MRI of awake rats

J. L. Ulloa[1], D. J. Barker[1], A. T. Doherty[1], and J. C. Waterton[1]
[1]AstraZeneca, Alderley Park, Macclesfield, Cheshire, SK10 4TG, United Kingdom

INTRODUCTION

The vast majority of rodent MR studies have employed anaesthesia for restraint. However, there remain concerns about anaesthetic effects on the animal [1], blood flow, thermoregulation, and effects of metabolism of the anaesthetic on the metabolism of drugs under study [2]. The interval between scans in imaging protocols involving repeated images are constrained by the time required to induce and recover from the effects of the anaesthesia, as well as anaesthesia-for-restraint may be incompatible with regulatory toxicology protocols. While anaesthesia-for-restraint has been avoided in some protocols by using neuromuscular blockers, sedatives, or preparative anaesthesia, these approaches pose other problems. Some previous MRI studies with tightly restrained awake rodents have been reported in cognition and muscle contraction studies [3]. However, tight restraint may also cause stress, suffering, and physiologic abnormalities, as well as motion artefact. Relatively few studies have reported MRI without tight restraint or pharmacologic support [4,5]. In this work we explore the conditions to establish a protocol for MR imaging of the kidney in awake wrapped rats, without pharmacologic support. Animals were acclimatised as proposed by [3, 6, 7, 8].

METHODS

6 healthy male rats were subject to an acclimatisation procedure for 17 days: 5 days of acclimatisation to the animal wrap restrainer, for increasing time periods, starting at 10 minutes on day 1 to 30 minutes on day 5. On days 3, 5 and 7, rats in restraint were placed in an opaque tube of similar dimensions to the MRI scanner. From day 8 onwards noise of the scanner was played while the animal was in the restraint. On days 12 and 15 animals were placed in the MRI scanner in the restrainer while running a dummy procedure. Images were acquired using an MRI system ('Inova', Varian), 4.7T magnet (Oxford Instruments), a 63mm quadrature birdcage volume transceiver, a high power gradient set (300mT/m, rise-time 0.3msec and outer bore 390mm), and software VNMR v1.1.D. Rats were imaged in 2 days: each rat imaging visit lasted no more than 30 minutes restrained in the magnet. Transverse FLASH images including both kidneys and adrenals were acquired (TE/TR=4/60 ms, 41 slices, FOV=64x64mm, matrix size=256x128 and slice thickness = 2mm). After the second period of awake imaging animals were humanely killed, and the imaging procedure repeated immediately post-mortem. Kidneys and adrenals were then removed and weighed.

Images were classified into 5 groups, according to their quality; the higher quality group (images taken post mortem, group 5) were the reference images. A total of 21 images belonging groups 3 (moderate), 4 (good) and 5, kidneys and adrenals were manually segmented in ImageJ. Reproducibility was assessed via Bland-Altman [9] using the average area (i.e. volume/slice) measurements, because the number of slices where the organs appeared was different between images.

RESULTS

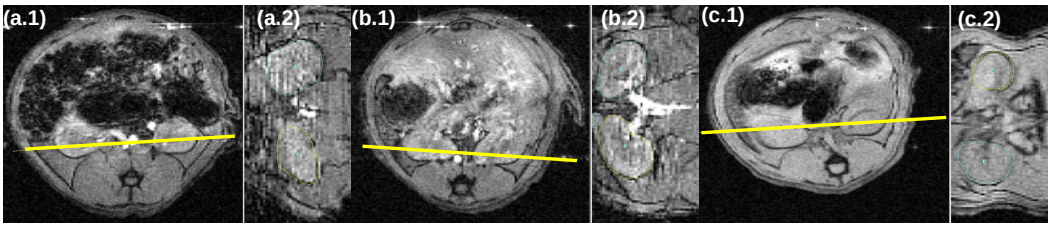

Figure 1: (a-b) Awake animals MR image compared with a (c) reference image taken after necropsy. Transverse slices (a1, b1, c1) were resliced in orthogonal views (a2, b2, c2) to see the motion effect in the whole volume. Despite the low CNR, it was possible to identify the kidneys and adrenals.

In Figure 1 a visual comparison between images of awake animals (1a and 1b) with the reference images (1c) is shown. To visualize the effect of the motion in the whole volume, multislice images were resliced in orthogonal views (1a.2, 1b.2 and 1c.2). Differences between the area estimated with awake animal images and the reference images were compared against the mean between both. In Table 1, the mean and the standard deviation of the differences and the bias in (mm^2) is shown. The average area estimated from the awake animals images were 77, 81, 35 and 44 mm^2 for the right and left kidney and right and left adrenals, respectively. Figure 2 shows the correlation between awake volume and post-mortem volume (dots) or mass (crosses).

Organ	Alive vs Dead			
	Mean	STD	Bias	
			Lower	Higher
Right Kidney	10.08	9.63	-9.18	29.34
Left Kidney	25.76	5.33	15.10	36.43
Right Adrenal	-1.36	11.88	-25.12	22.40
Left Adrenal	6.59	8.56	-10.53	23.72

Table 1: The bias (mean±2*std) indicates limit of agreements between awake animal images and post-mortem.

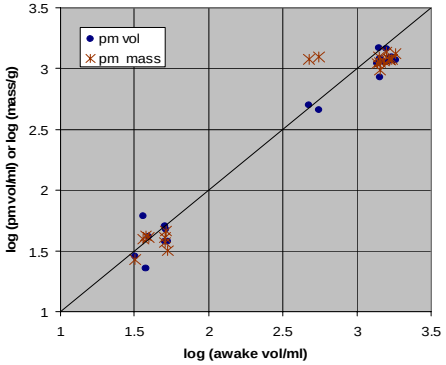

Figure 2. Awake vs post-mortem logarithmic correlation of the kidneys (right top corner) and adrenals (left bottom corner).

DISCUSSION

In this work we explored the feasibility and requirements to perform MRI studies in awake animals. Preliminary results indicate quantitative MRI appears possible in awake, wrapped rats.

REFERENCES: [1]Forsythe, *Lab Anim Sci* 42(1992):497-502;**[2]**Chaves, *Life Sci* 69(2001):213-22;**[3]**Ferris, *J Neuroendocrinol* 18(2006):307-18;**[4]**Checkley Proc SMRM 1984 (146);**[5]**Parzy, *NMR Biomed* 20(2007):615-23;**[6]**King, *J Neurosci Methods* 148(2005):154-60;**[7]**Cline, *Am J Physiol* 274(1998):E381-9; **[8]**Bjork 1999;**[9]**Bland, *Stat Method Med Res* 1999; 8:135-160.