IN VIVO MULTIPARAMETRIC MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF RODENT VISUAL SYSTEM

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The integrity of the neuronal connections between eye and brain plays an important role in the performance of the mammalian visual system. However, the developmental and pathophysiological mechanisms in the visual system are largely unexplored due to the lack of a sensitive technique for directly assessing both anterior and posterior visual pathways longitudinally under the same experimental conditions. This paper reviewed the recent use of magnetic resonance imaging and spectroscopic (MRI/MRS) methods (contrast-enhanced MRI, diffusion MRI, proton MRS and functional MRI) at high magnetic field strengths, for in vivo and global assessments of the structure, metabolism and function of the visual system in normal, developing and injured rodent brains. Using animal models of ocular diseases, optic neuropathies, developmental plasticity and neonatal hypoxic-ischemic brain injury, focus is put on the feasibility of MRI/MRS to evaluate axonal transport and cellular activity along segregated fibers of the visual pathways, to characterize lesion-induced neurodegeneration in the retina and the optic nerve and tract, to detect steady-state metabolite changes in the posterior visual nuclei, and blood-ocular dynamic exchanges in the eye, and to understand the neurovascular coupling and functions in the retina and the visual brain nuclei. These studies suggested the significant values of high-field multiparametric MRI/MRS for providing early diagnoses and comprehensive therapeutic strategies for promoting functional recovery upon partial vision loss.

Keywords: Manganese-enhanced MRI; gd-enhanced MRI; diffusion tensor imaging; proton magnetic resonance spectroscopy; functional MRI; rodents; visual system; glaucoma; optic neuropathies; neonatal hypoxic-ischemic brain injury.

1. Introduction

The neuronal connectivity between eye and brain is important for maintaining the performance of the mammalian visual system. In recent decades, two major questions arise regarding neuronal connections in the visual pathway: (1) How does the brain

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wire itself up during visual development, and (2) What are the exact mechanisms of neurodegeneration in the visual pathway in diseases and injuries involving vision loss. To date, the mechanisms of the formation of retinotopic orders between retina and primary visual targets, and the patterns of primary and secondary neurodegeneration upon ocular diseases and injuries remain controversial [1, 2]. A major obstacle of researching the developmental processes and new treatments for neurodegenerative diseases in the visual system is the lack of a precise and sensitive technique for directly assessing the spatial and temporal evolutions of both anterior and posterior visual pathways under the same experimental conditions. Although recent studies showed that treatment to both the eye and the brain for ocular diseases may result in better outcomes than treating the eye alone [3], current diagnoses of human ocular diseases and injuries are generally limited to the anterior visual pathway. Typical neuroanatomical experiments on the eye and brain connectivity in preclinical studies require animal sacrifice upon injection of chemical or viral tracers into the eye or the brain for histological sectioning [4–8], and do not allow direct investigation of the functional properties of anatomically identified areas. Recent advancement in \textit{in vivo} optical imaging of the retina and the visual cortex [9] are currently limited to unobstructed light paths and a relatively small field of view, rendering global, in-depth assessments of the entire visual pathway impossible [10]. It is of significant value to develop an \textit{in vivo} tool for understanding the regulations of the structure, metabolism, physiology and function of the primary visual system globally over time, which could help uncover the mechanisms of developmental and compensatory changes to lesions systematically along the entire visual pathway. This may, in turn, provide early diagnoses and comprehensive therapeutic strategies for promoting brain functional recovery upon ocular diseases and injuries that may lead to partial vision loss.

Magnetic resonance imaging (MRI) provides a non-invasive tool to study the structural, metabolic, physiology and functional details of the inner-depth of the body \textit{in vivo} in a single setting. In this paper, we review the recent use of high-field multiparametric magnetic resonance imaging and spectroscopic (MRI/MRS) methods (contrast-enhanced MRI, diffusion MRI, proton MRS and functional MRI) for \textit{in vivo} and global assessments of the visual system from the anterior chamber [11, 12], vitreous humor [11–16] and retina [10, 14–34] in the eye to the optic nerve, optic chiasm and optic tract [28, 29, 35–56], the superior colliculus (SC) [29, 36, 37, 51, 53, 54, 57–65], the lateral geniculate nucleus (LGN) [29, 54, 61, 63–65] and the visual cortex [60, 63, 64, 66–69] of the brain in rodent studies. Despite a relatively low visual acuity, rodents are an excellent model for understanding the developmental and pathophysiological changes in the visual system, given their relatively simple structures, short lifespan, rapid growth and the ability to produce precisely defined changes in gene sequence [70–75]. The retinocollicular projection between the retina and superficial layers of the rodent SC is a well-established model system to study the cellular and molecular mechanisms of retinotopic map formation and neurodegeneration in the brain [76]. The optic nerve injury model takes advantage of the
exclusive retinal ganglion cell centripetal axon projection, devoid of interneurons, so that the overt reactions of both retinal ganglion cell somata and axons to optic nerve transection are not complicated by either direct or indirect collateral damage to extraneous structures, which is a confounding issue in most other sites of CNS damage [77]. There are also increasing numbers of studies on experience-dependent plasticity in the retinogeniculate projection and the visual cortex in rodents [78–84]. Furthermore, rodents are phylogenetically closer to primates than carnivores are, thus may serve as a better model for understanding the human brain [85]. Their enhanced crossing of optic nerve fibers to the contralateral posterior visual pathway via the optic chiasm (e.g., >90% to the rat contralateral optic tract [86–89] compared to about 52% in humans and primates [90, 91]) generally also allows the visual components in the opposite hemisphere to serve as an internal control upon unilateral manipulation of the visual system.

2. *In Vivo* Manganese-Enhanced MRI (MEMRI) of Axonal Transport and Cellular Activity Along Segregated Fibers of the Visual Pathways

The functions of the central nervous system depend upon precisely organized neuronal connections [92–98]. Among the complex neural networks, the superior colliculus (SC) is a dome-shaped subcortical laminar structure in the mammalian midbrain, which is important in coordinating visual, somatosensory and auditory stimuli to guide animal behavior [99–101]. In particular, the superficial layers of the SC receive visual information from the retina in a topological order [4, 8, 102, 103], whereby the retinal ganglion cell axons emanating from superior, inferior, nasal and temporal retina projected to the contralateral lateral, medial, caudal and rostral SC respectively in rodents [4, 8, 102, 103]. Despite the increasing number of studies investigating the retinotopic projection in visual brain development and disorders [6, 76, 92, 103–109], the majority of *in vivo* techniques on brain organization, such as electrophysiology and optical imaging, focus on the cortex [9, 110–112]. The precise topological projection in the subcortical structures remains difficult to assess *in vivo*, due to the low spatial resolution of electrophysiological techniques, the depth limitation from optical imaging, the small sizes of the subcortical nuclei, their deep locations, and their closeness to surrounding large pulsating vessels [113, 114]. Development of a tool for *in vivo*, high-resolution 3D mapping of topographic organization in the subcortical visual nuclei would help open a new area for understanding the precise retinotopic organizations in the visual system globally, longitudinally and non-invasively in the same animal, which could be useful for monitoring the topographic changes in brain development, diseases, plasticity and regeneration therapies in a single setting.

Despite previous studies demonstrating the capability of blood-oxygenation level dependent functional MRI (BOLD-fMRI) for mapping gross retinotopic organizations in the subcortical visual structures and the visual cortex *in vivo* [113, 115, 116],
BOLD-fMRI cannot be readily used to map submillimeter-scale neural activities due to its relatively broad hemodynamic point spread function extending beyond the neuronally active area [117–120]. In addition, a large field-of-view for high-resolution mapping of the entire visual pathway is difficult to achieve in fMRI due to the trade-off of spatial resolution for fast imaging sequences to capture physiological responses with optimal signal-to-noise ratios. To overcome these problems, we recently used the alternative in vivo MEMRI technique [42], which relies on the entrance of Mn$^{2+}$ ions into voltage-gated calcium channel and fast axonal transport [40, 121], for global tract-tracing of segregated fibers and in vivo analyses of retinotopic orders in the optic nerve fibers and the SC [28, 29, 36, 37]. MEMRI has been increasingly used for neuronal tract tracing [28, 42, 53, 54] and functional brain mapping at lamina levels [20, 66] without the reliance on hemodynamic response. Mn$^{2+}$ ions are paramagnetic in nature and can shorten the T$_1$ relaxation time of the surrounding water protons. In addition, Mn$^{2+}$ ions accumulate at the target locations with a slow clearance rate, allowing sufficient time for high-resolution, 3D acquisition with a large field-of-view (FOV) and an optimal SNR using typical T1-weighted (T1W) imaging sequences [28, 40, 42, 51, 122–127]. Anatomical distortions from fast acquisition sequences such as echo-planar imaging in typical fMRI studies can also be avoided. The dosage of intravitreal Mn injection for optimally tracing the visual pathway in rodents with minimal toxicity have been extensively documented in the literature [34, 51–54, 62, 128]. To test whether there is sufficient sensitivity to detect a segregated amount of nerve fibers in the visual pathway, we examined the properties of anterograde axonal transport in the posterior brains upon total [37] and partial transections [29] of optic nerve fibers after intravitreal injection of the Mn$^{2+}$ contrast agent (Fig. 1).

In the untreated visual pathway [“Normal” in Fig. 1(a) and left eye-right SC in Fig. 1(b)], clear Mn$^{2+}$ contrasts were observed in the entire superficial layers of the SC (arrowheads in Fig. 1(a)) and the LGN (* in Fig. 1(a)) using 3D T1-weighted imaging (T1WI) at 200 µm isotropic resolution, whereas upon complete transection of the optic nerve fibers at the brachium of SC, no apparent signal enhancement was observed in the SC, and the Mn$^{2+}$ ions accumulated proximal to the transection site (arrows in Fig. 1(a)). Upon partial transection of right superior intraorbital optic nerve (arrowhead in Fig. 1(b)), the lateral region of the left superior colliculus (solid arrow in Fig. 1(b)) showed a significantly lower signal intensity compared to the medial left SC and the control right SC. A clear border was also observed separating the lateral and medial halves of the left SC, which appeared to colocalize with the topographic projections of the retinocollicular axons relative to the edge of the partial transection in the optic nerve. Signal reduction was also noted in the right LGN and optic tract, and the left optic nerve distal to the partial transection. In addition to MEMRI studies on optic nerve crush [52, 53] and radiation-induced optic nerve injury [44] models demonstrated by others, our previous MEMRI study using a rat model of chronic ocular hypertension also showed a reduced rate of Mn$^{2+}$ transport in the prechiasmatic optic nerve upon intravitreal Mn$^{2+}$ injection, in association with a diffuse partial loss of retinal ganglion cell axons [28].
Fig. 1. Typical Mn-enhanced MRI (MEMRI) (axial T1-weighted images (T1WI) in (ai); oblique T1WI in (aili); maximum intensity projection of axial T1WI in (aili) and (b)) of the adult hamster (a) and rat (b) visual pathways without transection [left column in (a)], with total transection of the left optic tract (OT) at the brachium of superior colliculus (SC) [right column in (a)], and with partial transection (yellow arrowhead) at the right superior intraorbital optic nerve (ONio) (b). Mn$^{2+}$ was injected into the right eye only in (a) and into both eyes in (b). In the normal animal in (a), T1W hyperintensity was observed in the SC (arrowheads), its brachium (asterisk), and the lateral geniculate nucleus (LGN) in the left hemisphere. Note the loss of T1W hyperintensity in the left SC distal to the site of total transection in (a). Note also the accumulation of Mn contrasts proximal to the lesion (arrows in a). In (b), partial transection of the right superior ONio resulted in reduced intensity in the right ON distal to the site of lesion (orange arrowhead), the left OT, as well as the left LGN compared to the contralateral components. Interestingly, there was a loss of hyperintense signals at the lateral half of the left SC (solid arrow) with a clear border separating the lateral and medial left SC, likely as a result of reduced anterograde axonal transport of Mn$^{2+}$ from localized regions of the anterior visual pathway after partial transection of the right superior ONio. Inset in (b) illustrates the graphical representation of retinotopic projection from right retina and ONio to left SC in rodents. It was suggested that retinotopic mapping could be performed using MEMRI upon partial lesioning of the ONio and/or the retina [29]. Note also the lower signal intensity in the medial half of the left SC compared to the right control SC, which likely indicated secondary degeneration surrounding the primary injury in the ONio [29, 129]. (OC: optic chiasm; PT: pretectum.) (Images modified from [29, 37].) For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

It has also been shown that partial transection of the superior intraorbital optic nerve resulted in primary injury predominantly in the superior but not the inferior retina and optic nerve [129]. The results of this study demonstrated the sensitivity of submillimeter-resolution MEMRI for in vivo, 3D imaging of segregated fibers after primary injury at localized regions of the anterior visual pathways. It also allowed mapping of the precise retinotopic projections in SC upon reduced anterograde axonal transport of Mn$^{2+}$ ions from the anterior pathways [29]. In addition to anterograde, retrograde and transsynaptic degeneration, optic neuropathies are associated with delayed neuronal death surrounding the lesions in the visual pathways.
similar to the penumbra in brain injury and ischemia [129]. In the current study, it is also noted that a lower signal intensity was observed in the medial half of the left SC compared to the right control SC, which likely represented secondary degeneration surrounding the primary injury in the intraorbital optic nerve [129]. Future experiments will determine the course of secondary degeneration in the intraorbital optic nerve, by comparing the signal enhancements in the optic nerve and medial left SC over time. A recent study has successfully demonstrated the use of MEMRI for retrograde tracing in the sciatic nerve [130]. Future experiments may also employ longitudinal retrograde-tracing MEMRI to evaluate the potential distal transport loss and transsynaptic degeneration in the SC, LGN and visual cortex in disease models such as glaucoma [2, 3, 5]. Upon intraperitoneal injection of Mn$^{2+}$ ions, previous studies using high-resolution MEMRI detected layer-specific retinal functional adaptation [20], retinal channel rhodopsin-2-mediated activity [31], ion channel dys-regulations [17, 22, 131], as well as changes in intraretinal signal intensities in rat models of optic nerve injury, hereditary glaucoma and choroidal melanoma [21, 25, 26]. Recent studies have also employed in vivo 3D MEMRI for detecting layer-specific activity in the visual cortex [66], and for longitudinal auditory functional mapping of tonotopic organization in the inferior colliculus from awake and freely-moving rodents [132]. Future experiments may also test the feasibility of activity-induced MEMRI [133] to map retinotopic activities in the visual nuclei during brain development, diseases, plasticity and regeneration therapies at high resolutions. The current in vivo MEMRI approach may help evaluate, at high resolutions, the mechanisms guiding the establishment of topographically ordered connections in the central nervous system [4, 8, 92, 103, 109, 134], the progressive changes in functional topology in diseases involving partial visual field loss, such as glaucoma, age-related macular degeneration, ischemic stroke and traumatic brain injury [28, 115, 135–140] and the effect of interventions on neuronal rewiring upon early retinal and SC lesions [6, 105–108, 141], all of which could eventually lead to new diagnostic techniques and therapies for improving visual impairments.

3. Diffusion Tensor Imaging (DTI) of Anterograde and Retrograde Neurodegeneration after Primary or Secondary Injury to Neonatal and Adult Visual Pathways

DTI is a method for the non-invasive assessment of white matter integrity and cellular organization in vivo [142–146]. In addition to examining neuronal connectivities using MEMRI, in vivo DTI has been used in combination with other MR modalities to determine the microstructural integrity along the visual pathways in rodents [39, 50, 52]. While the relaxation time constants and apparent diffusion coefficients of the retina have been quantified for adult rats and mice [147, 148], a recent study demonstrated the ability of in vivo DTI to reflect photoreceptor cell alignment in mouse retina with normal physiology or degenerative pathology [149].
Along the optic nerve and tract, directional [axial ($\lambda_\parallel$) and radial ($\lambda_\perp$)] diffusivities, measuring water diffusion parallel and perpendicular to the axonal tracts, were shown to be specific to axonal and myelin damage respectively in mouse models of retinal ischemia [45, 48–50] and experimental autoimmune encephalomyelitis [47, 55]. DTI has also been shown to be able to detect Wallerian degeneration after transient retina ischemia in adult mice [46]. In our laboratory, in vivo DTI has been employed to determine the course of microstructural alterations in postnatal brain development, glaucoma, radiation-induced injury and neonatal brain injury along the rat visual pathways [35, 38, 43, 56, 59, 150–152]. Using adult rat brains, in vivo DTI using fractional anisotropy (FA) and directional diffusivities have been employed for early detection of optic nerve degeneration in chronic experimental glaucoma [38], as well as detection of delayed retrograde degeneration in the optic nerve in the late stage of radiation-induced brain injury [43, 153]. Using a neonatal rat model of severe hypoxic-ischemic (HI) encephalopathy involving unilateral left common carotid artery ligation followed by 2-hour hypoxia at postnatal day (P) 7, our recent data further suggested the feasibility of in vivo DTI to detect and differentiate the co-existence of anterograde and retrograde neurodegeneration along the adult visual pathways in the same animals after primary and secondary neonatal brain injury [35, 56, 59]. As shown in Fig. 2, along the visual pathway projected from the ipsilesional eye, the differences in DTI metrics between age-matched controls and HI-injured brains at P60 were the largest in the ipsilesional prechiasmatic optic nerve (solid arrows) and the smallest in the contralesional posterior optic tract (arrowheads), whereas along the pathway from the contralesional eye, such differences were the largest in the ipsilesional posterior optic tract (open arrows) and the smallest in the contralesional optic nerve. While common carotid artery occlusion could lead to chronic retinal ischemia followed by secondary retina and optic nerve [154], the severe neonatal HI brain injury model was shown to result in a large porencephalic cyst as presented by hyperintensity in the diffusion trace map covering the ipsilesional hemisphere (asterisks in Fig. 2), including the primary and secondary visual cortices. It has also been shown that visual cortex damage caused trans-synaptic degeneration of the thalamus and the retinal ganglion cells [155]. The distinct spatial patterns of directional diffusivities in the visual pathways suggested that in vivo DTI can characterize both anterograde neurodegeneration in the visual pathway projected from the ipsilesional eye, and retrograde degeneration in the visual pathway projected from the contralesional eye in the same animals after neonatal HI brain injury. The DTI parametric pattern of FA decrease and $\lambda_\parallel$, $\lambda_\perp$ and Tr increase in the ipsilesional posterior optic tract may also represent primary degeneration in the chronic stage of neonatal HI brain injury, whereas FA and $\lambda_\parallel$ decrease, and $\lambda_\perp$ and Tr increase may suggest secondary degeneration in the ipsilesional prechiasmatic optic nerve [35, 56, 156] (Fig. 2). It has been shown that upon early visual cortex damage, the survivors continued to transmit visual information via the remaining routes to the superior colliculus of the midbrain, which could be enhanced as the result of extensive training [155]. Our DTI
Figure 2. (a) Color-encoded fractional anisotropy (FA) directionality, FA value and diffusion trace (Tr) maps of a normal rat and a neonatal hypoxic-ischemic (HI) injured rat at postnatal day (P) 60 at the level of the prechiasmatic optic nerves (PON), and anterior (AOT) and posterior (POT) optic tracts [35, 59]. The left common carotid artery was ligated at P7, followed by hypoxia at 8% O2 and 92% N2 for 2 hours. The expanded views of optic nerves and tracts in the FA value map are shown at the bottom. Note the apparent FA reduction in the ipsilesional left PON (solid arrows) and the contralesional right AOT (dashed arrows) and POT (arrowheads). The ipsilesional POT (open arrows) appeared to be displaced by the porencephalic cyst (asterisks) and was identified as a dorsoventrally-oriented fiber bundle in the color-encoded FA directionality map. (b) Comparisons of DTI parametric values (FA, axial diffusivity ($\lambda_{//}$), radial diffusivity ($\lambda_{\perp}$) and Tr) along the visual pathways projected from the ipsilesional (Ipsi-) left eye (left column) and contralesional (Contra-) right eye (right column) in the normal and HI-injured groups. Compared to age-matched normal brains, the HI-injured brains exhibited a significantly lower FA but higher Tr in the ipsilesional PON and ipsilesional POT; whereas significantly lower FA but not Tr were observed in the contralesional AOT and POT. The pattern of FA decrease and $\lambda_{//}$, $\lambda_{\perp}$ and Tr increase may suggest primary degeneration in the ipsilesional POT, whereas FA and $\lambda_{//}$ decrease, and $\lambda_{\perp}$ and Tr increase may suggest secondary degeneration in the ipsilesional PON [156]. No significant difference was observed in all DTI metrics between ipsi- and contra-lateral hemispheres in the normal group. Note also that along the visual pathway projected from the contralesional eye, the differences in DTI metrics between normal and HI-injured brains were the largest in the ipsilesional POT and the smallest in the contralesional PON; whilst along the visual pathway projected from the ipsilesional eye such difference was the largest in the ipsilesional PON and the smallest in the contralesional POT. These results appeared to characterize anterograde neurodegeneration in the visual pathway projected from the ipsilesional eye, and retrograde degeneration in the visual pathway projected from the contralesional eye. (Representative colors for different directions in color-encoded FA directionality map: blue, caudal-rostral; red, left-right; and green, dorsal-ventral). (Units for $\lambda_{//}$, $\lambda_{\perp}$ and Tr: x10$^{-3}$ mm$^2$/s; Two-tailed unpaired t-tests between two groups: *$p<0.05$, **$p<0.01$, ***$p<0.001$; Two-tailed paired t-tests between ipsi- and contra-lesional hemispheres in HI-injured group: # $p<0.05$; ##$p<0.01$; ###$p<0.001$ in left column). (Images modified from [35, 59].)
findings on the long-term outcome of the visual pathways after neonatal brain injury are potentially important in determining and improving the functional consequences of remaining nerve fibers after most compensatory and reparative phases have been passed.

Conventional MRI methods for measuring the diffusion of water, including DTI, assume that diffusion occurs in an unrestricted environment with a Gaussian probability distribution. These techniques can determine the apparent diffusion in three-dimensional space (i.e., directional diffusivity) but are unable to provide further information about the microenvironment of water molecules. In biological tissues, cellular microarchitecture constrains the movement of water, causing the diffusion displacement probability distribution to deviate substantially from a Gaussian form. Using our 7T MRI scanner, we have experimentally evaluated and
optimized diffusion kurtosis imaging (DKI) by means of higher-order (4th order) water molecule diffusion tensors for neural tissue characterization [150–152, 157]. Our DKI studies using directionally specific kurtosis for probing restricted water diffusion indicated its high sensitivity and comprehensiveness in characterizing brain maturations in the gray and white matters compared to conventional DTI techniques using 2nd order diffusion tensors [150–152] (Fig. 3). Our new DKI method

Fig. 3. Typical in vivo diffusion kurtosis imaging (DKI)-derived mean diffusivity (MD in $\mu$m$^2$/ms), axial diffusivity ($\lambda_\parallel$ in $\mu$m$^2$/ms), radial diffusivity ($\lambda_\perp$ in $\mu$m$^2$/ms), fractional anisotropy (FA), mean kurtosis (MK), axial kurtosis ($K_\parallel$), and radial kurtosis ($K_\perp$) maps in postnatal day 13 (P13), day 31 (P31) and day 120 (P120) rat brains at the level of the optic tract (arrows). (Images modified from [150].)
could substantially enhance the usefulness of MRI as a means of examining cellular microstructure in the visual system of the brain in vivo, and could greatly advance basic and clinical investigation of the brain by providing unprecedented microanatomical information in studies of white and gray matters, both in the normal and diseased brains in postnatal developing and adult subjects.

4. Evaluation of Steady-State Metabolisms in Posterior Visual Nuclei by Proton Magnetic Resonance Spectroscopy (1H-MRS), and Dynamics of Blood-Ocular Metabolite Exchanges by Gadolinium Contrast-Enhanced MRI

Rather than the conventional anatomical imaging by MRI, MRS measures chemical shift information of individual molecules or portions of molecules within a sample [158, 159]. It allows the study of the biochemistry and metabolism of developmental or disease processes within the subject without the need for invasive procedures such as biopsies [158, 159]. To date, in vivo 1H-MRS studies of the metabolic changes in human ocular diseases are limited [160, 161], possibly due to limited sensitivity and spectral resolution at low magnetic field strengths, limited regional specificity and inter-subject biological variations. Alternatively, high-field 1H-MRS has been becoming readily available to quantify the neurochemical profiles in selected volumes of the rodent brains in vivo with improved accuracy and precision [162–169]. For example, the most sensitive markers for developmental and regional variations in rat hippocampus, striatum, and cerebral cortex were observed to be N-acetylaspartate (NAA), myo-inositol, taurine, glutamate, and choline (Cho) compounds [166]. In the visual system, recent studies indicated that application of brain-derived trophic factor to both the eye and visual cortex resulted in increased levels of retinal ganglion cell survival and function that exceeded those seen following treatment of the eye alone [170]. While the majority of studies on ocular diseases and optic neuropathies focused mainly on the anterior pathways, using localized single-voxel 1H-MRS at 7 Tesla covering posterior visual brain nuclei, our recent studies indicated its feasibility in detecting alterations in major metabolites (e.g., Cho, glutamate, NAA, taurine, lactate and myo-inositol) in the steady state after complete or partial deafferentation of the anterior visual pathway with reference to creatine (Cr) level [58, 67–69, 169] (Fig. 4). In particular, upon unilateral chronic ocular hypertension to the right eye for 6 weeks, Cho (but not NAA) was found to be significantly reduced in the contralateral left visual cortex compared to the ipsilateral right visual cortex [67] (Fig. 4(a)). The results of reduced Cho level showed that glaucoma is accompanied by alterations in the metabolism of choline-containing compounds in the visual cortex contralateral to the glaucomatous rat eye, and potentially associated the pathophysiological mechanisms of glaucoma with the dysfunction of the cholinergic system in the visual pathway. Combining our previous DTI results using the same animal model [38], our findings of the loss in neuronal integrity in the optic nerve (as indicated by the decrease in FA and increase
Fig. 4. Single-voxel $^{1}$H-MRS of the visual cortex (a and b) and superior colliculus (c) in the posterior visual pathways upon chronic ocular hypertension to the right eye for 6 weeks in adult rats (a), 3 weeks after monocular enucleation to the right eye in neonatal rats at postnatal day 10 (b), and 3 days, 2 weeks and 4 weeks after complete transection (white arrow) of the optic tract (OT) fibers at the brachium of superior colliculus in adult hamsters (c). T2-weighted brain images on top of (a) and (c) indicate the localization of voxels (rectangular boxes) for $^{1}$H-MRS acquisitions. Relative to creatine level, $^{1}$H-MRS revealed a decrease in choline level in the left glaucomatous visual cortex compared to the right control visual cortex after unilateral chronic ocular hypertension (a). These results showed that glaucoma is accompanied with alterations in the metabolism of choline-containing compounds in the visual cortex contralateral to the glaucomatous rat eye, and potentially associated the pathophysiological mechanisms of glaucoma with the dysfunction of the cholinergic system in the visual pathway [67]. In the neonatal rat model of monocular enucleation, a reduction in taurine and NAA levels were observed in the visual cortex contralateral to the enucleated eye at juvenile age at P30 compared to the ipsilateral hemisphere and age-matched control [150] (b). In the longitudinal $^{1}$H-MRS study, upon deafferentation of the adult hamster optic tract, a consistent increase in lactate, and a transient decrease in NAA and glutamate followed by a delayed increase in myo-inositol were also found in the ipsilesional superior colliculus from 3 days to 4 weeks after lesion compared to the contralateral side [58] (c). (Images modified from [58, 67, 68].)
approximately 20% of retinal ganglion loss between Week 4 and Week 8 after glaucoma induction [7, 172]. While target cell loss represents perhaps the most extreme case of a reduction in trophic supply, decreases due to reduced synaptic connectivity or decreased LGN and visual cortex activity might also play an important role, especially during early stages of degeneration [170]. Our $^1$H-MRS results reflected the initial changes in Cho:Cr in the visual cortex that may indicate subtle disturbances of neurological function preceding the development of overt glaucoma in the visual cortex. On the other hand, in a neonatal rat model of early visual impairment by monocular enucleation at P10, a reduction in taurine and NAA levels was observed in the visual cortex contralateral to the enucleated eye at juvenile age at P30 (Fig. 4(b)) compared to the ipsilateral hemisphere and age-matched control [68]. Such metabolic changes measured in vivo likely reflected the cortical degeneration associated with the reduction of neurons, axon terminals and overall neuronal activity. These results also demonstrated that $^1$H-MRS approach has the potential to characterize neonatal monocular enucleation and other developmental neuroplasticity models (such as neonatal H1 brain injures [169]) noninvasively for the biochemical and metabolic processes involved. $^1$H-MRS has also been successfully applied to evaluate metabolic changes in subcortical visual nuclei upon complete deafferentation of the adult hamster optic tract, whereby a consistent increase in lactate, and a transient decrease in NAA and glutamate followed by a delayed increase in myoinositol were found in the ipsilesional superior colliculus from 3 days to 4 weeks after lesion compared to the contralateral side [58] (Fig. 4(c)). The results of the above studies further supported the recent issues on the need to investigate brain changes so as to look for better treatments to ocular diseases and optic injuries. More importantly, this in vivo spectroscopic method can be readily translated to study human glaucoma and early blindness, and can have direct clinical applications. In the human brain, the cortical sheet contains both gray and white matters in the range of a few microliters, and is vulnerable to larger partial volume effects than the rat visual cortex in both $^1$H MRS and proton chemical shift imaging ($^1$H CSI) using ordinary clinical MR scanners. Recent studies demonstrated the ability of $^1$H CSI to quantify metabolite concentrations in mammalian brains at microliter resolution under high magnetic field strengths [164, 173]. Future experiments would possibly enable the differentiation of subtle changes in metabolite concentrations in gray and white matters upon chronically elevated intraocular pressure or early visual impairment in humans. Note that the studies reported here are considered preliminary because we used a relative quantification method by measuring metabolite ratios utilizing Cr peak as the internal spectral reference, based on the concept that Cr was in chemical equilibrium and that its regional concentration was generally not affected by neurodegenerative processes. Future studies will employ the absolute quantification approach [174–176] for validation of the above findings.

Whereas $^1$H-MRS provide high spectral resolution of the metabolic and biochemical changes in the visual nuclei in the steady state, high spatial and temporal resolution MRI allows inner-depth localization of dynamic ocular processes in the
anterior visual pathway in vivo [12–14, 32, 177–192]. Recent studies using the arterial spin labeling (ASL) technique showed high basal blood flows in the ciliary body and retina of rat eyes [32]. The conventional route of aqueous humor outflow from the bloodstream into the anterior chamber via ciliary body stroma and iris root [193] has also been traced using dynamic contrast-enhanced MRI [177, 178, 180] after systemic administration of an exogenous MR contrast agent gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA). Despite the relatively small molecular mass of Gd-DTPA (938 Da), the blood-retinal barrier and aqueous–vitreous barrier are impermeable to Gd-DTPA in normal intact eyes [14, 178, 180, 182, 183]. Given such properties, Gd-DTPA enhanced MRI has been used to examine the ocular biotransport and pathology that altered the permeability of blood-aqueous [177, 181, 194] and blood-retinal barriers [14–16, 27, 182, 183, 195] in both human and animal eyes. Using the established chronic ocular hypertension model, we have demonstrated, for the first time, that systemic administration of gadolinium contrast agent could also provide early and sensitive detection of enhanced contrast leakage from the ciliary body into the anterior chamber and vitreous humor (Fig. 5) of the glaucomatous eye.

Fig. 5. (Top) Gadolinium (Gd) contrast-enhanced MRI allows visualization of the ocular components in the anterior visual pathway, and the blood-ocular dynamic exchanges in anterior chamber (AC) and vitreous body (VB) in the left normal and right glaucomatous rat eyes. [lens (Ln), ciliary processes (CP), posterior chamber (PC), optic nerves (ON)]. (Bottom) Windowed and zoomed serial T1-weighted images of the right glaucomatous rat eye (a), and the left control (b) eye of the same animal at 10–80 min following systemic Gd-DTPA administration after unilateral chronic ocular hypertension to the right eye for 8 weeks. Leakage of Gd contrast from the aqueous–vitreous interface into the vitreous body (arrows) were visible in the eyes undergoing chronic ocular hypertension, whereas no apparent leakage was observed in the vitreous humour of the control eye. These results suggest the compromise of the blood–aqueous or aqueous–vitreous barrier integrity upon chronic ocular hypertension. (Images modified from [11, 225].)
in the ipsilesional eye in the adult age (unpublished data). These results potentiated the non-invasive studies of aqueous flow and dynamic metabolic changes in ocular diseases and neonatal injuries by direct visualization of the untouched living eye, with improved accuracy and reliability as compared to traditional methods using fluorophotometry and post-mortem tracer studies [196].

In ocular diseases such as glaucoma, more evidence has been found suggesting the dissemination of damages in the posterior visual pathway in relation to transsynaptic degeneration [2, 3, 5, 115, 197–203], in addition to early lesions in the anterior visual pathway. Yet the primary sources of injury and their subsequent courses of anterograde, retrograde and transsynaptic neurodegeneration and recovery around injury sites in are still unclear. Our current procedures of $^1$H-MRS and gadolinium-enhanced MRI at high magnetic field strength may provide a sensitive and non-invasive measure of the metabolic processes and mechanisms of ocular diseases and injuries over time. They may also be potentially applicable to examine at high spectral resolutions the subtle alterations in small metabolites in other visual components, including the vitreous and LGN, and to assess the integrity of blood-aqueous, blood-retinal, aqueous-vitreous, blood-nerve and blood-brain barriers along normal and lesioned visual pathways in neonatal, juvenile and adult subjects. The aqueous flow and ciliary flow can also be more reliably quantified with the current global and non-invasive imaging protocols.


In recent years, fMRI has been successfully employed to identify the neurovascular coupling and hemodynamic responses in the normal and degenerated retina [10, 30, 32, 33, 102, 204–206] and in the visual nuclei of the posterior brain [60, 63–65] in adult rodents. Blood-oxygen-level dependent (BOLD) fMRI, blood-flow fMRI and blood-volume fMRI have been shown to differentiate layer-specific responses in the adult rodent retina upon physiological (hypercapnic/hyperoxic) challenges and anesthetic modulations [10, 30, 204, 205]. Recent studies by others [63–65] and by us [57, 59, 61, 207] also demonstrated that BOLD-fMRI can be used to visualize functional activations in the visual cortex [60, 63–65], the subcortical visual nuclei (SC, LGN, pretectum and substantia nigra) [57, 59, 61, 63–65, 207] and the cerebellum [65] in normal rodents upon visual flash illumination, with the SC responding faster than the LGN and substantia nigra in the subcortical structures under anaesthetized conditions [57, 61, 64]. However, the temporal dynamics and interactions among these components during development and plasticity are still
largely unknown. In normal postnatal visual development, our recent findings upon diffuse visual flash illumination suggested the presence of neurovascular couplings in the superficial layers of the rat normal superior colliculus since eye opening at about P14. We also demonstrated its progressive maturation in the amplitude and temporal BOLD responses up till adulthood at P60 [59, 207] (Fig. 6). BOLD-fMRI

![Image](image_url)

**Fig. 6.** (a) Typical T2-weighted images (T2WI) (top row) and BOLD-fMRI cross-correlation (cc) maps overlaid onto T1-weighted images (T1WI) (middle and bottom rows) of the normal rat brains from the day of eyelid opening at postnatal day (P) 14, to P21, P28, and adulthood at P60 (first 4 columns), and the neonatal hypoxic-ischemic (HI) injured brain at P60 (last column) at the level of the superior colliculus (SC). In the HI-injured group at P60, a porencephalic cyst was presented by T2W hyperintensity covering the ipsilesional hemisphere, including the entire primary and secondary visual cortices. Upon diffuse flash light stimulations to left (middle row) or right (bottom row) eye, BOLD-fMRI activations were observed in the contralateral SC in both normally developing and neonatal HI-injured brains (arrows). BOLD-fMRI activations were only occasionally observed in the visual cortex, which might be due to the fact that diffuse light had little effect on rat cortex as shown by 2-deoxyglucose autoradiographic technique [208]. (b) Averaged dynamic profiles of BOLD hemodynamic responses to visual stimulation in the normally developing brains from P14 to P60. Upon visual flash illumination, the normal SC underwent a systematic increase in BOLD signal amplitude with age especially after the third postnatal week. However, no significant difference in BOLD signal increase was found between P14 and P21. These findings implied the presence of neurovascular coupling at the time of eyelid opening, and the progressive development of hemodynamic regulation in the subcortical visual system. (c) Age-dependent changes in BOLD signal increase to visual stimulation in normal rat brains from P14 to P60 and in neonatal HI-injured brains at P60. In the HI-injured group at P60, the BOLD signal increases in both SC remained at the same level as the normal group at P28 though they were significantly lower than the normal group at P60. These observations suggested the residual visual functions on both sides of the subcortical brain, despite the presence of ipsilesional retinal ischemia [154], and damages to the entire ipsilesional visual cortex. (Two-tailed Students’ t-tests between age groups in the left superior colliculus: *p < 0.05, **p < 0.01 and ***p < 0.001; two-tailed Students’ t-tests between age groups in the right superior colliculus: #p < 0.05, ##p < 0.01 and ###p < 0.001.) (Images modified from [59, 207].)
activations were only occasionally observed in the visual cortex under our experimental conditions, which might be due to the fact that diffuse light had little effect on rat cortex as shown by 2-deoxyglucose autoradiographic technique [208]. Recently, we have extended the study to monitor subcortical visual responses in neonatal HI-injury at P7 involving total damage to the left ipsilesional visual cortex and different extents of lesioning in the left SC [59]. In the HI-injured group involving mild lesions to left SC at P60, the BOLD signal increases in both SC remained at the same level as the normal group at P28 though they were significantly lower than the normal group at P60 (Figs. 6(a) and 6(c)) [59]. These observations suggested the residual visual functions on both sides of the subcortical brain, despite the presence of ipsilesional retinal ischemia [154], and damages to the entire ipsilesional visual cortex. Interestingly, in the HI-injured brain involving severe lesions to left SC, our pilot data showed that BOLD activations were found in the SC of the right hemisphere upon right eye stimulation (data not shown) [226]. While infants with brain damages caused by neonatal HI injury often present cerebral visual impairment, a visual deficit associated with unilateral posterior cerebral lesions in the optic radiations and the visual cortex [209–212], increasing evidence suggested a role of the SC to visual recovery after visual cortex damage, a phenomenon called blindsight [155, 213–218]. The approach of BOLD-fMRI detection of SC functions after visual cortex damage may also help understand the mechanisms underlying the functional maturation and reorganization after cerebral visual impairment, which will ultimately lead to improved treatments of neurological disorders involving disruptions in neuronal circuitry along the visual pathway.

6. Conclusion

This review has shown that high-field MRI/MRS, in combination with recently introduced acquisition techniques, allows an in vivo multiparametric approach
to the rodent visual system and consequently, the study of early anatomical, metabolic and functional changes in animal models. In essence, the authors conclude that:

1. MEMRI significantly improves the sensitivity to study segregated fibers in the rodent retinocollicular and retinogeniculate pathways, allowing a submillimetric resolution for localization of lesions and for retinotopic mapping in the subcortical visual system.

2. DTI allows investigations into the microstructural integrity of the visual pathway. The use of FA and directional diffusivities can be successfully applied to distinguish anterograde and retrograde neurodegeneration in the optic nerve and tract after primary or secondary injury to both neonatal and adult visual pathways.

3. $^1$H-MRS allows early detection of metabolic changes in the posterior visual nuclei in response to complete or partial deafferentation of the optic nerve.

4. The use of Gd-DTPA as an exogenous contrast allows the exploration of the integrity of blood-aqueous and aqueous–vitreous barriers in the ocular hypertensive model.

5. Functional MRI based on BOLD contrast allows the detection of hemodynamic responses in the retina and visual brain nuclei during visual stimulation, and, consequently, the study of neurovascular coupling in healthy and damaged brains of both postnatal developing and adult rodents.

The current results suggested the significant values of high-field multiparametric MRI/MRS for uncovering the processes and mechanisms of developmental and pathophysiological changes systematically along both anterior and posterior visual pathways, which may in turn provide early diagnoses and comprehensive therapeutic strategies for promoting functional recovery upon neural diseases and injuries that may lead to partial vision loss. In addition, application of a specific technique could have a beneficial influence on handicaps attributed to others, improving respective sensitivity. This is the case of the relationship between changes in metabolites concentrations in MRS and BOLD effect in fMRI, eventually contributing to a better understanding of the neurovascular coupling [219–224]. Another example is the use of MRI-based tissue segmentation as a reliable approach for absolute metabolic quantification and for quantitative DTI at specifically localized regions [39]. The proposed method using Mn-contrasted acquisition could positively influence this approach. Our current MEMRI, Gd-MRI, DTI and $^1$H-MRS results in the ocular hypertension and optic nerve injury models [11, 28, 29, 36–38, 67, 225] can provide comprehensive indicators of the spatiotemporal dynamics of glaucoma mechanisms in the visual components in a single setting, whereas our Gd-MRI, DTI, IH-MRS and fMRI findings along the visual pathways in the neonatal HI-injured brains [35, 56, 59, 169, 207, 226] can also provide complementary information on the structural
metabolic and function integrity of the visual system after early lesions to the retina and the visual cortex. The studies reported here opened up several potential future research directions into the organizations of the visual pathway in the fields of neuro-ophthalmology, developmental biology, systems neuroscience, and pathophysiology, and laid the foundation in human studies.

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