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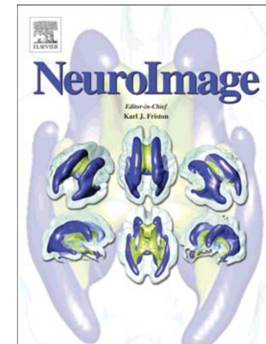

Propofol allows precise quantitative arterial spin labelling functional magnetic resonance imaging in the rat

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Propofol allows precise quantitative arterial spin labelling functional magnetic resonance imaging in the rat

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Abstract

Functional magnetic resonance imaging (fMRI) techniques highlight cerebral vascular responses which are coupled to changes in neural activation. However, two major difficulties arise when employing these techniques in animal studies. First is the disturbance of cerebral blood flow due to anaesthesia and second is the difficulty of precise reproducible quantitative measurements. These difficulties were surmounted in the current study by using propofol and quantitative arterial spin labelling (QASL) to measure relative cerebral blood volume of labelled water ($rCBV_{lw}$), mean transit time (MTT) and capillary transit time (CTT). The ASL method was applied to measure the haemodynamic response in the primary somatosensory cortex following forepaw stimulation in the rat. Following stimulation an increase in signal intensity and $rCBV_{lw}$ was recorded, this was accompanied by a significant decrease in MTT (1.97 ± 0.06s to 1.44 ± 0.04s) and CTT (1.76 ± 0.06s to 1.39 ± 0.07s). Two animals were scanned repeatedly on two different experimental days. Stimulation in the first animal was applied to the same forepaw during the initial and repeat scan. In the second animal stimulation was applied to different forepaws on the first and second day. The control and activated ASL signal intensities, rCBVlw on both days were almost identical in both animals. The basal MTT and CTT during the second scan were also very similar to the values obtained during the first scan. The MTT recorded from the animal that underwent stimulation to the same paw during both scanning sessions was very similar on the first and second day. In conclusion, propofol induces little physiological disturbance and holds potential for longitudinal QASL fMRI studies.

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Introduction

Functional magnetic resonance imaging (fMRI) is a non invasive method of registering cerebral vascular responses resulting from changes in neural activity following activation. Blood oxygen level dependent (BOLD) fMRI is the most commonly used technique in this field and arterial spin labelling (ASL) is also employed. Functional MRI techniques are based on the close relationship between local neural activity, metabolism and changes in the cerebral blood flow (CBF) (Raichle and Mintun, 2006). A major advantage offered by these techniques is the possibility for non invasive longitudinal studies of brain plasticity (Dijkhuizen and Nicolay, 2003) and functional recovery processes in models of brain injury (Mueggler et al., 2001; Schroeter et al., 2009). The tight coupling of regional CBF and neuronal activity was remarked upon by Roy and Sherrington more than a century ago (Roy and Sherrington, 1890). In 1948, Kety and Schmidt first described a method for quantification of CBF in humans using N_2O as a freely diffusible tracer (Kety and Schmidt, 1948). In 1979 Leniger-Follert and Hossmann, using a hydrogen clearance method, showed that stimulation of the cat forepaw resulted in an increase in the microcirculatory blood flow to the relevant sensory cortical area which accompanies enhanced neuronal activity (Leniger-Follert and Hossmann, 1979). This hyperaemia was later shown to be correlated to an increase in neuronal metabolism (Raichle, 1994).

When various peripheral nerves are stimulated electrically corresponding regions of the primary somatosensory cortex are activated. By applying stimulation to different areas of the body the somatosensory cortex can be mapped topographically. Previous studies of electrical mapping of the exposed brain have been confirmed using fMRI for cortical localisation of the body’s surface. In the rat, fMRI has been successfully used for the mapping of the forepaw (Hyder et al., 1994; Masamoto et al., 2007; Van Camp et al., 2006), the hindpaw (Bock et al., 1998), the tail (Spenger et al., 2000) and the barrel zones of the whiskers (Lu et al., 2005) .

Blood oxygenation level-dependent (BOLD) contrast, first described by Ogawa and colleagues (Ogawa et al., 1990), is the most widely used fMRI technique. BOLD fMRI is based on the diamagnetic properties of haemoglobin when oxygenated and its paramagnetic properties when deoxygenated (Laguenie et al., 1983). The BOLD

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signal relies on the blood concentration ratio of oxyhemoglobin to deoxyhemoglobin. During stimulation there is an increase in activation of the cerebral cortex, this leads to an increase in metabolic activity and an increase in the cerebral blood flow. Due to a mismatch between the cerebral blood flow and the metabolic rate, an increase in the ratio of oxygenated to deoxygenated haemoglobin occurs; this causes an increase in the BOLD signal. BOLD contrast is dependent upon many physiological parameters including cerebral blood flow, cerebral blood volume, venous system, vessel drainage, vessel size and oxygen consumption in a complex fashion (Kim et al., 1997).

Arterial spin labeling (ASL) is an MRI technique capable of providing a more quantitative assessment of cerebral perfusion. The technique uses either inversion or saturation pulses to label the magnetization of the water molecules of cerebral arterial blood (Detre et al., 1992; Williams et al., 1992). Labelled water molecules arrive by perfusion to the slice of the brain being imaged where they undergo capillary exchange with interstitial fluid. Consequently, the longitudinal magnetization of brain tissue is altered and contrast is provided between perfused and stationary tissue. In order to remove the contribution of tissue to the labelled image, a control image of the plane of interest is acquired in which the inflowing arterial blood is not labelled. Labelled and control images are generally acquired in a temporally interleaved fashion and difference images are obtained by subtracting adjacent pairs of control and labelled images. These difference images provide a qualitative assessment of perfusion (Petersen et al., 2006). ASL has been applied to functional imaging of the rat somatosensory cortex forelimb region (S1FL) during electrical stimulation of the forepaw under alpha chloralose anaesthesia (Kerskens et al., 1996).

ASL has advantages over other functional imaging methods; it has been reported to provide better localised mapping of neural activity compared to BOLD (Lee et al., 2002; Wang et al., 2003). ASL may also be superior to BOLD in tasks lasting longer than 1-2 minutes (Aguirre et al., 2002). Slow temporal drifts in BOLD signals are not seen with ASL signals, due to the interleaved nature of the acquisition of labelled and control images with this latter technique (Wang et al., 2003). One of the main advantages of ASL is its ability to provide quantitative measurements of cerebral blood flow changes (Detre and Alsop, 1999). It has been suggested that ASL methods may be superior to BOLD in tracking changes in neural activity (Silva, 2005). A

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recently developed method of bolus tracking ASL yielded data on mean transit time (MTT) and capillary transit time (CTT) (Kelly et al., 2010). MTT has been interpreted as the time taken for the labelled spins to traverse the vasculature. The CTT encompasses the effects that cause dispersion of the labelled bolus on its transit from the labelled plane to the imaging site (such as pseudo-diffusion within the microvasculature and water exchange between the capillaries and tissue). As all fMRI techniques are highly reliant on the blood perfusion state of the brain, the animal must be kept in a stable physiological state. With the employment of Kelly's methods the relative cerebral blood volume of the labeled water ($rCBV_{lw}$) can also be measured. The labelled water that is measured is dispersed in both intra and extra vascular spaces.

Under general anaesthesia blood pressure, respiration, and heart rate are depressed compared to the conscious state (Sicard et al., 2003). General anaesthetics also depress the metabolic activity of the central nervous system (CNS) and therefore reduce basal CBF (Ueki et al., 1992). Many inhalational anaesthetics cause cerebral vasodilatation, and therefore tend to increase CBF (Van Hemelrijck et al., 1993). All these factors frustrate the objective of imaging functional hyperaemic responses. One of the main difficulties of using fMRI is lack of a suitable anaesthetic agent.

Alpha chloralose is a commonly used anesthetic in ASL (Franke et al., 2000; Kerskens et al., 1996); it preserves neural activity and hemodynamic coupling (Ueki et al., 1992), and has been widely used during forepaw stimulation (Duong et al., 2000; Mandeville et al., 1998; Silva et al., 1999). However it decreases cerebral blood flow (Austin et al., 2005), and due to its toxic nature it cannot be used for recovery experiments making it unsuitable for longitudinal studies (Silverman and Muir, 1993). Urethane has been used during electrical whisker stimulation in the rat (He et al., 2007), but while it preserves neuronal activation it has adrenoceptor agonist action that disturbs CBF and, like alpha chloralose, it is non suitable for recovery experiments. Ketamine has been used in the monkey during hypercapnic challenges (Zhang et al., 2007), but when used alone it causes muscle hypertonicity and has undesirable effects on blood flow and blood pressure. Rat forepaw stimulation has been carried out under medetomidine sedation using BOLD (Weber et al., 2006) and ASL (Kelly *et al.* 2009b.), but a reproducible BOLD response is not seen until 60-90

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minutes after sedative administration (Weber et al., 2006). Isoflurane has been used during forepaw stimulation in the rat (Liu et al., 2004), but it acts as a cerebral vasodilator and raises basal CBF (Hendrich et al., 2001). Studies using only Mivaurium, a neuromuscular blocker, have been carried out in rats (Peeters et al., 2001) but paralysis without concomitant anaesthesia is unethical and prohibited by law. Experiments have also been carried out on restrained conscious animals using BOLD imaging (Ferris et al., 2001; Lahti et al., 1998; Logothetis et al., 1999; Wyrwicz et al., 2000) and ASL imaging (Sicard et al., 2003), in an attempt to preserve neuronal coupling. Not only can motion artefact can cause a problem with this approach, but there is also associated neural activation from stress and motor cortical activation (Willis et al., 2001).

Propofol is an intravenous short-acting anaesthetic agent (Vasileiou et al., 2009) introduced in the 1980's and positively modulates gamma-aminobutyric acid ($GABA_A$) receptors (Dong and Xu, 2002; Trapani et al., 2000). In a recent study, four anaesthetics; isoflurane, propofol, ketamine, and alpha chloralose, were studied for their effect on cerebral blood perfusion in the striatum. Both alpha chloralose and propofol anaesthesia decreased cerebral perfusion by more than the other two anaesthetics (Bruns et al., 2009). BOLD studies have been carried out using propofol anesthesia during hindpaw stimulation in the rat (Lahti et al., 1999), medial nerve stimulation (Scanley et al., 1997) and during visual stimulation in dogs (Willis et al., 2001), but there are no reports of its application to ASL imaging.

The aim of this study was to develop an optimal anaesthetic protocol for longitudinal animal fMRI studies. Both ASL and BOLD were employed during this study: BOLD provided qualitative evaluation of the activation, whereas ASL provided both qualitative and quantitative assessment of the haemodynamic response to neuronal activation. The quantitative ASL approach, when combined with a recoverable anaesthetic protocol, permits changes in perfusion parameters to be tracked over time.

Materials and Methods

Experimental animals

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All experiments were conducted in accordance with protocols approved by the animal ethics committees of both University College Dublin and Trinity College Dublin. These procedures were licensed by the Irish Department of Health and Children. A total number of 5 adult female Wistar rats (230 - 250 g) were used in the study. Animals were kept at a 12/12 hr light/dark cycle and had access to water and a standard diet *ad libitum*.

Animal preparation

All animals were anaesthetised with 5% isoflurane (Isoflo, Abbott, Queenboro, England) in oxygen (1L/min) and maintained with 1.5-2% isoflurane. The level of anaesthesia was regularly monitored throughout the procedure using the pedal withdrawal reflex to toe pinch and the corneal reflex. The tail vein was cannulated with a 22G intravenous cannula. The isoflurane was discontinued and the animal was allowed to recover from its effects before i.v. bolus administration of propofol (7.5mg/kg) (Rapinovet ®, Schering - Plough). Six minutes following the loading dose the animals were started on continuous infusion of the drug (45mg/kg/hr i.v.).
Under a full and stable surgical plane of anaesthesia, the femoral artery was cannulated. This allowed blood gas samples to be taken throughout the experiment. The femoral artery was not cannulated in the animals used for the repeat study. Once surgery was complete the animals were placed prone in a Plexiglas cradle with a three point-fixation system (tooth-bar and ear pieces). Electrodes (Grass subdermal electrodes, grass technologies, Rhode Island, US) were placed between the 2nd and 5th digit of the animal's right forepaw. Electrocardiographic electrodes (SA Instruments Inc., Stony Brook, New York, USA) were placed on the animal's chest and the animal's heart rate was monitored throughout the experiment. Temperature was monitored with a rectal thermometer and maintained constant at 37°C using a warming surface controlled by a water pump-driven temperature regulator. The respiration signal was monitored using custom hardware and software (SA Instruments Inc., Stony Brook, NY, USA). The animals where then placed in a 7T, 30 cm bore animal MRI system (Biospec 70/30, Bruker Biospin, Ettlingen, Germany) scanner with a circular polarized 1H rat brain RF coil (Bruker, BioSpin). A 7cm diameter volume coil was used for transmission of the ASL and FLASH excitation pulses. Signal detection was performed using a surface coil.

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Repeat study

Two animals were included in a repeat study and prepared as described above; study A and study B. A slightly higher bolus dose of propofol was administered in the repeat study (8mg/kg i.v), the infusion rate was the same as the first day (45mg/kg/hr i.v). The femoral artery was not cannulated on the first day; however arterial blood samples were only taken during the second study. Following the first scan, the animals were rescanned 72 hours later. Stimulation was applied to the right forepaw during the first and second scan in study A. Stimulation was applied to the right forepaw of the animal included in study B during the initial scan, the stimulation in the repeat scan was applied to the left paw. Only the QASL fMRI protocol was run on the second day of scanning, previous work carried out has shown that BOLD is not reliable for longitudinal studies (Siewert et al., 1996)

Stimulation protocols

In each experiment an ASL protocol was run during right forepaw stimulation, this was followed by a BOLD protocol during the same stimulation.

<u>ASL forepaw stimulation</u> A square pulse nerve and muscle stimulator (Grass Technologies Inc., West Warwick, RI, USA) was used to electrically stimulate the right forepaw (10V ± 0.5v, 10 Hz, 1 ms duration pulses) for the duration of the labelling phase of the ASL protocol (approx. 45 s). The current applied was approximately 2.3mA.

<u>BOLD forepaw stimulation</u> The same apparatus was used to electrically stimulate the right forepaw. A paradigm of 30 seconds resting period (10 images) followed by 30 seconds of an activation period (10 images) was used. This protocol was repeated 3 times resulting in acquisition time of 3 minutes.

Previous studies carried out using QASL methods tested various frequencies along with a range of voltages. The optimal stimulation protocol was applied in this study. Similar pulse duration and frequency was applied during direct nerve stimulation, with no effect on the physiological parameters of the animal (Cho et al., 2007).

A further study was carried out on the bench to determine the fibre type stimulated with the above protocol. One female Wistar rat was anesthetised with urethane (20% solution, 1.5g/kg). Stimulating electrodes (Grass subdermal electrodes, grass

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technologies, Rhode Island, US) were placed in the same position as described above. The brachial plexus was exposed; the nerve was place on a bipolar silver recording electrode. The velocity of the nerve was recorded following stimulation of 10V, 20V, 30V, 40V, 50V and 60V. As the length from the stimulation sight to the recording sight was 40mm latencies exceeding 20ms were considered to be associated with unmyelinated C-fibres (velocity <2m/s). Stimulation at 10V activated fibres with a latency of 1.04ms indicating that at this voltage myelinated fibers are stimulated (figure 1A). The voltage was increased by increments of 10V, at 30V all fibers stimulated have latencies shorter than 10ms, again indicating their myelinated nature (figure 1B). Only with application of 60 volts were long latency C-fibres recruited (figure 1C).

High Resolution Anatomical Scan

Forepaw somatosensory cortex (S1FL) was located in all animals using a rapid acquisition with relaxation enhancement (RARE) (Hennig et al., 1986) high resolution anatomical scan and compared to a rat brain atlas in order to locate the slice with optimal S1FL coverage (Paxinos and Wartson, 2005). Scan protocol: slice thickness = 2 mm, TE = 12 ms, TR = 3.134 s, FOV = 3.0 x 3.0 cm, image matrix = 256 x 256, RARE factor = 6. This imaging slice was then used for the subsequent ASL sequence.

ASL MRI sequence

The bolus-tracking ASL sequence was used to provide ASL signal-time curves (Kelly et al., 2009) The sequence consisted of a 5 s preparation interval containing the inversion pulse, followed by snapshot fast low angle shot (FLASH) gradient echo image acquisition. The protocol consisted of an inversion pulse and two varied delays (Kelly et al., 2009), allowing the signal-time curve to be plotted (Figure 2). The inversion pulse had a bandwidth of 2.8 kHz and the gradient strength was set to 14mT m^{-1}, this provided an inversion region thickness of 4.7mm. The imaging slice was centred on the S1FL region, at 0.2mm anterior to Bregma (Paxinos and Wartson, 2005). The tagging location was 2cm proximal to the imaging slice, and the pulse frequency was offset by -12kHz. The control image had an offset of +12kHz. The labelled images were subtracted from the control images to provide a perfusion map. The following parameters were used: slice thickness = 2 mm, repetition time (TR) =

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6.66 s, echo time (TE) = 2.99 s, RF flip angle = 30^0, field of view = 3.0 x 3.0 cm, image matrix = 128 x 64, receiver bandwidth = 50 kHz.

T1 measurement

The RARE sequence (Hennig et al., 1986) with variable repetition time (VTR) was used to generate T1 maps at the same imaging location as the ASL images. Maps from n=5 similar animals to those used in the ASL measurements were generated and subsequently averaged to provide an average T1 value of 1.74 s for the S1FL region. This T1 value was used when fitting the modified Focker-Planck equation (Kelly et al., 2010) to the btASL data. The following parameters were used for the RARE-VTR acquisitions: slice thickness = 2 mm, VTR = 0.3 s, 0.59 s, 0.94 s, 1.40 s, 2.03 s, 3.10 s and 8.00 s, TE = 25.27 ms, FOV = 3.0 × 3.0 cm, image matrix = 128 × 64, RARE factor: 6.

Qualitative ASL Data analysis

Arterial spin images were analysed using Scion image (Scion corporation™). For each experiment 16 images were captured, 8 images with spin labelling and 8 corresponding control images. The two stacks were averaged and the average labelled image subtracted from the average control image. The signal intensity within the S1FL region was calculated during activation and in the resting state.

Quantification of ASL

The region of activation from forepaw stimulation was selected using ImageJ ROI tool (Rasband W.S., Bethesda, MD, USA). The mean transit time and capillary transit time were quantified in the presence and absence of forepaw stimulation using the bolus tracking ASL method recently described by Kelly (Kelly *et al* 2009b). Briefly, a non-compartmental model of cerebral perfusion was fitted to the bolus tracking ASL data. The MTT and CTT were calculated from the first and second statistical moment of the signal-time curves, respectively (Figure 2). The relative cerebral blood volume of labelled water was also calculated from figure 1. The amplitude of the fitted curve is directly proportional to the area under the curve (Kelly et al., 2010). This parameter was therefore used to estimate the $rCBV_{lw}$ during resting state and neuronal activation.

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BOLD MRI sequence

A coronal T2*-weighted fast gradient echo (FLASH) sequence was acquired using the following parameters: 1.5mm, repetition time (TR) = 50ms, echo time (TE) = 10ms, RF flip angle = 30^0, field of view = 3cmx3cm, image matrix = 128 x 64, receiver bandwidth = 25,000Hz. Giving a resolution of 0.234 x 0.469 x $1.5mm^3$ and a total acquisition time of 3mins 12 sec.

BOLD Data analysis

BOLD data was analysed using Interactive Data Language (IDL; ITTVIS, Boulder, CO, USA); the percentage signal intensity change was calculated by averaging T2* signal intensity during activation (iterations 11-19, 31-39 and 51-59) and normalised to the signal intensity during rest (iterations 4-9, 21-29 and 41-49). Statistical parametric maps were generated by thresholding difference images between activated and resting repetitions of the same scan.

Statistical analysis

All statistical analysis was carried out using GraphPad prism (GraphPad software Inc., Avebuda de la playa, La Jolla, CA, USA). All results are presented as mean ± SEM unless otherwise stated. Statistical analysis was performed using Student's two-tailed paired t test, unless otherwise stated. The criterion for statistical significance was $p<0.05$.

Results

Cardio respiratory indices

The heart rate (HR), respiration rate (RR), and arterial blood gases were monitored during each experiment. The mean HR and RR in the animals were HR= 339.7 ± 19.3 (all results presented as mean ± S.E.M.) beats per minute, RR = 71.7 ± 2.7 breaths per minute. The arterial blood gases in the same animals were pCO_2 = 44.6 ± 1.1mmHg, pO_2 = 105.2 ± 3.3mmHg and pH = 7.4 ± 0.007. All animals were stable for the duration of the experiment and indices were within the normal physiological range. There was no change is respiration rate or heart rate during stimulation.

Assessment of activation following forepaw stimulation using arterial spin labelling and BOLD fMRI techniques

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This study employed both ASL and BOLD fMRI techniques to locate the activated region. Both of these methods successfully showed activation in the forepaw representation of the somatosensory 1 cortex following stimulation of the right forepaw, figure 3. Reproducible ASL activation maps were acquired approximately 5 minutes following the start of the continuous propofol infusion. Figure 4 shows the BOLD and ASL maps for all five animals. Four of the five animals scanned showed BOLD activation. Figure 5 shows the BOLD signal intensity graph for animal 2. The average percentage BOLD signal intensity increase following stimulation in the four animals that showed activation was 4.2%.

Signal intensity changes

The signal intensity in control image was 0.09 ± 0.004 (mean ± SEM) following stimulation, this intensity increased to 0.13 ± 0.003 ($p<0.0002$; Student's two-tailed paired t test) (figure 6). A percentage increase of 45% was recorded following activation. The percentage co-efficient of error for the signal intensity following activation was 29%.

Relative cerebral blood volume of labelled water

The $rCBV_{lw}$ increased following neuronal activation, from 0.106 ± 0.002 to 0.130 ± 0.003, ($p<0.0039$; Student's two-tailed paired t test) (figure 7). An average percentage increase of 12% following forepaw stimulation was recorded.

Mean Transit time and Capillary transit time

Both the MTT and the CTT decreased in all animals during forepaw stimulation. The MTT decreased from 1.97 ± 0.06 s to 1.44 ± 0.04 s ($p = 0.0012$, two-tailed paired Student's t test) and the CTT decreased during stimulation from 1.76 ± 0.06 s to 1.39 ± 0.07 s ($p = 0.0214$). The MTT decreased by 30% following activation and the CTT decreased by 21% following activation (figure 8).

Longitudinal study

The baseline values for signal intensity, $rCBV_{lw}$, MTT and CTT were remarkably similar when the same animals were re scanned after 72 hours under propofol anaesthesia (tables 1, 2 and 3). The signal intensity of both animals following activation was similar during the initial and the repeat scan. The MTT and the CTT

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were both slightly slower following activation in the second day compared to the results gathered during the first scan.

The rCBVlw in study A (same forepaw was stimulated during the first and second scan) increased from 0.102 a.u. to 0.126 a.u. following forepaw stimulation, a percentage increase of 23.5%. During the second the second scan day this increase was slightly lower: (14.3% an increase from 0.098 a.u. to 0.112 a.u). The animal in study B (different forepaws were stimulated during the first and second scan) also reflected a similar trend, during the first scan day the rCBVlw increased from 0.109 a.u. to 0.125 a.u. (14.7 % increase). The degree of change in the same animal on the second day was very similar (17.6% increase). The rCBVlw increased from 0.019 a.u. to 0.140 a.u.

The MTT in study A decreased from 2.10s to 1.48s, a decrease of 29.5% on the second day this decrease was slightly less at 25.9%, a decrease from 2.04s to 1.51s. The CTT during the first scan decreased from 1.90s to 1.41s a decrease of 25.7%. This degree of change was smaller on the second scan; the CTT decreased from 1.63s to 1.60s, a change of 1.8%.

The second animal (study B) included in the longitudinal study also shows a smaller degree of change in both the MTT and CTT on the second day of scanning compared to the first. The MMT during the initial scan decreased from 1.98s to 1.36s following activation a percentage decrease of 31.3%. Following stimulation on the second day, the MTT was slightly lower; a decrease from 1.90s to 1.66s a percentage decrease of 12.6%. The CTT in the same animal also showed a similar pattern, during the first day of scanning the time transit time decreased from 1.65s to 1.21s following activation a percentage decrease of 27.1%. The second day of scanning showed a decreased transit time: (1.70s to 1.56s) following activation, a decrease of 8.2%

Discussion

To the authors' knowledge this is the first report of ASL fMRI during propofol anaesthesia in the rat. Both ASL and BOLD fMRI data were acquired and compared in the same animal. In each of the five animals an ASL protocol was run during right forepaw stimulation followed by a BOLD block experiment. Figure 4 illustrates the activation in all five animals during both protocols. In all the ASL and BOLD

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activated images, the region of activation is well localised to the somatosensory 1 forelimb region of the contra lateral side of stimulation. ASL activation is present in all animals, images A-E. Bold activation is only present in images F-I. Previous studies have indicated that perfusion imaging techniques show a greater sensitivity to the hemodynamic response, compared to BOLD (Aguirre et al., 2002; Wang et al., 2003).

It has been suggested that ASL is more sensitive to feeding arteries in the capillary bed compared to BOLD which is influenced by large draining vessels (Tjandra et al., 2005; van Gelderen et al., 2005). Perfusion fMRI and BOLD has previously been compared in the human (Aguirre et al., 2002; Lee et al., 2002; Wang et al., 2003) and in isoflurane anaesthetised rats during forepaw stimulation (Liu et al., 2004), where it was reported that perfusion based fMRI provided better localisation of neuronal activation than BOLD.

The perfusion maps show low variability in signal intensity in the resting state, and the increase in signal intensity in response to activation is also consistent. This finding is in agreement with previous studies (Tjandra et al., 2005) that have shown low inter-subject variability when using perfusion fMRI. The MTT and CTT were both found to decrease significantly during electrical stimulation of the forepaw. The MTT is proportional to 1/CBF and the CTT is proportional to $1/CBF^2$ (Kelly et al., 2010). It follows that a decrease in both transit times is indicative of an increase in CBF. These findings are in agreement with previous studies, both MRI and non-MRI, that have shown focal increases in CBF during neuronal activation (Jones et al., 2001; Li et al., 2000). The consistency in these perfusion indices in both the resting and activated state suggests that the animals are maintained in a stable physiological plane, a fact borne out by the cardio respiratory parameters measured, which remained within normal limits. An advantage of this protocol is the very low variability between subjects across all measurable parameters (signal intensity, relative cerebral blood volume of labelled, MTT and CTT), this indicates that the use of this drug provides reproducible basal and evoked ASL states. A previous study employed the same quantitative arterial spin method which is described here, during fMRI acquisition the sedative, medetomidine was administered to the animal (Kelly et al., 2010). The $rCBV_{lw}$ in the control state in animals administered with medetomidine was 0.086 ±

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0.003a.u. which is less than the value in animals anesthetised with propofol: (0.106 ± 0.002a.u.). Kelly's study recorded an increase of in $rCBV_{lw}$ from 0.086 ± 0.003 a.u. to 0.096 ± 0.005a.u during forepaw stimulation. The work that is presented in the present study shows the $rCBV_{lw}$ is slightly higher when compared to the medetomidine study; an increase from 0.106 ± 0.002 a.u. to 0.130 ± 0.003 a.u. The MTT during the resting state under medetomidine was 1.94 ± 0.08s, following forepaw stimulation, this decreased to 1.62 ± 0.09s, a reduction of 16%. The CTT decreased from 1.76 ± 0.13s to 1.30 ± 0.09s following peripheral stimulation, a decrease of 26%. The MTT and the CTT during rest and activation recorded in Kelly's paper were very similar to the results presented here. The results presented here suggest that medetomidine causes a decrease in relative cerebral blood volume of labelled water during resting and activated states. The low $rCBV_{lw\ in}$ the control scan may be due to vasoconstriction caused by medetomidine, this constriction may also be sustained during stimulation. It also should be noted that different stimulation protocols were employed during both studies, this may also have an effect on the significant difference in $rCBV_{lw}$ following stimulation during both experiments. One of the main advantages of using propofol over medetomidine is that propofol provides better success rates during the acquisition of ASL data (unpublished observations).

The longitudinal study presented in this current study under propofol yielded very similar signal intensity changes. The signal intensity reflects the volume of labelled water in the activated S1FL, which is in turn an indicator of the blood volume in that region. The $rCBV_{lw}$ was quantified in both animals on both scan days and varied little from the initial scan to the repeat scan, during rest and activated states. In both animals there was an increase in the $rCBV_{lw}$ following peripheral stimulation. The $rCBV_{lw}$ recorded in both animals on both days were very similar. These results indicate good reproducibility in both basal and activated states over time. As previously mentioned; MTT is proportional to 1/CBF and the CTT is proportional to $1/CBF^2$ (Kelly et al., 2010). The MTT and the CTT were also similar in the resting state during the first and second day of scanning. This indicates that propofol kept the animal in a stable physiological condition during repeat doses of the drug. Both the MTT and the CTT decreased following stimulation to the forepaw, this decrease was recorded on both days of scanning. The degree of change following the application of stimulation in study A, on the first and second day of scanning was very similar. This

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suggests that the use of MTT might be a good parameter for tracking subjects over time. Interestingly the degree of CTT change in the same animal during the initial and the repeat scan was quite different. One possibility is that brain capillary permeability to water varies more than cerebral blood flow. The results recorded in study B on the second day of scanning show a smaller change in MTT and CTT compared to the initial scan. The mostly likely reason for the difference recorded following stimulation on both days is that different forepaws where stimulated. What is interesting at this point is the increase in signal change on both days is identical, along with the volume of labelled water delivered to the region of interest. This indicates no difference in blood volume during activation in the left or right somatosensory cortex, but a smaller functional hyperaemic response to the right hemisphere compared to the left. It should be noted at this point that the rats were administered with a loading bolus that was slightly higher on the second day (8mg/kg), although the infusion rate of propofol on both days was the same. It is unlikely that this had an effect on the results, as the animal signal intensity, MTT and CTT at rest were very similar on both days. The BOLD protocol was only run on the first day, previous work carried out has shown that BOLD is not reliable for longitudinal studies (Siewert et al., 1996)

In the past propofol has been used in BOLD fMRI studies. In 1999, a study was performed which compared cortical responses in conscious and propofol-anesthetized rats. There was a 1-6% increase in cortical signal compared to a 6-26% increase in the conscious animals, indicating some dampening of response by propofol (Lahti et al., 1999). The medial nerve in rats has also been stimulated and the cortical response imaged using a BOLD protocol, 2-2.7 % change was seen in signal intensity (Scanley et al., 1997). A study carried using visual stimulation during BOLD fMRI in dogs recorded no qualitative differences in images obtained in three anaesthetics; isoflurane, propofol and fentanyl (Willis et al., 2001).

Propofol anaesthesia preserves cardio respiratory status during experimentation and this may explain the high success rate with ASL fMRI imaging. The drug exhibits many useful properties including a rapid onset with minimal side effects (Glen, 1980) and is rapidly eliminated from the body. When continuously infused at a slow rate there is little change to heart rate and respiration rate (Koizumi et al., 2002). Propofol does not cause cerebral vasodilatation as do inhalational agents (Van Hemelrijck et

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al., 1993), but rather propofol induces vasoconstriction, decreases cerebral blood volume (Cenic et al., 2000; Werner et al., 1993) and relative cerebral blood flow (Kaisti et al., 2002). This latter effect possibly enhances the signal to noise ratio of functional hyperaemic responses as blood flow is elevated from a lower basal level. Propofol also decreases the cerebral metabolic rate of O_2 (Enlund et al., 1997) and cerebral metabolism (Alkire et al., 1995; Dam et al., 1990). However, it does not accumulate and this facilitates long term use (Hedenquist, Hellebrkers, 2003) and places it as the drug of choice for prospective animal fMRI studies. Currently propofol is used in longitudinal paediatric neuro-imaging; it has been described as a safe and effective method of sedation (Amundsen et al., 2005).

The use of anaesthesia in fMRI is not ideal as it suppresses basal cerebral blood flow (Sicard et al., 2003; Ueki et al., 1992), but without anaesthetics the images would be subject to movement artefact particularly in perfusion imaging. The user must be aware of the disadvantages when using anaesthetics; alpha chloralose decreases cerebral blood flow (Austin et al., 2005) and along with respiratory depression. Isoflurane acts as a cerebral vasodilator (Hendrich et al., 2001) and reduces the CBF response following peripheral stimulation (Masamoto et al., 2007).

Conclusion

This study has established a protocol for propofol anaesthesia during both ASL and BOLD fMRI in a spontaneously breathing rat during peripheral nerve stimulation. The protocol which has been described in this study may allow $rCBV_{lw}$, MTT and CTT to be tracked in longitudinal studies to investigate changes in the haemodynamic response to neuronal activation over time over time. The protocol holds much promise for future studies of cerebrovascular studies.

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Figure 1. Brachial plexus compound action potential. An Electroneurogram was recorded from the brachial plexus following stimulation applied to the forepaw of the rat. Trace A illustrates the action potential following 10V, B is an action potential recorded following 30V and trace C is following stimulation of 60V. The conduction distance was 40mm and therefore velocity measurements for individual peaks of the compound potential were calculated and shown in all 3 traces.

Figure 2. ASL signal-time curve. Least-squares fit of the ASL signal-time data (yellow and red asterisks for control and activation experiments respectively) to the non-compartmental model of cerebral perfusion (blue and green solid lines for control and activation experiments respectively).

Figure 3. Functional MRI of somatosensory cortex

(A) ASL fMRI activation in forepaw representation in the somatosensory cortex. (B) BOLD fMRI activation in forepaw representation in the somatosensory cortex. (C) High resolution image acquired following forepaw stimulation. Activation in both the ASL fMRI and BOLD fMRI are both in the same slice. Image coordinates: -0.2mm from bregma. (D) Coronal plate from "The Rat Brain" (Paxinos *et al.*, 1998). Plate coordinates: -0.2mm from bregma. Both the activation in the ASL and BOLD fMRI image is in the forelimb representation in somatosensory 1 cortex.

Figure 4. Comparison of ASL and BOLD for all animals

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Images A – E Arterial spin labelling fMRI images in 5 animals during forepaw activation. Images F-J BOLD fMRI images in same 5 animals during forepaw stimulation. ASL protocol was run first immediately followed by BOLD protocol. ASL activation is present in all animals. BOLD activation is present in animals F-I.

Figure 5. BOLD Signal intensity graph during rest and activated state.
This graph represents the BOLD signal intensity change of animal two during control and activate state, there was a 7.7% increase in the signal following stimulation to the forepaw.

Figure 6. Signal intensity change in control and activated image following forepaw stimulation using arterial spin labelling. Horizontal bar indicates the mean, n=5.

Figure 7. Transit times during activation of somatosensory cortex. MTT (A) and CTT (B) in propofol anesthetised animal during control and activated states. Horizontal bar indicates the mean.

Figure 8. Relative cerebral blood volume of labelled water. Horizontal bar indicates the mean, n=5.

Table 1. **Singal intensity in the longitudinal study** (n=2, study A and B); both animals were initially scanned at time point 0 and following 72 hours the animal was rescanned. The animal include in study A underwent electrical stimulation of the right forepaw on both days. The animal included in study B, underwent right paw stimulation on the first day and left paw stimulation on the second day. The signal intensity during the control image and activated image on both days were similar in value. These values correspond closely to the average group data.

Table 2. Relative cerebral blood volume of labelled water in the longitudinal study (study A and B corresponds to the same animal in the previous table). The

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relative cerebral blood volume of labelled water was measured during rest and activated state on 2 days.

Table 3. MTT and CTT in the longitudinal study (study A and B corresponds to the same animal in the previous table). The mean transit time (MMT) and capillary transit time (CTT) were measured during rest and activated state on 2 days.

Table 1

Study A

Experimental Day	Control (a.u.)	Activated (a.u.)
1	0.08	0.13
2	0.08	0.12

Study B

Experimental Day	Control (a.u.)	Activated (a.u.)
1	0.09	0.13
2	0.1	0.14

Table 2

Study A

Experimental Day	Control (a.u.)	Activated (a.u.)
1	0.102	0.125
2	0.098	0.112

Study B

Experimental Day	Control (a.u.)	Activated (a.u.)
1	0.109	0.125
2	0.119	0.140

Table 3

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Study A

	MTT		CTT	
Day	Control (s)	Activated (s)	Control (s)	Activated (s)
1	2.10	1.48	1.90	1.41
2	2.04	1.51	1.63	1.60

Study B

	MTT		CTT	
Day	Control (s)	Activated (s)	Control (s)	Activated (s)
1	1.98	1.36	1.65	1.21
2	1.90	1.66	1.70	1.56

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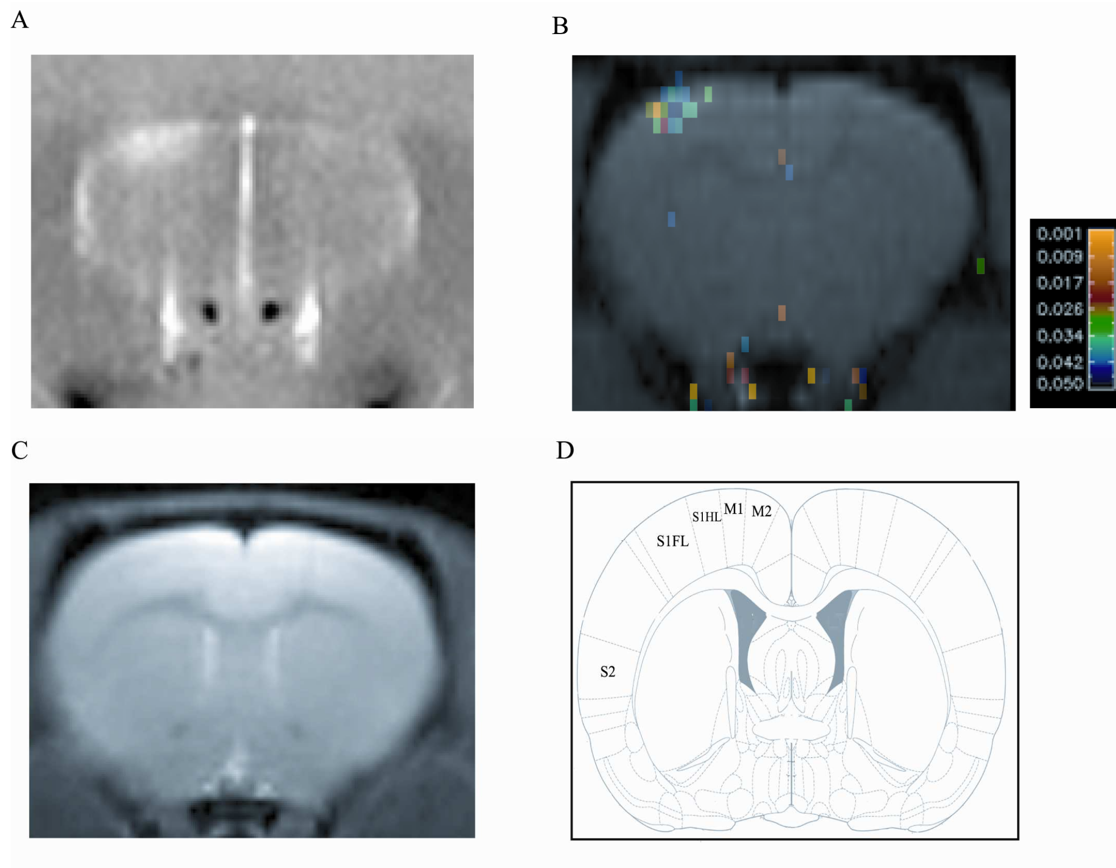

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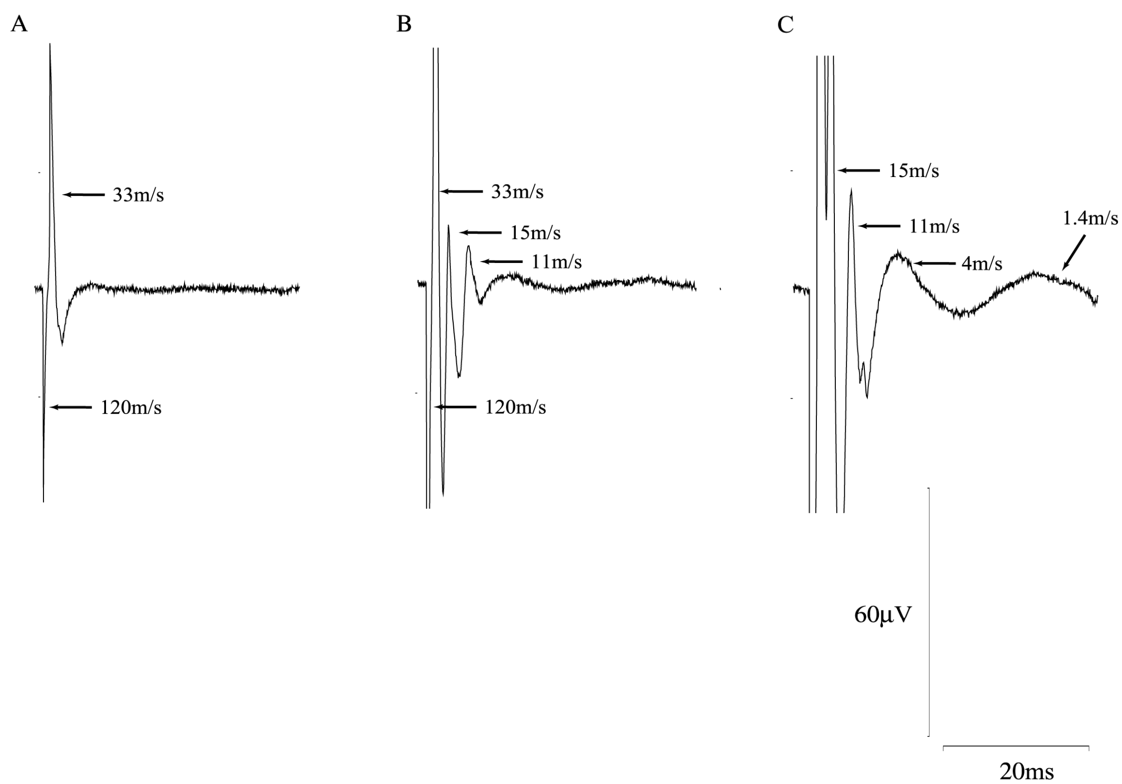

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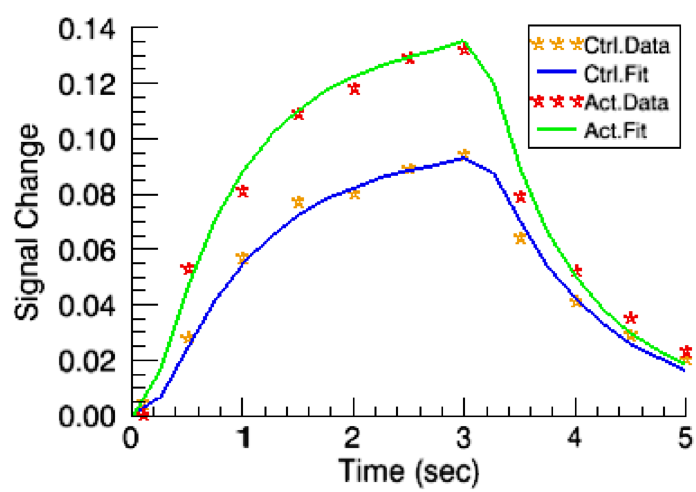

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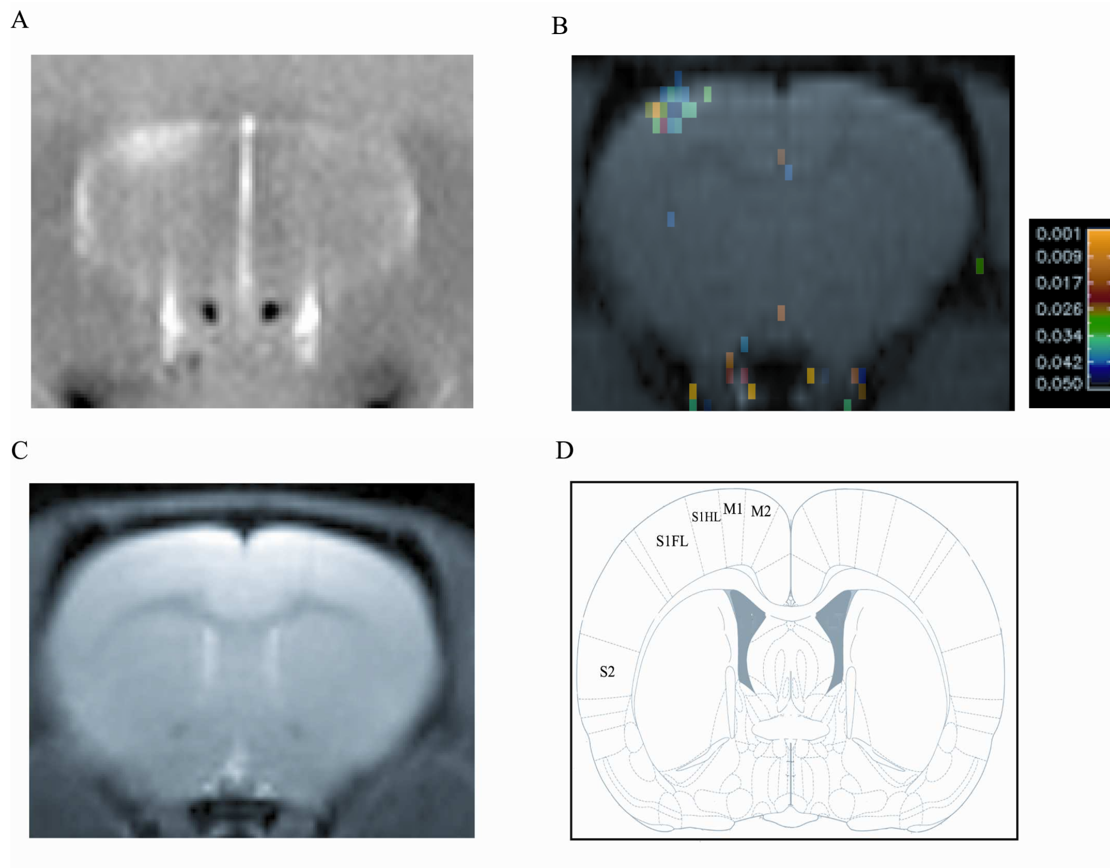

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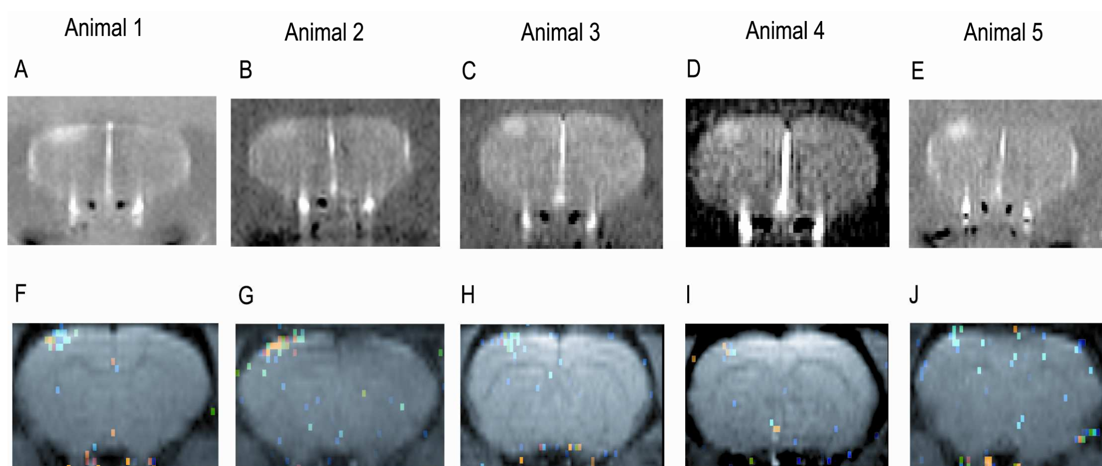

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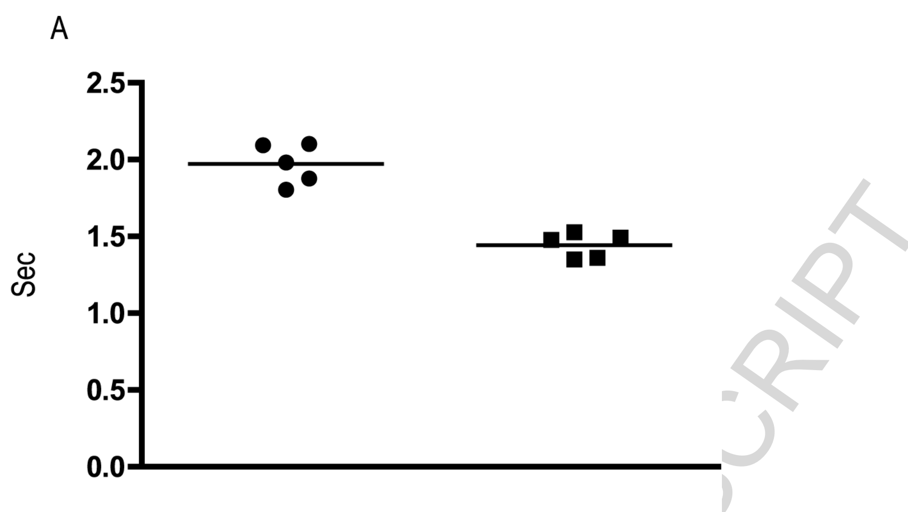

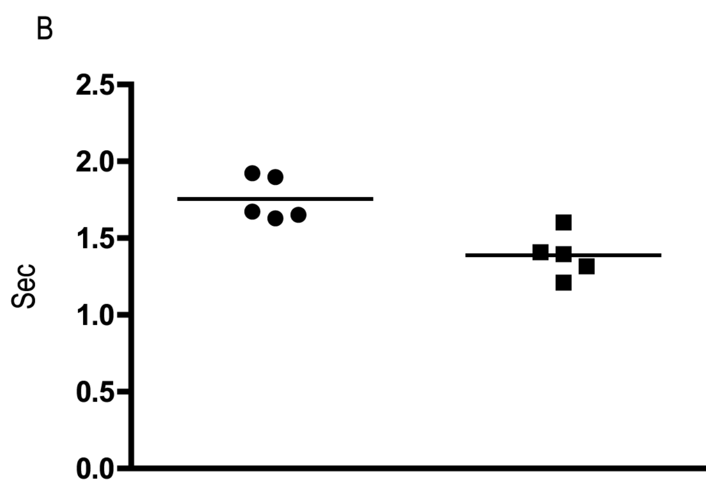

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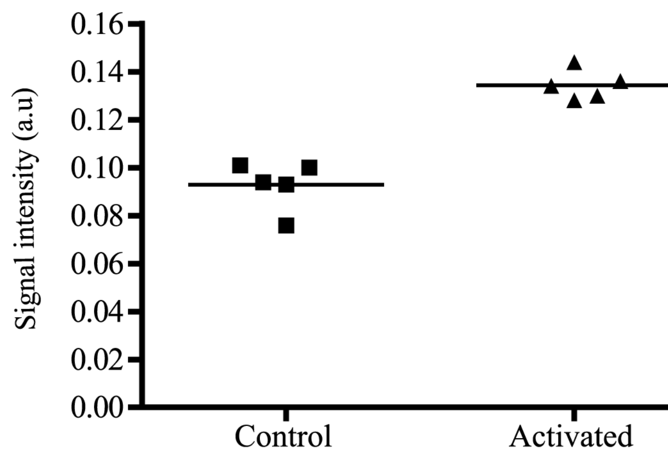

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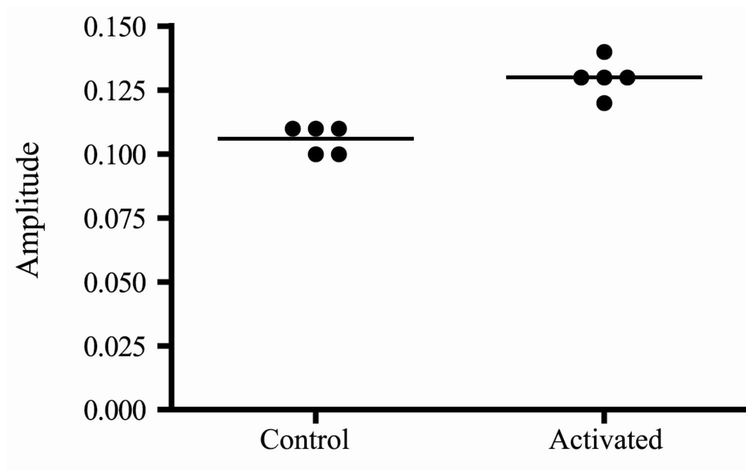

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