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Imaging birds in a bird cage: in-vivo FSE 3D MRI of bird brain

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Abstract

An in-vivo magnetic resonance imaging (MRI) procedure is described that allows one to obtain three-dimensional high quality images of the entire brain of small birds such as the canary (20 g) and the starling (75 g) with an image resolution of 0.1 mm (58–113 μm, dependent on the size of the imaged bird). The entire imaging procedure took about 2 h after which the birds recovered from anaesthesia uneventfully and could be reused for subsequent additional imaging. This non invasive MRI technique enables to correlate brain measures with behavioural or physiological data that are dynamic in nature and could permit significant progress for bird neurological research.

Keywords: Magnetic resonance imaging; In-vivo MRI; Bird brain; Canary; Starling

1. Introduction

Behavioural neurosciences represent a scientific discipline that has been recently growing at a rapidly increasing pace largely due to the recent availability of electrophysiological, chemical, molecular and imaging techniques that now permit the investigation of questions that could not have been addressed 20 years ago. It is now possible to correlate the behaviour of one individual with electrical recordings of single neurones, to identify the presence of a specific messenger RNA in single cells and to map at a cellular level of anatomical resolution the distribution of hormones, neurotransmitters and their receptors in the brain. During the last 50 years, avian species have consistently served as useful models in behavioural, neuroendocrine and neuroanatomical studies.

In particular, they have led to the first identification of major sex differences in the brain and also of changes in brain structure or chemistry that mediate seasonal changes in reproductive behaviour [1–3]. In the early seventies, studies of song birds such as the canary (*Serinus canaria*) identified a network of brain nuclei (the song control system) that controls both the acquisition during ontogeny and expression in adulthood of learned vocal behaviour [4,5].

This song control system was later found to be sexually dimorphic [6]. Furthermore, quantitative morphometry demonstrated that many of the nuclei that are part of this neuronal circuitry change in volume as a function of seasons [7,8]. Because these volumetric changes are associated with a marked neurogenesis, neuronal migration and incorporation of the newly formed neurones into long distance projection pathways, a lot of attention has been devoted recently to this avian model of seasonal plasticity in the adult brain [9]. A marked neural plasticity has also been identified in other avian model systems. Two prominent examples of this plasticity include the seasonal, individual and species differences in the volume of the hippocampus, in phase with natural changes in spatial behaviour

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[10,11] and the variations in response to changes in the circulating levels of testosterone of the volume of the medial preoptic nucleus of quail [12].

One recurrent problem in the studies of brain plasticity has been that with the traditional histological techniques, neuroanatomical features can only be measured once in a particular bird. The histological approach is therefore not optimal if one wishes to correlate brain measures with behavioural or physiological data that are dynamic in nature. Moreover, interindividual differences make it difficult to draw conclusions between variation in behaviour and variation in brain nuclei that are often smaller than 1 mm^3. An in-vivo non invasive technique that would enable the study of neuroanatomical features with a high resolution would permit significant progress in neurological research in birds.

Magnetic resonance imaging (MRI) is such a powerful and non-destructive microscopic tool that recently became an approved tool in medicine and experimental pharmacological research. This technique provides in-vivo morphological information with sub millimetre spatial resolution and a soft-tissue contrast that surpasses other imaging conditions. However, MRI has not to this date been used to study bird neurology. The main problems that have delayed the use of MRI in this field of research probably reside in the difficulties in obtaining anaesthesia of long durations (hours) in small avian species and in the high image resolution that is required to analyse in a meaningful way structures located in such small brains.

The present study assesses whether MRI can be used for in-vivo qualitative and quantitative analysis of morphology in small brains such as those of songbirds (oscines). MR microscopy at high field (7 T) should in theory allow spatial encoding with a resolution more than sufficient for imaging such small organisms with the requested details. It was our major aim to obtain 3D detailed information (ventricles, fibres and/or nuclei) on the brain of birds, as small as the canary (body weight, 20 g) or slightly larger such as the European starling (body weight, 75 g) by an in-vivo non invasive approach. To that end, MRI sequences had to be selected to permit the acquisition of images in a time that would be compatible with the anaesthesia and later survival of the subjects. This could be achieved with acquisition times of 1–2 h that allowed a 100% survival rate of the birds. The canary and European starling were chosen as subjects for our experiments for both scientific reasons and practical considerations. These species have been used in a large number of studies on the central control of behaviour and its seasonal plasticity [2,3,13,14] and a large amount of background data is therefore available concerning their endocrine cycle, behaviour and neuroanatomy. They can also be easily maintained and bred (canaries) in captivity, they are easy to handle and apparently quite resistant to surgical manipulations.

2. Material and methods

2.1. Anaesthesia, positioning and immobilisation of the bird

Canaries (*Serinus canaria*) weighing 22 g and European starling (*Sturnus vulgaris*) weighing 75 g were anaesthetised with an initial intraperitoneal injection of 5 ml kg^{-1} of a mixture containing 4.33 ml saline solution, 0.33 ml xylazine (Rompun: 20 mg ml^{-1}) and 2.10 ml ketamine (Ketalar: 50 mg ml^{-1}). Anaesthesia was maintained by administration of one fifth of the initial dose every 30 min through a catheter positioned in the chest muscle. The head of the animal was positioned in a Teflon custom build stereotaxic holder. The posterior fixation point was the ear bar placement in the external auditory canal of the animal which is quite easily located by wetting and lifting the feathers on the side of the head. The bill was blocked by a bill clamp and tightened with a screw restraining the upper mandible.

After placement of the head in the stereotaxic instrument, canaries were inserted in a custom build birdcage Radio Frequency coil (30 mm diameter), while starlings were inserted in a quadrature RF bird cage (50 mm diameter). We tried to standardise as much as possible the orientation of the head with respect to the horizontal axis of the stereotaxic holder, but it was not critical to achieve a perfect reproducible positioning because the direction in which the MR images will be acquired can be selected independent of the orientation of the object. The entire MRI protocol with positioning of the bird and preparation of the MRI instrument required an anaesthesia period of about 2 h, while the imaging procedure itself only took about 70 or 85 min, dependent on the MRI sequence used. All birds recovered uneventfully from this anaesthesia, and it was in fact possible to reanaesthetise one bird without incident on the day after the initial imaging.

2.2. In-vivo MRI protocol

MRI imaging was performed on a 7 T horizontal bore MR microscope from SMIS (Surrey Medical Imaging Systems, UK), provided with shielded gradients (8 cm width) with strength of 100 mT m^{-1}. Scouting spin echo images were acquired to guide the positioning of the 3D slab over the bird brain. 3D MR images were obtained in the sagittal and coronal planes. The coronal MRI slices were taken to conform the canary brain atlas of Stokes et al. [4].

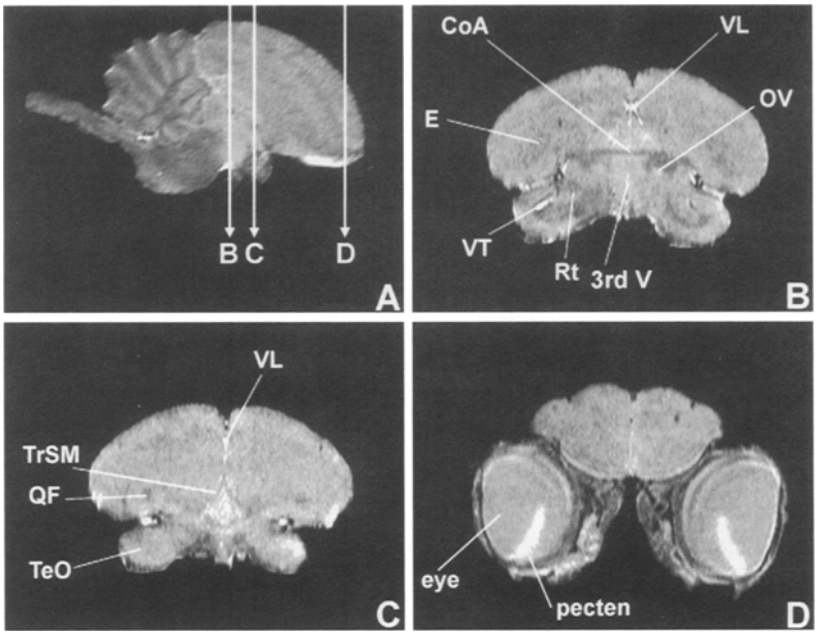

Fig. 1. Set of in-vivo sagittal (A) and coronal (B–D) 3D FSE MR images of the brain of a canary. The image resolution of the sagittal image is 78 × 78 μm^2 with a slice thickness of 58 μm. For the coronal slices, the image resolution is 86 × 86 μm^2 while the slice thickness is 78 μm. The vertical lines on the sagittal image indicate the section plane of the coronal images (B–D) and conform to the coronal plane of the canary brain atlas [4]. Section D also displays the eyes with the pecten. CoA, Commissura anterior; E, Ectostriatum; OV, Nucleus ovoidalis; Rt, Nucleus rotundus; TrsM, Tractus septomesencephalicus; TeO, Tectum opticum; QF, Tractus quintofrontalis; 3rd V, Ventriculus 3; VL, Ventriculus lateralis; VT, Ventriculus tecti mesencephali.

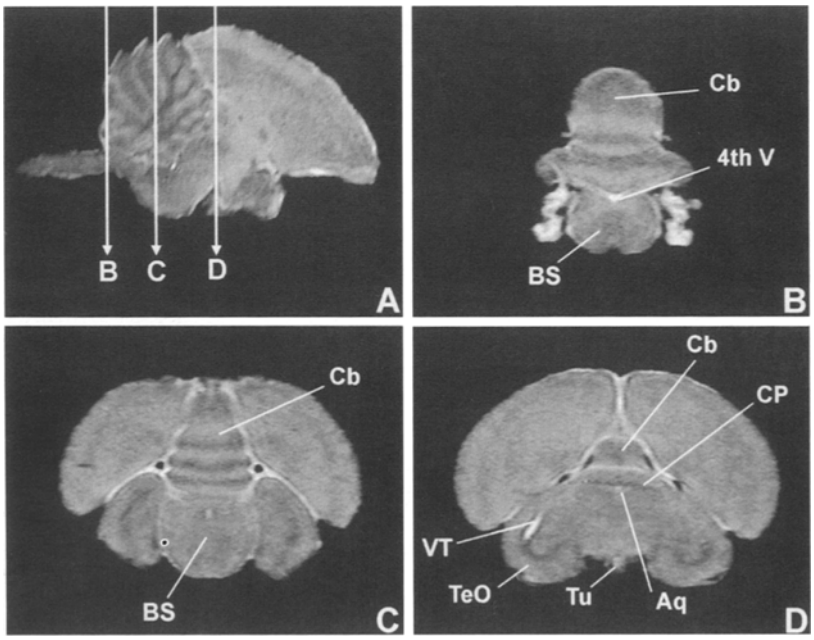

Fig. 2. Set of in-vivo sagittal (A) and coronal (B,C,D) 3D FSE MR images of the brain of a European starling. The image resolution of the sagittal image is 113 μm and for the coronal slices 94 μm in all directions. The vertical lines on the sagittal image indicate the section plane of the coronal images (B–D) and conform the brain atlas of the canary [4]. Aq, Aqueduct of Sylvius; BS, Brain stem; Cb, Cerebellum; CP, Commissura posterior; TeO, Tectum opticum; Tu, Nucleus tuberis; 4th V, Ventriculus 4; VT, Ventriculus tecti mesencephali.

To obtain high resolution slices of the bird brain within a reasonable experimental time, a 3D fast spin echo (FSE) sequence [15,16] was used with an echo train length of four, reducing the average imaging time as compared to a conventional 3D spin echo sequence, by a factor of four. To suppress unwanted stimulated echoes, crusher gradients of 3 ms were applied in the slice direction placed symmetrically around the 180° RF pulses, with increasing amplitudes (29, 32, 38 and 44) mT m^{-1}.

For the canary, MR signals of a 3D volume of $20 \times 20 \times 15$ mm^3 (coronal images) or $22 \times 22 \times 20$ mm^3 (sagittal images) were acquired within a $256 \times 128 \times 64$ matrix. For the European starling, signals of a 3D volume of $24 \times 24 \times 24$ mm^3 (coronal images) or $29 \times 29 \times 29$ mm^3 (sagittal images) were acquired within a $256 \times 128 \times 64$ matrix. The images were taken with a spectral width of 50 kHz and TR/TE = 2000/24 ms (proton density-weighted) for the canary and TR/TE = 2500/35 ms (T2-weighted) for the European starling. The central line of the k-space was sampled at the first echo.

The MR data was reconstructed to an image matrix of $256 \times 256 \times 256$ containing 256 coronal slices of 58 μm with spatial resolution of 78×78 μm^2 or 256 sagittal slices of 78 μm with spatial resolution of 86×86 μm^2 for the canary. For the starling, similar reconstruction procedures led to 256 coronal slices with a resolution of 94 μm in all directions and 256 sagittal slices with a resolution of 113 μm in all directions.

2.3. Image processing

To extract the entire brain from a set of in-vivo images of the bird, a semi-automatic 3D segmentation technique [17] was applied to the 3D MR images data set using an HP 720 workstation. The segmented images were combined to form a 3D volume rendered image. This 3D reconstruction was done with IDL (Interactive Data Language, Research Systems) on a PC.

3. Results

A very mild anaesthesia protocol was developed, as described in the material and methods section, which immobilised the animals sufficiently during the MRI acquisition period. Also a 3D FSE MRI sequence was applied which allowed to obtain an excellent resolution within a period of 70 min (proton density-weighted) or 85 min (T2-weighted). With the 3D FSE sequence, four echoes are scanned in one repetition time thereby acquiring the data 4 × faster than a conventional 3D spin echo. The signal of the high frequency components is lower since it is acquired with longer echo times (TE) resulting in a different Signal Intensity and contrast as compared to conventional 3D SE MRI.

This MR technique allowed the acquisition of detailed images of both canary and starling brains that were collected exactly in the plane of the canary brain atlas of Stokes et al. [4]. This plane of section is not consistent with established mammalian anatomical relationships. Because of the birds upright posture, the brain location or cerebral axis within the skull varies in its alignment to the bill axis. To illustrate this, sagittal images for both the canary (Fig. 1(A)) and the starling (Fig. 2(A)) are included for reference purposes. On these images the slice coordinates of the coronal images are indicated. For the canary, images are displayed from the frontal part of the brain (Fig. 1(B–D)) while the images displayed for the starling are chosen form the more caudal part of the brain, including the cerebellum (Fig. 2(B–D)).

The imaging parameters were chosen such that they highlight the difference between grey and white matter in order to optimise the identification of the different fibre tracts and fibre-associated structures. The major fibre tracts are visible and appear as dark structures on these images. The images displayed of both canary and starling reveal fibre tracts like the tractus septomesencephalicus (TrSM), the commissura anterior (CoA), the commissura posterior (CP), the tractus fronto-archistriatalis (FA) and the chiasma opticum (CO). With the current imaging parameter settings, some brain nuclei could be localised mainly because they are surrounded by fibre structures which allows an appropriate delineation on the MR images. This is for example the case for the nucleus rotundus (Rt), nucleus ovoidalis (OV). Other areas such as the hippocampus could be accurately localised because they are delimited by a clearly visible structure such as the lateral ventricles. Some structures were discerned because they actually appeared with a differential contrast in the images as was clearly the case for the ectostriatum (E).

The brain ventricles themselves were also discerned as hyperintense structures in the proton density weighted images and even better in the more T2 weighted images of the starling. At the levels described in Figs. 1 and 2, one can easily observe the third ventricle (3rd V), the ventriculus lateralis (VL), and at the most caudal levels, the ventriculus tecti mesencephali (VT), the Aqueduct of Sylvius (Aq) and the 4th ventricle (4th V).

Another advantage of the method is that the obtained MRI data are well suited for structural reconstructions as is illustrated in Figs. 3 and 4. The displayed 3D volumes of the different brains are the result of reconstructing the segmented 3D MR images obtained in-vivo at the level of the head of the birds. Fig. 4 also illustrates that the obtained 3D MRI data set can be transferred into images in any other desired direction and that this data set can be observed from any point of view in a volume rendered image. This processing of data allows one to obtain sections in the three orthogonal planes through a particular structure of interest. This provides an excellent 3D view on the structure which greatly facilitates the detailed understanding of its organisation in space and makes it easier to interpret sections that are not in the specific plane of the available brain atlases.

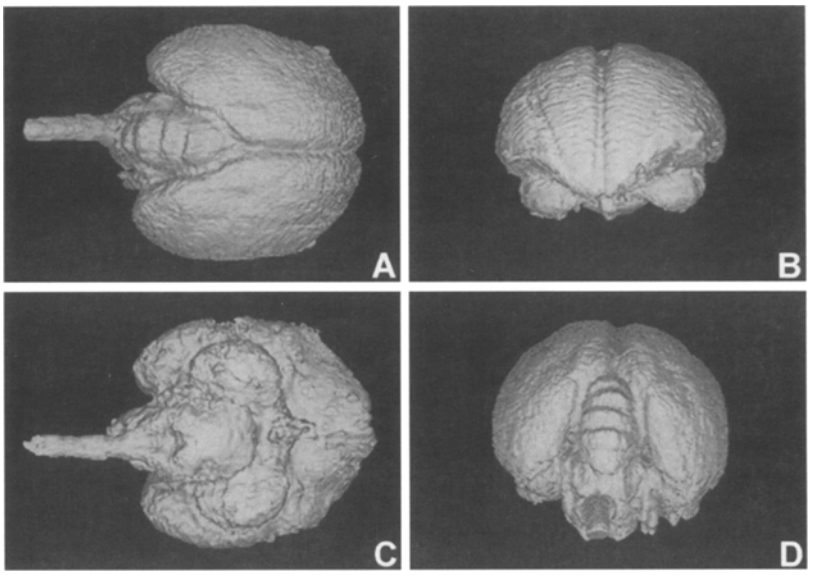

Fig. 3. Surface rendering of a 3D volume reconstruction of the brain of a canary based on an in-vivo 3D set of FSE MR images of the bird obtained in a sagittal plane with a slice thickness of 58 μm: A (top), B (frontal), C (bottom) and D (caudal) views on the 3D volume reconstruction.

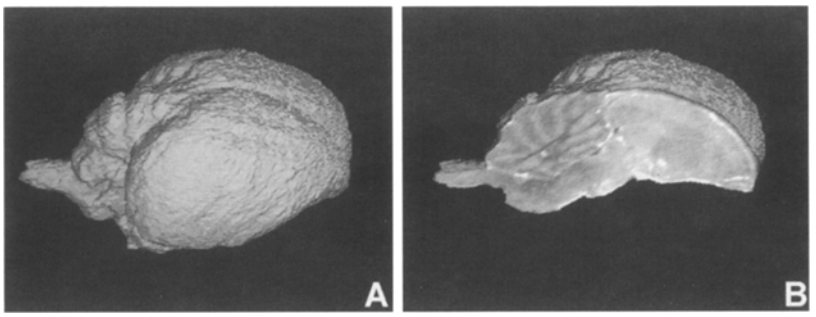

Fig. 4. Surface rendering of a 3D volume reconstruction of the brain of a European starling based on an in-vivo 3D set of FSE MR images of the bird obtained in a coronal plane with a slice thickness of 94 μm (A). Panel B illustrates the possibility to obtain sections in any desiredplane from the 3D data set.

4. Discussion and conclusions

The present results indicate that it is possible to obtain in-vivo, non-invasively 3D MR images from the brain of birds as small as a canary or starling. The entire imaging procedure can be carried out within about 2 h which include the relatively long period needed to position the bird and to adjust the imaging parameters. The birds recovered uneventfully after the anaesthesia and experimental manipulations. The anatomical resolution of the images obtained by the present method is excellent (tenth of millimetres). It allows an unequivocal identification of structures as discrete as the anterior commissure or tractus septomesencephalicus and would definitely be sufficient to perform volumetric reconstruction of specific brain regions such as the olfactory bulbs or optic lobes. A significant amount of comparative work has been devoted previously to anatomical comparisons of these structures in various orders of birds in an attempt to relate brain morphology to behavioural adaptations.

For example, Bang and Cobb [18] compared the size of the olfactory bulbs in 108 avian species and identified a high correlation of the bulb size with the olfactory capacity of the species. Also, Krebs et al. [10,19] found that the relative volume of the hippocampus is larger in bird species that store food than in ones that do not. It is self evident that research relating brain morphology and behaviour across species could easily be extended using MRI. However significant improvement in the anatomical resolution of the MR images and in the discrimination between brain nuclei would be required to make feasible studies of small brain nuclei and their change as a function for example of the season or sex of the subjects. A relevant example is the song control system containing the high vocal centre (HVC), the nucleus robustus archistriatalis (RA) and area X, all nuclei with volumes ranging between 1 and

7 mm^3. The relative volume of one particular brain nucleus in songbirds correlates with the number of different songs that individuals of species can learn [20]. In European starlings, males sing more frequently than females and their song nuclei are approximately 2–3 × larger than those in females [21]. Not only the volumes of these nuclei, but also their location and cell characteristics will determine whether they can be easily discerned with MRI. In this study we could only distinguish nuclei which are surrounded by fibres tracts, as is the case for the nucleus rotundus (Rt), the nucleus ovoidalis (OV), because the imaging parameters were chosen such that they highlight the difference between grey and white matter. However, other sequence parameters can be chosen for a preferential visualisation of particular structures. To that end, an exact determination of the NMR relaxation parameters of the desired structures is needed in order to choose the appropriate imaging sequence. Finally the size of the nucleus is also an important characteristic to consider if one wants to determine its volume and how this volume possibly changes under variable experimental circumstances. These measures will require, in addition to the appropriate imaging sequences providing the desired resolution, an accurate image segmentation procedure necessary to extract the structure of interest.

The in-vivo MR imaging technique, as described above, would also be extremely useful to researchers studying the behavioural impact of localised brain lesions. Electrolytic lesions are clearly visible with this technique and it is therefore conceivable that before engaging in tedious and time-consuming behavioural testing, a researcher may want to know in each individual bird the exact location of a lesion aimed at a given site. This would allow subjects for an experiment to consist only of the animals in which the lesion has been placed correctly. Placement of guide cannulae that will be used for repeatedly injecting drugs in a specific location and study the behavioural or physiological consequences could similarly be confirmed by the MR imaging technique on the live animal, provided however that the cannulae are made of non magnetic material. These capacities should make MRI a powerful new technique for studying brain morphology in birds.

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