoothed images (see Fig. 1). Therefore, a slight misalignment of the profile with the cortical normal did not result in incorrect values, as long as this misalignment was less then the width of a voxel. As the image quality varied between subjects, and hence the number of slices in which the stria of Gennari could be detected, a different number of profiles could have been obtained for every subject. However, to avoid any bias toward one of the subject groups, 10 profiles were obtained for every subject.

Grand averages were performed across positions, slices, and subjects of the profiles for the CB people and the SCs. Profiles were normalized to the image intensity of the gray matter on the side of the stria of Gennari adjacent to the pial surface. The procedure was repeated separately for the 2 different imaging protocols. Using the t-test, the statistical significance of the signal difference between the stria of Gennari and adjacent gray matter was assessed. The difference of the image intensity of the Gennari stripe between blind and sighted subjects was also statistically evaluated.

As subject motion is obviously a severe issue for the visualization of the stria of Gennari, an objective method for evaluating the amount of motion was applied (Gedamu et al. 2008). Here, signal intensities in the background of the acquired images were compared for 2 regions of interest (ROIs)—one localized in phase-encoding direction (left of the head) and one in frequency-encoding direction (superior to the head). If no motion occurred, both ROIs should show the same signal. Otherwise, motion-related artifacts manifest themselves in enhanced background signal in phase-encoding direction. Therefore, images where the ratio between both ROIs exceeded 3 were excluded from further analysis.

Results
Sufficient image SNR for quantitative analysis was obtained with a single acquisition using the parameters described, essential for

Figure 1. Section of a coronal view of the calcarine sulcus with examples of used profile positions (white lines).
the MR-naive blind subjects unable to tolerate the prolonged scan times required for extensive averaging. The most common source of image quality degradation was subject motion, which can affect the visibility of the stria of Gennari. However, the Gennari stripe could be reliably identified by visual inspection in all 15 SCs and in 11 of the 13 blind subjects. In the remaining 2 blind subjects, there was too much head motion, giving severe image artifacts and making the images uninterpretable. The ratio between background signal in phase- and frequency-encoding direction was 3.7 and 3.5, respectively (for all other subjects ≈1.8). These 2 subjects were excluded from further analysis, resulting in 11 blind subjects included in the quantitative analysis. To avoid any bias toward one of the subject groups, also 11 SCs were randomly chosen from the 15 subjects and were included in the quantitative analysis. Qualitatively, images of the SC subjects appeared slightly sharper than those of the blind subjects, who had no previous MRI scanning experience. Generally, the appearance of the Gennari stripe was similar to that reported in previous MRI studies of sighted subjects utilizing TSE (Carmichael et al. 2006) and inversion-recovery TSE sequences (Turner et al. 2008).

Figure 2 shows coronal, sagittal, and transverse views of part of the calcarine sulcus in a SC subject aged 23 years (Fig. 2A) and a CB subject aged 31 years (Fig. 2B). Some slice-to-slice image intensity variation can be seen in the transverse and sagittal views. While the shape and position of the calcarine sulcus differs between subjects, as expected, the appearance of the stria of Gennari is very similar. The extent of the Gennari stripe in the imaged section of the occipital lobe is also similar. Because the entire primary visual cortex was not imaged, no quantitative analysis of the extent of V1 could be performed.

Enlarged coronal TSE sections through the calcarine sulcus, demonstrating the stria of Gennari in 3 SCs (Fig. 3A–C) and 3 CB subjects (Fig. 3D–F), are shown in Figure 3. FLASH images of the same CB subjects are shown for comparison (Fig. 3G–I).

There was some variation in the profiles measured across primary visual cortex, for both subject groups. Figure 4A shows the average profiles obtained with the first TSE protocol for both subject groups (dashed for CB and solid for sighted). The results for the second TSE protocol are similar but have less statistical power as the corresponding images were not acquired for every blind subject. Figure 4B shows the differences between the mean of gray matter image intensity within each profile and the Gennari stripe intensity for CB subjects and SCs, respectively. The asterisk denotes statistical significance ($t = 4.2$ [CB], $t = 5.6$ [SC] → $P < 0.05$ for both). The intersubject differences in image intensity arising from variation in TR needed to minimize SAR, which affects the

Figure 2. Coronal, sagittal, and transversal view of a section of the calcarine sulcus of a SC (A) and a CB subject (B).

Figure 3. Coronal TSE sections showing stria of Gennari (arrows) in 3 SCs (A–C) and 3 CB subjects (D–F). FLASH sections of the same blind subjects (G–I).
TSE signal (Conturo et al. 1987; Meara and Barker 2005) as well as the acquisition order of the slices analyzed (Melki and Mulkern 1992; Thomas et al. 2004), were eliminated by normalizing profiles by their mean value before averaging. Additionally, for 1 subject 2 scans with different TR times were acquired while keeping the other parameters equal. The effect on the image intensity difference between the stria of Gennari and its surrounding gray matter after normalization was found to be negligible. The profile accuracy was more severely affected by subject motion and imprecision of the angle between the slice and the calcarine sulcus.

Furthermore, the detected signal difference between stria of Gennari and surrounding gray matter showed no apparent decrease with age in our subject group. Furthermore, no obvious difference was noted in the appearance of the Gennari stripe between blind people lacking eye and optic nerve development and those (blind or sighted) with normal visual equipment. However, the limited number of 11 evaluable data sets for the CB people in our study precluded intragroup statistical comparisons.

**Discussion**

We have demonstrated for the first time in vivo that CB people possess a stria of Gennari with MRI contrast that is not significantly different from that of SCs. Any differences in myelination are below the sensitivity of cutting-edge in vivo MRI techniques. Only a field strength as high as 7 T can reliably provide an adequate signal-to-noise ratio to allow high enough resolution images to visualize the Gennari stripe without the need for averaging. For MRI-naive subjects, a short scan time is essential for obtaining good-quality images. Still higher SNR might reveal subtle differences between blind and sighted subjects resulting from a differential myelination of the Gennari stripe (Scholtz et al. 1981), but this will require more effective methods for dealing with subject head motion, to enable further averaging.

The appearance of the stria of Gennari in both subject groups was similar to that observed in previous studies using similar MR protocols (Carmichael et al. 2006; Turner et al. 2008). Additionally, if a second or third scan with different MR parameters (e.g., bandwidth) was performed, the structure could always be identified at the same position. Thus a motion-related artifact mimicking the stria of Gennari could be ruled out. A second effect that could potentially interfere with the appearance of the stria of Gennari in MR images is Gibbs ringing (Haacke et al. 1989). As this phenomenon is caused by abrupt signal changes, it depends on the image contrast and resolution. For evaluating this possible artifact, a change of the image resolution was not possible as a decrease would make the visualization of the Gennari stripe impossible whereas an increase would lead to unacceptable long scan times and low SNR. However, a change in the contrast by applying a different MR sequence did not change the shape and extent of the detected structure identified as the stria of Gennari. Moreover, the structure could only be observed in the cortex of the calcarine sulcus and neighboring intrahemispheric fissure, and its position was consistent in sagittal, coronal, and axial views (see Fig. 2). Thus Gibbs ringing could also be ruled out, leaving the actual stria of Gennari as the only plausible origin for the detected structure.

Few other MRI studies have examined the structure of V1 and its connectivity to other brain regions in CB subjects in vivo (Kitajima et al. 1997; Shimony et al. 2006; Ptito et al. 2008; Bridge et al. 2009; Jiang et al. 2009; Park et al. 2009). They described increased cortical thickness in visual areas for CB compared with sighted subjects (Bridge et al. 2009; Jiang et al. 2009; Park et al. 2009). Significantly reduced surface area in the primary and associated visual areas has also been claimed (Park et al. 2009). The volumetric atrophy in the visual cortex combined with the increased cortical thickness in the blind subjects was explained as a result of reduced "pruning" of synapses in the visual cortex. A similar finding of calcarine atrophy was reported in patients with retinal degeneration (Kitajima et al. 1997). Diffusion tensor imaging studies have demonstrated white matter reorganization and alterations of the visual pathways in blind people (Shimony et al. 2006; Ptito et al. 2008). An overall preservation of brain architecture compared with sighted subjects was shown for anophthalmic subjects (people born without eyes) (Bridge et al. 2009). However, those studies did not investigate the presence or absence of the stria of Gennari, which is most likely due to the great difficulties in imaging intracortical structures at field strengths below 7 T.

Although we are the first to examine the consequences of congenital blindness at an intracortical level in vivo, several groups have investigated the organization of V1 in the absence of visual input in monkeys and humans ex vivo. It is known that in several ways the development of the human visual system is similar to that of monkeys (Polyak 1957; Rakic 1974; Burkharter and Bernardo 1989). It should therefore be unsurprising that similar findings have been obtained for both groups. Studies of monkeys that were prenatally enucleated showed a marked reduction in the extent of area 17 (Dehay et al. 1989, 1991). In
agreement with this finding, Bolton (1900) reported a reduced surface area of primary visual cortex in congenital anophthal-mia in humans. The plasticity of ocular dominance columns has been extensively investigated in monkeys and humans. In a normal developing brain, ocular dominance columns are formed before birth, prior to distinct visual experience (Horton and Hocking 1996). However, prenatal enucleation in monkeys causes ocular dominance columns to fail to develop (Rakic 1981). Similarly, enucleation performed in a critical period after birth causes column shrinkage (Hubel et al. 1977; Horton and Hocking 1997) to the point of complete obliteration of ocular dominance columns, shown in a human adult cadaver brain after enucleation at age 1 week (Horton and Hocking 1998). However, after a critical period (approximately 12 weeks for macaque), visual deprivation does not influence the size of the ocular dominance columns (Horton and Hocking 1997). Remarkably, in the case of a 94-year-old man where visual loss occurred at age 4 months, and hence, presumably near the end of the critical period in humans, the columns, albeit shrunken, survived after >90 years of blindness (Adams et al. 2007). The studies on ocular dominance columns show the complex interplay between genetic instructions and environmental factors. Therefore, it is important to mention that in our study, subjects with and without development of eyes and optic nerves were investigated.

While none of the above studies focused specifically on the stria of Gennari, none of them mention an abnormal laminar pattern of V1, the absence of the Gennari stripe, or a significant reduction of its myelination resulting from the absence of visual input. Rather, a normal laminar appearance of V1 in enucleated monkeys was emphasized by Rakic (1988) and Delhay et al. (1989, 1991) and in humans by Bolton (1900). In particular, the existence of the Gennari stripe was explicitly confirmed in anophthalmic patients by Cosmattos (1931) and Recordon and Griffiths (1938), although there is still controversy in the literature regarding the presence of the Gennari stripe in patients with this latter condition. In the light of the studies discussed above, it remains unclear why the stria was not detected in anophthalmic patients by 3 other groups (Duckworth and Cooper 1966; Haberland and Perou 1969; Brunquell et al. 1984). Except for those 3 studies, the majority of results obtained in animals and humans support our finding that the stria of Gennari is present in CB people. Moreover, our study is consistent with the radial unit model by Rakic (1988) that provides a framework for understanding the ontogenetic and phylogenetic development of cytoarchitectonic areas, for instance of primary visual cortex V1. According to that hypothesis, a protomap of prospective cytoarchitectonic areas is provided already in the embryonic state. Those areas can be modified through interaction with afferent input. However, the basic cytological, synaptic, and biochemical characteristics of V1 can develop in the absence of information from the retina (Rakic 1977, 1988).

Many fMRI studies have investigated the functional organization of primary visual cortex V1 in blind people (Pons 1996; Büchel et al. 1998; Burton et al. 2002; Röder et al. 2002; Ross et al. 2003; Raz et al. 2005; Garg et al. 2007). However, the possible function of the stria of Gennari, especially in blind people, is still unclear. Since the Gennari stripe does not require visual input to develop or to retain its structure, it may be involved, especially in blind subjects, in tasks other than processing visual input. It should be mentioned that all blind subjects in our study were experienced Braille readers. This resulted from the recruitment strategy, in which advertisements were placed in a newspaper for blind people that is readable by a special Braille reading device. Studies of Braille reading in blind subjects have shown that V1 plays a necessary role in this extreme test of haptic discrimination (Burton et al. 2002). Perhaps, it retains its structure in blind subjects, as we have observed, when it is put to such uses. Obviously, Braille reading cannot be the only reason for the retention of the Gennari stripe, since our subjects learnt this technique at the age of 6 or later. It is possible that during the first 6 years of a blind person’s life, other tasks require the stria of Gennari for optimal functioning and processing. Somatosensory tasks in general, including Braille reading as a particular case, might be likely candidates. For blind people, haptic discrimination (combined with auditory information) enables them to create a spatial concept of their environment that sighted people would achieve by vision. However, sighted people also use haptic discrimination to assist visual understanding of their surroundings, especially during infancy. Thus it is plausible to speculate that the Gennari stripe survives because it is involved in a broad range of haptic discrimination tasks, including, but not exclusively, Braille reading. Future fMRI studies may shed light on this question.

In conclusion, we have shown that the stria of Gennari exists in people who are blind from birth no matter whether eyes and optic nerves were developed or not. Clearly, the Gennari stripe does not require visual input to develop. Because it has very similar appearance in adult blind and sighted subjects and the appearance of the stria of Gennari did not depend on the age of the subjects, it is also clear that this structure does not degenerate when deprived of visual input.

The Gennari stripe appears to persist independently of visual processing. Further work is required to investigate more fully the functional processes in which it is involved, besides vision (for sighted people) or instead of vision (for the blind). This comment underlines the novelty of our study. Since our approach allows intracortical examination of human V1 in vivo, subsequent functional measurements in the same subjects can also be performed, and longitudinal comparisons are easily feasible. The latter is especially important because much remains to be learned about “the loss of visual function and how visual experience . . . can restore such function” (Chalupa 2004).

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