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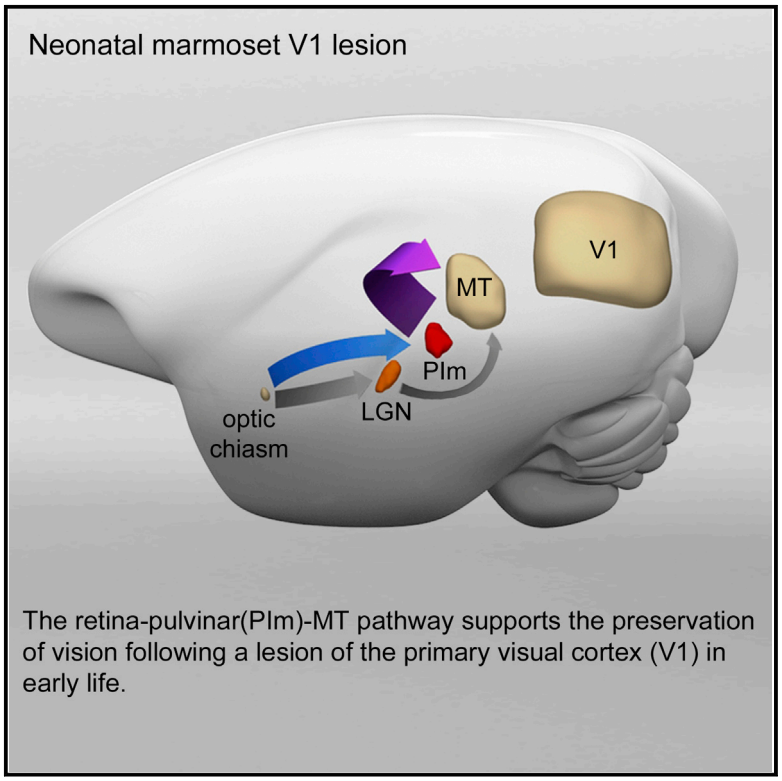
Preservation of Vision by the Pulvinar following Early-Life Primary Visual Cortex Lesions

Graphical Abstract



The retina-pulvinar(PIm)-MT pathway supports the preservation of vision following a lesion of the primary visual cortex (V1) in early life.

Highlights

- The pulvinar is responsible for preserved vision following early-life V1 lesions
- The retina-pulvinar-MT pathway remains unpruned following early-life lesions of V1
- The retina-pulvinar-MT pathway is an alternate pathway for visual information
- Retinal input to the LGN degenerates after an early-life V1 lesion

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In Brief

Warner et al. demonstrate in primates how, following early-life lesions of the primary visual cortex (V1), the disynaptic connection from the retina to the middle temporal area via the medial division of the inferior pulvinar is the likely neural substrate that affords the preservation of visual capacity. This phenomenon is often observed in humans.

Warner et al., 2015, Current Biology *25*, 424–434
February 16, 2015
http://dx.doi.org/10.1016/j.cub.2014.12.028

Current Biology 25, 424–434, February 16, 2015 ©2015 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2014.12.028

Article

Preservation of Vision by the Pulvinar following Early-Life Primary Visual Cortex Lesions

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Summary

Background: Conscious vision is believed to depend upon an intact primary visual cortex (V1), although injury in early life is often accompanied by the preservation of visual capacity, unlike in adulthood. The middle temporal area (MT) receives input from the retinorecipient koniocellular layers of the lateral geniculate nucleus (LGN) and the more recently described medial subdivision of the inferior pulvinar (PIm) of the thalamus, pathways that potentially contribute to preservation of vision after early damage to V1.
Results: We examined the potential of these pathways to the long-term preservation of vision after permanent lesions of primate V1 in early and adult life by using a combination of neural tracing and diffusion MRI. We show that early-life V1 lesions lead to less pruning of the retina-pulvinar-MT pathway than is observed in control or adult lesion animals.
Conclusions: These findings suggest that sustained visual input through the pulvinar to MT following a lesion of V1 in early life has the capacity to afford improved visual outcomes.

Introduction

The functional outcome of damage to the primary visual cortex (V1) depends upon the age at which injury occurs. Lesions to both human and monkey V1 acquired in adult life lead to cortical blindness, but this is not the case for similar lesions received in early life, where there is significant sparing of vision [1, 2]. However, the neural pathways that support this preserved vision remain unidentified.

In primates, the principal pathway facilitating visual perception passes from the retina via the lateral geniculate nucleus (LGN) to V1, beyond which it is distributed through a multiplex of connections to other visual cortical areas. In addition, the visual cortical middle temporal area (MT) also receives disynaptic input from the retinorecipient koniocellular layers of the LGN [3] and the more recently described retinorecipient medial subdivision of the inferior pulvinar (PIm) [4]. This is in contrast to the more commonly described trisynaptic pathway through the pulvinar via the superior colliculus [5]. In humans and monkeys, it has been demonstrated that neurons in MT can respond to visual stimuli in the absence of V1 [6–8]. Studies attempting to determine the neural substrate have focused on the retina-LGN-MT pathway and its role following lesions of V1 in adult life [9, 10]. However, the relatively new concept of the retina-pulvinar-MT pathway and its age-dependent competitive restructuring has not been taken into account, especially following early-life lesions of V1 [11].

To determine whether the retina-pulvinar-MT or the retina-LGN-MT pathway is responsible for the long-term preservation of vision following V1 damage in early life, we examined the integrity of both pathways later in life following permanent lesions of marmoset monkey V1 (0°–8° of contralateral visual field) induced in either early postnatal (postnatal day 14), when the retina-pulvinar-MT pathway is at its postnatal maximum [11] or adult life. Using a combination of neural tract tracing and diffusion magnetic resonance imaging (dMRI), we quantified retinal inputs to the LGN and pulvinar and their relays to MT and determined their functional activation by immunohistochemistry. Notably, we demonstrated that removal of V1 in early life results in greater connectivity between the retina and the pulvinar, as well as between the pulvinar and MT, when compared with both controls and V1 lesion adults. Conversely, in the early-life lesion animals, the retinal input to the LGN was significantly degenerated but the LGN relay to MT remained intact, most likely through the koniocellular and magnocellular layers [12]. Together, these findings indicate that the preservation of a pathway relaying visual information from the retina through the pulvinar to MT is responsible for the largely intact visual performance following lesions of V1 in early life.

Results

Early-Life Lesions of V1 Result in Degeneration and Reduced Volume of the LGN but Expansion of the PIm Subdivision of the Pulvinar

Previous studies have demonstrated significant retrograde transneuronal degeneration through the LGN following lesions of V1, which leads to changes in structural integrity and volume of the LGN [12, 13]. However, the structural integrity of the retinorecipient region of the pulvinar (PIm) has yet to be examined, and this analysis is necessary in order to determine whether it is the conduit for preserved vision following early-life lesions. To address the potential for the pulvinar to relay information to MT in V1 lesion animals, we compared the volumes of the pulvinar, PIm, and MT with the optic tract (postchiasm and ipsilateral to the lesion) and LGN, which are known to degenerate in adult monkeys following a permanent lesion of V1 in early or adult life. MRI and specific immunohistological and histochemical markers were employed to accurately demarcate regions of interest, and calculated volumes can be found in Table S1.

Interestingly, the volume of PIm was significantly larger than controls following a neonatal lesion of V1 (Figures 1A and 1E–1G) but remained unchanged in adult lesion animals (Kruskal-Wallis, $p = 0.0026$; Dunn's test for multiple comparisons to control, neonatal lesion, adjusted $p = 0.0255$; $n = 7$ control, $n = 6$ neonatal lesion, $n = 4$ adult lesion). The volume of the entire pulvinar was also calculated (Figure 1A) to see whether the increase in PIm volume resulted in a loss of territory of other pulvinar regions. Although not significant, the volume

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of the pulvinar was larger in the neonatal lesion group compared to controls (Kruskal-Wallis, p = 0.0543; n = 6 control, n = 4 neonatal lesion, n = 3 adult lesion), and we can therefore assume that no other pulvinar region has lost or gained territory. No change in the size of MT was observed in the neonatal or adult lesion animals compared to controls (Figures 1B and 1H–1J; Kruskal-Wallis, p = 0.4507; n = 7 control, n = 4 neonatal lesion, n = 4 adult lesion).

As expected, the optic tract was reduced in volume for both neonatal and adult lesion groups compared to controls (Figures 1C and 1K–1M), but the extent of degeneration was significantly greater for the neonatal group (Kruskal-Wallis, p = 0.0143; Dunn's test for multiple comparisons to control, neonatal lesion, adjusted p = 0.0369; n = 4 control, n = 2 neonatal lesion, n = 2 adult lesion).

A degeneration zone in the LGN relative to the retinotopic region of lesioned V1 [14, 15] was observed in both adult and neonatal groups (Figures 1O and 1P). The volume of the LGN, which included the degeneration zone, was significantly reduced in both neonatal and adult lesion groups compared to controls (Figure 1D) (Kruskal-Wallis, p < 0.0001; Dunn's test for multiple comparisons to control, neonatal lesion, adjusted p = 0.0002; adult lesion: adjusted p = 0.0356; n = 9 control, n = 7 neonatal lesion, n = 5 adult lesion).

These data highlight the potential of the pulvinar to be involved in relaying visual information to the neocortex following an early life lesion of V1.

The Pruning of the Retina-Pulvinar Pathway Is Prevented following an Early-Life Lesion of V1

While we observed a significant degeneration of the optic tract in adult animals that received a lesion of V1 in early life, it was unclear whether the component of the optic tract projecting to the pulvinar was affected. During development, there is evidence that significant pruning of the retina-pulvinar pathway occurs [11], but whether this process is prevented following the loss of V1 is unknown. In order to evaluate this, we utilized two independent techniques to visualize and assess the integrity of the retina-pulvinar pathway.

First, the retina-PIm pathway was analyzed following injections of an anterograde tracer (cholera toxin conjugated to AlexaFluor 488; CTb488) into the contralateral eye. The density of terminal fields in the PIm of the neonatal lesion group (Figure 2C) was greater than that observed in controls (Figure 2A) and in the adult lesion group (Figure 2E). For the neonatal group, the terminal fields were at a similar level to that previously described in normal development at postnatal day 14 [11], the approximate age at which the V1 lesion was performed. In the PIm, labeled retinal terminal fields converged with pulvinar-MT relay neurons, in the control (Figure 2B), neonatal (Figure 2D), and adult lesion (Figure 2F) groups. In contrast, V1 removal at either age triggered a large loss of labeled retinal input to the contralateral retinal recipient layers within the degeneration zone of the LGN (dzLGN). For the neonatal lesion group, sparse retinal terminal field labeling in the dzLGN could be observed in koniocellular layer 1 and 3 and the external magnocellular layer (Figures 2I and 2J). In the adult lesion group, only sparse retinal terminal field labeling could be observed occasionally in the external magnocellular layer (Figures 2K and 2L). However, even though labeled dzLGN-MT relay neurons could be found in layers with residual retinal input, retinal terminal fields did not colocalize with labeled dzLGN-MT relay neurons. This leads to the conclusion that the degeneration of the optic tract is largely due to the loss of the ganglion cells projecting to the parvocellular layers of the LGN via a process of transneuronal degeneration, also observed in other primates [12].

Second, dMRI was utilized to demonstrate changes in the connectivity between the retina and pulvinar following lesions of V1. The tracks from the optic chiasm followed an identical route to that identified from the neural tracing—tracks are a single streamline from seed to target region of interest generated from tractography—as opposed to a tract, which would be a bundle of nerve fibers e.g., the optic tract. A track or streamline must not be confused or considered an individual nerve fiber. Furthermore, it was possible to distinguish between tracks projecting to the pulvinar and LGN (Figure 3A). dMRI tracks destined for the PIm followed the optic tract posteriorly toward the LGN, where they passed along and through the inferior edge of the LGN before fanning out from its medial border across the inferior pulvinar into PIm (Figures 3A–3D).

Akin to the neural tracing data, dMRI analysis revealed that neonatal, but not adult, lesion animals exhibited increased connectivity between the optic chiasm and pulvinar. For the neonatal group, the number of tracks between the optic tract and PIm was 3.8 times (median) higher than that of controls, whereas the adult lesion group exhibited a 43% (median) decrease (Figures 3B–3D and 3H). We also revealed fewer tracks between the optic tract and LGN in both the neonatal and adult lesion groups, amounting to a 33% (median) and 28% (median) decrease when compared to controls, respectively (Figures 3E–3G and 3I). Comparing the optic tract input ratios of connectivity of the two thalamic nuclei, there were six times more tracks to the PIm than to the LGN for the neonatal group (Figure 3J) compared to control levels, suggesting that the there is a shift in the strength of connectivity between optic tract input to the PIm. The reduced retinal input to the LGN is analogous to the retrograde transneuronal degeneration described following neonatal and adult lesions of V1 [13].

This converging evidence suggests that the pathway leading from the retina to the pulvinar is at least as strong as, if not stronger than, normal in neonatal V1 lesion animals. This implies that the observed degeneration of the optic tract is secondary to the degeneration of the LGN following a lesion of V1.

Early-Life Lesions of V1 Prevents Pruning of Pulvinar-MT Relay

In addition to demonstrating an increase in the retinal input to PIm following a V1 lesion in early life, we wanted to determine whether this correlated with an increase in the pulvinar relay to MT by again employing both neural tracing and dMRI tractography. The validity of dMRI-identified pathways was confirmed by neural tracing (Figure S2).

For neural tracer based mapping of the thalamic relays to MT, we injected a retrograde tracer into MT (fast blue, FB). The population of MT afferent cells in the PIm of the neonatal lesion group (Figure 2C) was significantly greater than that observed in the adult lesion group (Figure 2E; Kruskal-Wallis, p = 0.0143; Dunn's test for multiple comparisons to control, neonatal lesion, adjusted p = 0.0369; n = 2 control, n = 4 neonatal lesion, n = 2 adult lesion; Table S2) and was at a similar level to that previously described in normal development at postnatal day 14 [11], the approximate age at which the V1 lesion was performed. A greater number of retrograde labeled cell bodies were also observed in the PIm compared

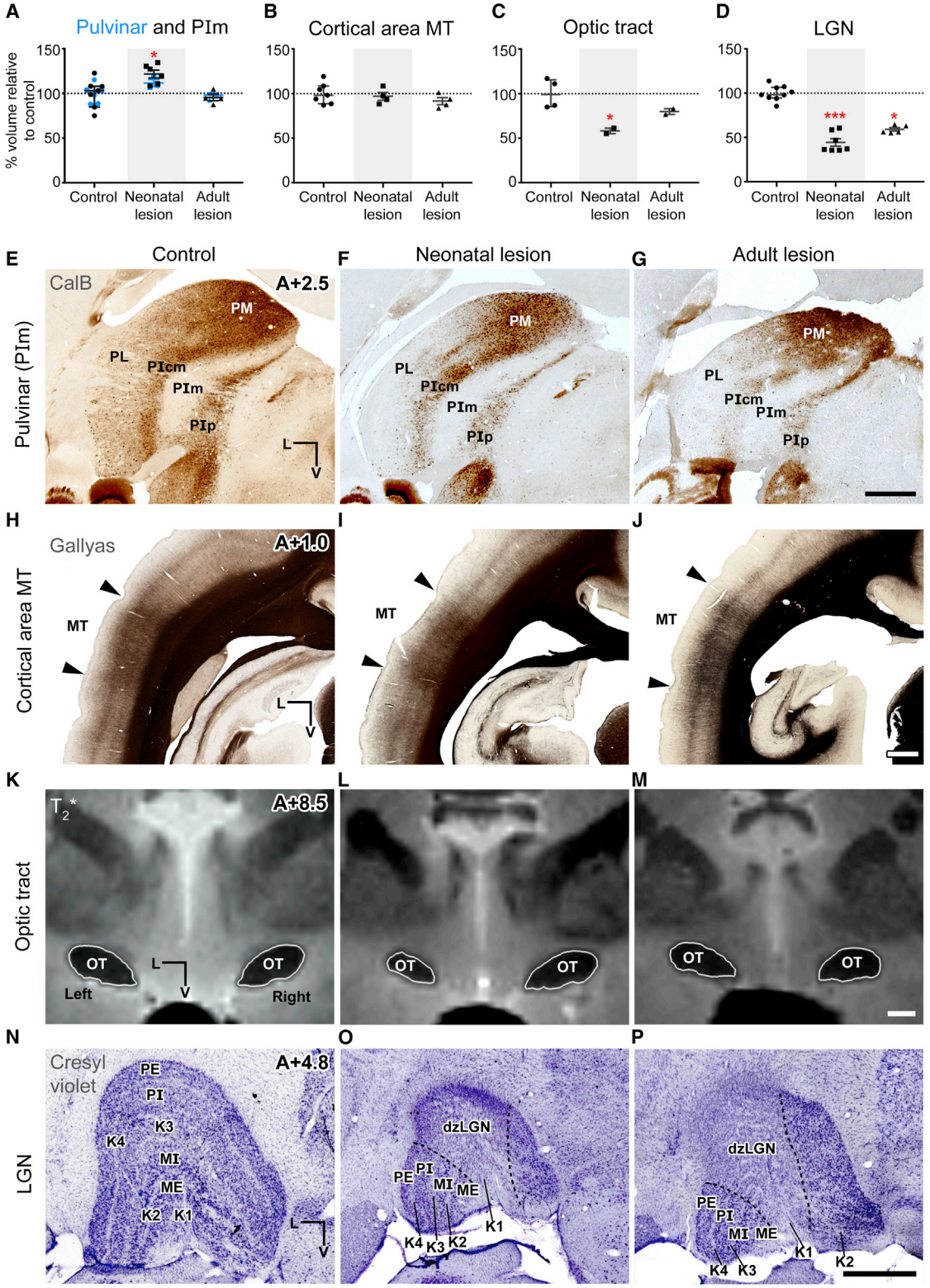

Figure 1. Volumetric Analysis of the PIm, MT, Optic Tract, and LGN

Volume of left (lesion hemisphere) pulvinar, medial subdivision of the inferior pulvinar (PIm), middle temporal area (MT), optic tract (OT), and lateral geniculate nucleus (LGN) calculated after long-term unilateral V1 ablation at approximately postnatal day 14 or adult.

(A) The volume of the PIm was significantly larger (median volume: 123% of control) for the neonatal lesion group (Kruskal-Wallis p = 0.0026; Dunn's test for multiple comparisons to control, neonatal lesion, adjusted p = 0.0255; n = 7 control, n = 6 neonatal lesion, n = 4 adult lesion hemispheres). The volume of the pulvinar was also larger for the neonatal lesion group, but this did not reach significance (p = 0.0543; n = 6 control, n = 4 neonatal lesion, n = 3 adult lesion hemispheres).

(legend continued on next page)

to the LGN in both neonatal and adult lesion groups compared to control animals (Figure 2A, 2C, 2E, 2G, 2I, 2K; Table S2).

dMRI demonstrated that the greatest increase in tracks was in the relay from the pulvinar to MT in the neonatal lesion group, with a more than 60-fold increase compared to controls, whereas in the adult lesion group it was reduced by 38% (Figures 3B–3D and 3K). In addition to a greater number of cells projecting to MT, this increase could also be due to reciprocal feedback from MT [16]. Tracks between the LGN and MT in the neonatal lesion group were also increased by 3.5 times. This is likely due to strengthening of cortical feedback to the LGN from MT as the number of labeled relay cells to MT in the dzLGN in the neonatal lesion group was similar to the number in the control or adult lesion group (Table S2). A decrease of 80% was observed in the adult lesion group relative to controls (Figures 3E–3G and 3L). The ratio of the number of tracks in the neonatal lesion group from the PIm to MT was significantly higher (14-fold; Figure 3M) than the number of tracks from the LGN to MT (Kruskal-Wallis, $p = 0.0143$; Dunn's test for multiple comparisons, neonatal lesion, adjusted $p = 0.0369$; $n = 4$ control, $n = 2$ neonatal lesion, $n = 2$ adult lesion).

Pulvinar-MT Relay Neurons Remain Visually Active Long-Term following a V1 Lesion in Early Life and Provide a Driving Input

If PIm-MT relay neurons of the neonatal lesion animals are compensating for the loss of input from V1, one would expect them to remain visually active and capable of driving input from the retina to MT. cFos immunolabeling can be used to determine visual activity in a tracer-labeled cell, and the cellular expression of either parvalbumin or calbindin-D28k can be used as an indicator of driver or modulator function, respectively [17–19]. In the neonatal lesion group, cFos expression in the PIm (Figure 4A) was at a similar level to that previously observed in control animals [11], and the MT projecting cells, identified by FB labeling, were all cFos positive (Figure 4C). However, there was a clear lack of cFos expression in the dzLGN (Figure 4B), and no MT projecting cells were identified as cFos positive (Figure 4D). This suggests that the pulvinar relay but not the LGN relay to MT was visually driven. All visually responsive FB-labeled PIm-MT relay neurons were parvalbumin positive (Figure 4C), indicative of a driver input, whereas FB-labeled cells in the LGN were calbindin-D28k positive (Figure 4D), which is indicative of a modulatory input to MT. These results suggest the retina-pulvinar-MT pathway is visually active following a lesion of V1 in early life with the potential to afford a route for driving visual input to the cortex in the absence of V1.

MT Is Structurally Unaffected by a Lesion to V1 in Early Life Unlike Other Areas that Are Not Recipient of a PIm Connection

Given the persistence of the retina-pulvinar-MT pathway following early life V1 lesions, we asked whether this pathway might selectively support MT, protecting it from the degenerative effects of a V1 lesion. To this end, we compared the chemoarchitecture of another visual cortical area, V3 ventral (V3v), that is recipient of a direct input from V1 [20, 21] and the LGN [22] but lacks input from PIm [23]. Of these two extrastriate areas, only MT is recipient of input from the retinorecipient PIm [24]. Following an early-life V1 lesion, we examined the chemoarchitecture and cellular phenotypes within the V1 lesion projection zone (LPZ) of both MT and V3v (Figures 5A and 5D).

Within the LPZ of both MT and V3v, there was no apparent cellular degeneration after early life lesion as evidenced by cresyl violet staining (Figures 5C and 5F) when compared to controls (Figures 5B and 5E). However, expression of the excitatory pyramidal neuron marker, non-phosphorylated neurofilament, reveals normal expression in MT (Figure 5C) but is either absent or markedly reduced in layers 3, 5, and 6 of V3v (Figure 5F) when compared to controls (Figures 5B and 5E). The density of the subset of inhibitory interneurons labeled by parvalbumin appeared normal in MT (Figure 5C) but was reduced across all layers in V3v (Figure 5F) compared to controls (Figures 5B and 5E).

Taken together, these results suggest that MT maintains a similar functional capacity in terms of both projection and local circuit neurons despite the loss of V1 in early life, which is most likely driven by the retained input from the PIm. However, areas that do not receive driver input from the PIm, such as V3v, have an altered cellular profile, symptomatic of altered function.

Discussion

Our data demonstrate that particular circuitry within the primate visual system is highly adaptable to competitive restructuring during development, enabling the enhancement and/or reduction of retinal inputs to the extrastriate cortex. While V1 is the primary source of input for the extrastriate visual cortical areas, many of these are recipient of their own direct visual thalamic input, making the system highly amenable to plasticity. Therefore, following a lesion of V1 in early life, these alternative routes have the potential to manipulate the flow of visual information from the eye to afford preservation of conscious perception. It is evident from our studies that the retinorecipient PIm and its connection with MT has the

(B) MT volume was comparable to that of controls ($p = 0.4507$; $n = 7$ control, $n = 4$ neonatal lesion, $n = 4$ adult lesion hemispheres).
(C) Volume of the left OT was significantly smaller in the neonatal lesion group ($p = 0.0143$; neonatal lesion, adjusted $p = 0.0369$; $n = 4$ control, $n = 2$ neonatal lesion, $n = 2$ adult lesion hemispheres), but not significantly reduced in the adult lesion group.
(D) However, the volume of the LGN was significantly smaller in volume in both lesion groups ($p < 0.0001$; neonatal lesion, adjusted $p = 0.0002$; adult lesion, adjusted $p = 0.0356$; $n = 9$ control, $n = 7$ neonatal lesion, $n = 5$ adult lesion hemispheres).
(A–D) Error bars indicate the median plus interquartile range. *adjusted $p < 0.05$; ***adjusted $p < 0.001$.
(E–G) Calbindin-D28k immunolabeling (CalB) was performed on coronal sections to demarcate the subdivisions of the inferior pulvinar nucleus, especially the CalB-negative PIm. At this level of the pulvinar, the central medial (PIcm) and posterior (PIp) subdivisions of the inferior pulvinar, and the lateral (PL) and medial (PM) pulvinar nuclei can be observed. Control (E), neonatal lesion (F), and adult lesion (G) are shown.
(H–J) Myelin staining was used to assist with demarcation of MT. Arrowheads point to borders of area MT. Control (H), neonatal lesion (I), and adult lesion (J) are shown.
(K–M) The OT can be identified as black ellipsoids in coronal T_2* MR images. Control (K), neonatal lesion (L), and adult lesion (M) are shown.
(N–P) Cresyl violet stained coronal sections of the LGN were used to demarcate the layers and degeneration zone (dzLGN) in lesion animals. Control (N), neonatal lesion (O), and adult lesion (P) are shown.
K1 and K3, koniocellular layers of the LGN; ME and MI, external and internal magnocellular layers of the LGN; PE and PI, external and internal parvocellular layers of the LGN; A, anterior position relative to interaural line; MR, magnetic resonance. All scale bars represent 1,000 μm. See also Table S1.

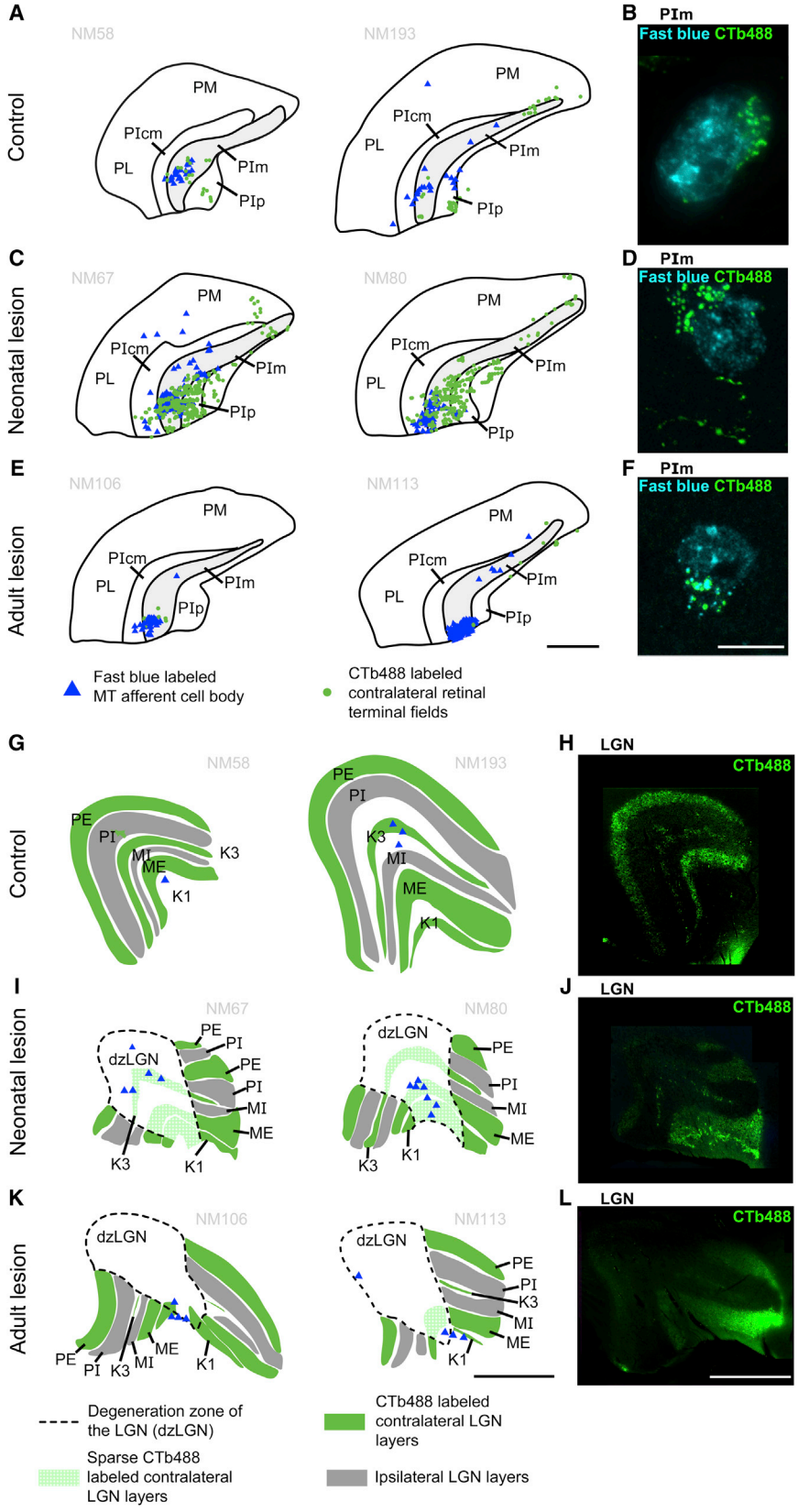

Figure 2. Neural Tracing of Afferent and Efferent Pathways of the PIm and LGN after Neonatal and Adult V1 Lesion

(A, C, and E) A greater amount of retinal terminal fields (CTb488; green dots) in the PIm was observed after a neonatal lesion (C), when compared to control (A) and adult lesion (E) animals. Retrograde-labeled relay neuron bodies projecting to MT (fast blue, FB; blue triangles) in the PIm were observed in control (A), neonatal lesion (C), and adult lesion (E) groups.

(B, D, and F) FB (cyan)-labeled cells in the PIm were surrounded by many contralateral retinal terminals (CTb488; green) in the control (B), neonatal lesion (D), and adult lesion (F) groups.

(G, I, and K) CTb488 labeled retinal terminal fields can be seen in PE, ME, K1, and K3 in the control (G). Sparse terminal fields were present in koniocellular 1 and 3 and ME within the dzLGN for neonatal lesion group (I), whereas in the adult lesion group (K) sparse terminal fields were occasionally observed only in the ME of the dzLGN. In the dzLGN, FB-labeled cells, bodies were more disperse in the neonatal lesion group (I) compared to the adult lesion group (K), where they were restricted to K1.

(H, J, and L) CTb488 labeling in control (H), neonatal lesion (J), and adult lesion (L) LGN.

dzLGN, degeneration zone of the LGN; K1 and K3, koniocellular layers of the LGN; ME and MI, external and internal magnocellular layers of the LGN; PE and PI, external and internal parvocellular layers of the LGN; PIcm, PIm, and PIp, centromedial, medial, and posterior subdivisions of the inferior pulvinar; PL, lateral pulvinar; PM, medial pulvinar. Scale bars represent 1,000 μm (A, C, E, and G–L) and 10 μm (B, D, and F). See also Figure S1 and Table S2.

ultimate potential for the preservation of vision, as increased connectivity of this visually functioning disynaptic pathway was observed in animals that received a lesion of V1 in early life and were subsequently examined in adulthood. The extent of connectivity was similar to that observed early in life before pruning of the pathway [11] and suggests a critical window during which restructuring is possible. While the LGN input to MT via the koniocellular layers has been implicated in the phenomenon of "blindsight" in monkeys that received a V1 lesion in adulthood [10], we suggest that this pathway is not responsible for conscious visual perception following early-life lesions of V1.

The Retina-Pulvinar-MT Pathway Supports Vision following Early-Life V1 Lesions

Behavioral and functional studies in adult humans and monkeys following early-life lesions of V1 demonstrate that they possess greater residual vision than adults that have received a similar lesion but these studies failed to interrogate the responsible pathway(s) [2, 25–27]. Other studies have concluded that visual signals can reach extrastriate cortex bypassing damaged V1 and that these signals are sufficient for visual discrimination and conscious awareness [1, 28]. Since motion detection is spared, residual vision has been linked to the functions of neurons in MT and its associated inputs [29], but few suggest an involvement of the retina-pulvinar-MT pathway. However, it is difficult to pinpoint exact type of visual perception this pathway is supporting after an early-life V1 lesion. Recent recordings from MT after long-term neonatal V1 lesions have demonstrated robust responses in MT [30], although directional tuning is absent, unlike an earlier study that showed improved motion discrimination in early-lesioned animals [26].

The two thalamic candidates investigated here, the pulvinar and LGN, are recipient of a retinal input and have a direct connection with MT. Our results revealed that early-life lesions to V1 resulted in an increase in functional connectivity between the retina and MT via the PIm, but not via the LGN. This is in contrast to a previous study in adult monkeys that demonstrated inactivation of LGN after a V1 lesion in adulthood results in a lack of response of MT neurons [10], and the spared visual performance after V1 lesion was credited to the LGN activation of MT. However, the lesion of V1 was at an age when the pruning of the retina-pulvinar-MT pathway would be complete but the retrograde transneuronal degeneration of the LGN and optic tract would likely be incomplete, thus affording the potential for transmission of visual information to area MT. To date, there is only one other study employing tractography to map the subcortical connections of MT after early V1 lesion [9]. This study identified connections between the LGN and MT in human "blindsight" subject GY, who was 8 years of age when he received trauma to V1. Given the relatively late timing of the trauma, it is unlikely that the retina-pulvinar-MT pathway would be enhanced, but the authors did not investigate its potential involvement. However, retrograde labeling of MT afferents in 5- to 6-week-old macaques demonstrated abundant labeling in the pulvinar, but not the LGN [31]. Although this study did not quantify the difference between the two thalamic nuclei or further demarcate the pulvinar nuclei, it was the first to highlight a potential role for the pulvinar following early-life lesions of V1.

Another potential pathway to MT also implicated in residual vision is the superior colliculus (SC)-pulvinar-MT pathway. A specific route through the PIm has recently been functionally and anatomically described in the adult macaque [32, 33] and is in contrast to [5], which previously described SC input did not terminate in the PIm but surrounding subdivisions (PIcm and PIp), which project to satellites of MT. In a previous study of adult macaques, it was also demonstrated that an SC lesion after the bilateral lesion of V1 resulted in inactivation of MT [34]. Therefore, there is certainly potential for the SC to be involved but studies in neonatal V1 lesion animals, and further examination of the interconnectivity of the pulvinar subdivisions and MT complex is needed to deduce its participation in preservation of vision following V1 lesion.

We also investigated whether the disynaptic relays through the thalamus were capable of providing visual input to MT. Although we did not undertake any electrophysiological analysis, we were able to ascertain information on the function of the pathways through specific protein expression in both adult and early-life lesion cases. First, the expression of cFos by the majority of pulvinar-MT relay neurons suggests that they are visually active. This is in contrast to the relay neurons in the dzLGN. Activity of just the pulvinar-MT relay neurons was akin to that previously observed in a normal 14-day-old animal [11]. Furthermore, the expression of the calcium-binding proteins parvalbumin and calbindin-D28k provides insight into the regulatory role of thalamic pathways. Like the principle relay neurons in the magnocellular and parvocellular layers of the LGN, the pulvinar-MT relay neurons expressed parvalbumin, suggesting that they are capable of driving visual input from the retina to MT, whereas the LGN-MT relay neurons expressed calbindin-D28k, which is more indicative of a modulatory role [17–19].

Taken together, these findings highlight that after early-life damage of V1 the normally transient projections between the retina and MT via the pulvinar are stabilized and can continue to function into adulthood. The degeneration of the LGN and lack of visual activity in the LGN-MT relay neurons suggests that the LGN plays a minor role, if any, in the reorganization of the visual system after early-life lesions of V1.

Developmental Plasticity of the Retina-Pulvinar-MT Pathway

Connections of the primate visual system, established initially by retinal waves and molecular cues in utero, provide a minimal operating system at birth where optimal performance is acquired through changes involving sculpting and retraction of retinothalamic, thalamocortical, corticothalamic, and corticocortical projections [35–37]. This refinement can be observed via the improved visual sensitivity and discrimination as the animal matures. Competitive restructuring during postnatal development of neurons originating from the PIm and V1 and terminating in MT could explain the retraction of retinal input from PIm during normal development. However, following the disruption of the normal course of events, in this case by permanently removing V1, it allows the PIm-MT projection to be integrated into the neuronal circuit during the period of cellular maturation, but not in the adult. The increase in the PIm-MT relay also enables stabilization of the exuberant retinal input to PIm, which continues to drive the pulvinar relay neurons.

For the disynaptic retinal input to MT through the thalamus, removal of V1 in early life results in the selective sparing of the pulvinar afferent pathway but results in the rapid degeneration of retinal ganglion cells projecting to the magnocellular and parvocellular layers of the LGN [12, 13]. Although we did observe sparing in the koniocellular layers of the dzLGN, these

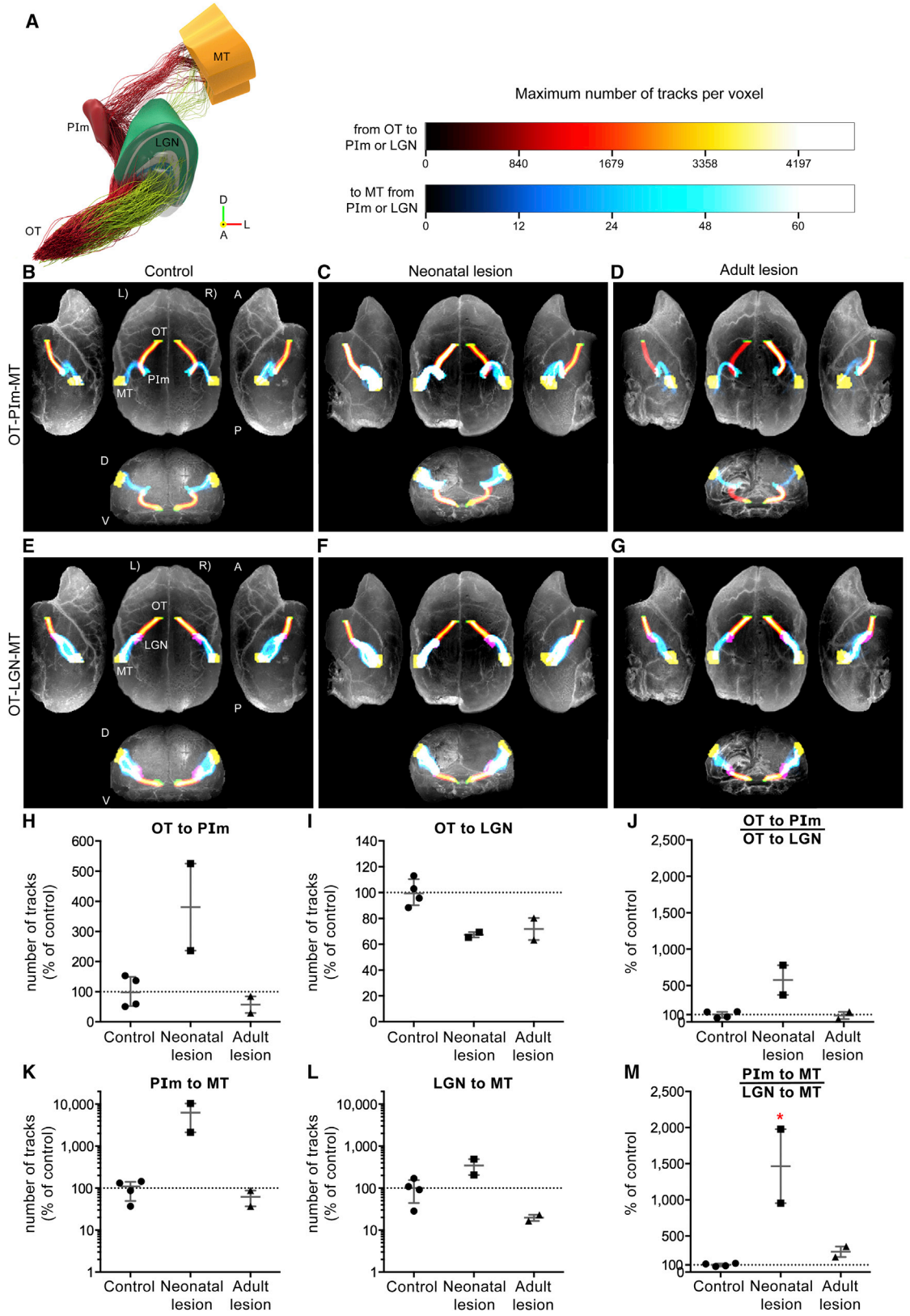

Figure 3. dMRI Reveals Increased Connectivity between Optic Tract, Visual Thalamus, and MT following a V1 Lesion in Early Life

(A) Three-dimensional representation of the OT-PIm-MT (red lines) and OT-LGN-MT (green lines) dMRI tracks in the left hemisphere of a control animal. (B–D) Example of maximum-intensity projection of the number of tracks per voxel in the OT to PIm (red-yellow gradient) and PIm to MT (blue gradient) pathways in control (B), neonatal lesion (C), and adult lesion (D) animals.

(legend continued on next page)

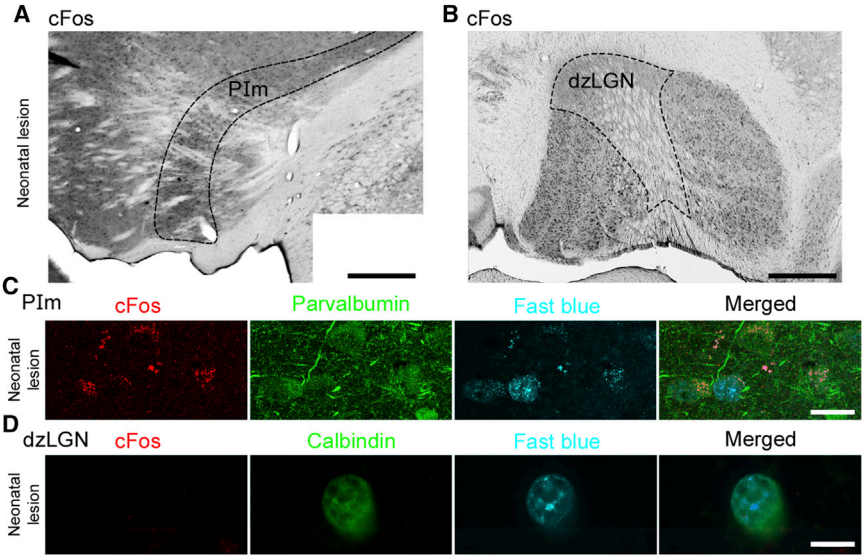

Figure 4. cFos Immunoreactivity Reveals Only PIm-MT Cells Are Active to Visual Stimulation following a Neonatal V1 Lesion

(A and B) The immediate-early gene product cFos was expressed by many cells in the PIm (A), but not within the dzLGN (B), following a visual stimulation protocol.

(C) A majority of the cFos (red)-positive cells in the PIm were also FB (cyan) labeled, indicating that they were PIm-MT relay neurons, and expressed the calcium-binding protein parvalbumin (green).

(D) FB-labeled MT afferent cells located in the K layers of the dzLGN expressed the calcium-binding protein calbindin-D28k (green), but no colocalization with cFos expression was observed.

dzLGN, degeneration zone of the lateral geniculate nucleus; PIm, medial subdivision of the inferior pulvinar. Scale bars represent 500 μm (A and B) and 20 μm (C and D).

afferents were not in the vicinity of the MT afferent relay neurons and may therefore synapse on the numerous relays to the extrastriate cortex that is recipient of geniculate input, which may survive following a V1 lesion [22, 38, 39]. A previous study has also shown that the remaining excitatory input to the dzLGN appears to be from the superior colliculus and not the retina [40].

Similar to thalamocortical pathways, connections between cortical areas are also initially broad during development, and the appropriate set of synapses is selected through activity dependent processes [41]. In this study, we were able to demonstrate that the loss of V1 in early life resulted in no apparent loss of specific subtypes of excitatory (projection) and inhibitory (local) neurons within the LPZ of MT or V3v. However, in the LPZ of V3v, there was an observed change in the cellular properties of the neurons indicative of a change in function. Unlike MT, V3v receives no direct input from PIm and is reliant on indirect afferents from V1 via the LGN and directly from the LGN [20, 22]. For the MT afferents, most likely via the PIm, these were able to provide sufficient drive to maintain neuronal function within MT, but the lack of an equivalent pathway in V3v resulted in restructuring of local and projection neuron circuitry. Although this study cannot directly answer what accounts for the preservation of the early retina-pulvinar-MT pathway after V1 lesion, we suggest that it is a result of reduced competition in MT for synaptic space.

The Pulvinar as a Developmental Organizer

Although it is apparent that the presence of a strong retina-pulvinar-MT pathway during development has significant advantage to the outcome of a lesion of V1 in early life, there is no identified role for this pathway in visual cortical development.

The expansion of the primate visual cortex has involved the addition of evolutionarily new areas rostral to the previous one. This fits with the hierarchical model of information processing and the suggested wave of development of extrastriate areas from V1 [42, 43]. However, area MT develops in concert with V1, which possibly results from the strong retina-pulvinar-MT pathway observed during development [11, 44]. In terms of a function, MT is implicated in the processing of motion [29], a specific feature of vision primates can process in early life [45, 46], which may be linked to an evolutionary necessity [47].

During the evolutionary expansion of the neocortex, a parallel expansion of the pulvinar has occurred. It has been noted that the size and differentiation of the pulvinar parallels the enlargement of the occipital, temporal, and parietal association cortex [48] that have many reciprocal connections

(H and K) In the neonatal V1 lesion animals, the OT-PIm (H) and PIm-MT (K) pathways in the lesion hemisphere were greater than those of controls (median absolute number of tracks: OT-PIm, 380%; and PIm-MT approximately 63 times greater than control), whereas there was a decrease in track numbers from OT-PIm and PIm-MT in the adult (OT-PIm, 43%; PIm-MT, 38% decrease).

(E–G) Examples of maximum-intensity projection of the number of tracks per voxel in the OT-LGN and LGN-MT in control (E), neonatal lesion (F), and adult lesion (G) animals.

(I and L) In the neonatal lesion animals, there was a decrease in track number from OT-LGN (33%; I) but an increase from the LGN-MT (348%; L) compared to controls, whereas in the adult lesion animals, there was a decrease in both the OT-LGN (28%; I) and LGN-MT (80%; L) track numbers compared to controls.

(J) Ratio of the number of tracks from OT-PIm divided by number of tracks from OT-LGN as a percentage of the control for the lesion hemisphere. A greater number of tracks in the OT-PIm than in the OT-LGN pathway was observed in the lesion hemisphere of neonatal group compared to controls, but not significantly.

(M) Ratio of the number of tracks from PIm-MT divided by number of tracks from LGN-MT as a percentage of the control for the lesion hemisphere. A significantly greater number of tracks in the PIm-MT than LGN-MT pathways was observed in the lesion hemisphere of neonatal group compared to contros (Kruskal-Wallis, $p = 0.0143$; Dunn's test for multiple comparisons, neonatal lesion, adjusted $p = 0.0369$).

(H–M) Error bars indicate the median plus interquartile range. *adjusted $p < 0.05$. In (I)–(J), $n = 4$ control group and $n = 2$ for each lesion group.

The color scale represents the maximum density of tracks per voxel. Red-yellow gradient, OT to PIm or LGN; blue gradient, PIm or LGN to area MT. LGN, lateral geniculate nucleus; MT, middle temporal area; OT, optic tract; PIm, medial subdivision of the inferior pulvinar; A, anterior; D, dorsal; L, lateral; P, posterior; V, ventral; L, left hemisphere; R, right hemisphere. See also Figure S2.

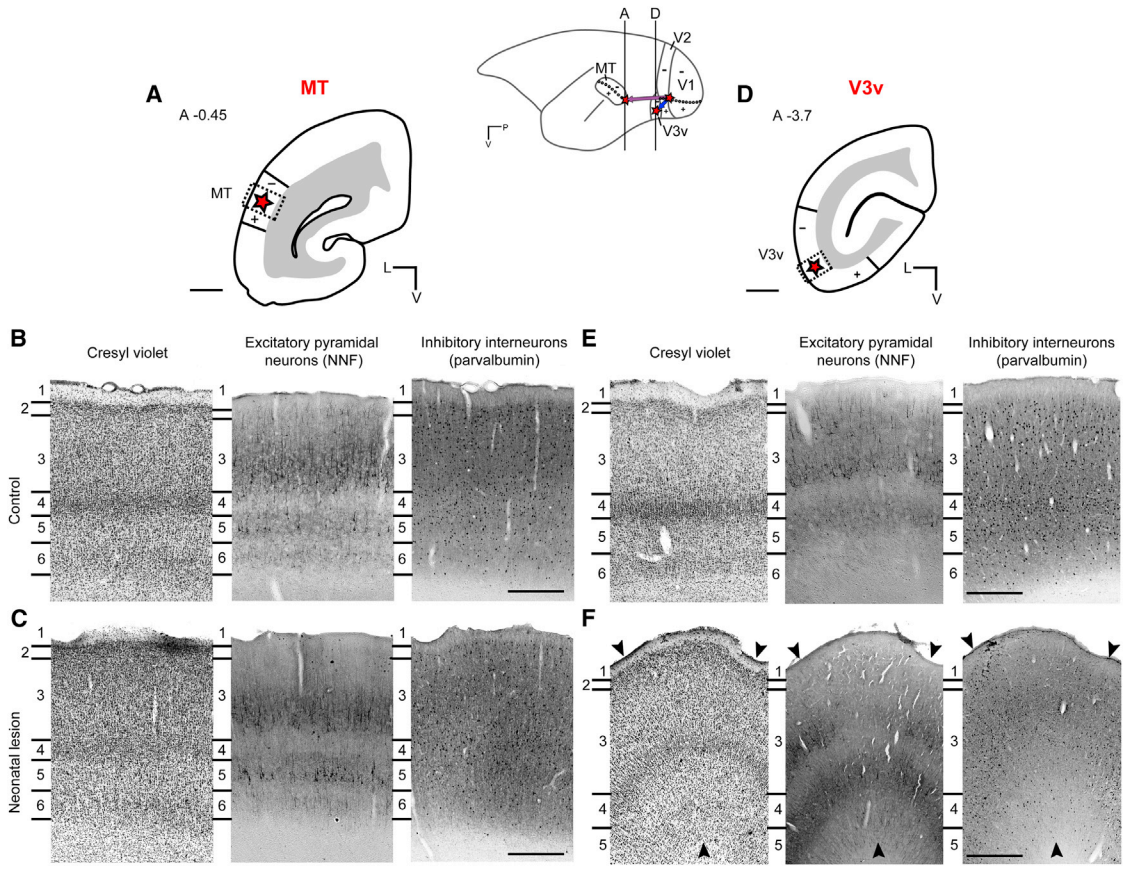

Figure 5. Visual Cortical Area V3v but Not MT Is Affected by Partial V1 Lesion in Neonatal Lesion Animals

(A and D) Comparison of the distribution of the excitatory pyramidal neuron marker, non-phosphorylated neurofilament (NNF), and the inhibitory interneuron marker, parvalbumin, in the V1 lesion projection zone (LPZ) in MT (A) and V3v (D). Red star, foveal representation; purple arrow, direct V1-MT connection; blue arrow, V1-V3v connection; dotted rectangles, estimated location of the V1 LPZ.

(B and C) For area MT, no discernible differences in either NNF or parvalbumin expression were observed (C) when compared to controls (B).

(E and F) But disturbances in NNF and parvalbumin was found in V3v (F; arrowheads). Specifically, expression of NNF in the supragranular layers of V3v was reduced, and a reduction of parvalbumin in all layers was observed (F) compared to controls (E).

MT, middle temporal area; V1, primary visual cortex; V2, second visual area; V3v, ventral part of visual area 3; A, anterior position relative to interaural line; D, dorsal; L, lateral; P, posterior. Scale bars represent 2,000 μm (A and D) and 500 μm (B, C, D, and F).

with the visual pulvinar. Therefore, one possibility is that the addition of a more direct pathway to MT during evolution, which has established MT as a developmental molecular anchor [49], has enabled the expansion of the visual cortex between MT and V1. Thus, the retina-pulvinar-MT pathway had potential to support the expansion of the motion related extrastriate cortical areas of the dorsal stream observed in primates.

Experimental Procedures

Animals

Neonatal marmoset monkeys (*Callithrix jacchus*; NL; n = 9, mean = 13.1 postnatal days old at time of lesion; range = P10–P19) or adults (AL; n = 5, mean = 2.74 years old at time of lesion; range = 1.37–3.41 years old) of either sex that had partial removal of left hemisphere V1 (operculum) were used in the present study (Figure S3; Table S3). In addition, there were eight adult controls (non-sham; >2 years old) whose material had been used in other studies. Two perfusion-fixed brains from each cohort of animals (NL, AL, and control) were used for dMRI. Eight of the lesion animals received a fluorescent retrograde tracer injection into the left hemisphere MT and contralateral eye fluorescent anterograde tracer injections. A light exposure protocol for the assessment of visual cell activity by immunohistochemistry was also applied (see Table S4 for animal allocation). Animals were housed in family groups (12:12 hr light/dark cycle, temperature 31°C, humidity 65%). All experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Monash University Animal Ethics Committee, which also monitored the welfare of these animals.

Full details of the experiments are provided in the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.12.028.

Acknowledgments

The authors would like to thank Rick Philipsen and Scott Kolbe for assistance with the MRI, Phil Owen for assistance with figures, and David Leopold and Cornelius Gross for comments on previous versions of the manuscript. The Australian Regenerative Medicine Institute is supported by grants from the State Government of Victoria and the Australian Government. J.A.B. is supported by National Health and Medical Research Council Project Grants (APP1002051 and APP1042893). A National Imaging Facility grant supported the MRI component of the project.

Received: September 17, 2014
Revised: November 14, 2014
Accepted: December 5, 2014
Published: January 15, 2015

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