THESIS OF DOCTORAL (PhD) DISSERTATION

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POSSIBLE INTRACRANIAL APPLICATIONS OF FUNCTIONAL AND DIFFUSION MAGNETIC RESONANCE IMAGING IN DOGS AND HUMANS
METHODOLOGICAL ASPECTS

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1. ANTECEDENTS OF THE RESEARCH, OBJECTIVES

In the last decades the development of diagnostic imaging is undiminished, the imaging studies in many areas of medicine today are not dispensable. Important parts of that development would not exist without the animal model experiments. However, it is important to realize that there are tests harmless to the human body where there is no need for animal tests it directly proven and apply in humans. Magnetic resonance imaging (MRI) is a kind of method. Most parts of the MR methods has been proven in animal studies after human application.

Two major "indicator" is observed of MR imaging in the animals: (1) studies with diagnostic purposes in veterinary and animal sciences and (2) animal model experiments.

By the applied research, it is possible to get more information about precise structural and functional organization of the species. The understanding of brain function localization stands behind the dog's behaviour is extraordinary challenge and opportunity at the same time.

Functional MRI (fMRI) allows to visualize on neurophysiological basis the neural activity of the brain. It is extremely sensitive to movements during the measurement, in this way the main question of the methodology is to minimize movement of the subject. The diffusion MRI gives information about the movement of water molecules. The two major powerful diagnostic parameter of the diffusion measurements are the apparent diffusion coefficient (ADC) and the fractional anisotropy (FA).

According to the literature a small number of dogs have functional and intracranial diffusion MRI, without exception, under general anesthesia.

OBJECTIVES

1.1. Our goal was to develop a new examination method, which allows MRI examination of alert dogs without any constraint (anesthesia or fixation).

1.2. To validate our new methodology by comparing the quality of MR images taken in awake and anesthetized state of the same dogs.

1.3. Functional MRI of alert, not fixed dogs to localize vital cerebral centers and if feasible, certain sociocognitive regions.

2. To perform the first human functional MRI prior to radiotherapy planning, in Hungary. Our goal was to achieve dose reduction in functional centers which have an effect on life quality.

3. To perform a human fMRI prior to neurosurgery with an aim to demonstrate how this routine human methodology maybe adapted in case of diagnostic veterinary imaging.

4.1. To make a diffusion MRI of a healthy dog’s brain. Furthermore, to calculate apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values, and analyze the data in regards to hemispheric asymmetry. Our further aim was to compare dogs’ data with similar human data and to examine the utilization of post-processing software.

4.2. To examine a diseased dog’s brain with diffusion MRI, and to compare the dog’s ADC- and FA-values with values from healthy dogs (mean value).

2. MATERIAL AND METHODS

MR examinations were performed at the Institute of Diagnostic Imaging and Radiation Oncology, University of Kaposvár using a 1.5T scanner (Siemens Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany).

2.1. Magnetic resonance imaging of awake dogs

Subjects: Four dogs were trained using our new methodology (two female, two male; one-one golden and Labrador retriever, two mixed breed).

Training: In order to prepare the dogs for the awake MRI a special step-by-step training (shaping) procedure was planned. Subjects were trained to stay in the wanted position and habituated to the noise and vibrancy of MR scanner. The training was based on positive reinforcement technique (clicker training) and on the very rare occasions when correction was needed it was only a verbal “no” for unwanted behaviour. The dogs were continuously treated and verbally praised for the desired behaviour, and were never punished. Preparation time of a dog for an MRI lasted about one—one and half months consisting of several minutes long training sessions per day. .

Image Acquisition: During the scanning we did not apply any constraint (anesthesia or fixation) to keep the dogs in the wanted position. Different T_1- and T_2-weighted sequences were acquired for structural scans.

Comparative analysis of MR images of awake and anesthetized dogs:

To verify the quality of anatomical images taken in case of alert dog we anesthetized our subjects once to acquire images during anesthesia. On these images we made ROI-based analysis (Region of Interest). We calculated three parameters to characterize the quality of MR images ("signal to noise value in gray matter", "signal to noise value in white matter", contrast). Statistical analysis was applied to answer whether significant difference exists between the quality of images taken in awake and anesthetized state.

2.2. Functional magnetic resonance imaging in awake dogs

Image Acquisition: Structural scans were acquired with a T_1-weighted MP-RAGE sequence (TR: 1160ms, TE: 4.24ms, slice thickness: 0.8mm, Flip Angle (FA): 15°, matrix: 384×512, 192 transversal slices). BOLD fMRI scans were acquired using a single-shot EPI (echo planar imaging) sequence (TR: 2000ms, TE: 50ms, slice thickness: 3.5mm, FA: 60°, matrix: 64×64, 19 transversal slices).

1. paradigm: images of reward. No stimulus was presented during the "rest" period and photos of dog's food and rewards were presented using a projector for the duration of the "active" period.

2. paradigm: somatosensory stimulus. No stimulus was presented during the "rest" period (dog was lying at rest). For the duration of the "active" period, the dog's back was petted by a person familiar to the dog.

fMRI image processing: All functional imaging data were processed using Statistical Parametric Mapping (SPM5) software. After post-processing we made general linear model (GLM), and after this significantly activated voxels were identified using a t-test.

2.3. Human functional magnetic resonance imaging

2.3.1. Integrating fMRI information into radiotherapy planning—case study

Patient: 32 year old female with epileptiform nausea, with malignus tumor in the left parietooccipital region. A residual tumor was found on the postoperative cranial MR scans, therefore the clinicians opted for radiotherapy.

Image Acquisition: BOLD fMRI scans were acquired using a single-shot EPI sequence (TR: 3140ms, TE: 50ms, slice thickness: 4mm, FA: 60°, matrix: 64×64, 30×170 axial slices). Structural scans were acquired with a T_1-weighted MP-RAGE sequence (TR: 1160ms, TE: 4ms, slice thickness: 0.8mm, FA: 15°, matrix: 512×432, 192 coronal slices).

Paradigms: Three different paradigms were applied. Our goal was localize the center of speech, the somatosensory cortex and one cognitive cerebral center.

fMRI image processing: All functional imaging data were processed using Statistical Parametric Mapping (SPM5) software. After post-processing we made general linear model (GLM), and after this significantly activated voxels were identified using a t-test.

Radiotherapy planning: The planning was made by radiation physicists using XiO (CMS Inc., St. Louis, MO, v.4.34) planning software. Three plans were made, for plan comparison dose-volume histogram analysis was used.

2.3.2. Integrating fMRI information into neurosurgery planning—case study

Patient: 31 year old male with a tumor located in the lateral ventricle, expanding into frontal and temporal lobe and into the posterior cranial fossa. This tumor was partially resected in two steps. Because of the increasing residual tumor, the neurosurgeons decided to perform further surgery, therefore, they requested fMRI prior to the intervention.

Image Acquisition: Structural scans were acquired with a T_1-weighted MP-RAGE sequence (TR: 1160ms, TE: 4ms, slice thickness: 0.8mm, FA: 15°, matrix: 512×384, 192 coronal slices). BOLD fMRI scans were acquired using a single-shot EPI sequence (TR: 3020ms, TE: 50ms, slice thickness: 5mm, FA: 60°, matrix: 64×64, 30×170 axial slices).

Paradigm: To localize motor cortex a modified "finger-tapping" paradigm was applied, where the patient was asked to move his carpus during the active scans.

fMRI image processing: Functional imaging data were processed using a specific technique which is further discussed in "Integrating fMRI information into radiotherapy planning—case study" section *(2.3.1. subsection).*

2.4. Diffusion magnetic resonance imaging in dogs

Subjects: 15 dogs with no intracranial disease were examined (three female, 11 male; mean age: 7.32 ± 3.85 year; no data about one dog).

Image Acquisition: Spin Echo EPI sequence was applied (TR: 10,000ms, TE: 118ms, slice thickness: 3mm, FA: 90°, matrix: 256×256, FoV: $300mm^2$, b: $700s/mm^2$). Number of measured directions was 12 + 1 (b: $0s/mm^2$).

Image processing: Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps was calculated with DTI Studio software. ROI-based analysis was applied in the right and left frontal whiter matter and in the right and left lateral ventricle, and ADC- and FA-values from these regions were compared statistically.

Comparative analysis of ADC- and FA-values from healthy and diseased dogs: To examine the possibly altered diffusion relation and the applicability of this method on diseased animals we compared the ADC- and FA-values from healthy dogs (mean value) and from a diseased dog.

3. RESULTS

3.1. Magnetic resonance imaging in alert dogs

3.1.1. The success of the training

The training series that we developed has proven to be effective: dogs learned to lying motionless in the desired position (without fixation and anesthesia). The average shifting was 0.5-1.0 mm during MR imaging in alert dogs and it ranged between 0.1-0.2 degrees, it was set up an average 5-6 minutes in the head position of the dogs.

3.1.2. Comparison the quality of records taken at alert and anesthetized state

We did not find any significant differences between the images taken at alert and anesthetized state.

3.2. Functional magnetic resonance imaging in alert dogs

3.2.1. Images of reward from a beamer

Activation was obtained at the nucleus caudatus (part of the rewarding system in humans), and also at the lobus occipitalis (visual cortex).

3.2.2. Somatosensory stimulation

Activation appeared in the right somatosensory cortex and in the nucleus caudatus.

3.2.3. Methodological experiences

Each of our subject behaved calmly during the fMRI measurements, they did not show an abnormal degree of fear. A number of factors had to be considered during the design and evaluation of the fMR study in alert dogs.

3.3. Human functional magnetic resonance imaging studies

3.3.1. Integrating fMRI information into radiotherapy planning—case study

Results of the fMRI:

First paradigm: we get the largest continuous activation in the area of the right middle temporal gyrus. In the left hemisphere affected by the tumor and oedema we found dislocational activation slightly offset from the oedema to cranial direction, in the middle temporal gyrus.

Second paradigm: The strongest activation was at the left hemisphere, at the underside of the gyrus postcentralis.

Third paradigm: the strongest activation was at the lower parietal, angular and supramarginal region, at the border area of the lobes, a bit dislocated due to the oedema, and also at the marginal part of the hippocampus.

Definition of organs at risk: According to the results of the functional MRI we determined four risk organs compared to the tumors contralateral: at the region of the superior temporal gyrus, the middle temporal gyrus, the lingual gyrus and the superior frontal gyrus.

Comparison of the plans of radiotherapy: Using fMRI information to 3D conformal, and also according to the IMRT plan the functional risk organs get 50% less radiation dose that the 3D plan that did not use fMRI information.

3.3.2. Integrating fMRI information into neurosurgery planning—case study

During the post-processing we could localize the motor cortex (precentral gyrus) in both hemispheres. It is clearly visible on the axial plan slices that the tumor, place occupying process did not affected the motor cortex.

3.4. Diffusion magnetic resonance imaging in dogs

3.4.1. Analysis of ADC- and FA-values

In the point of independent variables we did not find significant differences. We did not find any significant differences between the data of the two hemispheres in regards to the ADC- and FA-values.

Comparing our data measured in dogs with human data the ADC- and FA-values were equal in order of magnitude also in the human's and the dog's brain.

3.4.2. Comparative analysis of ADC- and FA-values from healthy and diseased dogs

We demonstrated that we can measure the ADC- and FA-values in the brain of diseased animals; the ADC- and FA-values of diseased dogs were different from the healthy mean values.

4. CONCLUSIONS

We developed a new examination method on dogs, it is possible way to do noninvasive MR imaging on alert not fixed dogs. We did not find any significant differences in the structural images taken from conscious, trained and anaesthetized dogs, and using different paradigms we made functional MRI on conscious dogs.

Examining the dogs with this method we get the answer for many questions where the answer may not be possible in anesthetized or anaesthetized animals.

The results of human studies showed that the fMRI information can be integrated in the planning of the 3D conventional radiotherapy, it makes possible to spare the functional areas besides the maximum supply of the targeted areas.

The other human fMR study was also effective because with the cooperation of the patient we could localize the motor cortex in both hemispheres that means additional information in the planning of neurosurgical intervention.

The results of the intracranial diffusion MR studies on dogs were similar in many aspects to the results of diffusion MRI on humans in our Institution: we did not find differences in the ADC- and neither in FA-values that refer to hemispherical asymmetry, the values measured in dogs correspond to the humans. DTI Studio was suitable to process the diffusion images of the dog's brain, however we found significant differences between the image quality of the three plane which makes it more difficult to localize the cerebral regions.

5. New scientific results

1.1. We worked out a new examination method, which allows MRI examination of alert dogs without any constraint (anesthesia or fixation). To our knowledge this is the first completely noninvasive MRI for dogs on the world.

1.2. We were able to acquire appropriate anatomical images of the trained conscious dogs' brain which were repeated with the same dogs under general anaesthesia. We did not find any significant differences in the images taken from conscious and anaesthetized dogs, so we validated our new methodology.

1.3. Using different paradigms we made functional MRI on conscious, not fixed dogs.

2. We examined the possible adaptation of routine human clinical functional and diffusion MRI within veterinary diagnostic imaging.

3. We collected important information and experience of the methodological aspects of human fMRI and DTI processing software's applicability for dogs.

4. We performed intracranial diffusion MRI on healthy dogs. We calculated the apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps, and placed two-two region of interest (ROI) points in the right and left hemisphere of the brain: in the region of frontal white matter and lateral ventricles. The diffusion values were measured in these points and were then statistically compared to detect hemispheric asymmetry, and we compared dogs' data with human data.

6. RECOMMENDATIONS

6.1. Theoretical and practical benefits of our research results

Based on our training methods, conscious dogs could stay motionless for an extended duration within the MR scanner without any need for use of restraint (fixation, anesthesia) allowing for long measurements using fMRI protocols and for applying so stimulations that we can not use in case of anesthetized animals. With this method we can avoid influential effect on neural activation of stress through fixation and anesthetic agents. Animal protection viewpoints do not speak against this methodology. We collected important information and experience of the methodological aspects during the training phase and MR examination of conscious dogs which are to be made available for other research groups (e.g. use in the planning and post-processing of fMRI on other animal species).

To demonstrate the possible adaptation of fMRI within veterinary diagnostic imaging we reviewed two human fMRI examinations.

DTI Studio proved to be successful in the evaluation of diffusion images of dog’s brain.

6.2. Suggestions for further studies

To adapt of human fMRI examinations within veterinary diagnostic imaging could be a realistic aim of our Institute. In intracranial cases fMRI could be a part of diagnostic imaging protocol. Diffusion sequences could help to detect certain pathological changes during diagnostic MRI of dogs. These sequences are widely usable in case of animal experiments also, for example to detect biological effects of radiotherapy.

7. Publications from the thesis studies

Scientific publications

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› Kovács, Á. – **Tóth, L.** – Glavák, Cs. – Lakosi, F. – Hadjiev, J. – Bajzik, G. – Vandulek, Cs. – Repa, I.: Integrating functional MRI information into radiotherapy planning of CNS tumors-early experiences. *Pathology and Oncology Research*, 2011. 17(2): 207–217. ***(IF$_{2009}$: 1.152)***

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Book, book chapter

Book chapter in Hungarian language

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- › KOVÁCS, Á. – LAKOSI, F. – GLAVÁK, CS. – ANTAL, G. – HADJIEV, J. – **TÓTH**, **L.** – LIPOSITS, G. – TOLLER, G. – REPA, I.: Integrating Functional MRI Information into Radiotherapy Planning of CNS Tumors. [Poster. 29th Congress of European Society for Therapeutic Radiology and Oncology. Barcelona/Spain, 12–16th September 2010.] *Radiotherapy and Oncology*, 2010. 96(Suppl. 1): 263. ***(IF$_{2009}$: 4.343)***

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- › **TÓTH, L.** – GÁCSI, M. – MIKLÓSI, Á. – BOGNER, P. – REPA, I.: The methodological aspects of awaken dog brain MRI. [Poster. Joint East and West Central Europe ISAE Regional Meeting. Bratislava/Slovakia, 15–17th May 2008.] In: *Proceedings of Joint East and West Central Europe ISAE Regional Meeting*. P. 59.
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