The CABMM's researchers detail a number of investigations into, and the results of, using proton magnetic resonance spectroscopy on dogs with various brain conditions

More than just imaging

Modern diagnostic imaging techniques are used in veterinary medicine, similar to those in human medicine, playing an important role in clinical and research applications. Diagnostic imaging principally includes radiology, ultrasound, computerised tomography (CT) and magnetic resonance imaging (MRI).

Historically, new developed imaging modalities have always been established in veterinary medicine with some delay in comparison to human medicine. However, radiology and ultrasound have become widely available in many private veterinary clinics, while advance imaging modalities, such as CT and MRI, are more often restricted to referral centres, university institutions and veterinary research centres. Research in veterinary medicine itself, and with the background of translational medicine, has an important clinical impact for humans and animals.

Research and imaging

Numerous interdisciplinary projects relevant to human health issues have already been performed successfully at the Vetsuisse Faculty in Zürich. A lot of translational studies were done in collaboration with the Musculoskeletal Research Unit (MSRU) and the Center for Applied Biotechnology and Molecular Medicine (CABMM).

Issues in imaging play an increasingly important role, which is why the Clinic of Diagnostic Imaging at the Vetsuisse Faculty Zürich has now established a department for clinical research under the name Diagnostic Imaging Research Unit. The DIRU pursues a vision as an innovative and reliable research partner, which can enable the pursuit of innovative ideas through quality assurance and transparency in project-based collaborations. In this function, the DIRU represents a bridge between academic research and industry, which is well integrated into the network of the MSRU and the CABMM.

The aim of the DIRU is therefore to support projects based on the tracking of imaging research and imaging services, both for internal research groups as well as external partners. The interdisciplinary exchange and implementation of a network of several other professionals are a major locational advantage; a close co-operation with the MSRU and membership in the CABMM can be considered an example. In our organisation, we have experts from different fields of interests, including congenital diseases, cardiovascular imaging, liver imaging, perfusion imaging, musculoskeletal imaging, neuroimaging, interventional imaging and translational medicine.

A variety of tools

Research projects about spectroscopy in dogs, several diffusion and perfusion techniques, dynamic examinations of the heart, and highly detailed representations of particularly small structures in the 3 Tesla MRI represent only a selection of the options that can be implemented at our clinic. We offer modern imaging modalities like CT or a 3 Tesla MRI (Philips Ingenia).

In comparison to other low-field MRIs, we work with a better SNR (signal to noise ratio) in the 3 Tesla MRI. Consequently, there is a significant improvement in the image quality of standard MRI sequences, where we reach excellent resolution. In addition, we follow the approach of molecular imaging (spectroscopy) as well as functional MRI. A set of different coils provides the possibility to scan material of different sizes (from rodents up to the large parts of a horse). More advanced sequences, such as DIXON, are also possible.

In recent years, the demand for documentation and standardisation of preclinical animal models has increased. To reach this high level, different quality assurance programmes have been introduced, such as good laboratory practice (GLP), good manufacturing practice and good clinical practice. In close collaboration between DIRU and MSRU, it is also possible to run projects with GLP certification to fulfil high quality standards of regulatory affairs.

The aim of this clinically applied research is to translate scientific evidence into commercial developments. Whether new surgical techniques, medical implants or treatment methods are addressed, the use of modern imaging techniques and modalities can make an important contribution.

In Fig. 1, the x-axis represents the signature chemical shift of each metabolite concentration,

and the y-axis represents the signal intensity. Tissue concentration of a metabolite is related to the integrated amplitude of the MRS (magnetic resonance spectroscopy) signal it generates, which is the area under the ^{1}H MRS (proton magnetic resonance spectroscopy) signal curve. Both peak area and height are contributing factors for determining the concentration of each metabolite. In relation to Fig. 1, Cho is choline; Cr, creatine; Glx, glutamine-glutamate complex; MI, myo-inositol; and NAA, N-acetylaspartate.

^{1}H MRS

MRS is a non-invasive technique that provides specific biochemical information on numerous intracellular metabolites. ^{1}H MRS uses measurements of signals emitted by proton nuclei because of their high magnetic sensitivity and presence in all tissues of the human or animal body. Instead of a morphological image of the brain, the result of MRS can be visualised as a graph of signal intensity with respect to its frequency.

Proton signals of different metabolites, or even different protons of one molecule, can occur at different positions (frequencies) within the MRS spectrum. The shift of peaks in their relationship to one another on the frequency axis is called chemical shift. Instead of a frequency scale, which is dependent on the magnetic field's strength, a parts per million (ppm) is commonly used to describe the position of the spectral peaks on the x-axis.

Each metabolite has a characteristic set of chemical shift values in its signal. The concentration of each metabolite is related to the corresponding signal amplitude, which is the area under the curve. The relative concentration of the metabolites can be measured by numerical integration (normally using metabolite ratios) or by sophisticated software such as LCModel, which is the one used in the Diagnostic Research Unit at the Vetsuisse Faculty of Zürich.

Glutamate and glutathione

Proton MRS can detect several metabolites such as *N*-acetylaspartate, choline, creatine, glutamate, glutamine, myo-inositol, and glutathione in the brain of clinically normal subjects. *N*-acetylaspartate, which resonates at

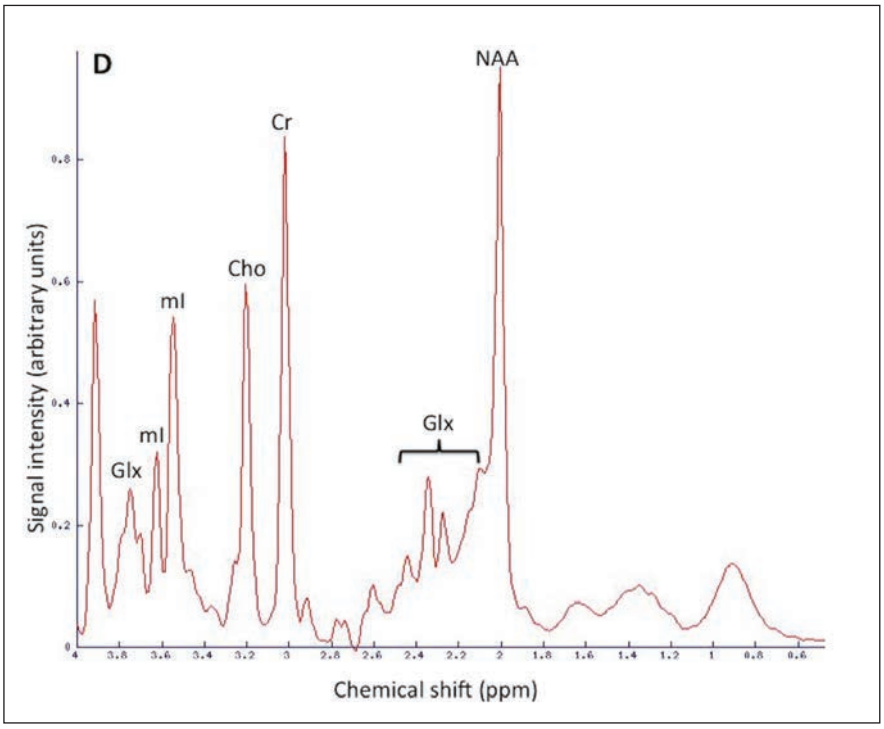


Fig. 1 Single voxel short echo time magnetic resonance spectroscopy performed on the basal ganglia of a healthy three-year-old Beagle dog

2.01 ppm, is considered a neuronal marker and is present only in neurons, axons and dendrites. Choline resonates at 3.2ppm and is involved in membrane synthesis and degradation, whereas creatine resonates at 3.0ppm and is involved in energy metabolism. Glutamate and glutamine metabolites resonate closely at 3.75ppm and between 2.1 and 2.5 ppm, respectively.

Glutamate is an excitatory neurotransmitter that plays a role in mitochondrial metabolism; glutamine is involved in detoxification and regulation of neurotransmitter activity. Myo-inositol is a pentose sugar that resonates at 3.5-3.6ppm and is part of the inositol triphosphate intracellular second messenger system. It is considered an astroglial cell marker since it is predominantly found in glial cells.

Glutathione resonates at 3.77ppm; it is an antioxidant and is essential for maintaining red blood cell structure and maintaining haemoglobin in a ferrous state. In pathological conditions, these metabolites may be found in abnormal concentrations (absent, lower, or higher concentrations), and other metabolites (e.g. lipids or lactate) that aren't typically present in a healthy brain may be detected.

Fig. 2 shows single voxel short echo time MRS performed on the basal ganglia region of a seven-year-old golden retriever with chronic liver disease and hepatic encephalopathy. In particular, the high peak for the glutamine-glutamate complex and the lower peak for myo-inositol in this dog differ when compared with results shown in Fig. 1.

Dog's brain

At the Clinic of Diagnostic Imaging at Vetsuisse Zürich, active work employing ^{1}H MRS has been developed for the last three years. The first step was to define an optimised protocol and to assess the metabolite concentration in the brain of healthy dogs. Single voxel short echo time ^{1}H MRS was performed on ten healthy Beagle dogs in different regions

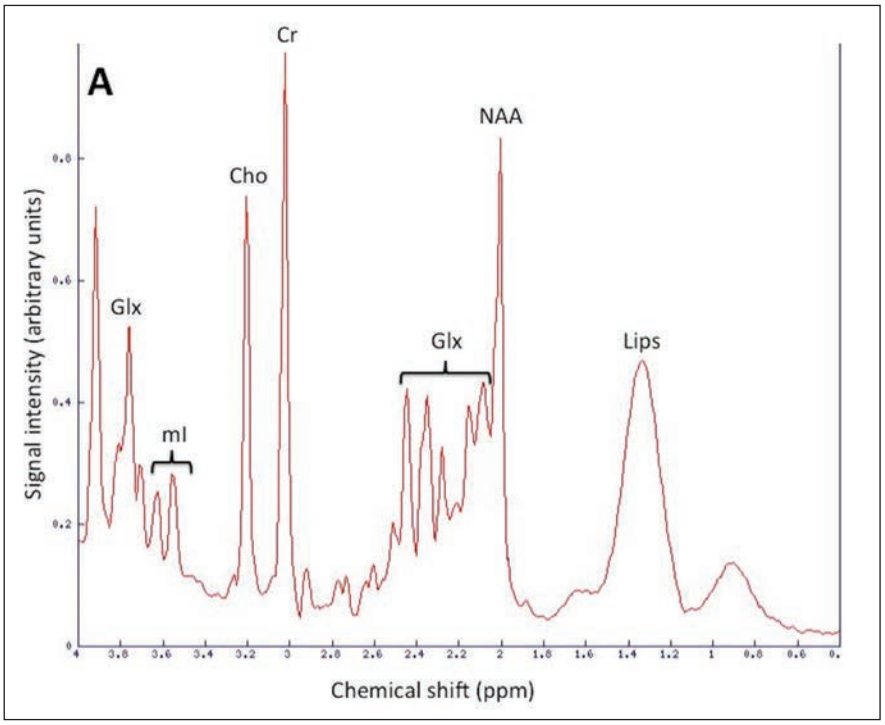


Fig. 2 Single voxel short echo time magnetic resonance spectroscopy on the basal ganglia region of a seven-year-old golden retriever with chronic liver disease and hepatic encephalopathy

of the brain, including thalamus, parietal lobes, temporal lobes and occipital lobes (Fig. 1), basal ganglia and cerebellum.

This study showed no differences between right and left hemispheres and no differences between sexes. Statistically, significant metabolite concentration in different regions of the brain was found. For instance, the concentration of *N*-acetylaspartate was highest in the parietal lobes and lowest in the cerebellum, whilst the choline concentration was highest in the basal ganglia and lowest in the occipital lobe. This study provided reference values for clinical and research studies employing similar ^{1}H MRS technique. What follows are various clinical applications.

Brain metabolite abnormalities in dogs

Hepatic encephalopathy is a neurological condition associated with failure of the liver to detoxify neurotoxins. The pathogenesis is complex, but it appears that ammonia plays a central role in the damage of the brain. Ammonia is produced primarily in the gastrointestinal tract. Typically, a high amount of ammonia is extracted by the liver via the urea cycle. When the liver function is not correct, the ammonia accumulates in the brain, and, once there, the astrocytes are responsible for ammonia detoxification. The ammonia gets metabolised and converted into glutamine. Too high levels of glutamine in the brain produce brain oedema and the neurological signs characteristic of hepatic encephalopathy.

^{1}H MRS was performed in the brain of six dogs with hepatic encephalopathy (because of portosystemic shunts or chronic hepatitis), and they were compared to 12 normal dogs. We found characteristic differences between these groups: dogs with hepatic encephalopathy showed high levels of glutamine and lower levels of myo-inositol (Fig. 2). Furthermore, choline and *N*-acetylaspartate concentration were slightly lower in dogs with hepatic encephalopathy than in the control dogs. In some dogs, the concentration of ammonia in blood was normal. This highlights the importance of ^{1}H MRS to prove the presence of hepatic encephalopathy, even when the blood analyses are normal.

Future studies include the investigation of the metabolic profile in patients with hepatic encephalopathy before and after treatment, either medical or surgical. Another future project would include the investigation of hepatic encephalopathy in feline patients. It is suspected that the pathophysiology in cats with hepatic encephalopathy may be slightly different from that found in dogs, since they respond in very variable ways to medical and surgical treatments.

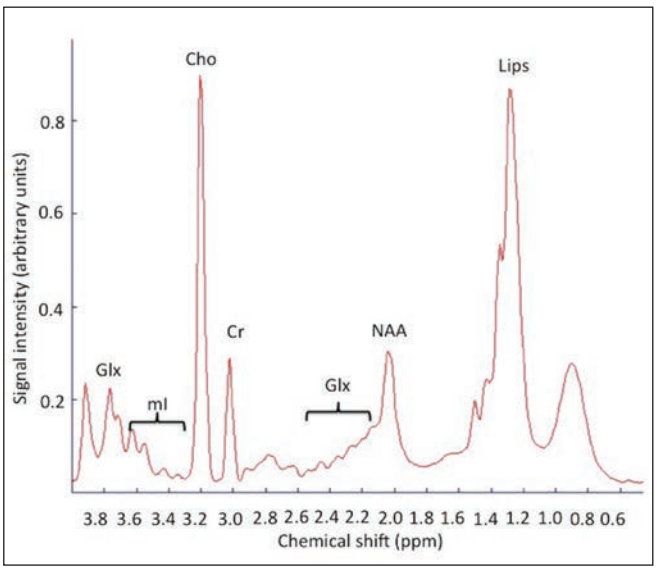
Shown in Fig. 3 is the typical short echo time magnetic resonance spectra of a malignant tumour (glioblastoma multiforme) in a nine-year-old dog. The high peak of choline, extremely low *N*-acetylaspartate and the high amount of lipids correlate with a high degree of necrosis.

Neoplastic and inflammatory brain diseases

Intracranial neoplasias and meningoencephalitis are common diseases in dogs. Conventional MRI allows characterising the morphology of the lesion; however, it is not always possible to distinguish between a neoplastic process and an inflammatory one. This is crucial because the treatment, as well as the prognosis, may be quite different.

We performed ^{1}H MRS in the brain of dogs with neoplasia and inflammatory meningoencephalitis in order to study the metabolic profile of these diseases and to investigate if there were any metabolites that could serve as indicators for

Fig. 3 Typical short echo time magnetic resonance spectra of a malignant tumour in a nine-year-old dog



each disease. In this study, we included 15 dogs with proven neoplasia and 15 dogs with inflammatory meningoencephalitis, which were then compared to normal dogs.

There were statistical differences between diseased and normal dogs. Differences were also noted between neoplasia *versus* inflammation. The concentration of *N*-acetylaspartate is lower in neoplasia than in meningoencephalitis, whilst the concentration of choline is higher in neoplasia than in meningoencephalitis.

In Fig. 4 is a typical short echo time magnetic resonance spectra of a dog with granulomatous meningoencephalitis. In this type of meningoencephalitis, the presence of lactate is common due to hypoxia. Choline is slightly elevated, whilst *N*-acetylaspartate is moderately reduced.

A follow-up ^{1}H MRS of four dogs with meningoencephalitis showed recovery of *N*-acetylaspartate to normal values, correlated with the clinical improvement. This proves that the reduction of *N*-acetylaspartate in meningoencephalitis may be due to neural dysfunction, rather than neural death. Interestingly, in ten out of 15 patients with meningoencephalitis, taurine was found; this metabolite was only found in one neoplasia. Potentially, taurine may serve as a metabolic marker for patients with meningoencephalitis. Future projects involving tumours and meningoencephalitis are the investigation of the response after treatment.

Tick-borne encephalitis

This investigation focused on the brain metabolite abnormalities in dogs infected with tick-borne encephalitis (TBE). Central European TBE is an infection caused by a *Flavivirus.* It is transmitted by tick bites (*Ixodes ricinus)* to various species, including humans, dogs, horses, sheep and goats; the disease is endemic in 27 European countries. TBE virus is a neurotropic RNA virus which causes a non-suppurative encephalomyelitis in dogs, characterised by widespread neurophagia and gliosis throughout the grey matter of the central nervous system, but mainly involving the brainstem, cerebellum and ventral horn of the spinal cord.

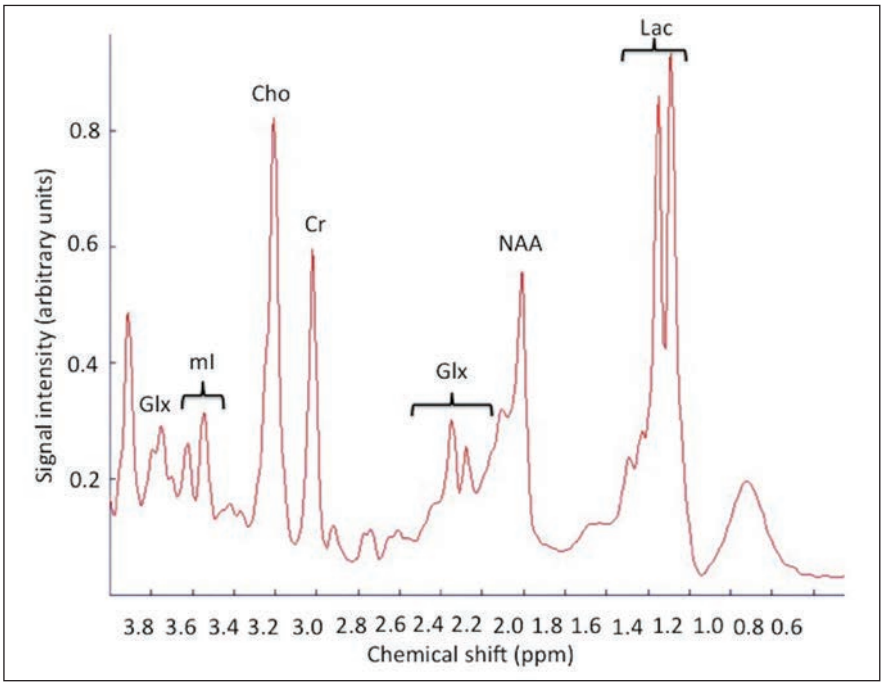


Fig. 4 Typical short echo time magnetic resonance spectra of a dog with granulomatous meningoencephalitis

Diagnosis of TBE in dogs is very challenging. During the first viremic phase of the disease, the virus can only be isolated from the blood or from cerebrospinal fluid (CSF) by reverse-transcriptase-polymerase chain reaction. During the second phase, when neurological signs become clinically evident and humoral immune response starts, the virus clears up from the blood and CSF and cannot be detected anymore.

MRI has been described in both people and dogs. The MRI changes are very characteristic, showing bilateral symmetrical lesions of the thalamus, hippocampus and grey matter ventral horn of the spinal cord. However, MRI may be negative in some patients, even with evident clinical signs. We are investigating the metabolic changes in dogs with confirmed TBE, especially those in which there are no evident morphological lesions.

When compared to normal dogs, patients with TBE show a marked decrease in *N*-acetylaspartate concentration and higher myo-inositol. These findings may help to detect brain abnormalities at a metabolic level, even though conventional MRI is normal.

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