Published in final edited form as:

Int J Dev Neurosci. 2008 February ; 26(1): 13–25.

Application of Magnetic Resonance Imaging in Animal Models of Perinatal Hypoxic-Ischemic Cerebral Injury

Gregory A Lodygensky, MD¹, Terrie E Inder, MD PhD^{1,2,3}, and Jeffrey J Neil, MD PhD^{1,2,3}

¹Departments of Pediatrics, Washington University School of Medicine, St. Louis, Missouri, USA

²Departments of Neurology, Washington University School of Medicine, St. Louis, Missouri, USA

³Departments of Radiology, Washington University School of Medicine, St. Louis, Missouri, USA

Introduction

This article will outline the current utility of magnetic resonance imaging (MRI) in relation to animal models of perinatal brain injury. This will be presented in two parts. In the first part the technical aspects of small animal imaging are reviewed. In the second, we discuss the application of specific MR methods to animal models of perinatal hypoxic-ischemic injury. The most common animal model reviewed is that of hypoxic-ischemic injury as described by Rice et al. (Rice et al., 1981) consisting in the occlusion of a common carotid artery followed by a variable period of hypoxia.

Technical Aspects

Image spatial resolution

Obtaining images of sufficiently high spatial resolution from small animals is both challenging and critical, especially in rodent pups. The brain of a 7-day-old rat pup has a volume ~0.45 ml, whereas the volume of a mouse brain at the same age has a volume of ~0.2 ml. These volumes are 1200 and 2700 times smaller, respectively, than a human term infant brain. Typical high resolution MRI of a human neonate has a spatial resolution of $1 \times 1 \times 1 \text{ mm}^3$. In contrast, the average resolution for rodent imaging ranges from $0.078 \times 0.156 \times 1 \text{ mm}^3$ to $0.234 \times 0.234 \times 1 \text{ mm}^3$. Even this remarkably high resolution is an order of magnitude lower, relative to brain volume, than that achievable in human infants. The undesirable effects of low spatial resolution are related mainly to partial volume averaging. This effect consists of inclusion of various tissue types, such as white matter, grey matter, and/or CSF, in a single image element or voxel. This leads to blurring of the image, and in some cases, small structures may not be detectable at all. The primary determinant of achievable spatial resolution is signal-to-noise ratio (SNR), which scales directly with voxel volume. Thus, increasing spatial resolution from $2 \times 2 \times 2 \text{ mm}^3$ to $1 \times 1 \times 1 \text{ mm}^3$ is associated with an 8-fold reduction in SNR. Interestingly, image contrast can sometimes be better at higher spatial resolution, despite the reduction in SNR, due to the associated reduction in partial volume averaging effects with less image blurring.

There is a variety of approaches to increasing SNR. One method is to signal average by repeating the image acquisition several times in succession and averaging the signals. SNR increases as the square root of the number of averages, but this approach can significantly lengthen imaging time. In the example above, restoring SNR after increasing spatial resolution

from $2 \times 2 \times 2 \text{ mm}^3$ to $1 \times 1 \times 1 \text{ mm}^3$ would require a 64-fold increase in imaging time. Another means of increasing SNR is the use of radiofrequency (RF) imaging coils that are better matched to the size of the subject and provide better “filling factor.” The use of MRI systems with higher field strength also provides an increase in SNR. Practically speaking, SNR scales roughly proportionally with magnetic field strength.

Motion artifact

Animal motion is a common artifact, which can be very detrimental to image quality, particularly at long imaging times, which are required for the high resolution imaging in the small immature brain. It typically arises from respiratory motion, spontaneous movement or from vibration of the MR scanner caused by torque on the magnetic field gradient set during image acquisition. Anesthetizing the animal is usually not sufficient to completely remove animal motion, making it is necessary to employ special restraint devices during imaging. For adult animals, such devices typically include tooth and ear bars. Newborn animals with soft skulls, no teeth, and very small ear canals present a special challenge. We designed an MR compatible holder with lateral bars that can be tightened around the head with a distance equivalent to the measured head diameter and a small front platform for the palate with an adjustable height. Standard T2 weighted images can be fairly forgiving in the case of motion, which is not the case for diffusion imaging where the slightest motion will greatly impair the computation of relative anisotropy.

Field strength

As noted above, higher field strength provides higher SNR, but at a cost. As field strength increases, so does the frequency at which RF coils must operate to obtain the images. RF inhomogeneity becomes a significant issue at higher frequency. In our experience using an 11.74-T scanner operating at 500 MHz, dielectric effects cause significant image signal inhomogeneity for samples larger than the size of an adult rat brain. While there are means of partially compensating for these effects, such as the use of multi-element coils, they add an additional level of complexity to image acquisition and analysis. Magnetic susceptibility effects are also more severe at high field strength. These effects are related to inhomogeneity in the local magnetic field arising at boundaries between structures of differing magnetic susceptibility, such as the air-tissue interface of cranial sinuses. This effect causes significant distortions in images obtained with fast, gradient echo imaging methods such as echo-planar imaging. On the other hand, gradient effects also form the basis for signal intensity changes associated with brain activation in functional MRI. Thus, contrast in functional MRI studies is better at higher magnetic field strength.

Animal monitoring

MR-compatible monitoring devices are available from a few companies (Small Animal Instrument Inc., Biopac Systems Inc., FISO Technologies). Such systems allow monitoring of respiratory rate, body temperature, heart rate, EKG and blood pressure. In addition to providing information on the health of the animal under study, these data can be used to gate MRI acquisition to respiration and/or heartbeat, which may further reduce the effects of animal motion. Also, feedback circuits can be employed in conjunction with body temperature measurements and heating devices to maintain animal body temperature within a set range. Maintenance of body temperature is particularly crucial in small rodent pups that have a high body surface-to-volume ratio and are prone to rapid loss of body heat with associated hypothermia.

Clinical Applications in the Models of Cerebral Injury

The model utilized - Animal age and model employed

The age of the animal used for the experiment is an important aspect of the model, as the developing brain goes through differing stages of tissue vulnerability – both in the extent and the pattern of tissue injury. A difference of just one day has been shown to have a profound impact on the severity of the injury as assessed by spectroscopy and histology (Malisza et al., 1999).

In relation to the distribution of the tissue injury, hypoxia on pregnant Sprague-Dawley rats will cause a predominant white matter injury with white matter cell death, increase number of macrophagic and microglial cells resulting in a gliotic scar with delayed myelination. Early imaging of these pups showed a T2 signal increase and a T1 signal decrease of the white matter with an associated increased ADC (Baud et al., 2004). In contrast, the same insult in the same rat model at 7-day-old will produce a predominant severe cortical insult (Nedelcu et al., 1999), with MRI and especially diffusion alterations being easily detected.

The duration of hypoxia will also have a great impact on the size and localization of injury. Qiao et al. (Qiao et al., 2004) found that 45 minutes of hypoxia following unilateral carotid artery ligation in 7-day-old Wistar rats produced predominant white matter injury as compared to 90 minutes of hypoxia which produced a cortical and white matter injury.

An important aspect in the consideration of the model and the application of MRI is the equivalency of the animal age to human gestation. Most of the studies referred to in this review article are performed on rodent day of life 7, which is commonly accepted to be equivalent to the human term newborn, though there are publications offering a wide variation in the calculation of the equivalent age between human and rodent (Hagberg et al., 1997, Clancy et al., 2001, Clancy et al., 2007). Craig et al. have compared oligodendroglial maturation in both human and rodents. They showed that a P2 mouse or rat had an age equivalent to preterm infants at high risk of periventricular leukomalacia (Back et al., 2001, Craig et al., 2003). Hagberg et al. compared neurochemical and metabolic data, EEG pattern, synapse formation, cell growth and proliferation, patency of the blood-brain barrier. Taking all those elements into consideration, they concluded that a 7 to 14-day-old rat brain is equivalent to a human brain at term (Hagberg et al., 1997).

Application of MR Imaging

T1 imaging: In conventional MR imaging, the signal detected arises from ^1H in $^1\text{H}_2\text{O}$. For T1-weighted images, tissue contrast based on the T1 relaxation properties of the ^1H atoms. The T1 relaxation time constant for $^1\text{H}_2\text{O}$ in CSF, for example, is greater than that for $^1\text{H}_2\text{O}$ in white matter due to differences in chemical environment. As a result, CSF appears dark relative to white matter on T1-weighted images. It is important to remember that both the T1 and T2 relaxation time constants of tissue water are significantly different between newborn and adult animals. Further, they change rapidly during early development. Consequently, grey/white contrast for newborn brain is actually reversed from that of adult brain. In humans, grey/white contrast takes on the adult form during the first year of life. A second effect of rapid developmental changes in T1 and T2 relaxation time constants is that the optimum MR image acquisition parameters, those at which tissue contrast-to-noise ratio is greatest, varies during development. In newborn rodents, the optimum parameters vary every few days during the first week of life. Thus, they must be carefully determined for the animal of interest for the age at which the imaging will be done. This is usually achieved through measurement of tissue T1 and T2 relaxation time constants and calculation of the best acquisition parameters (Haacke, 1999).

As noted above, it is desirable to obtain small animal images at high magnetic field strength for improvements in SNR. However, T1 varies with magnetic field strength, and image contrast in T1-weighted images suffers at higher field strength as the differences in T1 between water in white and grey matter are smaller. One solution to regain T1 contrast at high field was published in 1993 by Ugurbil et al. (Ugurbil et al., 1993). They designed an MR image acquisition sequence called MDEFT (Modified Driven Equilibrium Fourier Transform) that provides a broader range of acquisition parameters over which optimized image contrast can be obtained (Fig. 1).

Manganese enhanced MRI (MEMRI) also can be employed to enhance T1 contrast. $MgCl_2$ is typically administered intra-peritoneally, where it is absorbed into the vascular system and passes through the blood brain barrier to enter the brain. Contrast in the hippocampus, pituitary gland, olfactory bulb and cerebellum is enhanced. There are no hypoxic-ischemic studies in rat pups to date using MEMRI or MDEFT, but there is a very elegant description of enhancement of cerebellar cortex in mice pups following manganese administration. (Wadghiri et al., 2004) (Fig 2).

T2 imaging: T2 relaxation time constants are less field dependent than T1, though there is a tendency for T2 values to be lower at higher field (de Graaf et al., 2006). Similarly to human newborns, immature rodent have an inverse grey-white matter contrast (Fig. 3). T2 relaxation time constants change quickly during maturation, with a normal contrast already present by three weeks of age (Fig. 3). It is therefore necessary to optimize image acquisition parameters to the model being used. Even with this optimization, it is often difficult to optimize acquisition parameters for both grey/white contrast and normal/injured tissue contrast (Fig. 4). As a result, the acquisition parameters used are often a compromise between optimization of these two contrasts.

T2 changes following hypoxia-ischemia

An increase in the local tissue water T2 relaxation time constant, which causes areas of injury to appear bright on T2-weighted images, is often detectable in a matter of hours following ischemia in the immature rodent brain (Fig 5 and Fig 6) (Table 2) (Albensi et al., 1998, Aden et al., 2002). This contrasts greatly with changes observable on T2-weighted images from human neonates, which are usually evident only after 7 days following a perinatal ischemic insult (Rutherford et al., 2006).

It is important to appreciate that the lesion size as well as the T2 values change over the first 7 days following an ischemic insult in the immature brain (Aden et al., 2002). This is presented in Figure 6 where both the lesion size as well as the T2 values rise rapidly from 6–24 hours following an ischemic insult. Thus, delaying MR imaging until 24 hours after the injury may increase the visibility of lesions, particularly if the injury is more subtle or diffuse in nature.

The correlation of lesion size from MR T2 weighted imaging with histology has been studied. In newborn rodents, the area of T2 hyperintensity measured 3 and 6 days after injury has been shown to correlate with infarct size evaluated by histology (Albensi et al., 1998, Stieg et al., 1999). Aden et al. showed that T2 changes as early as 3 to 6 hours post injury correlate well with the histopathological score at 4 weeks (Aden et al., 2002). Antier et al. (Antier et al., 1999) used both TTC staining and T2-weighted imaging to evaluate injury size in 7-day-old rat pups subjected to unilateral carotid ligation and hypoxia. Their data showed good qualitative agreement between TTC staining at 72 hours and T2-weighted imaging at 5 weeks. Thus, lesion size on MR imaging early appears to be well defined in relation to histology.

Diffusion

In diffusion-based MR imaging, image contrast is based on the microscopic displacements of water molecules rather than the T1 or T2 relaxation properties of water in different chemical environments. The displacements measured are remarkably small, on the order of 10 μm , and are affected by cell membranes and constituents in a way that can be applied to learn about local tissue microstructure.

The basic measure of water displacements is the apparent diffusion coefficient, or ADC. ADC has units of mm^2/s and varies during development. In general, ADC values decrease as the brain matures and water content decreases. ADC values also decrease within minutes of brain injury, providing a robust and widely used means for the early detection of brain injury. It is important to note that water ADC values decrease with both reversible and irreversible injury. For example, ADC decreases in response to administration NMDA in a mouse pup, but returns to normal when the excitotoxic action of NMDA is blocked pharmacologically (Dijkhuizen et al., 1996). Water ADC values are also anisotropic, meaning that they are not equivalent in all directions in space. In white matter, for example, water displacements parallel to myelinated fibers are greater than perpendicular to them. This is likely because water motion perpendicular to fibers is hindered by layers of myelin. To move in this direction, water molecules must pass through or around myelin layers. Similarly, water ADC values are anisotropic in immature cerebral cortex. In this case, displacements are greatest in a radial direction, reflecting the radial organization of the apical dendrites of pyramidal cells and radial glia. As the brain matures, anisotropy increases in white matter and decreases in cortex.

In practice, diffusion images are usually acquired using “diffusion tensor imaging” or DTI. With this approach, a series of diffusion measurements are made for each imaging slice. For each measurement, diffusion is evaluated along a different spatial orientation. These measurements are then combined to create a 3-dimensional representation of water displacements for each image element or voxel. This representation is described mathematically as a 3×3 matrix or tensor – hence the name. For areas of high anisotropy, the shapes tend to be cigar-like. In white matter, the long axis of the cigar is oriented parallel to the direction of the axons. In immature cerebral cortex, the long axis is oriented radially or perpendicular to the cortical surface. For areas in which diffusion is isotropic, such as in mature cortex or CSF, the shapes are spherical. For area of high anisotropy, it is common to show the long axis of the ellipsoid as a short line or “whisker” (Fig. 9).

ADC for detection of injury

The change in ADC values following brain injury is a dynamic process. For both newborn infants and rodent pups (Fig 7 and Fig 8), ADC values tend to decrease quickly following injury and then return to normal after a period of time. This return to normal is known as “pseudonormalization.” Following pseudonormalization, ADC values increase to higher than normal and stay elevated as cell debris is cleared away by macrophages. Thus, as with conventional MR imaging, the sensitivity of DTI to injury varies with time after injury. As shown on figure 8, pseudonormalization takes place more rapidly in animal models of perinatal ischemia than in human newborns (Fig. 7).

Application of diffusion methods to animal models of perinatal injury has been fairly limited. Tuor et al. (Tuor et al., 1998) reported a reduction of ADC values in neonatal rats following hypoxic/ischemic injury. In contrast to humans, ADC and T2 changes occurred in parallel (Fig. 6). Sizonenko et al. (Sizonenko et al., 2007) evaluated the effects of unilateral carotid ligation and exposure to hypoxia on the cerebral cortex of newborn rats. They showed a loss of cortical anisotropy in association with injury (Fig. 7). Drobyshevsky et al. (Drobyshevsky et al., 2007) in a model of rabbit uterine ischemia showed a correlation between low fractional

anisotropy in corpus callosum, internal capsule and corona radiata in hypertonic kits which coincided in immunostaining with a loss of phosphorylated neurofilaments.

Spectroscopy

Spectroscopy offers information about metabolite levels, which in turn reflect the metabolic state of the brain. The greatest difference between spectroscopy and imaging lies in the relative concentrations of the molecules under study. The concentration of ^1H in brain water is on the order of 100 M, whereas the concentrations of metabolites such as lactate are in the tens of mM range. As a result, a typical “voxel” for spectroscopy is much larger than that for imaging, often 2 cm on a side. Spectroscopy benefits markedly from high field strength with better SNR and improved spectral dispersion. Better spectral dispersion provides better separation of resonance peaks in MR spectra and allows for more accurate quantitation of resonance amplitudes, from which metabolite concentrations can be calculated. One potential challenge for spectroscopy at high field is that it can be difficult to eliminate magnetic field distortions related to magnetic susceptibility effects. There are automatic shimming techniques like FASTMAP (Gruetter, 1993), which help greatly in obtaining a homogeneous field. The line-width of the water peak is used to assess the quality of the shim and should be reported. It is also worth noting that a variety of nuclei are MR detectable in addition to ^1H , including ^{31}P , ^{23}Na , ^{19}F and ^{133}Cs .

Proton spectroscopy

There are several metabolites that can be detected by proton spectroscopy. Changes in N-acetylaspartate (NAA) and lactate following a hypoxic-ischemic insult have been analyzed in detail. NAA is considered as a marker of neuronal integrity, whereas lactate is a marker of anaerobic metabolism (Malisza et al., 1999). Following hypoxic-ischemic injury, spectroscopy typically shows a decrease of NAA and an increase of lactate. Malisza et al. (Malisza et al., 1999) showed in the rat pup that increased lactate concentration can be detected during hypoxia-ischemia and remains high during 48 hours. NAA has a different profile. It initially decreases during hypoxia-ischemia, recovers in the following hours and decreases again at 24h and 48h. Vial et al. (Vial et al., 2004) in a piglet model found that lactate remains elevated over a longer period with lactate/NAA lactate/choline and lactate creatine ratio increased up to 7 days after injury.

Proton spectroscopy can also be used to measure brain temperature as the water resonance frequency is temperature dependent. Since the NAA resonance frequency is not temperature dependent, the chemical shift between the water and NAA resonances is usually used to measure temperature (Corbett et al., 1995).

^{31}P spectroscopy

^{31}P spectroscopy measures phosphorylated metabolites. ^{31}P spectroscopy in the rat pup and in the piglet has been used to quantify acute changes of brain energy metabolism following hypoxic/ischemic injury. In a piglet model of hypoxia, high energy metabolite levels are monitored during hypoxia via ^{31}P spectroscopy in an effort to maximize the reproducibility of the model. The duration of hypoxia is guided by nucleoside triphosphate and phosphocreatine levels (Thornton et al., 1997, Nedelcu et al., 1999, Vial et al., 2004). ^{31}P spectroscopy during hypoxic/ischemic injury shows a rapid reduction of the ratio of phosphocreatine to inorganic phosphate that normalizes in a few hours and is followed by a more prolonged reduction of the ratio (Nedelcu et al., 1999) that correspond to secondary energy failure (Fig. 10).

Peeters-Scholte et al (Peeters-Scholte et al., 2003) applied both T2-weighted imaging and ^{31}P spectroscopy to a newborn piglet model of hypoxia ischemia. They found significant

improvement of MR parameters following allopurinol or deferoxamine, *i.e.* no decrease the phosphocreatine/inorganic phosphate ratio and less T2 increase after injury (Table 3). There was no improvement in their histopathology evaluation with no difference in caspase-3 activity, TUNEL labeling or light microscopy. This suggests that MR methods may be more sensitive than histological approaches under some circumstances.

Cell tracking

Cell tracking can now be undertaken with MRI by labeling the cells with ultrasmall superparamagnetic particles of iron oxide (USPIO) particles. USPIO causes distortions of the local magnetic field that cause signal dropout in MR images obtained with T2* weighting. This allows one to detect the cells. While there are no studies so far in neonatal models of hypoxic/ischemic injury, Wiart et al. have shown the distribution and evolution of inflammation of adult mice with stroke (Wiart et al., 2007), (Fig. 11). USPIO can also be incorporated in selected cell populations such as stem cells, thus broadening the utility of the method.

Functional MRI

Functional MRI (fMRI) is possible in small animals although quite challenging. It relies on regional changes of cerebral blood flow occurring in concurrent changes of neuronal activity. Signal intensity varies in relation to the local concentration of paramagnetic deoxyhemoglobin. fMRI requires higher resolution for immature brains. Shen et al. (Shen et al., 2005) have shown loss of activation in somatosensory cortex after forepaw stimulation in a model of ischemic brain injury in adult rat at 4.7 T. Considerable work will be required to adapt those sequences to the developing rodent brain, as a spatial resolution 5 to 10 times higher than that used by Shen et al. would be required to identify cortical structures with precision. However, it is likely this technical hurdle will eventually be overcome.

It is also worth mentioning a form of functional MRI – functional connectivity or fcMRI – that has strong potential for application in animals and young children. For fcMRI studies, the subject does not perform a task during scanning, and data are collected with the subject at rest. The “noise” in fMRI data is of physiologic origin, and this aspect of the “resting” signal is analyzed. Correlations are sought between low frequency (< 0.1 Hz) signal fluctuations in the fMRI signal from a seed region (e.g., motor cortex) and other brain regions. For regions that are functionally connected, the signal fluctuations are temporally correlated (Biswal et al., 1995). When the signal in the seed area shows a positive fluctuation, the signal in the connected area does as well. Further, it has been shown that the fcMRI signal fluctuations in regions that undergo task-related deactivation are temporally anticorrelated with those of regions that undergo task-related activation (Fox et al., 2005). When the signal in the seed area shows a positive fluctuation, the signal in the connected area shows a negative fluctuation. In this fashion, fcMRI shows both correlations within networks and anticorrelations between networks. In addition, functional connectivity MRI effects persist under a variety of conditions. During the performance of low-level tasks, correlations corresponding to networks can be detected during both rest and stimulus epochs (Arfanakis et al., 2000; Greicius M.D., Menon V., 2004). The effects are also present, becoming more prominent, under midazolam sedation (Kiviniemi et al., 2005). To date, this method has been applied primarily in human studies, but will likely be adapted to animal studies in the near future.

Conclusion

Imaging of neonatal animal models following hypoxic-ischemic injury remains a challenge, but there are already an extensive number of studies in this domain. Recent access to very high magnetic field has improved image quality and the potential for defining the changes occurring after a hypoxic-ischemic insult. Moreover MRI is a fast developing field with new approaches

being implemented that may also help in better defining injury and regeneration. Overall, diffusion studies (particularly ADC measurements) are effective for showing injury in the acute stage (within hours of injury). At later time points, conventional T1- and T2-weighted imaging shows the extent of injury and subsequent atrophy. Spectroscopy can be employed for evaluating changes in metabolic state, particularly energy status with ^{31}P spectroscopy. Microstructural changes can be evaluated through measurement of diffusion anisotropy. In the future, changes in neural circuitry may also be approachable through fcMRI, and the presence of inflammatory or stem cells may be detected with T2*-weighted imaging. MR imaging also provides an important bridge between human clinical studies, for which developmental outcomes are often available, and animal studies, for which histology data are often available. It is likely that MRI will play a pivotal role in the evaluation of neuroprotective agents in both animal models and human trials.

REFERENCES

- Aden U, Dahlberg V, Fredholm BB, Lai LJ, Chen Z, Bjelke B. MRI evaluation and functional assessment of brain injury after hypoxic ischemia in neonatal mice. *Stroke* 2002;33:1405–1410. [PubMed: 11988622]
- Albensi BC, Schweizer MP, Rarick TM, Filloux F. Magnetic resonance imaging of hypoxic-ischemic brain injury in the neonatal rat. *Invest Radiol* 1998;33:377–385. [PubMed: 9659589]
- Albensi BC, Schweizer MP, Rarick TM, Filloux F. Unilateral hypoxic-ischemic injury in the neonatal rat brain evaluated by in vivo MRI. Correlation with histopathology and neuroprotection by MK-801. *Invest Radiol* 1999;34:249–261. [PubMed: 10196716]
- Antier D, Franconi F, Sannajust F. Idazoxan does not prevent but worsens focal hypoxic-ischemic brain damage in neonatal Wistar rats. *J Neurosci Res* 1999;58:690–696. [PubMed: 10561697]
- Arfanakis K, Cordes D, Haughton VM, Moritz CH, Quigley MA, Meyerand ME. Combining independent component analysis and correlation analysis to probe interregional connectivity in fMRI task activation datasets. *Magn Reson Imaging* 2000;18:921–930. [PubMed: 11121694]
- Ashwal S, Tone B, Tian HR, Chong S, Obenaus A. Comparison of two neonatal ischemic injury models using magnetic resonance imaging. *Pediatr Res* 2007;61:9–14. [PubMed: 17211133]
- Back SA, Luo NL, Borenstein NS, Levine JM, Volpe JJ, Kinney HC. Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. *J Neurosci* 2001;21:1302–1312. [PubMed: 11160401]
- Baud O, Daire JL, Dalmaz Y, Fontaine RH, Krueger RC, Sebag G, Evrard P, Gressens P, Verney C. Gestational hypoxia induces white matter damage in neonatal rats: a new model of periventricular leukomalacia. *Brain Pathol* 2004;14:1–10. [PubMed: 14997932]
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* 1995;34:537–541. [PubMed: 8524021]
- Chang YS, Mu D, Wendland M, Sheldon RA, Vexler ZS, McQuillen PS, Ferriero DM. Erythropoietin improves functional and histological outcome in neonatal stroke. *Pediatr Res* 2005;58:106–111. [PubMed: 15879287]
- Clancy B, Darlington RB, Finlay BL. Translating developmental time across mammalian species. *Neuroscience* 2001;105:7–17. [PubMed: 11483296]
- Clancy B, Kersh B, Hyde J, Darlington RB, Anand KJ, Finlay BL. Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics* 2007;5:79–94. [PubMed: 17426354]
- Corbett RJ, Laptok AR, Tollefsbol G, Kim B. Validation of a noninvasive method to measure brain temperature in vivo using ^1H NMR spectroscopy. *J Neurochem* 1995;64:1224–1230. [PubMed: 7861155]
- Craig A, Ling Luo N, Beardsley DJ, Wingate-Pearse N, Walker DW, Hohimer AR, Back SA. Quantitative analysis of perinatal rodent oligodendrocyte lineage progression and its correlation with human. *Exp Neurol* 2003;181:231–240. [PubMed: 12781996]

- D'Arceuil HE, Crespigny AJ, Rother J, Moseley M, Rhine W. Serial magnetic resonance diffusion and hemodynamic imaging in a neonatal rabbit model of hypoxic-ischemic encephalopathy. *NMR Biomed* 1999;12:505–514. [PubMed: 10668043]
- de Graaf RA, Brown PB, McIntyre S, Nixon TW, Behar KL, Rothman DL. High magnetic field water and metabolite proton T1 and T2 relaxation in rat brain in vivo. *Magn Reson Med* 2006;56:386–394. [PubMed: 16767752]
- Derugin N, Wendland M, Muramatsu K, Roberts TP, Gregory G, Ferriero DM, Vexler ZS. Evolution of brain injury after transient middle cerebral artery occlusion in neonatal rats. *Stroke* 2000;31:1752–1761. [PubMed: 10884483]
- Dijkhuizen RM, van Lookeren Campagne M, Niendorf T, Dreher W, van der Toorn A, Hoehn-Berlage M, Verheul HB, Tulleken CA, Leibfritz D, Hossmann KA, Nicolay K. Status of the neonatal rat brain after NMDA-induced excitotoxic injury as measured by MRI, MRS and metabolic imaging. *NMR Biomed* 1996;9:84–92. [PubMed: 8887373]
- Drobyshevsky A, Derrick M, Wyrwicz AM, Ji X, Englof I, Ullman LM, Zelaya ME, Northington FJ, Tan S. White matter injury correlates with hypertonia in an animal model of cerebral palsy. *J Cereb Blood Flow Metab* 2007;27:270–281. [PubMed: 16736047]
- Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A* 2005;102:9673–9678. [PubMed: 15976020]
- Grate LL, Golden JA, Hoopes PJ, Hunter JV, Duhaime AC. Traumatic brain injury in piglets of different ages: techniques for lesion analysis using histology and magnetic resonance imaging. *J Neurosci Methods* 2003;123:201–206. [PubMed: 12606068]
- Greicius MD, Menon V. Default-mode activity during a passive sensory task: uncoupled from deactivation but impacting activation. *J Cogn Neurosci* 2004;16:1484–1492. [PubMed: 15601513]
- Gruetter R. Automatic, localized in vivo adjustment of all first- and second-order shim coils. *Magn Reson Med* 1993;29:804–811. [PubMed: 8350724]
- Haacke, EM. *Magnetic resonance imaging : physical principles and sequence design*. New York: Wiley; 1999.
- Hagberg H, Bona E, Gilland E, Puka-Sundvall M. Hypoxia-ischaemia model in the 7-day-old rat: possibilities and shortcomings. *Acta Paediatr Suppl* 1997;422:85–88. [PubMed: 9298801]
- Kiviniemi VJ, Haanpaa H, Kantola JH, Jauhiainen J, Vainionpaa V, Alahuhta S, Tervonen O. Midazolam sedation increases fluctuation and synchrony of the resting brain BOLD signal. *Magn Reson Imaging* 2005;23:531–537. [PubMed: 15919598]
- Malisza KL, Kozlowski P, Ning G, Bascaramurty S, Tuor UI. Metabolite changes in neonatal rat brain during and after cerebral hypoxia-ischemia: a magnetic resonance spectroscopic imaging study. *NMR Biomed* 1999;12:31–38. [PubMed: 10195327]
- McKinstry RC, Miller JH, Snyder AZ, Mathur A, Schefft GL, Almli CR, Shimony JS, Shiran SI, Neil JJ. A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. *Neurology* 2002;59:824–833. [PubMed: 12297561]
- Meng S, Qiao M, Foniok T, Tuor UI. White matter damage precedes that in gray matter despite similar magnetic resonance imaging changes following cerebral hypoxia-ischemia in neonatal rats. *Exp Brain Res* 2005;166:56–60. [PubMed: 15968456]
- Nedelcu J, Klein MA, Aguzzi A, Boesiger P, Martin E. Biphasic edema after hypoxic-ischemic brain injury in neonatal rats reflects early neuronal and late glial damage. *Pediatr Res* 1999;46:297–304. [PubMed: 10473044]
- Niblock MM, Luce CJ, Belliveau RA, Paterson DS, Kelly ML, Sleeper LA, Filiano JJ, Kinney HC. Comparative anatomical assessment of the piglet as a model for the developing human medullary serotonergic system. *Brain Res Brain Res Rev* 2005;50:169–183. [PubMed: 16043226]
- Peeters-Scholte C, Braun K, Koster J, Kops N, Blomgren K, Buonocore G, van Buul-Offers S, Hagberg H, Nicolay K, van Bel F, Groenendaal F. Effects of allopurinol and deferoxamine on reperfusion injury of the brain in newborn piglets after neonatal hypoxia-ischemia. *Pediatr Res* 2003;54:516–522. [PubMed: 12815112]

- Qiao M, Meng S, Scobie K, Foniok T, Tuor UI. Magnetic resonance imaging of differential gray versus white matter injury following a mild or moderate hypoxic-ischemic insult in neonatal rats. *Neurosci Lett* 2004;368:332–336. [PubMed: 15364422]
- Rice JE 3rd, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic-ischemic brain damage in the rat. *Ann Neurol* 1981;9:131–141. [PubMed: 7235629]
- Roohey T, Raju TN, Moustogiannis AN. Animal models for the study of perinatal hypoxic-ischemic encephalopathy: a critical analysis. *Early Hum Dev* 1997;47:115–146. [PubMed: 9039963]
- Rutherford M, Srinivasan L, Dyet L, Ward P, Allsop J, Counsell S, Cowan F. Magnetic resonance imaging in perinatal brain injury: clinical presentation, lesions and outcome. *Pediatr Radiol* 2006;36:582–592. [PubMed: 16770663]
- Shen Q, Ren H, Cheng H, Fisher M, Duong TQ. Functional, perfusion and diffusion MRI of acute focal ischemic brain injury. *J Cereb Blood Flow Metab* 2005;25:1265–1279. [PubMed: 15858531]
- Sizonenko SV, Camm EJ, Garbow JR, Maier SE, Inder TE, Williams CE, Neil JJ, Huppi PS. Developmental Changes and Injury Induced Disruption of the Radial Organization of the Cortex in the Immature Rat Brain Revealed by In Vivo Diffusion Tensor MRI. *Cereb Cortex*. 2007
- Stieg PE, Sathi S, Warach S, Le DA, Lipton SA. Neuroprotection by the NMDA receptor-associated open-channel blocker memantine in a photothrombotic model of cerebral focal ischemia in neonatal rat. *Eur J Pharmacol* 1999;375:115–120. [PubMed: 10443569]
- Taylor DL, Mehmet H, Cady EB, Edwards AD. Improved neuroprotection with hypothermia delayed by 6 hours following cerebral hypoxia-ischemia in the 14-day-old rat. *Pediatr Res* 2002;51:13–19. [PubMed: 11756634]
- Ten VS, Wu EX, Tang H, Bradley-Moore M, Fedarau MV, Ratner VI, Stark RI, Gingrich JA, Pinsky DJ. Late measures of brain injury after neonatal hypoxia-ischemia in mice. *Stroke* 2004;35:2183–2188. [PubMed: 15272130]
- Thornton JS, Ordidge RJ, Penrice J, Cady EB, Amess PN, Punwani S, Clemence M, Wyatt JS. Anisotropic water diffusion in white and gray matter of the neonatal piglet brain before and after transient hypoxia-ischaemia. *Magn Reson Imaging* 1997;15:433–440. [PubMed: 9223044]
- Tuor UI, Kozlowski P, Del Bigio MR, Ramjiawan B, Su S, Malisza K, Saunders JK. Diffusion- and T2-weighted increases in magnetic resonance images of immature brain during hypoxia-ischemia: transient reversal posthypoxia. *Exp Neurol* 1998;150:321–328. [PubMed: 9527902]
- Ugurbil K, Garwood M, Ellermann J, Hendrich K, Hinke R, Hu X, Kim SG, Menon R, Merkle H, Ogawa S, et al. Imaging at high magnetic fields: initial experiences at 4 T. *Magn Reson Q* 1993;9:259–277. [PubMed: 8274375]
- van Pul C, Jennekens W, Nicolay K, Kopinga K, Wijn PF. Ischemia-induced ADC changes are larger than osmotically-induced ADC changes in a neonatal rat hippocampus model. *Magn Reson Med* 2005;53:348–355. [PubMed: 15678540]
- Vial F, Serriere S, Barantin L, Montharu J, Nadal-Desbarats L, Pourcelot L, Seguin F. A newborn piglet study of moderate hypoxic-ischemic brain injury by 1H-MRS and MRI. *Magn Reson Imaging* 2004;22:457–465. [PubMed: 15120164]
- Wadghiri YZ, Blind JA, Duan X, Moreno C, Yu X, Joyner AL, Turnbull DH. Manganese-enhanced magnetic resonance imaging (MEMRI) of mouse brain development. *NMR Biomed* 2004;17:613–619. [PubMed: 15761950]
- Wagner BP, Nedelcu J, Martin E. Delayed postischemic hypothermia improves long-term behavioral outcome after cerebral hypoxia-ischemia in neonatal rats. *Pediatr Res* 2002;51:354–360. [PubMed: 11861942]
- Wang Y, Cheung PT, Shen GX, Wu EX, Cao G, Bart I, Wong WH, Khong PL. Hypoxic-ischemic brain injury in the neonatal rat model: relationship between lesion size at early MR imaging and irreversible infarction. *AJNR Am J Neuroradiol* 2006;27:51–54. [PubMed: 16418355]
- Wiar M, Davoust N, Pialat JB, Desestret V, Moucharaffie S, Cho TH, Mutin M, Langlois JB, Beuf O, Honnorat J, Nighoghossian N, Berthezene Y. MRI monitoring of neuroinflammation in mouse focal ischemia. *Stroke* 2007;38:131–137. [PubMed: 17122417]

Figure 1.

A: 9 day old healthy rat pup, T1 weighted image with a TR of 0.7 s TE of 0.013 s, and resolution: $140 \times 140 \times 500 \mu\text{m}$ obtained at 11.74 Tesla. B: MDEFT at the same resolution with a TR 2 s of and a TE of 0.015 s and an equivalent TI of 518 ms. Note the presence of contrast between gray and white matter and a better delineation of the hippocampus in figure B in comparison to figure A.

Figure 2. Axial, T1-weighted image of a mouse after an intra-peritoneal administration of manganese (Wadghiri et al., 2004). Note the enhancement of the olfactory bulbs, the hippocampus and cerebellum following manganese administration.

Figure 3.

T2 map of a healthy rat at 7 days (A) and at 20 days of life (B) acquired at 11.74 Tesla. Note the difference of contrast between grey and white matter. At 7 days of life (A) white matter is white and grey matter is gray. At 20 days of life (B) grey matter appears brighter than white matter.

Figure 4.

Eight-day-old C57BL6-J mouse pup 24 h after left common carotid ligation followed by 8% oxygen for 45 min. Images were obtained at 11.74 T, 24 hours after injury. Spatial resolution was $93 \times 93 \mu\text{m}^2$. A, TE of 45 ms to optimize grey/white contrast B, TE of 80 ms to optimize normal/injured tissue contrast. A longer TE will provide lower signal to noise but it will be more sensitive to injury. Surgery performed by Tim West.

Figure 5.

Temporal evolution of T2 of CD1 mice pups in a selected region of the ipsilateral cortex after a common artery ligation followed by oxygen deprivation. con: controls at postnatal age 7 to 8 and 12 days (Aden et al., 2002).

Figure 6.
T2W, T2 map and ADC map of CD1 mice pups after a common artery ligation followed by oxygen deprivation (Aden et al., 2002).

Figure 7. Scatter plot of ADC values in function of time after injury in term infants following perinatal brain injury (McKinstry et al., 2002).

Figure 8. Scatter plot of the ADC in function of time after ligation of the common carotid artery and 90 min of 8% oxygen in 7-day-old rat pup (Nedelcu et al., 1999).

Figure 9. Whisker plot computed from a DTI, showing a disruption of radially organized cortical eigenvectors 24 hours after hypoxia-ischemia in the parietal cortex. B, FJB coloration showing degenerating neurons in the same area (Sizonenko et al., 2007).

Figure 10.

³¹P magnetic resonance spectroscopy on 7-day-old rats following a hypoxicischemic injury. Note the biphasic decrease in the PCr/Pi ratio (Nedelcu et al., 1999).

Figure 11. Gradient echo imaging of an adult mouse 6 hours after occlusion of the middle cerebral artery, 1 hour after USPIO administration and gadolinium. Arrowheads, signal dropout in the perilesional area related to local accumulation of inflammatory cells (Wiart et al., 2007).

Table 1

Maturation age equivalence table

Studies	Specie/ strain	Animal post natal age in days	Human age equivalence	Method of comparison
Roohey et al., 1997	Rat	7 (range from 0–28)	Term newborn	Review article
Roohey et al., 1997	Piglet	3 (range from 0–30)	Term newborn	Review article
Roohey et al., 1997	Fetal sheep	120 (range from 52–150)	Term newborn	Review article
Craig et al., 2003	Rat, Mouse	P2	23–32 weeks of gestation	Quantitative assessment of oligodendrocytes lineage
Clancy et al., 2001	Rat	P7	~22 weeks of gestation for cortical development	Statistical model
Clancy et al., 2007 Clancy et al., 2001	Mouse	P7	~24 weeks of gestation for cortical development	Statistical model
Clancy et al., 2007 Clancy et al., 2001 Clancy et al., 2007	Rabbit	P7	~25 weeks of gestation	Statistical model
Grate et al., 2003 Hagberg et al., 1997	Piglet Rat	Birth 7–14 days 21 days	Birth Term newborn 1 year of age	Set of references Review of growth/proliferation, periventricular germinal matrix, neurochemical data, EEG, synapse formation, patency of the blood-brain barrier
Niblock et al., 2005	Piglet	4 days	1 month	Equivalent postnatal ages for 5-HT development

Table 2

MRI as a marker of injury

Studies	Species/Strain Days of life (DOL)	Surgery	Times points	MRI sequences Magnetic Field (T; Tesla)	MRI findings	Histopathology	Behavioral testing
Tuor et al., 1998	Rats, Wistar, 7 DOL	Carotid artery ligation and 2 hours in 8% oxygen	Before hypoxia and first 2 hours post hypoxia then at 24, 48 and 72 hours after injury At 3 and 6 DOL	T2W, DWI, T2 maps, 9.4 T	DWI and T2W intensity changes following surgery	Hematoxylin and eosin 24–72h after surgery. Correlation of injury with MR images.	
Sizonenko et al., 2007	Rats, Wistar, 3 DOL	Carotid ligation and oxygen 6% for 30 min		DTI, 4.7 T	Reduction of cortical ADC and FA following injury	Hematoxylin and eosin, Fluoro-Jade B (FJB), Nestin, GFAP, MAP2, showing neuronal death, radial glial disruption and astrogliosis.	
Baud et al., 2004	Pregnant Rats, Sprague-Dawley	Hypoxia during gestational day 5 to 19	0, 3, 7, 14, 21 DOL	DWI, T1W, T2W 1.5 T	Evolution of ADC in correlation with histopathological markers. T1 hypointensity and T2 hyperintensity in the white matter after delivery	Nissl stain, Glut1, GFAP, S100B, OX42, Tomato lectin, O4, MBP, RT97, PSA-NCAM, TUNEL and Western blotting, in situ hybridization, DNA fragmentation analysis. Histopathology reveals the presence of white matter cysts, increased lipid peroxidation, white matter cell death, increase activated macrophage counts, delayed myelination.	
Wang et al., 2006	Rat, Sprague-Dawley 7 DOL	Carotid artery ligation and 2.5 hours in 8% oxygen	1.5h, 4 days after injury	DWI, T2W, 1.5 T	Infarct volume measured on the initial ADC map in correlation with the final infarct volume	Triphenyltetrazolium chloride at 10 days post-HI. Correlation of injury with DWI images at 1–2 hours post insult	
van Pul et al., 2005	Rats 8–12 DOL	Dissected hippocampus and perfused	Consecutive scans of 45 min started within the 1 hour for 6–8 hours	DTI, 4.7 T	ADC changes in relation to changes of osmolality, glucose and oxygen deprivation	Calcein-AM and propidium iodide for cell viability at the end of the MRI	
Meng et al., 2005	Rats, Wistar, 7 DOL	Carotid artery ligation and 1.5 hour in 8% oxygen	10min, 1 and 24 hours after injury	DWI, T2 maps, 9.4 T	Comparison of ADC and T2 early changes between grey and white matter	Hematoxylin and eosin, TUNEL labeling at 0.1h or 24h after surgery showing the kinetic of injury with initially selective white matter injury.	
Malisz et al., 1999	Rats, Wistar, 6 and 7 DOL	Carotid artery ligation and 2 hours in 8% oxygen	0, first hour, 24 and 48 hours after injury	Spectroscopy T2W, DWI 9.4 T	Lactate and N-acetylaspartate (NAA) changes in response to irreversible injury	Hematoxylin and eosin 48h after surgery	

Studies	Species/Strain Days of life (DOL)	Surgery	Times points	MRI sequences Magnetic Field (T: Tesla)	MRI findings	Histopathology	Behavioral testing
Albensi et al., 1998	Rats, Wistar, 7 DOL	Bilateral carotid artery ligation and 45 min in 9% oxygen	1–6, 24, 48, 72 hours following injury	T2W 2 T	Temporal evolution of T2W following injury with in correlation to the size of injury assessed by histopathology	Hematoxylin and eosin 9– 10 days of life	
Derugin et al., 2000	Rat, Sprague-Dawley 7 DOL	Occlusion of the middle cerebral artery	Before, 20 min., 2.5 hours after injury, and 24 hours after injury	DWI, perfusion imaging 2 T	Decreased ADC in the territory of the infarct in correlation with the infarct volume assessed by histology	Triphenyltetrazolium chloride at 24h, cresyl violet, GFAP, ED-1, MAP2 at 4, 8 and 24h 72h.	
Nedelcu et al., 1999	Rat, Sprague-Dawley 7 DOL	Carotid artery ligation and 1.5 hour in 8% oxygen	0, 2, 12, 18, 24, 32, 38 and 52 hours and 5 days after injury 0.3, 6, 13, 20, 24, 28, 32, 38, 52, 80 hours for ³¹ P spectroscopy	DWI, ³¹ P T2W, ³¹ P spectroscopy 2 T	Temporal evolution of the ratio of phosphocreatine to inorganic phosphate and of the ADC	Hematoxylin and eosin, GFAP and TUNEL at 0, 2, 8, 15, 24, 52 hours and 5 days	
Qiao et al., 2004	Rats, Wistar, 7 DOL	Carotid artery ligation and 45– 90 min in 8% oxygen	24 hours after injury	T2 maps, T1 maps 9.4 T	Increase in T1 and T2 proportional to the severity of injury with cortical involvement only in moderate hypoxic/ischemic insult	Hematoxylin and eosin	
Ashwal et al., 2007	Spontaneously hypertensive rats, 10 DOL	Temporary occlusion of the middle cerebral artery or carotid artery ligation with 1.5h of oxygen 8% Carotid ligation and 30 min of oxygen 8%	1, 3, 7, 14, 28 days after injury	T2 maps, DWI 11.7	Difference in volumes of infarct depends on the type of injury	Cresyl violet	
Aden et al., 2002	CD1 mice, 7 DOL,	Carotid ligation and 30 min of oxygen 8%	3, 6, 24 hours, 5 days after injury	T2 maps, DWI 4.7 T	Evolution of T2 and ADC following injury	Cresyl violet at 35 days	Open field, beam walking test, locomotor activity, 28 days Neurofunctional deficit worse when presence of porencephalic cyst assessed by spatial memory using the Morris Water Maze motor cortical function using the rota-rod test. Behavior in correlation with the degree of injury assessed by MRI and histopathology
Ten et al., 2004	Mice, C57/BL6J, 7 DOL	Carotid artery ligation with 2 hours of oxygen 8%	11 weeks of life	T2W 9.4 T	Ipsilateral brain atrophy with or without porencephalic cyst and contralateral ventriculomegaly.	Triphenyltetrazolium chloride at after MRI.	

Studies	Species/Strain life (DOL)	Surgery	Times points	MRI sequences Magnetic Field (T: Tesla)	MRI findings	Histopathology	Behavioral testing
D'Arceuil et al., 1999	Rabbit, New Zealand white, 8–12 DOL	Carotid ligation with up to 3h of variable amount of oxygen deprivation	0 to 3 hours during hypoxia	DWI, T2W relative cerebral blood volume 2 T	Evolution during hypoxia of the relative cerebral blood volume and of the ADC.		using the open field test at 7 to 9 weeks after injury.
Drobyshevsky et al., 2007	Rabbit, New Zealand white, 22 days of gestation	Temporary occlusion of the descending aorta of the pregnant rabbit	1 and 5 DOL	DTI 4.7 T	Lower fractional anisotropy of corpus callosum, internal capsule and corona radiata of hypertonic kits	Immunostaining showing a reduction of phosphorylated neurofilaments in the corpus callosum and internal capsule	Locomotion, tone, righting reflex, suck and swallow
Thornton et al., 1997	Piglet, Large White newborn	Clamping of the carotid arteries with oxygen 12% until energy depletion assessed by spectroscopy	During hypoxia and the following 48 hours	³¹ P spectroscopy, DTI 7 T	Temporal evolution of the ratio of phosphocreatine to inorganic phosphate showing an immediate transient decrease during hypoxia followed by a more prolonged decrease due to secondary energy failure. It was accompanied by a reduction of the ADC throughout the brain and a reduction of anisotropy in the white matter.		
Vial et al., 2004	Piglet, large white newborn	2 episodes of occlusion of the common carotid arteries with oxygen 4%	During injury, 2 hours, 3, 5, 7 days after injury	³¹ P, ¹ H spectroscopy T2 maps 2.35 T	Transient reduction of the ratio of phosphocreatine to inorganic phosphate during the injury. Persistent increase in lactate following the injury. Increase in T2 in the parietal white matter.		

T1W: T1 weighted imaging T2W: T2 weighted imaging, DWI: Diffusion weighted imaging, ADC: apparent diffusion coefficient, FA: fractional anisotropy

Table 3

Studies evaluating a neuroprotective strategy

Studies/ treatment	Species/ Strain	Surgery	Times points post injury	MRI sequence Magnetic Field (T: Tesla)	MRI findings	Histopathology	Behavior
Wagner et al., 2002 Post ischemic hypothermia	Rat, Sprague-Dawley 7 DOL	Carotid artery ligation and 75 min in 8% oxygen	2, 12, 24 hours, 10 days, 42 days	T2W, DWI 2 T	Reduction of infarct volume		Spatial memory improved by cooling using the Morris Water Maze until 5 weeks. Sensory motor function, circling behavior were also evaluated.
Taylor et al., 2002 Post ischemic hypothermia	Rats, Wistar 14 DOL	Carotid artery ligation and 90 min of oxygen 8%. Brain temperature measurement	24h	³¹ P spectroscopy 7 T	No decrease of phosphocreatine/ inorganic phosphate in cooled animals following hypothermia	Hematoxylin and eosin at 5 days with a reduction of the infarct size after hypothermia	
Stieg et al., 1999 Memantine	Rats, Long-Evans, 7-10 DOL	Focal stroke caused by photochemical thrombosis	6 days	T2W, 4.7 T	Correlation of infarct size with histopathology, Reduction of size of infarct after treatment	Hematoxylin and eosin at 6 days. Reduction of size of infarct after treatment	
Antier et al., 1999 Idazoxan	Rats, Wistar, 7 DOL	Carotid artery ligation and 2 hours of oxygen at 8%	5 weeks	T2W 7 T	Increase in size of infarct in the treated group	Triphenyltetra-zolium chloride at 72 hours post injury showing an increase in size of the infarct of the treated group	
Albensi et al., 1999 MK-801	Rats, Wistar, 7 DOL	Carotid artery ligation and 2.5-3 hours of oxygen at 8.2%	1-6, 24, 48, 72 hours	T2W 2 T	Reduction of hippocampal injury	Hematoxylin and eosin in correlation with MRI	
Chang et al., 2005 Erythropoietin	Rat, Sprague-Dawley 10 DOL	Temporary occlusion of the middle cerebral artery	During injury	DWI during occlusion to document the injury 2T	Documentation of the injury	Cresyl violet with stereologic volumetry showing a reduction of the injury and a decrease of the expansion of the subventricular zone	Sensorimotor function with the cylinder test
Peeters-Scholte et al., 2003 Allopurinol, Deferoxamine	Piglet, Dutch store, DOL 1-3	Occlusion of the common carotid arteries with reduction of the FiO2	During hypoxia	³¹ P spectroscopy and T2 maps during injury and at 24 hours 4.7	T2 decrease after treatment and improvement of the ratio of phosphocreatine to inorganic phosphate	Assay of caspase-3 activity, TUNEL, light microscopy showing no difference between the treated and non treated groups	

T2W: T2 weighted imaging, DWI: Diffusion weighted imaging, ADC: apparent diffusion coefficient