# Design and Use of Novel Non-invasive Head Immobilisation Method for Investigation of Behavioural and Functional Asymmetries in Non-human Primate Auditory Cortex

HEATHER SLATER

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INSTITUTE OF NEUROSCIENCE

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# Abstract

This project was initiated with two goals in mind. The first, to refine methods of head immobilisation for rhesus macaques participating in experiments which do not require direct access to the brain, and the second to investigate the effect of attention on lateralisation in auditory cortex. Head immobilisation is often necessary for neuroscientific procedures. A number of Non-invasive Head Immobilisation Systems (NHIS) for monkeys are available, but the need remains for a feasible integrated system combining a broad range of essential features. This thesis details the development of an individually customised macaque NHIS which addresses several animal welfare and scientific needs. The system comprises a customised facemask that can be used separately or combined with a back piece to form a full head helmet. The system was evaluated during performance on several auditory or visual behavioural tasks with testing sessions lasting 1.5-2hrs. To investigate the effect of attention on lateralised processes, four male rhesus macaques were trained to perform an active auditory spatial discrimination task (two of which used the NHIS) using either conspecific "coo" vocalisations or a coo vocalisation from a different individual which had the phase information scrambled, but preserved the spectral components (sCoo). Behavioural results indicated a directional bias during the task with coos, with the animals performing the task with ease when the coo initially appeared on the left but performance being hindered when the coo first appeared on the right. No bias was observed with an animal initially trained with the noise. Attention effects on hemispheric laterality were then studied using fMRI with the trained animals and, as a point of reference, a naïve animal who was passively presented with the task stimuli. The results shown have implications for the control of attention when investigating lateralised processing in both human and non-human species. Additionally, it is conclusively shown that auditory fMRI and behavioural experiments can be conducted without the need for invasive head immobilisation techniques in rhesus macaques.

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# Contents

**List of abbreviations:**

| | |
|---|---|
| A1 | Primary auditory cortex field |
| AC | Auditory Cortex |
| AL | Anterior Lateral auditory cortex field |
| ANOVA | Analysis of Variance |
| AST | Asymmetric Sampling in Time |
| BOLD | Blood Oxygen Level Dependent |
| CL | Caudo Lateral auditory cortex field |
| CM | Caudo Medial auditory cortex field |
| CPB | Caudal Parabelt auditory cortex field |
| CR | Correct Rejection |
| CT | Computerised Tomography |
| *d′* | D prime |
| EEG | Electroencephalography |
| EPI | Echo Planar Image |
| EV | Explanatory Variable |
| FA | False Alarm |
| FEAT | FMRI Expert Analysis Tool |
| fMRI | Functional Magnetic Resonance Imaging |
| FSL | FMRIB Software Library |
| GLM | General Linear Model |
| ITD | Inter-aural Time Difference |
| ITL | Inter-aural Level Difference |
| LI | Lateralisation Index |
| LO | Left Originating |
| LONT | Left Originating Non-Target |
| LOT | Left OriginatingTarget |
| MDEFT | Modified Driven Equilibrium Fourier Transform |
| ML | Medio Lateral auditory cortex field |
| MM | Medio Medial auditory cortex field |

| | |
|---|---|
| MRI | Magnetic Resonance Imaging |
| NHIS | Non-invasive Head Immobilisation System |
| PEEK | Polyether Ketone |
| PET | Positron Emission Tomography |
| R | Rostral auditory cortex field |
| RM | Rostro Medial auditory cortex field |
| RO | Right Originating |
| ROI | Region of Interest |
| RONT | Right Originating Non-Target |
| ROT | Right Originating Target |
| RPB | Rostral Parabelt auditory cortex field |
| RT | Rostro Temporal auditory cortex field |
| RTL | Rostral Temporo Lateral auditory cortex field |
| RTM | Rostral Temporal Medial auditory cortex field |
| sCoo | Phase Scrambled Coo Vocalisation |
| TE | Time to Echo |
| TPJ | Temporo-parietal Junction |
| TR | Time to Repeat |
| Ts1 | Temporalis Superior 1 |
| Ts2 | Temporalis Superior 2 |

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# Chapter 1 : Introduction

This project was initiated with two objectives in mind. The first, to refine methods of head immobilisation in primate neuroscience where direct access to the brain is not necessary, and the second to use this method to investigate the divergence of hemispheric functions of the brain resulting in lateralisation of certain cognitive processes. Given these two different, but complementary aims, I will first discuss the current methods in use for head immobilisation of non-human primates during neuroscience studies, and the need for further refinement, before discussing my scientific question and hypothesis.

## 1. Use of Non-Human Primates in Neuroscience Research

Rhesus macaques (*Macaca mulatta)* are a longstanding and commonly used animal model in scientific research (Weatherall, 2006). This is due in part to the ability of the species to successfully adapt to a range of environments, including the laboratory, and also their wide repertoire of behaviour. Macaques are able to learn to perform many behavioural tasks with relative ease, and are closely physiologically related to humans, sharing many of our cognitive and social abilities. This allows experimental research relevant to humans to be conducted where it is not possible to use human subjects.

Work with non-human primates is protected under ethical regulations which need to be met in order for the work to continue. In the UK, primates are protected under the Home Office Animals in Scientific Procedures Act, as are "all living vertebrates, other than man and any living cephalopod" (Guidance on the Operation of the Animals (Scientific Procedures) Act 1986). Any scientific procedure involving an animal therefore must meet strict ethical guidelines which vary according to the country the research is carried out in, and only those who hold the appropriate license are able to carry out such procedures. Additionally, all research projects must demonstrate the need to use animal models rather than any other method of research. Establishments and license holders are also responsible for application of the principles of the 3Rs of animal research: replacement, refinement and reduction, which were first identified and defined in 1952 by Russell and Burch (William M. S. Russell & Rex L. Burch 1952). Replacement entails implementation of methods of testing which replace animal testing with non-sentient alternatives. Refinement requires that during all procedures, methods which remove or reduce discomfort, pain or distress to the animals must be explored and implemented. Finally, reduction means as few animals as possible should be used in order to meet the goals of the project (Flecknell 2002). Research involving non-human primates is based on data from as few animals as possible in order to meet these goals while also producing data which is robust. In fact, it is not uncommon for data from two individuals to support the hypothesis of the intended project (Milne et al. 2017). Many research projects are ongoing which specifically address refinement of animal welfare through proposed changes to scientific procedures, husbandry and welfare monitoring. The project outlined in this thesis aimed to address the principle of refinement by providing a non-invasive alternative to the current methods of head immobilisation used with non-human primates, and reducing reliance on implanted methods.

## 2. Refinement of Head Immobilisation for Non-Human Primates

*Chapters 3* and *4* of this report concern the development of a novel non-invasive head immobilisation system which was implemented during the course of the project. Much of the behavioural research involving non-human primates in neuroscience requires some form of head immobilisation. Generally this is necessary for data collection methods including behaviour, eye tracking and fMRI, where movement can cause artefacts resulting in unusable data. One method which is used extensively for fMRI is a head post implant which is attached to the skull with ceramic screws and dental acrylic (Georgopoulos and Acuna 1974), although other methods using titanium (Betelak et al., 2001) and more biocompatible materials (Lanz et al. 2013) are also used. The implant fits into attachments on the training setup which hold the head in place. This has the advantage that the animal’s head is otherwise unobscured; meaning access to the eyes, ears and mouth for stimulus presentation and reward is not an issue. In addition to limiting head movement, the implant can accommodate chambers used for direct neuronal recordings.

A major drawback of these methods, however, is that the nature of non-biocompatible implants means that they may not integrate well with the surrounding tissue, and significant numbers of implanted animals in research facilities in the UK have low-level infections around the implant (Pickard 2013). Additionally, if the implant fails, there is a risk of bone damage and distress to the animal. Additionally if the animal cannot be re-implanted and further options are unavailable, data collection cannot continue and the animal may have to be replaced.

Research is ongoing into the refinement of head implants in order to improve biocompatibility and integration with the tissue (Johnston et al. 2016). However, several alternatives to surgical methods for head immobilisation in primates have also been proposed. These methods aim to counteract some of the limitations of implanted immobilisation methods and increase levels of animal welfare whilst also providing similar levels of head immobilisation. Currently, many of the proposed methods lack certain features which can be attained with an implanted headpost, making them less desirable than the surgical method. For example, Howell et al, (2001) were able to obtain high quality positron emission tomography (PET) data using a non-invasive head immobilisation system (NHIS) constructed of Lexan and rigid foam enclosing the subject's head to prevent movement. They noted similar fluctuations in cortisol levels in restrained monkeys as those seen in control animals required to sit in a primate chair, indicating that there was no detriment to the welfare of the animal. However, the apparatus is large in size and there seems to be little to no access to the eyes, ears and mouth for the presentation of auditory or visual stimuli, or to provide juice or food rewards.

Another non-invasive method was described by Srihasam et. al, (2010) which consists of a vacuum suction system which was further developed by Hadj-Bouziane et al., (2014), who used a similar method to obtain resting state MRI data in macaques. Their system comprises a cap which fits over the top of the animal's head and is attached to a vacuum pump. The authors show similar results to those obtained with a surgical implant, however, they do note that larger and less co-operative animals are able to pull away from the system. Additionally, in the latter publication, pain relief was given to the animals following scanning sessions to counteract any discomfort which may have resulted from the vacuum suction, indicating that this method may have a negative impact on the health of the animal.

The aim of the project detailed in this report was to contribute to the ongoing effort to develop and refine non-invasive head immobilisation options, identifying several scientific and animal welfare considerations which have not been wholly addressed by previous non-invasive options. Table 1.1 provides a summary of the existing non-invasive methods assessed in relation to several identified criteria for an effective alternative to implantation. Most recent systems are individually customisable to better fit the animal's head, however, surprisingly little is known about the impact of the existing systems on levels of distress or discomfort experienced by the animals during habituation to or use of the system.

| | **Customisable** | **Access** | **Minimise Pressure Points** | **Tested with:** | **Minimal Distress** | **Lab Adaptable** | **Voluntary Engagement** | **Animal Sizes** |
|---|---|---|---|---|---|---|---|---|
| **Howell et. al. 2001*** | ✓ | ✗ | ? | PET | ✓ | ? | ✗ | 6-11kg |
| **Amemori et. al., 2015*** | ✓ | ✗ | ? | Ephys, TMS | ? | ✗ | ✗ | 6-7kg |
| **Itoh et. al., 2015*** | ✗ | ✓ | ? | EEG | ? | ? | ✗ | 5-8kg |
| **Drucker et. al., 2015 +** | ✓ | ? | ? | Eye tracking, Ephys | ? | ? | ✗ | 5-13kg |
| **Machado and Nelson, 2011 +** | ✓ | ✓ | ? | Eye tracking | ✓ | ? | ✗ | 10-14kg |
| **Srihasam et. al., 2010 ★** | ✓ | ✓ | ? | fMRI | ? | ✗ | ✗ | 5-10kg |
| **Hadj-Bouzaine et. al., 2014★** | ✓ | ✓ | ? | fMRI | ? | ✗ | ✗ | 5-6kg |
| **Fairhall et. al., 2006 ♦** | ✓ | ✓ | ✓ | Eye measurement | ✓ | ✗ | ✓ | 9-11kg |
| **Kiorpes et. al., 2012 ♦** | ✓ | ✓ | ✓ | Eye tracking | ✓ | ✗ | ✓ | ? |

**Table 1.1 Recent developments in Non-invasive Head Immobilisation Systems (NHIS) for macaques and key features.** Columns identify eight scientific and animal welfare needs in relation to recent NHIS. Customisable: Cannot be ‘one-size-fits all’. Access: for the animals to hear, see and obtain rewards. Minimise pressure points: to avoid pain, sores and infection. Tested with: seems to offer comparable head immobilisation to surgically implanted approaches for certain applications. Minimal distress: Should minimise distress during immobilisation. Lab adaptable: to a variety of settings/setups. Voluntary engagement: Option for voluntary engagement with the system, to help with habituation and minimise distress. Animal size: Should work with a broad range of small (5-6kg) to large and strong animals (>10kg).

To address these needs, a system that combines the essential requirements of the available options while also extending the range of features was developed and evaluated. The system uses techniques modified from so called “beam direction shells” which are routinely used when treating cancer patients with radiotherapy. When immediate radiotherapy treatment is required, an impression of the patient’s head is taken and used to create a head model and a customised helmet immobilisation system, which is usually produced by the following day. This system is used to immobilise the head for extended periods of time in order to minimise damage to neighbouring tissue while high-energy radiation is used to target cancerous tissue.

For the project outlined here, a collaboration with the Cancer Radiotherapy Unit at the Freeman Hospital in Newcastle was established. Out of several approaches currently being used in radiology, one was considered to have the best potential for overcoming the limitations of previous head immobilisation options for animals. We aimed to combine our expertise with primate work with the methods currently in use with humans to develop an innovative system for monkeys of different sizes, which could be used for behavioural testing and fMRI data collection. *Chapter 3* of this report describes the development and implementation of the system for behaviour and eye fixation training.

### *2.1 Non-Invasive Primate fMRI*

In addition to eye fixation training, movement is a limiting factor in the quality of fMRI data. Therefore for use with fMRI, any non-invasive system would need to provide equivalent levels of motion restriction to that which can be achieved with a surgical implant. Movement during an fMRI scan can cause artefacts and distortions such as ghosting and false activation which can render the scan useless (Wu et al. 1997; Pfeuffer et al. 2007). Even a rotation of 1° will cause noise in the resulting scan (Jezzard and Clare 1999). Measures have been developed to correct for movement artefacts such as phase correction algorithms and k-space analysis with global frequency correction (Pfeuffer et al. 2007). These techniques are designed to be used in conjunction with restricted movement and their effectiveness is limited when larger movements occur.

Many of the motion correction techniques employed for fMRI data analysis are retrospectively applied to data once acquisition is complete, with limited success. Some prospective methods of motion correction have been developed for MRI which counteract motion induced artefacts by tracking the movement of the head within the scanner, adjusting the magnetic field homogeneity and compensating for the head movement in real time. This has been used with some success with paediatric participants (Brown et al., 2010) and at higher magnetic fields (Stucht et al., 2015). Additionally, real time motion correction can be used to image freely moving objects by updating the position of the imaging volume prior to volume acquisition (Zaitsev et al., 2006). Successful prospective methods of motion correction are also being developed for functional MRI (Todd et al., 2015; Zaitsev et al., 2016).

Although these methods have proven successful when collecting data from human participants, they have yet to be introduced to the primate research sphere, and even in humans they sometimes fail, resulting in loss of data. Once these approaches are improved and become standard for human imaging, they can begin to be adopted for scanning non-human animals, which will pose additional challenges (Logothetis et al., 1999; Petkov et al., 2006; Poirier et al., 2017). Some methods also require additional sensors and equipment to be placed around the head, and/or a dental retainer which the participant is required to bite on to immobilise the head while being scanned (Stucht et al. 2015). This would be problematic with non-human primates as scanning animals during behavioural paradigms requires administration of reward, such as juice, which would be blocked by a dental bite bar.

Until these methods are perfected and can be implemented for non-human primates, head movement in the scanner should be kept to a minimum, and any successful non-invasive head immobilisation method would need to be comparable in terms of movement possible with the commonly used implanted method. Therefore, to contribute to the ongoing research into NHIS which is suitable for fMRI recording, the initial design for behavioural training was modified in order to allow functional neuroimaging data collection, focussing on auditory stimulation and tasks. *Chapter 4* of this report concerns the adjustments made and assessment of the effectiveness of the restraint for fMRI data collection. During the development of the system, two animals were trained on an auditory spatial location task. This formed the first part of investigation into the lateralisation of behaviour during the task. The system was then further developed, and the animals were scanned to provide insight into the functional activity which related to the behavioural data. The two projects, therefore, ran alongside each other.

## 3. Lateralisation of Function in the Primate Auditory Cortex

*Chapters 5* and *6* of this report describe the use of an auditory attention task which was used to assess both behavioural and functional lateralisation in rhesus macaques. The task involved attention to the spatial location of a stimulus which was either communicative or non-communicative in nature. It was hypothesised that the communicative aspect of the sounds would influence successful behavioural performance, and that attention would influence auditory cortex (AC) responses to the same sounds. Here a review of the research which provides background information to the processing of auditory stimulus is provided, which forms the basis for the proposed hypotheses.

### *3.1 Laterality*

Laterality refers to the bias of behaviours or functions to one side of the body. This can manifest in several ways, such as the common trait of handedness in humans and other animals, whereby one hand is preferred for manual tasks over the other. Behavioural laterality in an individual can be used to infer the lateralised organisation of the cerebral hemispheres. For example, in most cases those who are behaviourally right handed for the most part have language regions mainly associated with the left hemisphere, whereas those who are left handed may present with more bilateral or right hemisphere dominant organisation.

### *3.2 Hemispheric Lateralisation*

Inter-hemispheric transfer of information is mediated largely by the corpus callosum, a large bundle of nerve fibres which connect the two hemispheres in all placental animals. The presence and strength of brain lateralisation has been shown to be affected by this structure in several ways. For example, differences in callosal anatomy have been identified in strongly right handed individuals compared with those with mixed and left handed abilities (Witelson 1985; Witelson 1989) such as musicians (Schlaug et al., 1995), although more recent studies have suggested that the link is smaller than first thought, and related to the degree of handedness rather than direction (Luders et al. 2010). Additionally, factors such as gender and age have been shown to influence the anatomy of the structure (Driesen and Raz 1995). Some studies have shown that greater mass of the corpus callosum correlates with greater inter-hemispheric connectivity, resulting in differences in behavioural lateralisation.

However, disagreements have arisen as to whether the corpus callosum mediates interhemispheric transfer, or maintains independent processing between the two hemispheres (van der Knaap and van der Ham 2011), or whether it has an inhibitory or excitatory influence on the opposing hemisphere (Bloom and Hynd 2005). This has implications for higher order functions such as inter-hemispheric inhibition and the bilateral representation of language (Clarke and Zaidel 1994), and differences in the structure have been linked to cognitive processing (Hinkley et al. 2012). Further insight into the role of the corpus callosum can be gleaned from patients with disorders of the structure, and structural differences are seen in a number of disorders, including attention deficit disorder (Dramsdahl et al. 2012), Huntington's Chorea (Rosas et al. 2010), and mental health conditions (Matsuo et al. 2010) to name a few. Hemispheric differences are most apparent in those who have undergone a collosotomy, a procedure which severs the corpus callosum, disconnecting the two hemispheres of the brain in order to prevent the spread of seizures in intractable epilepsy. This procedure blocks the transfer of information between the two hemispheres and allows each to be studied in relative isolation. Patients having undergone this procedure experience perceptual issues which seem to be related to the lack of communication between hemispheres. This allows researchers to identify processes which are more strongly associated with one hemisphere over the other (for a review see Gazzaniga, 2000).

Since lateralisation is seen across many species, (Dreosti et. al. 2014, Quaranta et. al., Vallortigara, 2002) it would seem a reasonable assumption that there is an evolutionary benefit to this property of organisation in the brain (Rogers, 2000), especially given that the phenomenon persists in evolutionarily separate lineages to mammals, e.g. birds, fish and reptiles (Vallortigara, 1999). Indeed, lateralisation is seen even in lower vertebrates, such as sharks (*Scyllium stellare*), eels (*Anguilla Anguilla*), frogs (*Rana esculenta*) and lizards (*Lacerta sicula*) at the diencephalic level (Harris et al., 1996). However, evidence for the advantage of symmetrical brains has also been reported in fish (Dadda et. al., 2009), as lateralisation can interfere with decision making when information is presented to one eye or the other, and in toads, where the individual is more likely to respond to a predator on the left (Lippolis et. al., 2002).

However, for this feature to become so common across so many species it must have evolutionary advantages which outweigh the disadvantages. A number of theories involve the idea of dual processing and division of labour. Some studies posit that functional lateralisation is a result of overcrowding (Cai et al. 2013), meaning that with competition for cortical representation, similar but separate functions diverged to occupy topographically similar areas in opposite hemispheres. As an evolutionary feature, it is possible that the segregation may have occurred to allow information about possible predator location to be handled by one hemisphere while the other dealt preferentially with animal specific behaviours, such as feeding and communication. It has been shown in chicks (*Gallus gallus domesticus*) that lateralisation improves speed of detection of predators (Rogers 2000) and in yellow belly fish (*Girardinus falcatus*), those selected for lateralisation consume prey more rapidly in the presence of a predator than do their non-lateralised counterparts (Dadda and Bisazza 2006). This seems to be indicative of the ability of the lateralised fish to monitor the predator with one eye while co-ordinating feeding behaviour with the other, whereas the non-lateralised fish must switch attention between the two activities.

Inter-hemispheric differences in performance of several cognitive functions in humans have been well established and have been investigated in several ways. Greater degrees of lateralisation have been associated with improved cognitive functioning (Gotts et al., 2013; Mellet et al., 2014; Powell et al., 2012), and, specifically for spatial verbal skills, weaker levels of lateralisation have been shown to impair performance (Mellet et al. 2014). However, this is contrary to previous findings showing that, while smaller degrees of lateralisation may improve cognitive performance, those with more extreme lateralisation patterns show poorer cognitive performance on some tasks (Hirnstein et al., 2010). It has been argued that these differences in performance may be dependent on whether or not the tasks used involve similar processes (Boles and Barth 2011), with greater similarity of the processes involved producing a negative correlation between asymmetry and task performance.

### *3.3 Lateralisation in Non-human Primates*

Lateralisation of function in the human brain is well established. However, this property is less clear in non-human primates. Chimpanzees have been observed to prefer the right hand when producing food begging behaviours (Meguerditchian et. al., 2010), and tool use (Lonsdorf and Hopkins 2005), however a recent meta-analysis revealed this link to be weak in general (Fitch and Braccini 2013). Previously, analyses involving new and old world monkeys as well as apes and pro-simians also showed variable or weak results (Papademetriou et. al., 2005).

Research has shown some level of laterality in new and old world primates which is comparable to humans, and correlates with human related behaviour (Iturria-Medina et al., 2011; Wey et al., 2014; Lindell, 2013). However, some functional research finds this not to be the case (Petkov et al., 2008; Wilson & Petkov, 2012). Additionally, some fundamental anatomical differences have been observed. The planum temporale, a structure involved in speech processing in Wernicke's area in humans, has been shown to be larger in the left hemisphere in right handed humans (Foundas et al., 1994) and in apes such as chimpanzees (*Pan troglodyte*) and gorillas (G*orilla gorilla*). In contrast, this structure is not seen at all in old world monkeys such as rhesus macaques (Hopkins et al., 1998). However, greater activity has been observed in left hemisphere temporal pole regions when an animal is exposed to conspecific vocalisations as opposed to other natural sounds (Poremba et al. 2004), and lesions in left temporal lobes of macaques disrupts perception of conspecific vocalisations (Heffner and Heffner 1984). Greater left hemisphere activation has been shown in response to vocalisation sounds, possibly due to suppression of the right temporal pole by the left hemisphere (Poremba and Mishkin 2007). Asymmetries have also been seen in vervet monkeys (*Cercopithecus aethiops*). However, in this genus of old world monkey, lateralisation for conspecific vocalisations seems to be right rather than left lateralised.

One way in which lateralisation of language processing can be demonstrated is via dichotic listening paradigms. A right ear advantage for the perception of verbal stimuli, first reported by Doreen Kimura (1961), has been well established. Since then the property has been exploited by researchers in order to better understand lateralisation and linguistic processing. This property has also been observed in monkeys (Hauser et al., 1998; Hauser & Andersson, 1994) and chimpanzees (Szymanska et al. 2017) during experiments during which animals are played conspecific vocalisations and orienting behaviour is observed.

Lateralisation of other processes in non-human primates is less clear. A contributing factor to this may be the physiological differences in the corpus callosum of different primate species, which impact on the speed of interhemispheric excitatory and inhibitory conduction. Larger brains result in larger axons, which induce delays in the conduction of activity, however, this may be countered to some extent by the larger diameter of axons in the human brain (Caminiti et al. 2009). As mentioned previously, a right hemisphere dominance for spatial processing seems to exist in humans, but this is not reliably seen in studies with non-human primates. In fact, some studies show that in split brain macaques, visual tasks which involve motion direction and orientation discrimination show left hemisphere dominance rather than right (for review see; Oleksiak et al., 2011). Additionally, there is little research providing insight into the lateralisation of primate auditory processing, other than those relating to conspecific vocalisation responses, and fewer still which require attention to the auditory stimulus, which may be the source of variability in the findings of previous studies. Research into these aspects may help to close the gap in our understanding of how human brains came to be lateralised for these functions.

### *3.4 Sound Processing in Auditory Cortex*

Primary and non-primary regions in AC have been shown in humans to be larger in the left hemisphere, which also shows dominance for processing simple auditory stimuli (Devlin et al. 2003). Further anatomical differences have been seen in the volume of white matter between the left and right cortices, due to increased thickness of the myelin sheath in left AC neurons (Anderson et al., 1999). This is further supported by findings of larger cortical surface area and cortical volume in left Heschel's gyrus and sulcus, and a rightward asymmetry for cortical thickness (Meyer et al., 2014).

In humans, primary AC is found in Heschel's gyrus, also known as the transverse temporal gyrus. Additional auditory regions span the superior temporal sulcus and gyrus. This can be further delineated in non-human primates where AC is known to consist of three major regions, the core, belt and parabelt, which are subdivided into several smaller regions. The core region consists of primary (A1), rostral (R) and rostro-temporal (RT) regions, which are surrounded by the medial belt regions: caudo-medial (CM), medio-medial (MM), rostro-medial (RM), rostral temporal medial (RTM) and lateral belt regions: caudo-lateral (CL), medio-lateral (ML), antero-lateral (AL) and rostro temporal lateral (RTL). The caudal and rostral parabelt (CPB and RPB) regions are positioned to the ventral side of the lateral belt (Arnott et al., 2004) Figure 1.1.

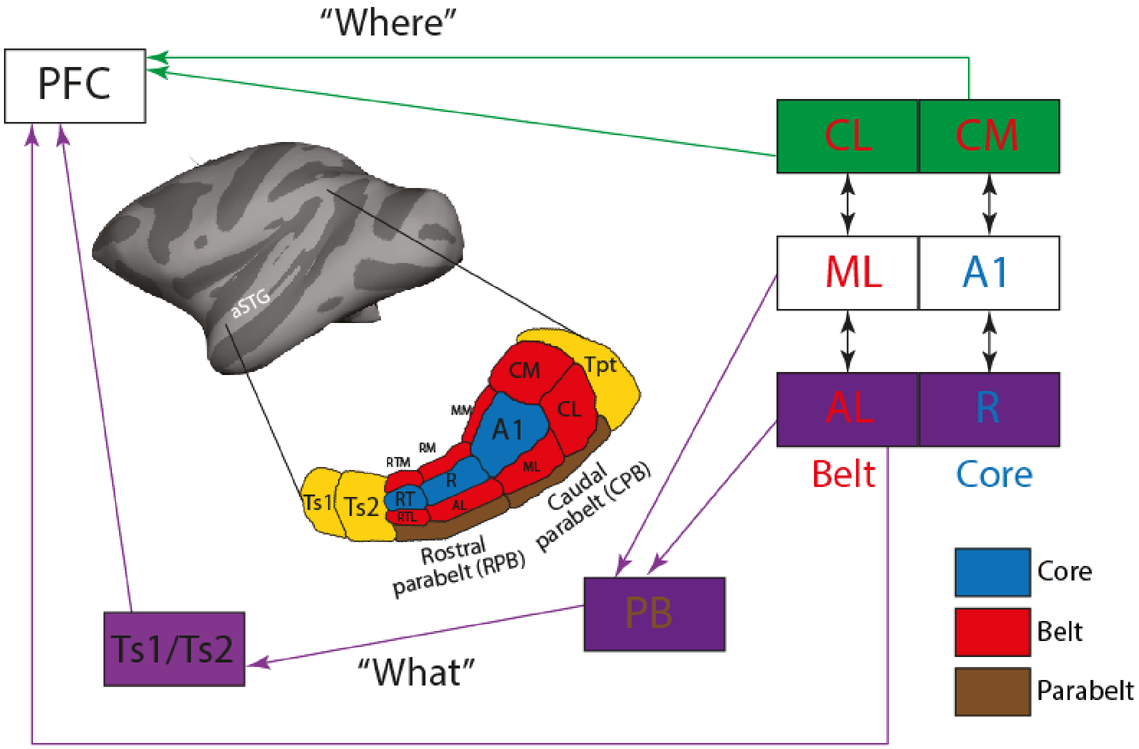


**Figure 1.1. Regions and Processing Streams in Primate Auditory Cortex.** Based on Rauschecker and Tian, 2000; brain image and map modified from Petkov et. al., 2015.

One way in which sounds are evaluated has been shown to depend on “what” or “where” streams in AC (Figure 1.1) which differentiate auditory stimuli based on their location and identity (Kaas & Hackett, 1999; Rauschecker & Tian, 2000). Further to this, spectral and temporal features can make the auditory object more salient to areas located in the right or left hemisphere preferentially (Zatorre and Belin 2001) and facilitate further processing.

Functional differences have been seen in hemispheric specialisation for different types of auditory stimuli, with the left hemisphere showing preference for temporal changes in acoustic stimuli, and spectral changes eliciting greater activity in the right hemisphere, although responses are seen bilaterally in both cases (Zatorre and Belin 2001). This has been further expanded upon to develop a hypothesis of hemispheric asymmetry as “asymmetric sampling in time” (AST) (Poeppel 2003), which posits that bilaterally represented neural responses to sounds in core auditory regions are preferentially proliferated to the left or right non-primary AC, depending on temporal features. The model suggests that left auditory regions preferentially respond to short temporal integration windows of about 20-40ms, such as those present in verbal input, and the right auditory regions respond preferentially to longer windows of about 150-250ms which are present in sounds such as music. However, while some studies support this theory to some extent (Luo and Poeppel 2012; Morillon et al. 2012), others argue that the theory does not account for other factors related to the characteristics of speech (McGettigan and Scott 2012).

Visual processing in the brain has been shown to involve two distinct pathways (Ungerleider et. al. 1982), and this was further shown to be the case in the auditory system (Arnott, 2004, Romanski 1999). The core areas of AC receive inputs from the medial geniculate nucleus and form a point at which cortical processing begins. The core regions are heavily interconnected, but also connect to the adjoining belt regions, which are thought to represent the next stage of auditory processing. Belt areas are also interconnected and have connection with the parabelt regions. the site of the third stage of auditory processing, and to the pre-frontal cortex (Kaas and Hackett 2000). These areas differ in their preference of specific types of auditory stimuli, and because of this can be identified and delineated with fMRI (Petkov et al. 2006) and targeted with electrophysiology (Kikuchi et al., 2010; Perrodin et al., 2011).

Information processing in AC can proceed in different ways depending on the properties of the stimulus. For example, information on the identity of an auditory object can be gleaned from input from one cochlea, whereas spatial processing requires the convergence of information from both cochleae. This is due to the fact that localisation depends on perception of the difference in time of the sound reaching one or the other ear, known as inter-aural time difference (ITD), and the relative intensity of sounds arriving at both ears, known as inter-aural level difference (ILD). Therefore the two aspects require processing from structurally different areas from the outset (Kaas and Hackett 1999), and once auditory information reaches AC, representations of the incoming auditory object are created in order to assign it to the correct processing route (Nelken et al., 2003).

Further to this, information relating to the identity and features of an object or sound preferentially activates ventral streams of processing, such as ML, AL, R and parabelt regions, whereas the perception of the location of the object or sound seems to involve dorsal cortical streams involving areas CL and CM. Because of this feature, these dorsal and ventral streams are commonly referred to as the “what” and “where” pathways of processing (Kaas & Hackett, 1999; Rauschecker & Tian, 2000). These two streams differ in their interhemispheric connectivity, with the ventral stream being more bilaterally organised, and the dorsal stream being more left lateralised (Hickock and Poeppel 2007). The left lateralisation of the dorsal stream may therefore be a contributing factor to the left hemisphere dominance for communication.

### *3.5 Perception of Communication Signals in Auditory Cortex*

Processing of language related components has received most attention in lateralisation studies due to the fact that language dominance must be measured before proceeding with therapeutic neurosurgical treatment of epilepsy (Bradshaw et al., 2017). Research into the cortical underpinnings of language production and comprehension has supported the idea that the left hemisphere is heavily involved in this process, seemingly more so than the right. This is also true of a number of non-human species, including mice (*Mus* *musculus)* (Ehert 1987), and frogs (R*ana pipiens*) (Bauer 1993) and recent findings show that in dogs (*Canis lupus familiaris*), human vocalisations cause asymmetric activation independent of perceived reward content (Andics et al. 2017), although in this case activity was right lateralised.

Lateralisation of language dominance has been reliably seen in the left hemisphere in 90% of right handed individuals (Springer et al. 1999), and several models attribute this to the temporal features of speech, with the left hemisphere preferring information presented during short time windows, such as phonemes, and the right hemisphere preferring information over longer time windows, such as syllable length and intonation. As verbal information has highly varying temporal properties, the left hemisphere auditory regions are better suited to processing this information. This is supported by findings that increased temporal variation in non-speech acoustic stimuli preferentially activate posteromedial Heschel's Gyrus in the left hemisphere (Boemio et al., 2005; Jamison et al, 2006) in a similar way to speech stimuli (Obleser et al., 2008). This may be due to processing of a more focal nature in the left hemisphere, which may be better suited to time sensitive stimuli such as speech (Boemio et. al., 2005; Poeppel, 2003; Joly et. al., 2012). The left hemispheric preference may relate to physiological connections, as the left anterior temporal lobe has been shown to be heavily asymmetrically connected to other language associated regions (Hurley et. al., 2015).

Another theory as to the possible reasons for the evolution of language lateralisation is based on a change in motor abilities in primates. The postural origins theory (MacNeilage, 1987) posits that primates evolved a left limb preference for reaching and grasping, while the possibly stronger right limb provided postural support, or held on to trees. Once primates descended from the trees and no longer required postural support from the right hand, it became free for other purposes, such as social and communicative gestures. This has been contested, due to the fact that it implies that asymmetry precede handedness, however, the motor aspect of social cue production with the right hand ties in with motor theories of language and speech perception (Kolb and Whishaw, 2003).

The processing of natural sounds, especially communicative sounds, is vital to animal survival. The ability of animals to recognise warning or mating calls, especially in social species, has a huge bearing on the success of both the individual and the other members of the social group. This is a possible reason why auditory regions are consistently found to respond preferentially to natural over artificial sounds and to communicative over non-communicative natural sounds (Petkov et al. 2008; Carrasco and Lomber 2011), and why regions in the AC exist which specifically deal with processing of conspecific vocalisations (Perrodin et al., 2011; Poremba et. al., 2013).

Individual regions in AC are known to respond to vocalisation and speech signals to a greater degree than other fields. In humans, greater activity is seen in regions of the upper bank of the STS in the presence of vocal sounds compared to non-vocal sounds (Belin et. al., 2000), a finding which, in non-human primates, can be further localised based on functional and electrophysiological data. The auditory core region A1 responds preferentially to conspecific vocalisations over natural sounds and other animal vocalisations, as do the superior temporal pole and temporalis superior 1 and 2 (Ts1 and Ts2) (Petkov et al. 2008; Perrodin et al. 2011). Additionally, the AL field has been shown to respond to the type of vocalisation (Tian et. al., 2001). Core regions of AC are, however, known to respond to specific acoustic features or combinations of acoustic features, whereas processing of conspecific vocalisations elicits activity in regions which are thought to be more hierarchically advanced, such as RT, RTp (Kikuchi et al. 2010) and RM (Kusmierek et. al., 2012).

Although humans are the only species to have acquired language, it is becoming increasingly evident that non-human primates, especially old world species, possess some basic ability to process simple characteristics of human language (Attaheri et al., 2014; Milne et al., 2017; Wilson et al., 2013), although perhaps not on a semantic level. Therefore, although direct comparisons between the processing of communication sounds in non-human primates and humans may not be possible, the presence of these similarities indicate that some comparisons may be valid in species with which we share a common ancestor.

### *3.6 Spatial discrimination*

In human studies, research has revealed that the perception of the spatial location of a given stimulus is more heavily associated with the right hemisphere (Kaiser et al., 2000; Anourova et al., 2001; Griffiths et al., 1996; Karnath, 2001). Additionally lesion studies have shown that functions involving evaluation of location in the visual domain are interrupted by lesions in the superior temporal gyrus in the right hemisphere with patients left unable to identify information in the contralateral visual field, while the visual system itself remains intact (Robertson et al., 1997).

A vital role in auditory spatial perception is the identification of the location in space from which a sound originated. Many species rely on this ability to locate food, detect predators and locate mates producing mating calls. Two of the cues used to locate a sound are the ILD and the ITD, which are detected by the superior olive (Joris and Yin 1995) which processes the differences in the time delay and intensity of sounds reaching the left and right ear. Following this, neurons in the inferior colliculus receive excitatory input from the contralateral ear and inhibitory input from the ipsilateral ear and integrate the spectral and temporal information into a spatial auditory map. Information then proceeds to the AC via the medial geniculate nuclei (Langers et. al., 2005).

The role of AC in sound localisation has been studied with lesion and single unit recording studies in non-human animals. The primary auditory area A1 is thought to be the point of initiation of auditory processing in cortex, and is known to be involved in spatial processing of sounds. Lesions to this region result in deficits in localisation of sounds to the contralateral hemifield in several species, including ferrets *(Mustela putorius)* (Kavanagh and Kelly 1987), cats *(Felis catus)* (Jenkins and Merzenich 1984) and rhesus macaques (Heffner 1997), indicating that primary AC indeed plays a part in localisation of a sound. In human AC, specific regions respond more strongly to moving over static sounds (Baumgart et. al., 1999), specifically in the right hemisphere. These regions are functionally delineated into three areas. T1 and T2 span areas close to Heschel's gyrus, and T3 is located on the rostral planum temporale (Scheich et al. 1998). Right hemisphere dominance for spatial perception in humans has also been shown in further studies (Brechmann & Scheich, 2005; Zatorre et. al., 2002).

In non-human primates, the caudal part of the superior temporal gyrus (STG) contains neurons that are spatially tuned to the location of a sound stimulus presented in free field (Rauschecker et. al., 1997; Recanzone, 2000; Tian et al., 2001). Recent studies have supported the view that auditory motion preferentially activates areas CL and CM, which, along with Tpt, are thought to be homologous to the human planum temporale. Areas A1, ML and CPB are also activated by motion (Poirier et al. 2017). However, unlike the lateralisation effects seen in humans, in animal studies activity is seen contralaterally to the presented sound (Ortiz-Rios et al. 2017), while ipsilateral regions show little activity. This is in line with findings that unilateral ablation of the macaque AC disrupts sound location discrimination in the contralateral hemifield space (Heffner 1997). This may indicate that non-human primates and humans differ in terms of lateralisation of spatial discrimination.

### *3.7 Processing of Spatial and Communicative Sound Features in Combination*

Differences in the spatial location of speech sounds have been shown to assist in the segregation of speech streams in the cocktail party problem (Cherry 1953), whereby, an individual is alerted to the sound of their name being spoken in amongst a room full of other talking individuals and other background noises. Loss of hearing in one ear can inhibit a person's ability to perform such tasks. However, spatial location of speech sounds seems to matter little to auditory processing due to schemas which exist for speech (Bregman, 1990).

In humans, processing of spatial and communicative auditory objects occurs in topographically similar areas separately in the right (spatial) and left (communication) hemisphere (Karnath et. al., 2001). However, those same areas in the left hemisphere are capable of spatial processing on a sub-dominant level (Suchan and Karnath 2011). This lends credence to the theory that, on lateralisation of the brain, spatial processing became focussed in the right hemisphere, possibly due to an increase in demand for cortical representation of communication in left hemisphere regions (Cai et al. 2013). It is possible that the left hemisphere, being tuned toward stimuli with faster variation in time signals, made it better suited to communication, and the right hemisphere, which shows a higher degree of inter-hemispheric connection, made it better suited to stimuli of a more general and dichotic nature (Gotts et al. 2013) allowing for the incorporation of bilaterally presented stimuli such as spatial cues.

We know through various studies that multiple demands placed on similar regions in one hemisphere can inhibit adequate performance on one or both of the tasks being performed, and dual task interference on processes involving similar areas is regularly used to assess the degree of lateralisation which a person displays (e.g. Kosaka et al. 1993). One evolutionary theory posits that lateralisation of communication abilities came at a cost to our left hemisphere spatial processing, at least in visual processes (Corballis et. al., 2000). In this case, visuospatial processing was lost in the left hemisphere and the regions previously dedicated to these processes were allocated instead to language, resulting in a left hemisphere impairment for spatial processing. This may explain findings that left hemisphere spatial processing is seen, but as a sub-dominant process in humans (Suchan and Karnath 2011), and at an inferior level to other organisms. This effect may also be seen in other processes, such as temporal judgements (Brown and & Nicholls, 1997), which may have been lost by the right hemisphere as the left hemispheric superiority developed (Corballis et al. 2000).

Research involving non-human primates has suggested that communication signals are conveyed to regions implicated in spatial localisation in AC, namely caudal regions of the STG (Tian et al. 2001). More specifically, while spatial selectivity increases from A1 to ML then to CL, communication sounds elicit activity travelling from A1 to ML, and then to both CL and AL (Rauschecker & Tian, 2000). Therefore, CL represents a point of convergence for the two types of auditory information, which then allows an auditory object to be processed on the basis of both types of cues.

Lateralisation of spatial processing may be an evolutionarily recent function in humans, a view which is supported by research into the lateralisation of spatial abilities in non-human primates (for review see: Oleksiak et al. 2011). It is possible that as we developed linguistic abilities, competition for cortical representation meant that the left hemisphere auditory regions prioritised processing of communication, and areas in the right hemisphere continued to specialise for spatial processing. This may be possible to investigate further by studying processing in an animal species with less complex cognitive abilities, such as non-human primates, and with incorporation of other processes, such as attention.

### *3.8 Attention*

Following the detection of a sound, or any other stimulus, further processing requires the allocation of attention to its presence. The attention network of the brain can select the relevant stimuli from the sensory environment in order to fulfil the behavioural requirements of the situation that the organism faces, such as the task described in *Chapter 5*. The regions of the brain which are most heavily associated with these processes are the frontal and parietal lobes, which together form the fronto-parietal attention network (Ptak 2012).

The visual system provides a lot of the information we currently have about how attention works. When we attend to something in the sensory environment our natural reaction is to turn to look at the stimulus which has attracted our focus, an effect referred to as oculomotor capture. Therefore issues relating to attention in the visual field are more easily identified than other sensory modalities. Allocation of attention can happen automatically on detection of a salient stimulus (automatic), or on a conscious basis whereby attention is deliberately deployed in order to complete a specific goal (voluntary) (Buschman and Miller 2007). These two processes form a dichotomy in processing pathways, with bottom-up sensory regions associated with automatic attention, and top-down processes associated with voluntary attention. Bottom-up attentional processing is known to occur faster than top-down, and theories suggest that top-down processing relies on initial information from the bottom-up processes in order to bias attention based on expectancy and goal set (Theeuwes 2010). However, this idea has been challenged more recently (Awh et. al., 2012).

The salience of an object is defined by its distinctiveness in comparison to the surrounding environment, and the salience of its properties determine the speed with which attention is directed towards it. However, the salience of the same stimulus can change depending on additional factors. For instance, when a salient stimulus is presented twice in the same location, it produces slower reaction times than when the same stimulus is presented in two different locations. This is thought to be due to so-called inhibition of return processes, which suppress a second response to the stimulus in the same location. This has been demonstrated both with eye saccades and with neuronal activity (Bichot and Schall 2002). A saliency map for shifts of visual attention has been defined detailing which aspects of a stimulus will generate a more pronounced attentional response. This allows attentional processes to prioritise certain aspects of the input as most relevant to the organism (Itti and Koch 2000).

The attributes which constitute the allocation of attention based on the saliency map are mainly focussed on the bottom-up model of attention, with the object saliency capturing the attention of the animal. However, others have also proposed a priority map which implicates both bottom-up and top-down streams in the allocation of attention to behaviourally relevant stimuli. This process is heavily associated with the lateral intraparietal area, which has been shown to be involved in target selection and generation of attention (Bisley and Goldberg, 2012).

It has been suggested that attention is a process which is dealt with primarily by the right hemisphere. Flöel et. al., 2005 documented hemispheric dissociation between attention and language areas in humans using transcranial Doppler ultrasonography while participants performed a visuospatial attention task and a word generation task. They noted that in right handed participants there was a right hemisphere attentional dominance and left hemisphere language dominance. Additionally, research has shown that the temporo-parietal junction (TPJ), which forms connections with the bottom-up attention network, is more strongly connected in the right over the left hemisphere (Kucyi et. al., 2012). Downar et. al. (2001), showed that areas of the TPJ were more highly activated with increased saliency of the presented stimuli and noted that this area is frequently found to be interrupted in patients showing hemineglect, reducing attention and awareness.

In contrast, top-down voluntarily allocated attention in humans seems to produce a shift in attention to the right side of space, and it has been shown that higher cognitive load increases this effect. When performing a task, increased difficulty and reduced alertness is associated with higher levels of shift to the right side of space (Bareham et. al., 2014; Malhotra et. al., 2009; Manly et. al., 2005; Pérez et al., 2009). Additionally, participants performing poorly on a sustained attention task show more bias to the right side of space than those performing well on a spatial attention task (Bellgrove et. al., 2004), with participants with poor sustained attention showing significantly less left bias than those with good sustained attention. However, differences have also been noted in terms of task requirements. During a visual selective attention task, participants asked to shift their attention between local and global features of the same image revealed that processing of the local features induced increased event related neural activity in the temporal-parietal cortex of the left hemisphere, and global features produced greater activity in right hemisphere (Yamaguchi et. al., 2000). Taken together, these findings suggest that lateralisation of attentional processes may depend on a range of factors relating to the task and stimulus used.

### *3.9 Attention in Auditory Cortex*

The mechanisms which underlie the attribution of attention have been explored extensively in the context of visual processing. However, less is known about how attention affects activity in the auditory cortex. Much of the activity seen in AC has been studied in humans or animals with passive presentations of sounds, or even under anaesthesia. However, it has been shown that variations in attention and context of the stimuli presented can alter the way in which the fields in AC are activated (Petkov et al. 2004).

The processes involved in attention allow us to filter the sensory environment for information which is pertinent to the current behavioural goal. A good example of this is seen in the cocktail party effect, (Cherry 1953). Since the discovery of the effect it has been used in various ways to investigate how the presence of speech-like qualities in, and relevance of, the stimulus can automatically commandeer our attentional processing (for review see: Bronkhorst, 2000).

Certain sounds are necessarily better at capturing attention than others. Natural sounds and conspecific vocalisation have been shown to better engage attentional processes and, similarly to the visual system, an auditory saliency map has been determined in both humans and non-human primates (Kayser et. al., 2005). However, allocation of attention in response to a sound produces different results to attention which is directed prior to sound onset. Studies involving dichotic listening tasks demonstrate increased attention related modulations in the contralateral hemisphere to the attended ear when fast rate streams of broadband noise bursts were presented to the left ear or the right ear (Rinne, 2010). This suggests that, at least in terms of auditory tasks, there is a bilateral attention process. However, even with pre-allocated attention to a stimulus, bottom-up processing can still interfere if there is a task irrelevant salient feature to the stimulus, suggesting that top-down processes have limited control over interfering bottom-up attentional capture, especially if the features are unpredictable (for a review see: Sussman et. al., 2003).

A right ear advantage for speech stimuli during dichotic listening tasks has been long established (Kimura 1961), with participants showing a performance bias on tasks involving spoken stimuli when they are presented to the right ear over the left ear. Several studies have shown a right ear advantage present for attention to communication signals (Alho et al., 2012; Asbjørnsen et. al., 1990; Hiscock & Kinsbourne, 2011). It has also been suggested that there is active suppression of the right hemisphere in response to communication signals by the left hemisphere, which has been successfully abolished following collosotomy (Scott 2005). This effect is hypothesised to be due to a greater connection of the right cochlea to the left temporal lobe than the left cochlea. However, when attention is allocated to one ear or the other prior to stimulus onset, different effects are seen (Mondor and Bryden 1992). This may indicate that involuntary allocation of attention in response to a stimulus elicits different processes than pre-allocation of attention to a given stimulus (Kinsbourne 1970), or that there is a bias towards the right ear when attending to speech sounds presented dichotically, even when there are no instructions to do so (Alho et al. 2012). In fact, this may not be an effect which is confined to verbal material, as tones have been shown to elicit faster responses to presentations to the right ear when the direction of stimulation is unknown, but not when the side of stimulation is known in advance (Simon 1967).

In contrast to the AST model, which assumes that information captured by the AC regions is an architectural feature of the processing stream, it has been shown that lateralisation of AC for a specific stimulus can be context dependent, with attention to different aspects, such as duration and direction, inverting the pattern of activation from left to right hemispheres respectively (Brechmann and Scheich 2005). Here it was shown that, for the same set of stimuli, if the participants were required to report the pitch direction of the sound (rising or falling), right hemisphere regions were more highly activated, while reporting the duration of the stimulus produced left hemisphere activity. Further, performance on a task has been shown to modulate activity present in the AC depending on the task specific features of the stimuli (Ahveninen et al. 2006), in this case, either the phonetic or spatial changes in the presented sound stimuli. When participants were instructed to attend to the phonetic content effects were seen in AC “what” regions, whereas attending to the spatial location produced effects in the “where” associated regions of AC.

Learning induced plasticity of AC has been shown in a number of animal species, as well as humans (Ohl et. al., 2001; Ohl & Scheich, 2005) showing that the same sound can induce changes in cortical activity depending on how the stimulus relates to the required behaviour. Mongolian gerbils (*Meriones unguiculatus*) show differing patterns of spatial activity when passively presented with sounds as compared to animals who have been trained to categorise the sounds as either rising or falling, producing electrophysiological correlates of category learning (Ohl et al., 2001). Further to this, it has been shown in cats that task performance sharpens spatial sensitivity in AC, which may be due to an increase of responses in neuronal firing to sounds presented in preferred locations, and suppression of neuronal firing in response to least preferred locations (Lee and Middlebrooks 2011). Additionally, in humans, task dependent responses to the same sound have been seen to differ between the hemispheres of the brain (Jamison et al. 2006). When the sound was interpreted in terms of pitch direction, increased activity was seen in right AC, while categorisation of the sound duration elicited stronger activity in left AC.

### *3.10 Conclusion*

A number of functions of the mammalian brain have been shown to be associated with one hemisphere or the other. Various theories exist as to the evolutionary advantage of this feature and how it developed. For many years lateralised processing was thought to be a uniquely human feature, but this has been conclusively shown not to be the case. By investigating lateralised processing in the non-human primate brain, we may be able to gain a unique insight into how human functions of lateralisation arose and the influence of this process on our development of language.

In rhesus macaques and humans, the lateralised effects of vocalisation sounds and attention have been reported. However, the lateralisation of spatial processing seems to differ between the two species. In auditory regions, it may be possible that certain aspects of spatial processing were outcompeted for cortical representation in the left hemisphere by our increasing need for communication processing. In addition, if there is bilateral representation of these aspects of spatial processing in the macaque brain, the apparent left hemispheric preference for communication signals may affect the animals’ ability to detect the spatial qualities of the sound. We have seen that experiments involving tasks that are lateralised can be affected by the addition of a task associated with functions which are lateralised to the same hemisphere, therefore possibly competing for resources within the same hemisphere. This may point towards an evolutionary advantage for lateralisation of a once bilateral processes.

Combining theories suggesting that the left hemisphere has a faster time integration window than the right hemisphere and that communication signals are processed on a pre-attentive timescale (Rinne et al., 1999), it may be possible to infer that suppression of the auditory regions in the right hemisphere would interrupt task-dependent processing of stimuli thought to be dominant in the right hemisphere, such as spatial attention. If this were the case, we may expect that task dependent behaviour requiring allocation of attention to spatial location would demonstrate greater behavioural accuracy when initially dealt with by right hemisphere auditory regions, where there is a communicative aspect to the stimuli.

The hypothesis guiding the research detailed further in this report was that monkeys would be hindered in learning and performing an auditory spatial discrimination task if the stimuli used were communicative in nature. The right ear behavioural bias described earlier in humans during dichotic listening tasks, and monkeys on passive presentation of vocal cues, may result in a hindrance to correct performance on right-originating stimuli. This could potentially cause interference between discrimination of the spatial location of the sounds and communication areas in similar regions of the auditory cortex during competition for cortical dominance. This effect would not be present with a non-communicative stimulus. Although spatial processing as a whole is dependent on a range of cognitive processes, by looking specifically at auditory regions it may be possible to identify some of these differences when animals are presented with a simplified spatial task. Further, the allocation of attention to the sounds would produce differences in the lateralisation of functional responses to the same sounds. This will be discussed further in the following chapters.

# Chapter 2: General Methods

All work was conducted by the author unless otherwise stated.

## 1. Subjects

Nine male Rhesus macaques (*Macaca mulatta*) from a group of pair housed animals provided data used in this thesis. All animal procedures performed were approved by the UK Home Office and complied with the Animal Scientific Procedures Act (1986) on the care and use of animals in research, and with the European Directive on the protection of animals used in research (2010/63/EU). All methods were prepared in line with the Animal Research Reporting of In Vivo Experiments (ARRIVE) principles on reporting animal research. All persons involved in this project were Home Office certified and the work was strictly regulated by the U.K. Home Office.

The floor area of the pens in the monkey colony range from 130x240cm to 215x240cm. All are 230cm high, and hatches between neighbouring cages are used to increase the space available to the animals. Two monkeys (MT, 6 years, 12kg and MW, 5 years, 7kg) were naïve to behavioural and head immobilisation training, not having previously had an implanted head post. Two other monkeys (ME, 11kg; MC, 16kg, both 8 years old at the time of testing) did not have implanted head posts at the time of assessment, but had previously had head post implants. These implants had become unstable and were removed at 7 months and 4 years after implantation, respectively. The other animals (MK, 6 years, 12.5kg; MA, 6 years, 14kg; MP, 8 years, 15kg; MM, 4 years, 6kg and MD, 14 years, 16kg) had existing implants. Table 2.1 and Table 2.2 summarise which animals took part in each section of the report and the procedures they were involved with.

| | MT | ME | MC | MK* | MA* | MP* | MM* | MW |
|---|---|---|---|---|---|---|---|---|
| **MRI for 3D head model** | ✓ | ✓ | ✓ | | | | | |
| **Auditory task performance** | ✓ | ✓ | | | | | | |
| **Habituation time to immobilisation** | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **Thermal imaging** | ✓ | ✓ | ✓ | | | | | ✓ |
| **Number of sedations** | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |
| **Simple eye-tracking** | ✓ | | ✓ | | | | | ✓ |
| **Functional Imaging comparison** | ✓ | ✓ | | ✓ | ✓ | | | |

**Table 2.1. Summary of animals involved in the NHIS development and testing. *** indicates an animal with an implanted head immobilisation device

| | MT | ME | MK | MA | MD |
|---|---|---|---|---|---|
| **Behavioural Data for Auditory Coo Task** | ✓ | ✓ | ✓ | ✓ | |
| **Behavioural Data for Auditory sCoo Task** | ✓ | ✓ | | | |
| **fMRI Data for Auditory Coo Task** | ✓ | ✓ | ✓ | ✓ | |
| **fMRI Data for Auditory sCoo Task** | ✓ | ✓ | | | |
| **Passive fMRI Data for Coo and sCoo Stimuli** | | | | | ✓ |
| **fMRI Data for Audio-visual Task** | | | ✓ | ✓ | |

**Table 2.2. Summary of animals involved in the lateralisation project section of the report.** Coo and sCoo refer to the stimulus used for the experiment, further details of which can be found in *Chapter 5*, section 3.

### 2. Motivation for Task Performance

Food or fluid restriction is commonly used to motivate animals to perform a given task (Rowland 2007; Prescott et al. 2010). In the case of this study, the animals were fluid restricted during training. This entailed withholding water from the animal until they were in the lab environment, at which point preferred fluids were provided in return for correct trials. The amount of fluid given to an animal during a training session was individually determined, although a minimum amount must be given during this time. In line with the project license guiding the welfare of the animals, the minimum amount required is determined as a percentage of the animal's average daily water intake measured over seven days. The animal must be given at least 50% of the average intake, or 20ml per kilogram of body weight (depending on which value is higher) during a training session. Initial training began with the animal being given as much as they would like to drink during a session and the amount was reduced over several sessions to find a point at which the animal was motivated to perform the task. This ensured that they stayed motivated, healthy and not overly thirsty (Gray et al. 2016). The animals had unrestricted access to fluid on days when they were not being trained, including holidays and weekends.

To attempt to increase the amount of fluid which could be given in the lab without detriment to performance, more recently, for MT and MW a mixed fluid control with delayed food access has been successfully employed. Under these conditions the animals are restricted from fluid in the manner described above, and only have access to fine forage for enrichment during the day. They are then given the bulk of their diet late afternoon, once training has complete. In the lab they are given fluid for the first half of the session, then a smoothie mixture consisting of a banana and a pot of yoghurt blended with a little water as food reward to satiate them until they are fed at a later point.

### 3. Lab Habituation and Operant Training

Initial training of a naïve animal began with acclimatisation to the primate training chair. The animals were trained using special treats and fluids to come into the chair from their home cage and put their head up through a hole, which prevented them from escaping from the chair. Following this they were taken to the lab, where again, they were given treats in order to familiarise them with the new environment. Calming music was also played in an effort to make them feel more relaxed.

The animals were then given a basic task, being rewarded with Ribena or other special fluids in response to lever presses or for looking towards a screen. Operant training protocols using juice rewards were used to motivate the animals to correctly perform the task. Following initial habituation to the laboratory environment, head immobilisation training began. For implanted animals a magnetic resonance imaging (MRI) compatible head post was implanted under aseptic conditions during general anaesthesia. After a recovery time of 6 weeks, the animals began slow habituation to head immobilisation using positive reinforcement training. For MT, ME and MW the animals were trained to use a novel non-invasive head immobilisation device (described in *Chapter 3*) which was used throughout behavioural and fMRI training and data collection. Initially, MT and ME were trained without any form of head immobilisation. Full details of habituation to the non-invasive system is described in *Chapter 3*, section 2.4.

## 4. Behavioural Training

During the course of this project, three tasks were used: an audio-visual task, an auditory location change detection task and a basic eye fixation task. Training on the audio-visual task was part of a collaborative initiative with Helsinki University, therefore MK and MA were trained by the author, Ross S. Muers and Emma Salo. The fMRI data collection for these animals was completed by the author and Ross S. Muers. The animals trained on the auditory location task (MT and ME) were trained and scanned by the author, and for the visual fixation task, MC was trained initially for another project by Ben Wilson, and MT and MW were trained by the author. All tasks in the lab and scanner were controlled by Cortex software (Salk Institute) unless otherwise specified.

Initial training of MK and MA began with short sessions in the lab to familiarise them with the environment. They were then introduced to the touch bar sensor and a reward was delivered each time they made contact with the touch bar. The number of touch trials completed during each session increased from around 35 to 600 trials. Emphasis during each session was on allowing the animal to succeed at the task and all sessions were concluded with additional reinforcement, in the form of extra treats and upbeat music. This allowed the animal to end the session with a positive experience. If the animal struggled with a new aspect of the task, training was returned to a remedial stage to build up good performance and avoid frustration, and more simple intermediate steps were implemented to aid the transition to the next part of the task.

MT was initially trained to press and hold the lever via a voluntary engagement system which was attached to his home cage in the colony. This consisted of a regular training chair which had a lever attachment and mobile unit with a presentation screen and laptop to control the task (Figure 2.1). The task was controlled via Matlab's psychtoolbox. A juice reward was dispensed after the lever was held for increasing amounts of time before release. This successfully expedited initial lab training. Once in the lab MT had the same familiarisation procedure as MK and MA before proceeding with the lever release for reward, followed by initial presentation of the stimuli.

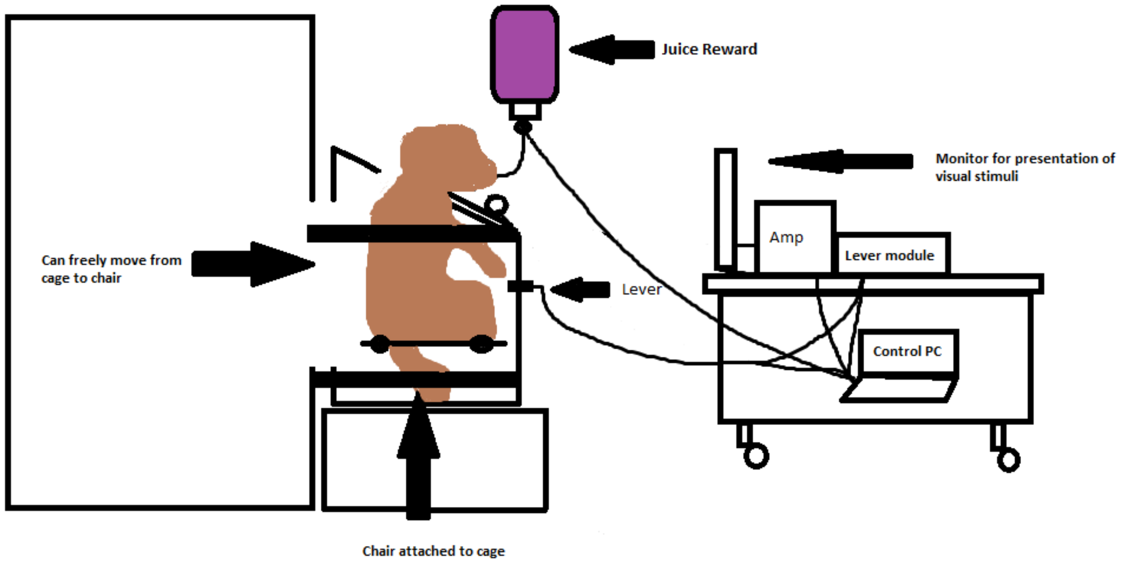


**Figure 2.1. Colony self-training setup.** A regular primate training chair was attached to the home cage in such a way as to allow the animal to move freely in and out of the chair. The animal was able to put his head up through the neck plate and press a lever to obtain a juice reward. This had the advantage of allowing him to become familiar with the training chair and paradigm before lab training began.

ME had been trained previously by another research group to respond to auditory cues with a lever release response, therefore we inverted the responses from the task for MK and MA so that he would have to maintain contact with the lever until a target was presented, at which point he would release the lever. Additionally, MC and MD had previously been trained for other projects to perform an eye fixation task.

## 5. Measure of Good Performance

As a measure of the animal's performance on the auditory and audio-visual tasks, Signal Detection Theory (Green and Swets, 1966) was used to assess correct responses (correct rejections (CR) and hits) against incorrect responses (false alarms (FA) and misses). D prime (*d')* is a measure of discrimination, in this case between moving and static auditory stimuli, calculated using the proportions of hits and FAs. Increasing values correspond to greater levels of behavioural sensitivity to the target stimuli, with a *d'* of zero reflecting a lack of sensitivity. Negative values are also possible when an animal produces more FAs than hits (as may happen during initial stages of training). As an additional level of performance measure, we calculated a bootstrap measure based on the score which would be observed on that trial run if the animal had responded at chance levels. Chance *d'* performance was evaluated by shuffling the relationship between responses given and the stimulus identity to simulate a monkey that gave the same responses but was oblivious to the stimulus conditions. A null distribution of *d'* values was created from 1000 permutations. Chance *d'* performance was defined as the 95% (one-tailed) point in distribution. In this way we were able to determine when the animal was consistently performing above chance and viewed this as successful performance.

## 6. fMRI Habituation and Training

Once consistently successful performance on the required task was established (performance breaching bootstrap *d'*), MT, ME, MK and MA were habituated to the scanner environment, initially by replicating aspects of scanning in the lab, such as introduction of scanner noises to the task. As far as possible the scanner environment was emulated in the lab to familiarise the animals with the experience in order to minimise the impact of re-locating the task to the scanner. Simulated scanner sounds recorded from the primate scanner were introduced to the task between trials to simulate sparse imaging acquisition. These were initially barely audible so as not to distract the animals from the task, and over several sessions the sound levels were increased. Following this, the animals were introduced to the scanner chair which, rather than a touch bar, had an MRI compatible button press lever activated by an infra-red proximity sensor. The animals were rewarded for lever responses until they began to respond reliably, at which point the main task was reintroduced. Recordings of the initial scans (tripilot, shim etc.) were then implemented at the beginning of the training session, initially during head immobilisation and task setup. Initiation of the sounds was delayed over several sessions so that the animals became accustomed to waiting for the noises to complete before the task would begin. A similar procedure was used to introduce the animals to the structural scan which would complete the scanning sessions. This began with the sounds playing while removing the headphones, juice tubes etc. which completed the session, and delaying the point at which removal of equipment was initiated so that the animal became accustomed to waiting for the sounds to complete before being released. During the habituation period the animals were also taken to the scanner lab after training sessions and given fruit rewards to familiarise them further with the setup before training was re-located to the scanner environment.

Once in the scanner setup, several habituation sessions were used prior to scanning. The animals were rewarded for remaining calm during setup to acclimatise them to the process. Following this, they were trained to perform the task in the scanner as they would in the lab, with only the virtual scanner sounds which had been used for lab habituation. We then slowly replaced the virtual sounds with the actual scanner sounds and continued with fMRI data collection. Additionally, in early sessions, slice numbers were kept to a minimum to reduce the noise of the volume acquisition. Further, to maintain good task performance, scanning sessions were alternated with lab sessions over the course of a week.

## 7. fMRI Data Collection and Analysis

The animals were scanned in a primate dedicated vertical 4.7 Tesla MRI scanner (Bruker Biospec 47/60 VAS, GA-38S gradient system; Bruker Medical). Functional images were acquired using a gradient-echo echo-planar sequence (GE-EPI; 7.6s inter-volume time (TR), volume acquisition time (TA) = 2s, TE = 22ms, flip angle = 90°, matrix = 96×96, field of view (FOV) = 9.6×9.6cm$^2$, slice thickness = 2.0mm with no gap, in-plane resolution =

1×1mm$^2$, 20 axial slices covering most of the brain). In each trial, one functional volume was acquired 4s after completion of the stimulus presentation (i.e., at the expected peak of the blood oxygen level dependent (BOLD) response to stimulation (Baumann et al. 2010) Figure 2.2). This imaging paradigm allowed stimulus presentation during a silent period to avoid the effects of the scanner noise on the BOLD response (Petkov et al. 2008). Two structural scans were acquired in each session aligned with the functional volumes. One of these was a full-head EPI with extra slices, which was used to improve the registration of the functional volumes to the higher resolution anatomical image. The other image was a higher resolution anatomical volume (MDEFT; TE = 6ms; TR = 20ms; matrix = 192 × 192, FOV = 9.6 × 9.6 cm$^2$, slice thickness = 2.0mm with no gap, in-plane resolution = 0.5 x 0.5mm$^2$).

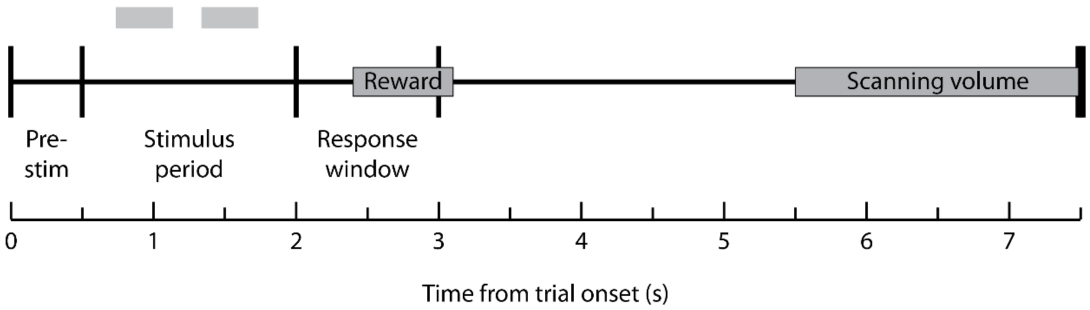


**Figure 2.2. Sparse imaging paradigm for the auditory task described further in *Chapter 5***. Sparse imaging allowed auditory data collection without interference from scanner noise. During passive stimulation the same timings were used with the addition of a fixation period preceding the stimulus period.

The functional data from each scanning run were pre-processed in MATLAB and analysed using FEAT analysis in FMRIB's Software Library (FSL), version 5.0.8. The data were motion corrected, high-pass filtered (100s cutoff) and spatially smoothed (Gaussian kernel of 3mm; full-width half maximum, FWHM). A first level general linear model with FMRIB's Local Analysis of Mixed Effects (FLAME) and the required number of explanatory variables for the experiment (detailed in methods sections for each experiment) was defined (Winkler et al. 2014) and performed. Functional data of each scanning run was co-registered via the intermediate anatomical scans to a template monkey brain that is in register with a macaque brain atlas in stereotactic coordinates (Saleem and Logothetis, 2007). A higher level group FEAT analysis was then performed incorporating the individual scanning runs. Following this, activity was projected to the cortical surface using Freesurfer for clearer presentation of the activity (McLaren et al. 2009). Each animal had one additional scanning session with passive band passed noise and tone stimuli to locate auditory cortex (AC) regions of interest (ROIs), and create an individual functionally determined approximation map of AC (Petkov et al. 2006). This allowed the task related functional data to be localised to specified regions of AC. This is described further in *Chapter 4*.

For further details relating to specific experiments, see the methods section of the relevant chapter.

# Chapter 3: Development of Non-Invasive Head Immobilisation Option for use with Non-Human Primates; Production and Training Methods

Based on **"Individually Customisable Non-Invasive Head Immobilisation System for Non-Human Primates with an Option for Voluntary Engagement"** Journal of Neuroscience Methods, 2016.

**Heather Slater**, Alice E. Milne, Benjamin Wilson, Ross S. Muers, Fabien Balezeau, David Hunter, Alexander Thiele, Tim Griffiths, & Christopher I. Petkov.

The contributions of the authors to the above publication were as follows. The author initiated the collaboration with the Freeman Hospital mould team in the Radiology department. Here various immobilisation techniques were evaluated, and the beam direction shells were considered to be the most adaptable for use with the monkeys. Stephen Thompson of the mould team trained the author on acquisition of the mould and creation of the beam direction shells and also visited the facility to provide advice on adapting the procedure for the monkeys. He, however, did not wish to be included as an author on the publication. The monkey shells were then adapted for our use by the author with invaluable assistance from our machine shop technician, Vincent Willey, who also declined to be included as an author.

Alice Milne assisted with habituation training with MC, and Benjamin Wilson assisted with the analysis of eye fixation data from MC. Ross Muers provided assistance on numerous occasions during mould procedures with the animals, and provided the suggestion to use thermal imaging for identification of pressure points. Fabien Balezeau and David Hunter assisted with fMRI data collection, and provided advice on adaption for scanning. Alexander Thiele and Tim Griffiths were kind enough to support the application to the NC3Rs for the pilot grant which funded the initial development of the system, and allowed the use of their animal, ME. All data collection and analysis (other than analysis of the eye tracking) was completed by the author. The manuscript was written by the author and Chris Petkov.

## 1. Abstract

This chapter describes the development of an individualised macaque non-invasive head immobilisation system (NHIS), which addresses several animal welfare and scientific needs. The system comprises a customised-to-fit facemask that can be used separately or combined with a back piece to form a full-head helmet. The system permits presentation of visual and auditory stimuli during immobilisation and provides mouth access for reward. The facemask was incorporated into an automated voluntary training system, allowing the animals to engage with it for increasing periods leading to full head immobilisation. The system was evaluated during performance on several auditory or visual behavioural tasks with testing sessions lasting 1.5-2hrs and thermal imaging was used to identify and prevent pressure points. A comprehensive evaluation of the system is provided in relation to several scientific and animal welfare requirements. Behavioural results were often comparable to those obtained with surgical implants. Cost-benefit analyses were conducted comparing the system with surgical options, highlighting the benefits of implementing the non-invasive option. The system has a number of potential applications and could be an important tool in neuroscientific research, when direct access to the brain for neuronal recordings is not required, offering the opportunity to conduct non-invasive experiments while improving animal welfare and reducing reliance on surgically implanted head posts.

## 2. Introduction

In collaboration with the Freeman Hospital Cancer Radiotherapy Unit at Newcastle upon Tyne, UK, the author prototyped and developed a non-invasive head immobilisation system for nonhuman primates, using similar design approaches as those in use in human radiotherapy cancer treatment units. In developing the nonhuman primate NHIS, the experience of the Freeman Hospital Unit in developing and using highly customised whole head or limb immobilisation in human patients was combined with our experience working with non-human primates on neuroscientific procedures.

The system was designed to achieve head immobilisation for macaques of different sizes, providing a highly customised fit, and allowing for visual and auditory stimulation and for the animals to receive fluid rewards as positive reinforcement (Figure 3.3). The transparent plastic allows the animals to see through the facemask while it is being placed, which makes placement of the facemask less intimidating or distressing. The plastic can be greatly modified while retaining structural strength; air holes can be created and the plastic can be thinned in problem areas to prevent overheating and to alleviate pressure points. It can be easily modified to incorporate fittings for a wide range of scientific and laboratory attachments, which can readily be integrated into the facemask or full-head helmet system.

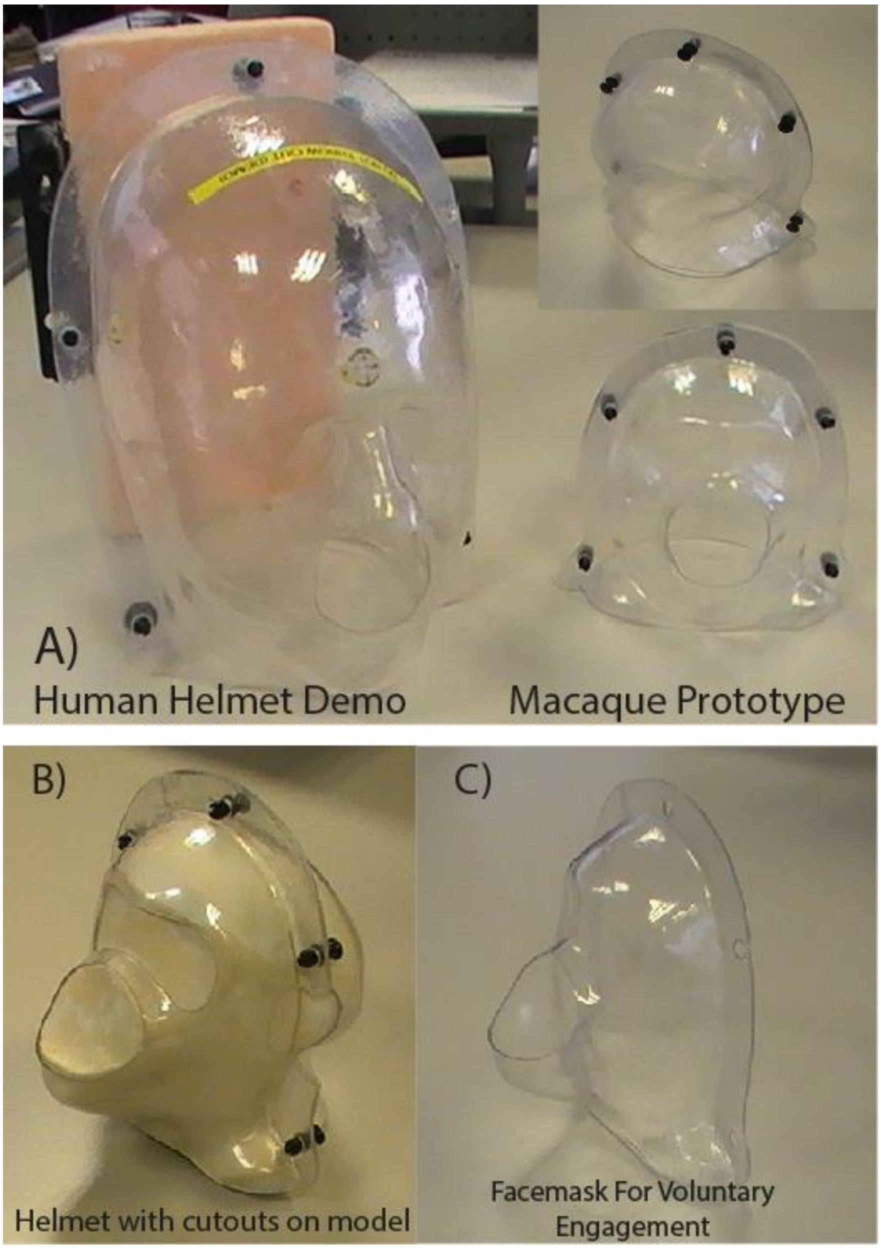


**Figure 3.3. A monkey non-invasive head immobilisation system, based on head immobilisation methods used with human head or neck cancer patients being treated with radiotherapy.** A) Shows a transparent demo of a human helmet that is customised for a human cancer radiotherapy patient. To the right is shown the prototype that was developed here for neuroscientific research with macaques. B) The transparent plastic helmet can be easily modified to include cut outs allowing the animal to see, hear and make small mouth movements to drink fluid rewards. C) The two piece helmet system can be separated so that the facemask can be used alone for initial habituation training to whole head immobilisation, with periods of voluntary immobilisation of the animal.

## 3. Methods

### *3.1 Creating the head model*

Two different methods were used to create a head model from which the helmet system could be made.

***Head impression using plaster bandages and alginate***

For one approach, an impression of the whole head was created using plaster bandages and alginate moulding putty (BabyRice Chromatic Alginate Moulding Material mixed with water). Once the head impression had been made, it was filled with plaster to create the head model.

First the animal was sedated, e.g., with ketamine (0.1ml/kg; Henry Schein, trade name Narketan 10) while blood oxygen saturation was monitored and maintained by providing additional oxygen if needed, and ensuring that the breathing pathways were unobstructed. The eyes were protected by closing the eyelids and covering them with gauze and plastic cling film. Excess hair was trimmed from the areas to be moulded and aqueous cream was applied to prevent the impression material from sticking to remaining hair. The mould of the back of the head was made by gently lowering the animal's head into a suitable container, containing a cutaway section to accommodate the neck, filled with alginate to take the impression. Plaster bandages were applied to the face and allowed to set (Figure 3.4A), ensuring the mouth and nose were not obstructed. This procedure took about 15-25 minutes to complete. Once the bandages and alginate had set, they were removed and any remaining cream or moulding material was removed by hand from the monkey's head and neck. The animal was then monitored in a recovery unit until fully conscious before being returned to the home cage.

The plaster facemask and alginate back piece were joined together using additional bandages. Plaster of Paris was then poured into the impressions and allowed to set to create the head model (Figure 3.4B). Following setting of the plaster, the bandages were removed and the rough edges of the head model were filed down. The helmet was then created by halving the model (Figure 3.4C and D) and placing each half into a vacuum forming machine (C.R. Clarke Vacuum Former 1210). Sheets of 4mm thick Polyethylene terephthalate (PETG) thermoplastic (Bay Plastics) were heated in the machine and these were vacuum formed around the head model (Figure 3.4E). A band saw and hand held rotary tool (Dremel) was used to trim excess plastic from the shell and shape the front and back pieces of the helmet as desired (Figure 3.4F).

The two halves can additionally be made in isolation rather than producing a full model. For example, once a full helmet has been created, if only a new front or back piece is needed, the other half can be used to position the animal while an impression is retaken of the desired area. However, for greatest accuracy in creating the initial model it would be recommended to create the whole head impression. Straws inserted into the alginate impression either side of the head can help in realigning the front and back pieces after the alginate sets and the impression is taken.

Additionally, alginate putty was used to improve the fit of helmet pieces. For example, if the head immobilisation requires adjustment to further refine the fit, or create a model for a smaller animal, alginate putty can be applied to the inside of an existing mask. The mask plus the alginate putty applied to the inside are then placed over the animal’s head while sedated. Once the alginate has set, the facemask is removed and the set alginate is left inside the plastic. This can then be used to create a new model of the head to create a better fitting or more accurate facemask for the helmet. A similar procedure can be performed to improve the fit of the back piece.

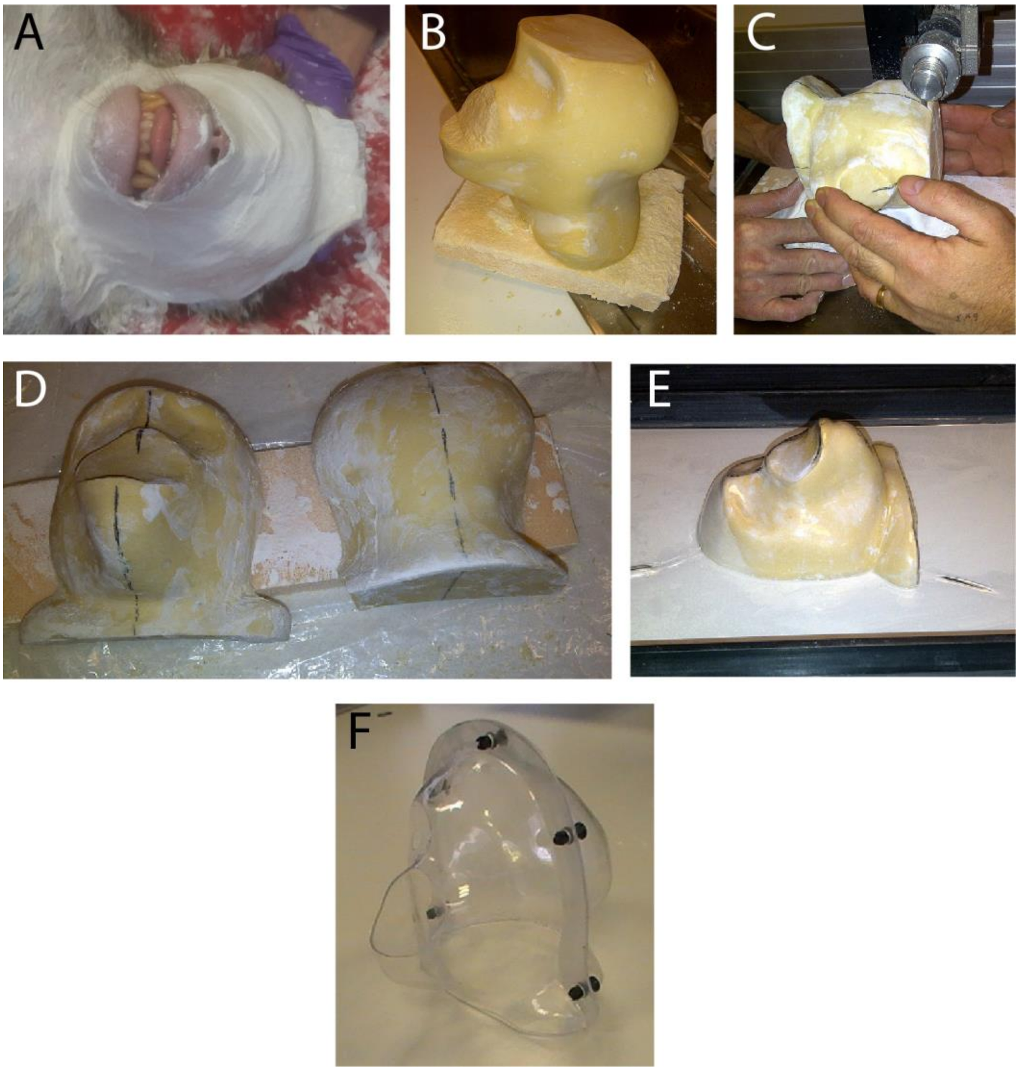


**Figure 3.4. Creating the thermo-plastic shell for the helmet.** (A) Plaster bandages are used to take an impression of the animal’s head and a model is created (B). (C) The model is then halved to create a face and back piece (D). Thermal plastic is heated and moulded around the head model using a vacuum forming machine (E). A hand drill is used to cut out areas for the ears, eyes and mouth and to place attachments as needed (F).

### *Head model creation using MRI*

A model of the head was also acquired using MRI imaging under anaesthesia. For each procedure the animal was initially sedated with ketamine (i.m. 10mg/kg) before being pre-oxygenated and prepared for intubation (Propofol, typically 3-4mg/kg i.v.). The trachea was intubated and the lungs ventilated (at 25 strokes/min) to maintain expired $CO_2$ within the physiological range. Anaesthesia was maintained with sevoflurane 2.5-3.0% mixed with 100% oxygen. Lactated Ringer's solution was given intravenously at a maximum rate of 10 ml/kg/h. Physiological parameters (heart rate, blood pressure, blood oxygenation, and expiratory $CO_2$) were monitored and kept in desired ranges with volume supplements. When the animals were fully anaesthetised, they were then transferred to a primate MRI scanning chair, where they were held in place using body supports, ear bars and padding to support the head.

MRI-based T1 weighted Modified Driven Equilibrium Fourier Transform (MDEFT) and T2 weighted Rapid Acquisition with Relaxation Enhancement (RARE) structural images of the whole head were taken on a non-human primate dedicated, vertical 4.7 Tesla research MRI scanner (Bruker BioSpin, Ettlingen, Germany). The processed scans were converted into Analyze format to be loaded into a Medical Image Data Examiner (AMIDE, SourceForge, Slashdot Media; Figure 3.5) and converted into 3D format. These were then 3D printed (Rogue Research Inc. or in-house) to produce the head model. As before, the model was halved and each half was used to create the thermoplastic shell of the helmet on a vacuum forming machine.

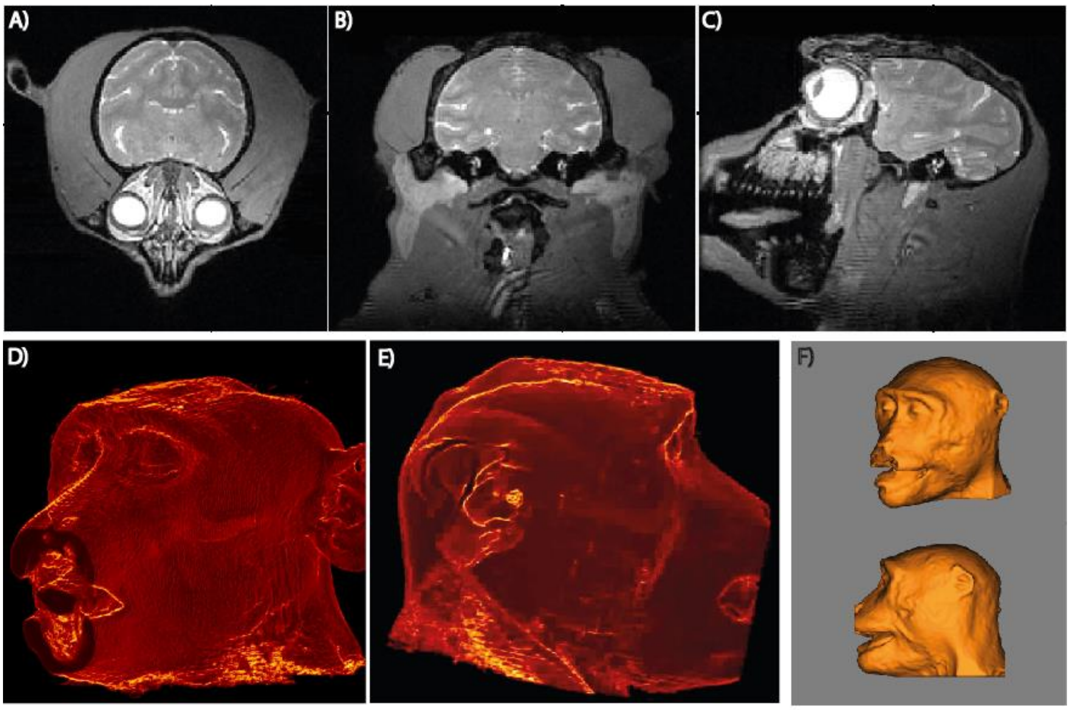


**Figure 3.5. Acquiring a head model using MRI.** A whole head MRI is taken of the animal (A, B and C) and converted into a 3D surface using Amide 3D software (D-F; shown for ME, MT). The head 3D image is separated into two halves in post processing and the image files were sent to Rogue Research Inc. for 3D printing or printed locally on a 3D printer to create the model of the head. Images D and E taken from Supplementary Video 1 (available in online version of Slater et al., 2016).

Creating a model using plaster bandages is relatively easy and cheap to perform, and does not require general anaesthesia or complex imaging techniques. It could therefore be used in most primate labs. The second approach has the potential to provide more anatomically accurate head models, but is more demanding in terms of equipment and resources. A similar procedure as the one described for creating the head model using MRI, but instead using Computerised Tomography (CT) could in principle also be used if the equipment is available. Generally, the models created with either the plaster bandages or MRI provided sufficiently accurate models of the animals' heads for creating the NHIS.

### *3.2 Design of the facemask and helmet system for use in the laboratory*

The helmet system comprised a mask and a back piece which was placed over the back of the head. Initially the animal was seated in a standard vertical primate training chair and restrained via the neck plate attached to the chair (see Figure 3.6 and Figure 3.7). No additional body restraint was used. The facemask was attached to the training chair via a frame which fits over two attachment bolts protruding from the neck plate. Thumb screws were used to secure the facemask to the bolts in the chair (Figure 3.6). The frame can be moved back and forth and has hinges which allow the mask to be tilted to accommodate the natural position of the monkey's head. The facemask was then secured in the desired position. The back piece was attached from behind to meet with the front piece and was secured to the frame via thumb screws which fit through the back and front of the device. Plastic snaps can also secure the two pieces. Additional rigidity was achieved by incorporating the metal head bar attachment which is commonly used in laboratories to attach to the animal's surgically implanted head post, but in this case it was attached to the helmet. Using the metal bar to provide additional stability to the system from above did not seem to be critical, but can provide additional stability when the facemask is used alone, e.g., for voluntary engagement (see Figure 3.7). During training the animal received reward for correct task performance through the mouth piece. The mouth cut out allows the monkey to breathe, drink and move the lips.

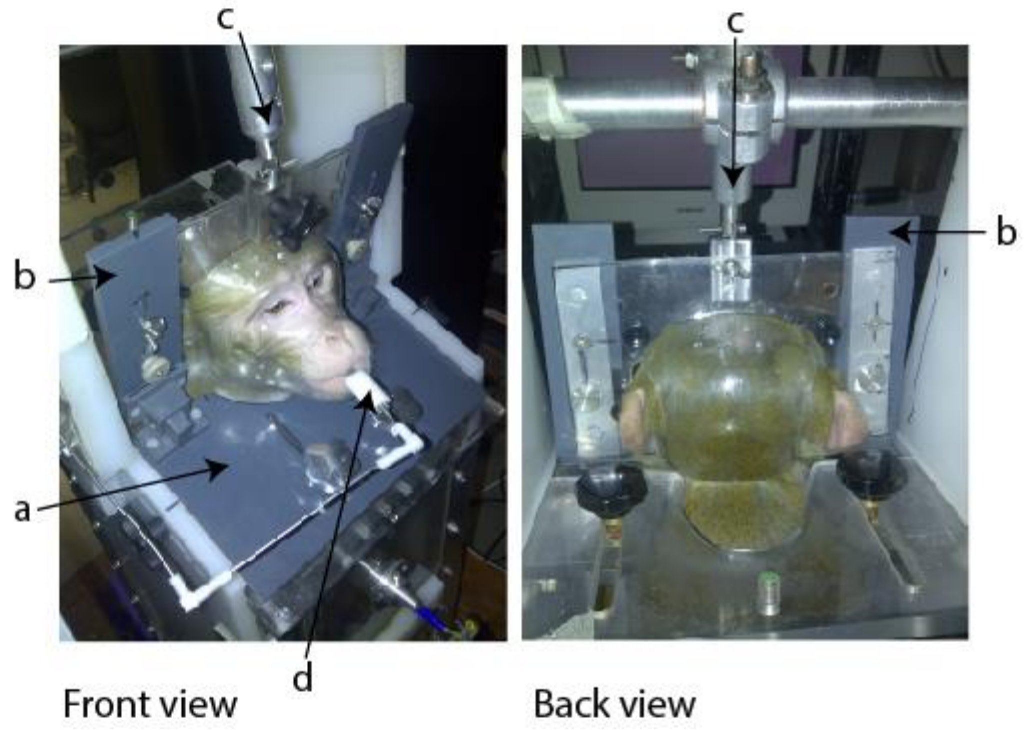


**Figure 3.6. The helmet system within a typical laboratory working chair.** The front of the transparent mask is fitted to the frame (a and b) and attached to the training chair. The initial attachment is through the base plate of the frame (a). The brackets to the side (b) are fixed via hinges which allow the mask to be tilted as needed. The head bar attachment (c) can provide further rigidity. The animal receives reward for his task through a juice reward system and can breathe, drink and move the lips (d). Several air holes can be added to the helmet to help with ventilation. The translucent facemask and back piece provide an individually customised fit for each animal.

## *3.3 Voluntary facemask engagement training for auditory behavioural and simple eye fixation tasks*

Two different behavioural tasks were used to assess performance during voluntary engagement. The front piece of the helmet system (the facemask) was initially used to gradually habituate monkeys to head immobilisation.

Two of the animals (MT and ME) were trained to perform an auditory spatial discrimination task, initially without any head immobilisation (see *Chapter 5*, section 2). Early in training, the facemask alone was attached to the front of the chair and voluntary engagement training began. The animals performed their task while engaging with the mask in order to receive reward for correct trial completion. Over the course of 4-5 testing sessions the mask was moved closer to the animal's face to encourage voluntary engagement. Initial training on the task did not require complete head immobilisation, but as the animals were required to identify a change in direction of a sound, it was important to have them face forwards and engage with the facemask for more accurate perception of the spatial location change in the stimuli. Full head immobilisation was later required for the placement of headphones for more accurate stimulus presentation and for habituation to other aspects of the scanner environment which the animals would progress to. Once performance was stable and the animal was willing to keep their face in the mask, the back piece was gradually introduced and fixed in place. Initially the back piece was held by hand over the back of the animal's head. Once this was tolerated, the back piece was attached to the front piece loosely so that some movement was possible, but not enough for the macaque to fully remove their face from the mask. Finally the back was attached for increasing lengths of time with full head immobilisation.

A third animal (MC) had been trained to perform basic eye fixation prior to the loss of his implant at age 8 years (for methods on eye fixation training used for this animal, see Wilson et al., 2013). To assess the relative quality of eye-tracking data using this NHIS, the animal was gradually habituated to the facemask using voluntary engagement. Eye-tracking typically requires the head to be immobilised, therefore it was not possible to allow the animal to habituate to immobilisation while performing a behavioural task as above with MT and ME. Instead, habituation training began using an infra-red proximity sensor (OPB733TR; OPTEK Technology) which was placed on the outside of the mask (Figure 3.7). When the sensor was activated by the presence of the animal's face in the facemask, juice reward was dispensed. The reward was then delayed, e.g., 1000-2000ms, to encourage him to hold his face within the mask for longer periods. The reward could also be delivered continuously for as long as the monkey's face was present in the mask and stopped when the sensor detects that they have removed their face (see Supplementary Video 3, online version of Slater et al., 2016). Once the animal readily engaged the mask, the sensor was relocated to the back piece of the helmet, and this procedure was repeated while placing the back piece over the back of the head as the animal engaged the facemask. The level of free movement was then slowly reduced during full helmet attachment. Following this, eye fixation training was able to resume.

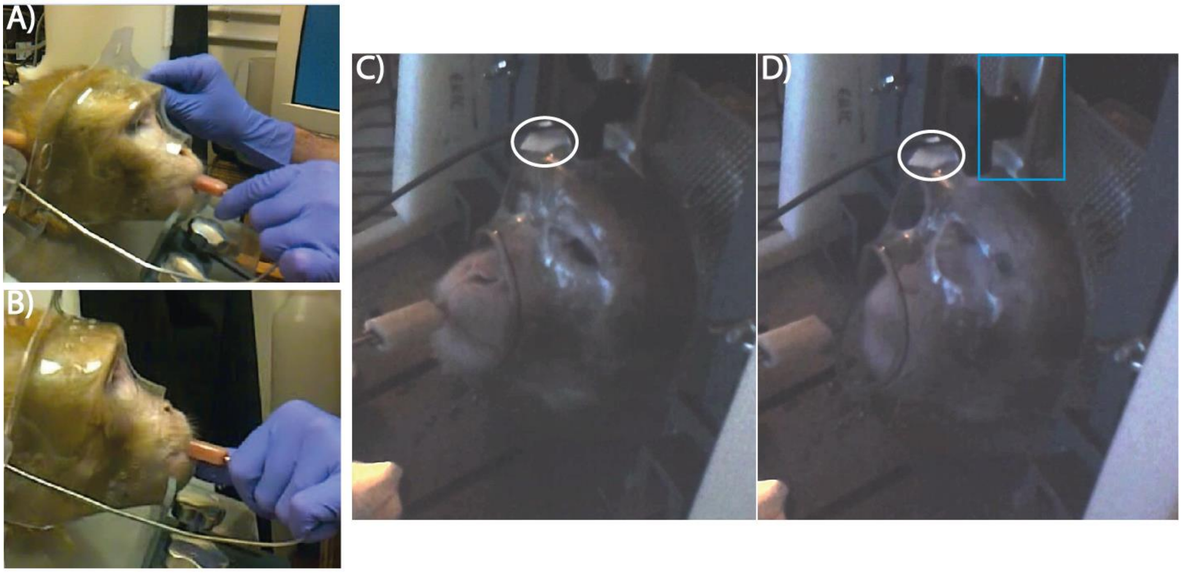


**Figure 3.7. Voluntary facemask engagement during initial habituation training.** Initially the animal is introduced to the mask and receives reward while placing his face inside the customised facemask (A and B, ME). The face mask is then attached to the training chair (C and D, MC) with extra stability for the helmet system provided by the head bar usually used for attaching to a surgical implant, as an option (indicated with a blue box in D.). An infra-red sensor is placed at the top of the mask (indicated with a white circle) which is activated by the presence of the animal's face in the facemask. On sensor activation, juice is dispensed via the reward system. Following this, the sensor is placed on the back piece of the helmet system and the animal is rewarded for as long as the face is in the facemask with the back piece touching the head, until the back piece is completely attached. Images shown here are frames from Supplementary Videos 2-3 (see online version of Slater et al., 2016).

For comparative data collection, we used the same experimental design as had been used for a previous experiment involving MC performing a fixation task, using an infra-red eye tracking system (Arrington Research). Briefly, MC was seated in a primate chair 60 cm in front of a computer monitor. A fixation spot was displayed at the centre of the computer monitor and he was rewarded for visually fixating on it for 4 seconds within a fixation window of $5^o$ visual angle. Trials in which he failed to fixate on the spot for 4 seconds were classed as aborts and were restarted after a brief inter-trial interval. In the initial head-posted experiment auditory stimuli were infrequently (25% of trials) presented from audio speakers located to the left or right of the computer monitor. We used the same experimental setup for data collection with the NHIS, however, no auditory stimuli were used. For the analysis, only trials for which no additional stimuli were presented in the original experiment were used; therefore the data represent a 4 second fixation period and a 3 second period during which no auditory or visual stimuli were presented, and the animal was free to look around. In the second experiment using the helmet, the monkey was again presented with a fixation spot for 4 seconds, following which eye-tracking data was recorded for an additional 3 seconds in the absence of any other stimuli. In both experiments, 10 testing runs, each containing 16 trials were collected.

Further to this, a fourth animal, MW was trained to perform a fixation task while habituating to the mask. MW was naïve to training and had never been implanted. Once he had been familiarised to the mask and it was attached to the chair, he was rewarded for putting his face into the mask and looking at a video playing on a computer monitor placed in front of him. Over a number of sessions the size of the video window was reduced and he was rewarded for as long as he was looking at the centre of the screen. Further to this, the back piece was placed over his head for increasing amounts of time while he continued to fixate on the video. After 5 days of this, the back piece was attached and he was introduced to the fixation spot. This was presented for 3000ms using Matlab's psych tool box. He was rewarded for the duration of time which the spot was on the screen and he was fixating in order to reinforce the required behaviour. Following this, the spot was presented for 2 seconds, with the animal waiting until after the fixation period before being rewarded. The time he was required to fixate was then increased and the reward following the fixation period was delayed.

More recently, MT has also been successfully trained to fixate using the same method but starting with full head immobilisation as he had previously been habituated.

## 4. Results

### *4.1 Task performance and behaviour during voluntary engagement and non-invasive head immobilisation.*

Performance on an auditory spatial discrimination task (*Chapter 5*, section 2.2) was measured over the different stages of habituation to the system (Figure 3.8). The initial stage of training with MT and ME involved no immobilisation. The facemask alone was then introduced for voluntary engagement training in combination with task performance, and this was followed later by attaching the back piece of the helmet. Figure 3.6 shows that performance on the task improved or remained stable throughout the three stages. In MT, who was already performing at a good level, performance was relatively stable across the different procedures (no significant difference in behavioural performance across the conditions; ANOVA; $F_{2,66} = 2.19$, $p = 0.12$). ME's performance significantly improved during the facemask and helmet immobilisation procedure ($F_{2,79} = 8.66$, $p < 0.001$), as the animal learned the task. Thus, there was no overall detrimental effect on auditory task performance by implementation of the non-invasive head immobilisation procedures, demonstrating that this is an effective method for training macaques and collecting data on auditory spatial location tasks.

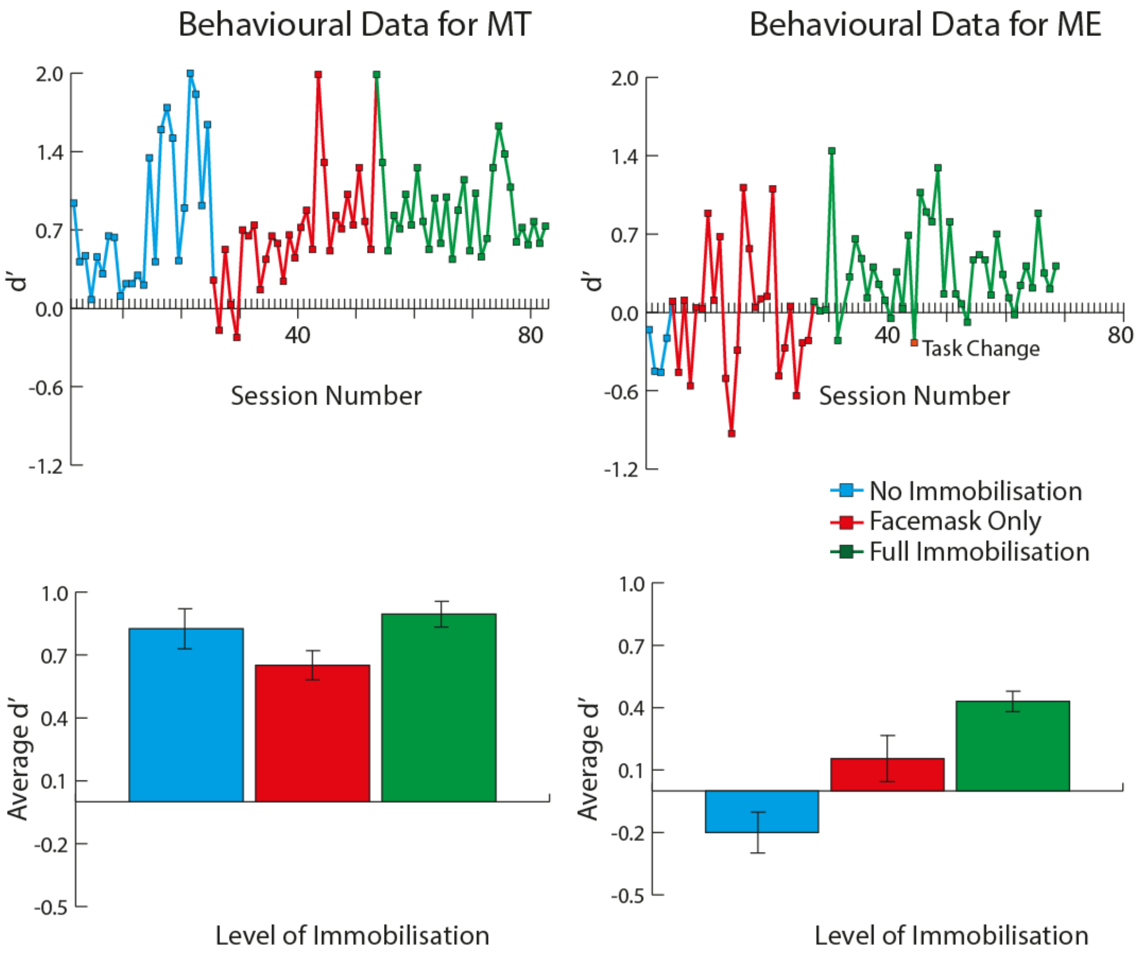


**Figure 3.8. Performance on an auditory spatial discrimination task during different stages of habituation to facemask or helmet systems.** Average *d'* during the testing session was used to assess performance on the task for these two monkeys (MT, ME).

The period of habituation to achieve full head immobilisation is summarised as the number of daily testing sessions required from the point at which we began to immobilise the animal’s head in any way, to the point at which they work for a full training session head immobilised (>30mins). For the animals with a surgical implant this refers to touching or holding the head post to allow the animal to become accustomed to movement restriction. For the animals using the helmet system, this refers to the point of initial introduction of the back of the helmet. For the helmet system (red bars in Figure 3.9) full immobilisation was achieved in 3-19 daily testing sessions (mean = 10; standard error mean, SEM = 3.4) and for the implant in 5-22 sessions (mean = 10; SEM = 2.7). Figure 3.9 suggests that habituation to the non-invasive system with full head immobilisation without distress requires at least as much time as habituating the animal to head immobilisation using an implanted head post, not including habituation training with the facemask which can take an additional 2-3 sessions. However, unlike the implant, no recovery period is required before immobilisation training begins when using the NHIS.

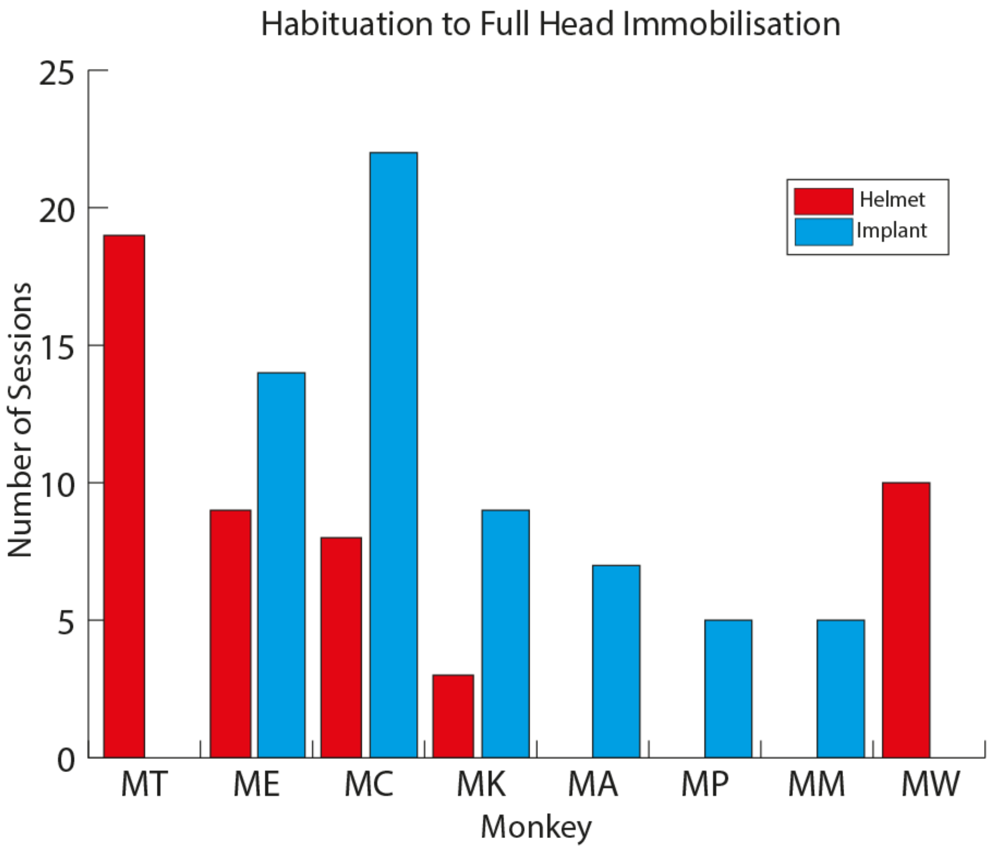


**Figure 3.9. Initial habituation period for the two immobilisation methods: helmet versus implant.** Monkeys ME, MC and MK had previously been trained with head immobilisation using their implanted head post.

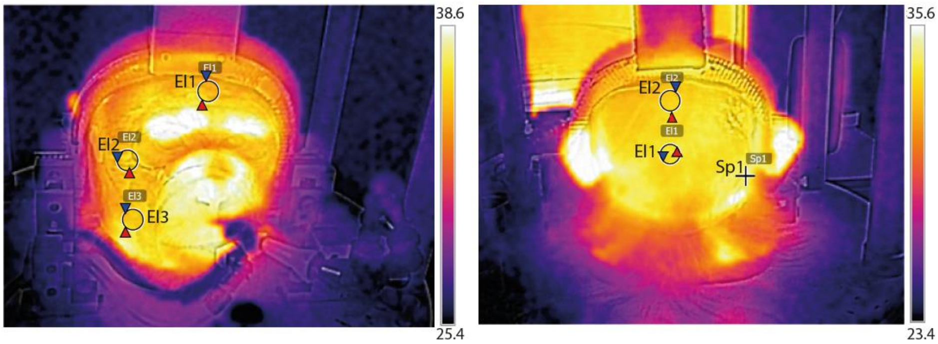
### *4.2 Thermal imaging to monitor for hot-spots*

An area of increased pressure or contact between the animal's head and the plastic can result in an increase in temperature or a "hot spot" in that area, which if not properly ventilated or depressurised could become sore and potentially infected. Being able to measure hot-spot formation in head immobilisation systems could identify potential problem areas that can be addressed by thinning or removing the plastic in that area, provided that the remaining pressure between the head and the immobilisation device is well distributed over a relatively large remaining area.

An infra-red thermal imaging system (FLIR Systems E4 camera, FOL7 lens with 80 x 60 IR resolution) was used to assess the potential for the formation of hot spots. Readings were taken before and after a training session and the images were processed with the FLIR software to identify areas of increased temperature. These images were then used to guide the placement of ventilation holes in the plastic, if needed. The effectiveness of these modifications in dissipating heat was measured over the course of subsequent training sessions (Figure 3.10A). Figure 3.10 shows that heat within the helmet can increase within a range of 0.7-4.5°C from the beginning to the end of the testing session (in this case 50 minutes). Any spots that increase more than 3°C during the course of the session reveal the need for modification of the helmet system in those areas to decrease potential discomfort and reduce pressure point formation. A full helmet system like this can accommodate many ventilation air holes without reducing stability in head immobilisation or rigidity of the system.

**A:**

**Images Post Training Session Only (50 mins):**



| Front | | | | Back | | | |
|---|---|---|---|---|---|---|---|
| **ROI** | **Before Temp** | **After Temp** | **Change** | **ROI** | **Before Temp** | **After Temp** | **Change** |
| **El1** | 33 | 35.4 | 2.4 | **El1** | 31.7 | 33.8 | 2.1 |
| **El2** | 31.2 | 35.7 | 4.5 | **El2** | 32.6 | 33.3 | 0.7 |
| **El3** | 33.5 | 36 | 2.5 | **Sp1** | 32.5 | 34.9 | 2.4 |

**B:**

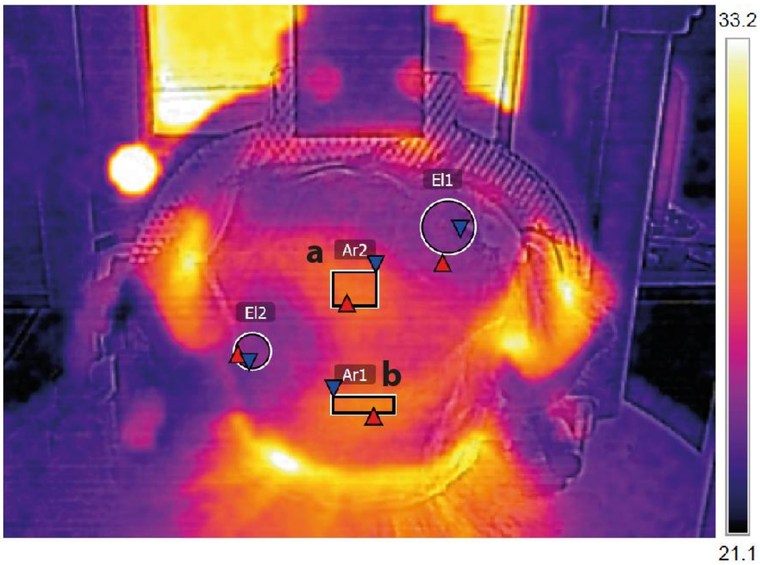


**Figure 3.10. Thermal imaging to identify hot spot formation.** (A) Thermal measurements before and after training. Images were taken of the face and back of the head before and after training (50 minutes of immobilisation at room temperature). For brevity, only post training images are shown. Hotspots can be identified and labelled (B: rectangles a and b) and ventilation holes placed in the plastic at those points to allow for better ventilation of the area and less pressure on the underlying part of the head/face. All temperatures reported are in degrees ($^{o}$) Centigrade.

### *4.3 Numbers of sedations*

Short term sedation with ketamine (0.1mg/kg, circa 30 mins) is required both for the maintenance of a surgical implant and for obtaining a model of the head to produce the NHIS. For the MRI procedure, general anaesthesia is required (initial sedation with ketamine followed by propofol and sevofluorane, c.a. 1-2 hours; Methods). If the animal grows, loses weight or the helmet becomes uncomfortable, the helmet may need to be replaced, requiring another procedure under sedation to obtain a new head model. The number of occasions when an animal was sedated for implant maintenance or for head model creation is shown in Figure 3.11. The figure shows that over the course of a year with these 7 macaques 2-3 sedations are needed for the non-invasive system, and anywhere between 0-8 sedations for surgical implant maintenance.

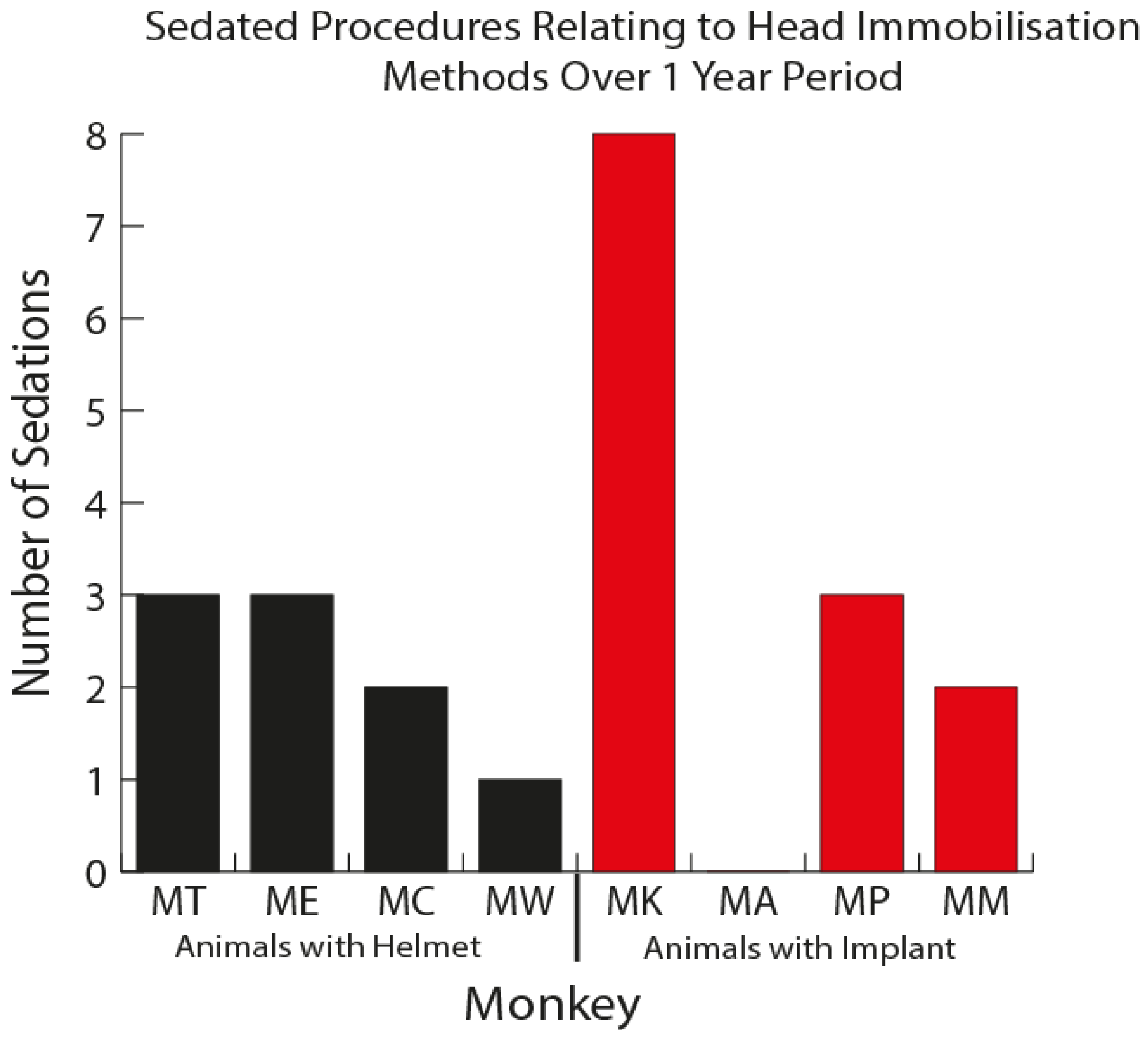


**Figure 3.11. Number of sedations for each of the seven animals in this study during a one year period for immobilisation related procedures with the helmet system or to maintain surgical implants.** For animals with an implant this refers to implant procedure (if it occurred within the year of monitoring) and sedations required for margin debridement (MK, MA and MP had their implant procedure in a previous year). For animals without a surgical implant this refers to sedations for obtaining head impressions or MRI based models of the head. Some animals, like MK were sedated more regularly for implant debridement and maintenance procedures if these would distress the animal to conduct while awake.

### *4.4 Number of helmet replacements over a 1 year period*

An increase or decrease in weight of the animal has the potential to impact on the fit of the helmet. If the animal begins to display signs of discomfort such as reluctance to engage with the helmet, or the level of immobilisation provided is insufficient, it may be necessary to update the model of the head and produce a new facemask and helmet. Over the course of a year, three animals on study with the helmet system required 1-3 replacements, even in animals whose weight was relatively steady (Figure 3.12). This highlights that the system might need updating ~2 times a year and that body weight can, but does not always, predict when an animal might be due for a helmet replacement.

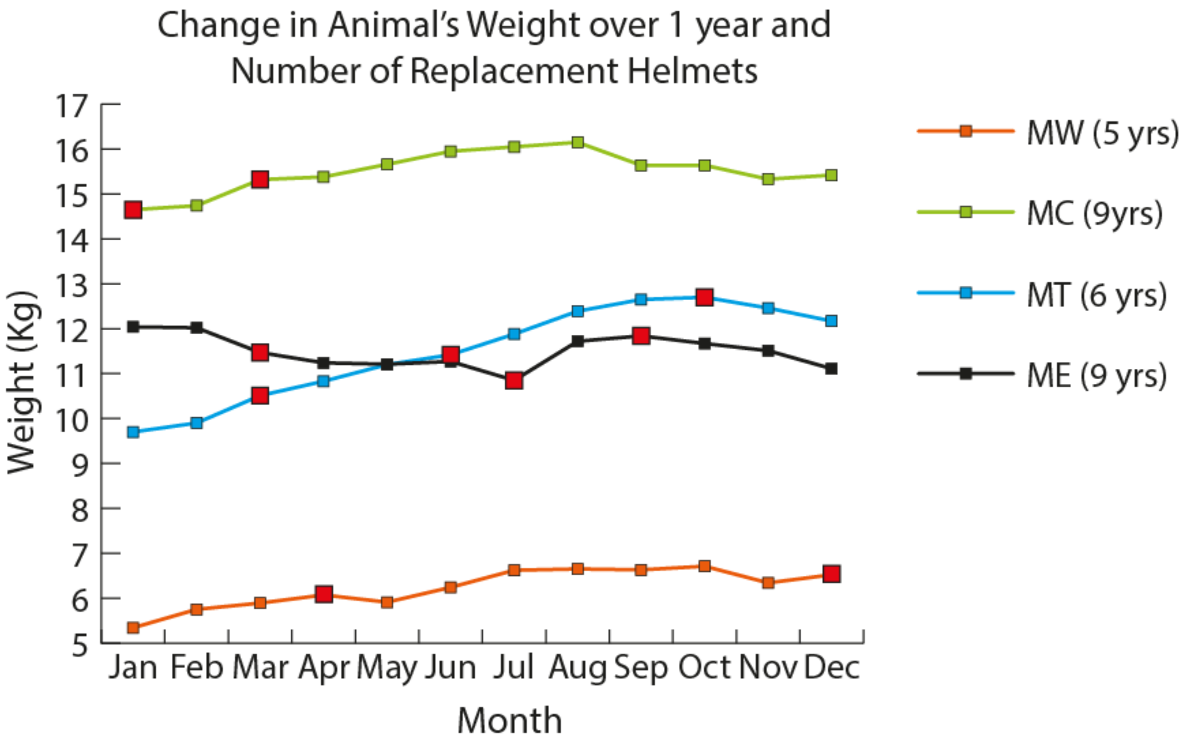


**Figure 3.12. Weight change of animals and number of occasions when each animal had a helmet replacement during a one year period.** Red markers indicate the point at which the helmet was replaced. As the weight of the animal changes the helmet/facemask may need to be adapted to eliminate discomfort, in the case of weight gain, or to improve the fit, in the case of weight loss.

### 4.5 Simple eye fixation stability during head immobilisation

To assess the stability during a simple eye-fixation task conducted with the helmet system, data gathered from MC when he was tested using a surgically implanted head post was compared with data gathered using the helmet system. To assess how well MC fixated, in relation to his prior training and testing with his surgically implanted head post, we initially calculated the variability in looking at the fixation spot as the average distance between his eye position and the fixation spot throughout each trial, including 4s of fixation and the following 3s of silence, where he was not required to fixate. A comparison of eye tracking data acquired with both methods is shown in Figure 3.13. While MC appeared to fixate more tightly using the head-post system (*t*-test comparing the average eye position of the animal during fixation period; $t_{18} = 7.37$, $p < 0.001$), both methods showed significantly less eye movement during the fixation period than during the non-fixation period ($F_{1,36} = 190.35$, $p < 0.001$, helmet system: $t_{18} = 6.96$, $p < 0.001$).

It is, however, important to note that while this level of fixation is adequate for our experiments, where we needed general fixation followed by unrestricted viewing towards the location of sound sequences (Wilson et al., 2013), further assessment is required before this method could be applicable to visual research where tighter levels of fixation (<1° visual angle) are required. It is possible that the additional movement during fixation may be a combination of some movement in the helmet system and an animal that is not fixating as well. Since we see somewhat comparable level of eye movement for both methods after the fixation period ended, this may suggest that the variation has more to do with the animal's performance than head movement. In any case, combining eye-tracking with MRI (the latter of which can provide a measure of head movement in the helmet system, as detailed in *Chapter 4*) could help to tease apart the differential contributions to eye fixation performance.

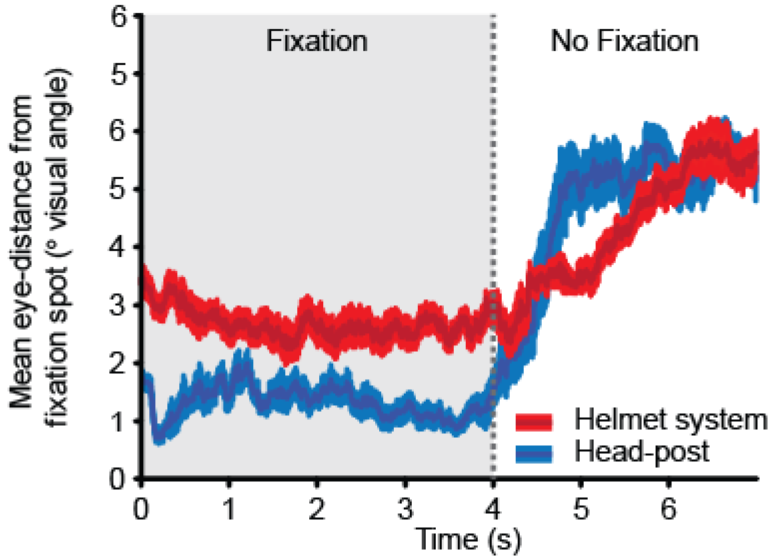


**Figure 3.13. Comparison of eye-tracking data acquired in MC with implanted head post and the helmet system.** The mean distance between the monkey's eye position and the centrally located fixation spot (±SEM) within a 5° fixation window rejection area was calculated during the 4 second fixation period and the subsequent 3 second period during which no stimuli were present and the animal was free to look around.

### *4.6 Monetary cost of procedures*

The monetary cost of the surgical implant was compared with non-invasive head immobilisation procedures in our facility. Representative data on our cost for a surgical implant procedure, maintenance of the skin margin and the implant post-surgery maintenance were collated. The costs for these are compared with those for the procedures required for producing the helmet. An itemised list of the costs is shown in Table 3.1. The initial setup for the helmet production, including equipment and consumables, is £2,111 GBP ($3,223 USD). After the initial equipment investment, a large number of helmets can be produced at a cost of approximately £139 ($212) per helmet. In comparison, a single implant procedure costs ~ £1,919 ($2,930), with additional costs of ~£145 ($202) for each implant maintenance procedure.

The cost of two replacement helmets per year for a period of four years would be £588 ($898). In contrast, a surgical implant procedure, with 4 implant maintenance procedures per year (average for the animals listed) over the four years would, in our lab, amount to £4,239 ($5,924). Therefore, under similar conditions to those used in our facility, there is a clear financial benefit to the use of this system.

| **Helmet** | |
|---|---|
| Plastic | £5/sheet |
| **One off (Initial Setup)** | |
| Vacuum former | £1200 |
| Thermal Camera | £796 |
| Frame for square chair | £15 materials<br>£100 labour |
| **Consumables** | |
| Plaster | £20 for 25kg |
| Alginate | £30 for 2kg |
| Bandages (per procedure) | £1.00 |
| Ketamine | £12/10ml |
| Oxygen | £25 |
| Use of prep room | £100 |
| **Other** | |
| Vets/Theatre Cost | £100.00 |
| Anaesthesia | £65 |
| Scanner | £295/hour |
| 3D models | $910 (£544) each |
| **Total for equipment:** | £2,111 |
| **Total for MRI Procedure:** | £1,004 |
| **Total for impression mould procedure:** | |
| Basic: | £139 |
| With Alginate: | £147 |

| **Surgical Implant** | | **Implant Maintenance** | |
|---|---|---|---|
| PEEK post | £40 | X-rays | Husb/Vet |
| Workshop costs | £100 | Chemical Cauterization | Husb/Vet |
| Theatre cost (1 day) | £822 | Dermasol cream | £8 |
| Vet costs | £232 | Wonder Dust | £17 ( per 113g bottle) |
| Dental acrylic | £65 | Ketamine | £12/10ml |
| MRI compatible screws | £400 | Other Consumables | £25 |
| Consumables and post-op care (analgesia etc.) | £259 | Oxygen | £25 |
| | | Use of Prep Room | £100 |
| **Total for Surgical Implant Procedure:** | £1,918 | **Total for Implant Maintenance** | £145 |

**Table 3.1. Comparison of monetary costs for surgical implant procedure and helmet production.** Husbandry costs are not included. Anaesthesia refers to procedures following short term ketamine sedation.

## 5. Discussion

An individually customised non-invasive head immobilisation system was developed for non-human primates. The approach includes the use of a facemask, which is shown to be useful for habituation and initial behavioural training to help reduce animal distress when using the system. The option for automated voluntary facemask engagement can be used separately, where full head immobilisation is not required, or in combination with whole-head immobilisation to help the animals to habituate to using the full system. An approach for monitoring and addressing pressure point formation using a relatively inexpensive device for thermal imaging was also developed, which, since no occurrences of pressure points were encountered, seemed to be successful. The feasibility and quality of the data for auditory behavioural experiments and for basic eye-fixation training is also demonstrated, which for our purposes with auditory tasks does not require sub-degree visual angle fixation. Overall, the system is robust, versatile and can be flexibly incorporated into a number of laboratory setups, being easily modified to suit the individual animals and the requirements of the experimental procedures. The results obtained with the system in relation to the scientific and animal welfare criteria identified in Table 1.1 are summarised here.

1) Customisable: The system is individually customised for each animal. It is produced by creating a model of the animal's head and using this to create a thermoplastic facemask and shell. The aim of creating individual facemasks and helmets for each monkey is to improve the fit for each animal (an important feature in recent systems). Additionally, this is not a cumbersome process in terms of cost or the time needed to create the facemask and helmet, even if a replacement of these might be needed. The other aspects of the system (attachments, fittings etc.) can be created once and used with different animals' facemasks or helmets.

2) Access: The system ensures access for auditory and visual stimulation as well as the delivery of fluid to the animal. Due to the versatility of the system, adjustments can easily be made to the plastic material to allow access to the ears, eyes and mouth.

3) Minimising pressure points: At no point did any animals experience pressure sores as a result of the helmet. The system successfully strikes a balance between coverage of an

area which is large enough to distribute pressure around the head, while allowing enough room to modify as needed to open up air holes and spaces to improve the comfort of the animal. Additionally, the fit of the head immobilisation device was monitored with thermal imaging which allowed the identification of any hots spots, highlighting areas where the helmet may be too tight. An approach for monitoring hot spots using thermal imaging data to reduce pressure points and sores from forming is also shown.

4) Comparisons to implanted head posts: Behavioural performance data, even on a difficult auditory task, were encouraging in the two monkeys tested (MT and ME) during performance while using the facemask or full helmet. The habituation time for achieving immobilisation for >30 mins is comparable in the 5 animals tested in relation to the use of a surgically implanted head post. Additional assessment is needed before the system can be recommended for tasks which require more rigorous control over eye-movements than what was required for these purposes, and further comparison of the two methods for use in fMRI data collection is explored in *Chapter 4.*

5) Minimise distress: There were no obvious behavioural signs of distress exhibited by the animals, and evidence that habituation to the device has no lasting effect on performance is provided. Habituation was aided by the transparent plastic, allowing the animal to see through the mask, drawing their attention to rewards rather than the enclosure.

6) Adaptable: The system is highly adaptable and can be implemented in a more common laboratory testing chair (such as those produced by Crist Instruments or Rogue Research), and can be further adapted for use with fMRI data collection, which is shown in *Chapter 4*, where the helmet is adapted to avoid the implant for MK, indicating further versatility and we are now exploring options for implementation for the collection of EEG data.

7) Voluntary engagement: A key feature of the system is its ability to be used as a facemask attached to any training setup. The facemask can incorporate a sensor to automatically identify if the animal has engaged the facemask, at which point a reward is provided to encourage longer periods of self-immobilisation. This can expedite training and the transparent nature of the mask allows the animal to see rewards through the plastic, encouraging continuing engagement to obtain the reward. The use of a sensor to

detect the face during habituation was adequate for these purposes, but the option also exists for the use of eye trackers to automate identification of engagement with the facemask in combination with a visual task (Kiorpes et al., 2012; Fairhall et al., 2006).

The results presented here have confirmed that the animals will readily engage with the mask for reward and can progress from habituation to full immobilisation while working on different types of tasks. Thus, the two parts of the system (the facemask and the helmet) can be used flexibly as needed and can be used in conjunction with training and behavioural data collection on tasks, even while the animals habituate to the helmet system. The system may also be useful for increasing the training potential in animals, such as those more prone to moving their head around and not attending to the sounds or screen in front of them. Using the facemask alone in this case can also mean that the animal self-immobilises by placing the face in the facemask while working on the task. This would potentially increase the quality of the auditory or visual behavioural data than if the animal is free to move its head around.

8) Larger animals: The system works well with larger animals (6 to 16kg) and is therefore likely to be a viable method for use with most rhesus macaques, and possibly other species of primates, although this would need to be separately tested.

In addition, over a one year period the number of sedations required to obtain a model of the head for the NHIS were monitored and compared to animals that required sedation to maintain or monitor surgical implant stability. Consistently, animals with the helmet required 2-3 sedations. In contrast, the number of sedations for implanted animals ranged from 0-8 during a one year period. In addition, since the head impressions can be obtained relatively quickly (15-25mins), they could be combined and obtained during other planned sedation or veterinary procedures which is also true of implant maintenance procedures.

Finally, implementation of this NHIS is cost effective for our lab. The combination of implant and implant maintenance costs are much higher than the cost of producing multiple helmets, once the required equipment and materials for the helmet system are in place.

## 6. Conclusions

An individually customisable non-invasive head immobilisation system was designed and systematically tested showing that it is robust and flexible to implement, and, for the first time, combines a facemask with a whole head immobilisation approach, providing the option for voluntary engagement. Additionally, an approach was developed for monitoring hot spots with thermal imaging to prevent pressure points or sores from forming. The system was evaluated with behavioural tasks, including an eye-fixation task. Moreover, we show that the system is not time consuming to create, generally does not take much longer to train the animals to use and is far cheaper to implement than traditional surgical implant approaches. The system offers the opportunity to conduct non-invasive scientific experiments with head immobilisation, while reducing the reliance on surgically implanted head posts and improving animal welfare. This work and that of other recent efforts provide information that may be useful for laboratories to consider as they weigh the costs and benefits of using non-invasive head immobilisation for certain procedures.

# Chapter 4: Non-Invasive Magnetic Resonance Imaging in Non-Human Primates

## 1. Abstract

Head immobilisation is necessary for a number of scientific procedures, including functional magnetic resonance imaging (fMRI), where head movement would disrupt data acquisition and/or result in injury to the animal. In this project, the system described in *Chapter 3* was further developed and tested in terms of movement and the quality of fMRI that could be obtained using the new method, drawing comparisons with data obtained from implanted animals wherever possible.

Here, MRI data from three animals using the non-invasive head immobilisation system (NHIS) is presented. One of the animals provided data using an implanted head post and with a NHIS which was adapted to avoid the implant, allowing direct comparison between the two methods. We compared fMRI data obtained during a passive listening paradigm, which did not require a response from the animals, and an active auditory task, which required the animals to press a lever to make a response during scanning. Data showed that the head movement levels using the NHIS can be up to twice those obtained by using implants, although on average the movement rarely breached 1mm with the NHIS in trained animals, which is typically the point at which head movement severely affects fMRI data quality. Further training was able to reduce the movement levels during immobilisation using both the NHIS and implanted head post. Further, auditory cortex tonotopic maps were produced using both methods of head immobilisation and signal strength in two auditory conditions was assessed. Data analyses suggest that the auditory fMRI signal is in some cases stronger with the implant, however in most cases fMRI data is comparable between the two methods.

## 2. Introduction

To progress with the development of the system for further application in the lab, the NHIS was modified so that it could be attached to the primate scanner chair. It was then tested to assess the feasibility of fMRI data collection using this method. In the initial training setup the remaining plastic sheet left after forming the facemask/helmet was retained for the attachment of the facemask/helmet to the frame of the chair. The surrounding plastic can, however, be removed or flexibly modified to allow attachment of the system as needed for different laboratory setups. The system was implemented on a primate dedicated vertical bore MRI scanner chair using attachments made specifically from MRI compatible polyether ether ketone (PEEK) material. These attached to the top of the mask in a similar way as the metal head post holder in the laboratory setting, where using ferromagnetic materials is not an issue.

For MRI, all ferromagnetic materials need to be replaced with non-magnetic materials, such as PEEK plastic. The bottom of the mask is given extra support by two MRI compatible PEEK legs, which attach the base of the mask to the chair (Figure 4.1). The system could, in principle, also be flexibly modified for use in a horizontal bore scanner. The helmet was further modified to create a NHIS for an implanted animal (MK) which avoided contact with the implant. This was used to compare data quality within one animal. The levels of motion seen during scanning with implanted head immobilisation were compared with the animals using the NHIS with both passive and motor tasks. The quality of the data gained with the two methods was then compared in terms of signal strength, motion artefacts and functional activity patterns.

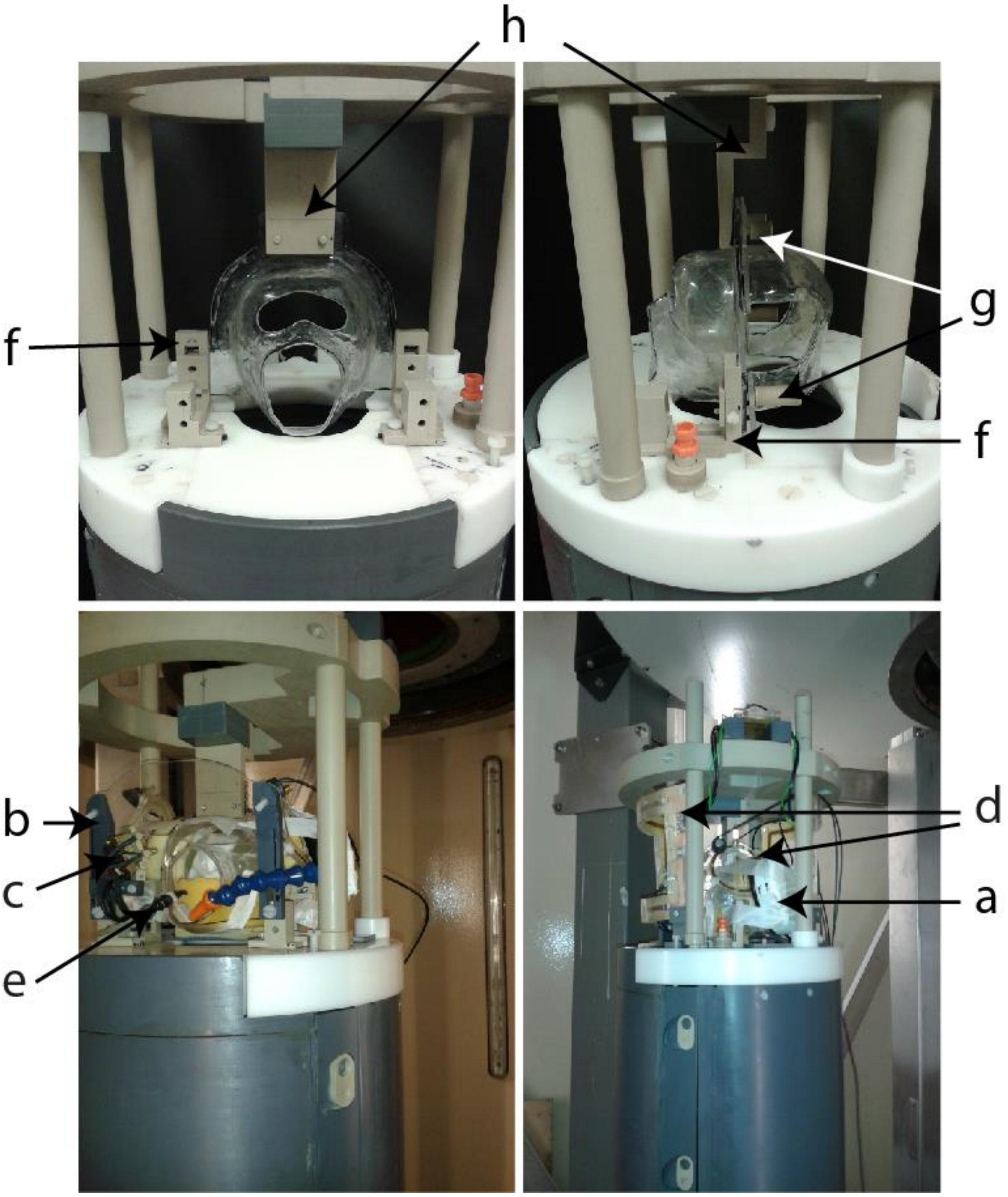


**Figure 4.1. Alternative setup for MRI data collection in a vertical primate-dedicated MRI scanner.** Removal of the plastic surrounding the face and head pieces allows for more room around the head. This accommodates placement of headphones (a), mirror (b), camera (c) and coils (d) needed for scanning, and the juice tube (e) needed for providing reward. The mask is initially secured to the chair with customised fittings (f) before the back piece is attached via thumb screws (g) and the helmet is secured to a point at the top which is where an implanted head post would usually be fixed (h). This has been implemented for use with our single and multi-channel (shown here) MRI imaging coil setups.

## 3. Methods

### *3.1 Training*

Once monkeys MT, ME and MC were performing their respective tasks to a high standard, they were habituated to the scanner environment and setup (for details see *Chapter 2*, section 6). MT and ME were completely naïve to the scanner environment and required slow habituation. MC had previously been scanned when implanted, which would have allowed us to collect data with the NHIS and compare this with previous data collected with the implant. Unfortunately it was not possible to progress with this animal due to a combination of behavioural issues and time constraints and he was subsequently re-implanted for another project. However, the experience gained from working with MC prompted further adaptation to the scanner chair setup which allowed the training of the subsequent animals for this procedure to be expedited.

### *3.1 Task and Stimuli*

Two experiments were conducted for comparison of data quality between the invasive and non-invasive method. The first of these was an active task which involved the animal making a response with a lever depending on the stimulus condition, which is described in full in *Chapter 5*, section 2. Briefly, the animals were trained to make a lever response following a pair of conspecific coo sounds which were presented in two different spatial locations in azimuth, or withhold the lever response to a pair of sounds which were presented in the same spatial location. The second was a passive listening paradigm, which involved the animal (MK) being exposed to various artificial noises without requiring a behavioural response. First, band passed noise bursts ranging from 1-, 2-, and 3-octave band-passed to broadband noise (0.250-19kHz) were presented during 10 scanning runs consisting of 90 volumes each. Further, single frequency tones were presented during 10 scanning runs consisting of 100 – 120 volumes each. The stimulus sounds were 50ms in duration sampled at 44.1 kHz and presented at 8Hz with a 75ms inter stimulus interval.

### *3.2 fMRI Data Collection*

During fMRI data collection, where testing sessions are longer (~1hr setup time; 2 or more hours of scanning/testing) potential increases in temperature inside the helmet become a concern due to the less efficient air circulation inside the bore of the magnet. Therefore, for our longer MRI sessions gel cooling packs were placed behind the animal's head in a position that would not interfere with the setup. This seemed to be an effective solution for reducing the temperature in the helmet during these sessions. The cooling pack in combination with some of the other approaches described (such as placing additional ventilation holes in the helmet), are promising measures for progressing to longer testing sessions. However, the use of the system with full head immobilisation testing sessions longer than were tested (i.e., >2 hours) would need to be assessed.

### *3.3 Active Task Data*

For the lever press task, each testing run consisted of ~100 trials. A total of 18 scanning runs were included in analysis for the two implanted monkeys (MK and MA) and 18 were included for the animals scanned with the NHIS (MT and ME). Of all of the trials presented, 20% contained no stimuli and were used as a baseline for sound versus silence comparisons. The criteria for data inclusion in further analysis were based on the behavioural measure *d'* and only sessions where behaviour breached pre-determined performance levels (see *Chapter 2*, section 5) were entered into further analysis. Two categories of movement were assessed, one relating to the motor response made using a lever, and one relating to jaw movements when the animal received a reward. The EVs for the GLM analysis for the movement assessment were hit, false alarm (FA), miss and correct rejection (CR) with silence as an implicit baseline. Here, hit and CR (both correct responses) were compared with miss and FA (both incorrect responses) for reward related movement, and hit and FA (lever response conditions) were compared with miss and CR (no response conditions) for lever related movement.

### *3.4 Passive Listening Paradigm*

For each method of immobilisation, 30 scanning runs of 70-100 volumes were recorded with MK. The animal was presented with band passed or tone stimuli on every other scanning volume, with dummy volumes taken in between stimulus trials. The band passed noise condition also contained purely silent trials. The data was analysed as per the methods described in *Chapter 2*, section 7. The GLM for the tone experiment had each of the 6 tones presented as an explanatory variable (EV), and for the band passed noise comparison the two types of band passed noise (high and low) were entered as EVs with silence as an implicit baseline.

## 4. Results

### *4.1 Movement within the NHIS measured during fMRI*

For all datasets, where the data showed normal distribution, parametric tests are used. Where the data was not normally distributed, non-parametric equivalents were used instead. In order to compare levels of movement in the helmet in relation to head posts, the MCFLIRT motion detection algorithm in FSL (Jenkinson et al., 2002) was used after acquisition of functional Echo Planar Imaging (EPI) scans taken with the animals in the scanner awake, and performing an auditory task. MT, ME, MK and MA had all been trained to perform a lever press task in the scanner, allowing us to compare the head movements in two animals with an implant (MK and MA) with two animals using the NHIS (MT and ME) (Figure 4.3). For each comparison 20 runs of 100 trials (10 runs per animal) were included in analysis (full head immobilisation durations typically of 1.5-2 hours).

Initially, movement levels during scanning were compared during early sessions (first ~30 scanning runs for each animal), when the animals were first introduced to the scanner, and more recent sessions (most recent ~30 scanning runs for each animal), where the animals had more experience with scanning (Figure 4.2). A repeated measures ANOVA revealed a significant effect of time point (early vs later session) ($F_{1,113} = 65.32$, $p < 0.001$), an interaction of time point and method ($F_{1,113} = 17.59$, $p < 0.001$) and an interaction of monkey and time point ($F_{1,111} = 33.99$, $p < 0.001$). A pairwise comparison of method (helmet vs headpost) revealed significant effects ($p < 0.001$), and that movement levels reduced significantly from the early to later time points with both methods ($p < 0.001$), which is to be expected as the animals habituate to the environment. Therefore, although the helmet may allow more movement in the beginning, movement can be reduced with both methods following careful habituation and positive experiences.

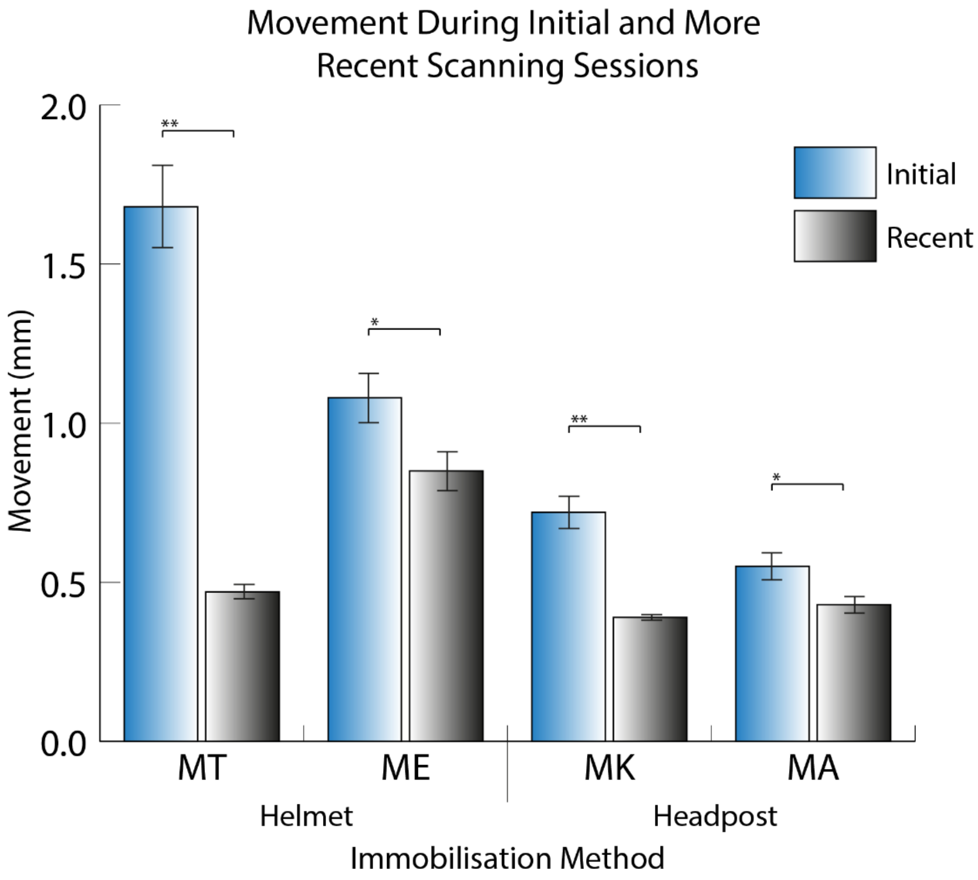


**Figure 4.2. Movement levels pre and post habituation to the scanner.** Early scanning sessions (first 30 sessions in the scanner) with both methods show more movement than later sessions (most recent 30 sessions in the scanner). However movement with the helmet system can be reduced to comparable levels with training. Animals in both conditions were performing a lever response task. ** $p < 0.001$, *$p < 0.05$.

At a later time point, when all of the animals were fully habituated to scanning and performing a lever task, a significant difference was seen between the two immobilisation methods, with less movement for the implanted head post ($t_{81} = 6.0$, $p < 0.001$; mean movement, helmet: 0.61mm; head post: 0.40mm). However, while a one-way ANOVA of movement by monkey also showed significant variation ($F_{3,113} = 33.14$, $p < 0.001$) post-hoc Bonferroni analysis revealed that the significant effect was driven by ME ($p < 0.001$ when comparing ME to each of the other three animals), and there was no significant difference in movement between MT and the animals immobilised with the implant (MT compared to MK: $p = 0.865$, MT vs MA: $p = 1.0$, Bonferroni corrected). In all cases the movement levels on average rarely breached 1mm, which in our experience is the point at which motion correction algorithms are unable to effectively correct for movement distortions. This includes animals performing a task for which they are required to perform a motor response (lever press). In this comparison, 1 scanning run of 100 trials from ME was discarded due to excessive movement levels which could not be corrected for (1.37mm).

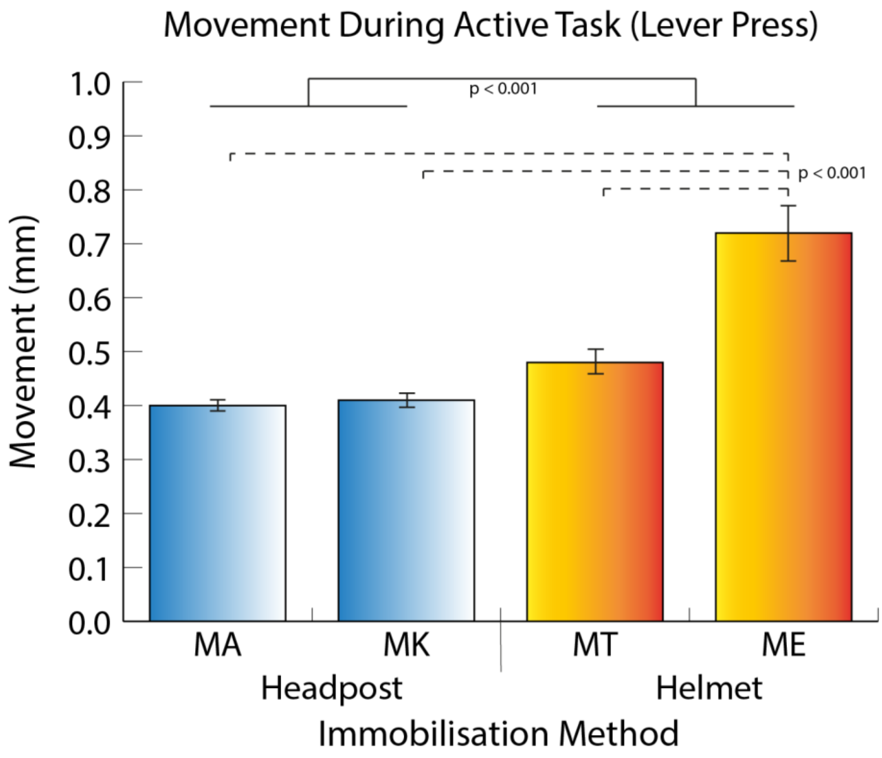


**Figure 4.3. Comparison of movement measures between the NHIS and the headpost during lever press task**. MRI-based data from behavioural scanning sessions were taken for each animal to compare across methods. Overall the movement with the helmet is more, but only for one animal.

In order to compare levels of movement in the helmet in relation to the head post within the same animal, the same method was used with an animal with an implant (MK), first using the helmet which was modified to avoid the implant, and then using the implanted head post. For both methods EPI scans were taken with the animal awake and being stimulated passively. Thirty scanning runs of 70-100 volumes were collected over 10 scanning sessions (full head immobilisation durations typically of 1.5-2 hours). The resulting comparison of the movement measures taken using the helmet with the equivalent number of scanning runs using the head post are shown in Figure 4.4. An independent samples *t*-test showed that for this animal there was a significant difference between the motion recorded using the two methods ($t_{57}$ = 2.33, $p$ = 0.023; mean movement in helmet: 0.38mm, mean movement with head post: 0.27mm). None of the scanning runs had to be discarded for either immobilisation method due to movement.

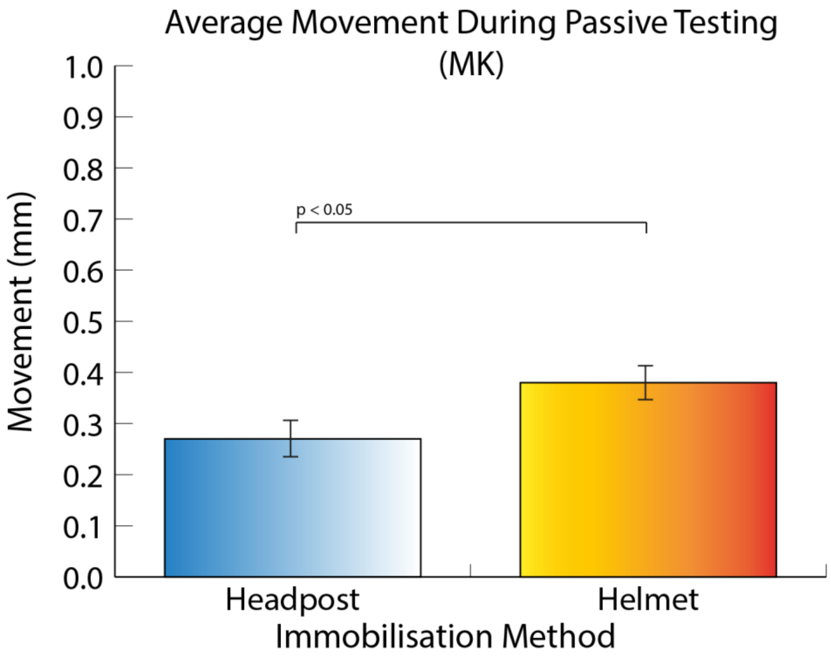


**Figure 4.4. Comparison of movement measures using headpost versus helmet: within animal comparison**. Thirty scanning runs of 70-100 imaging volumes each were compared between the two immobilisation methods: helmet or implanted head post.

As with all comparisons it is important to consider that the effects may also be due to the affective state of the animal rather than the method of immobilisation used. This may provide an explanation for the differences between ME and MT, however, affective state is difficult to account for with the methods used.

### *4.2 fMRI Activity During an Active Task*

During the active lever press task, the animals were trained to respond following two sounds which changed in location, and withhold the response following two sounds presented in the same location. To assess movement related effects due to the lever press (MK and MA) and release (MT and ME), lever response was compared with no response with two animals in each immobilisation condition. Figure 4.5 shows that motor areas associated with arm and hand movement are visible in the response condition for both comparisons, although the activity seen for the helmet comparison did not survive cluster correction. The animals differed in their response method, with MK and MA responding with a lever press and MT and ME responding with a release. This may have resulted in some variability between the data, as motor activity may also be present in the "no response" condition for the animals immobilised with the helmet, which would be removed by this contrast. Press versus silence analyses, however, were inconclusive for both immobilisation methods, showing little activity across motor cortex.

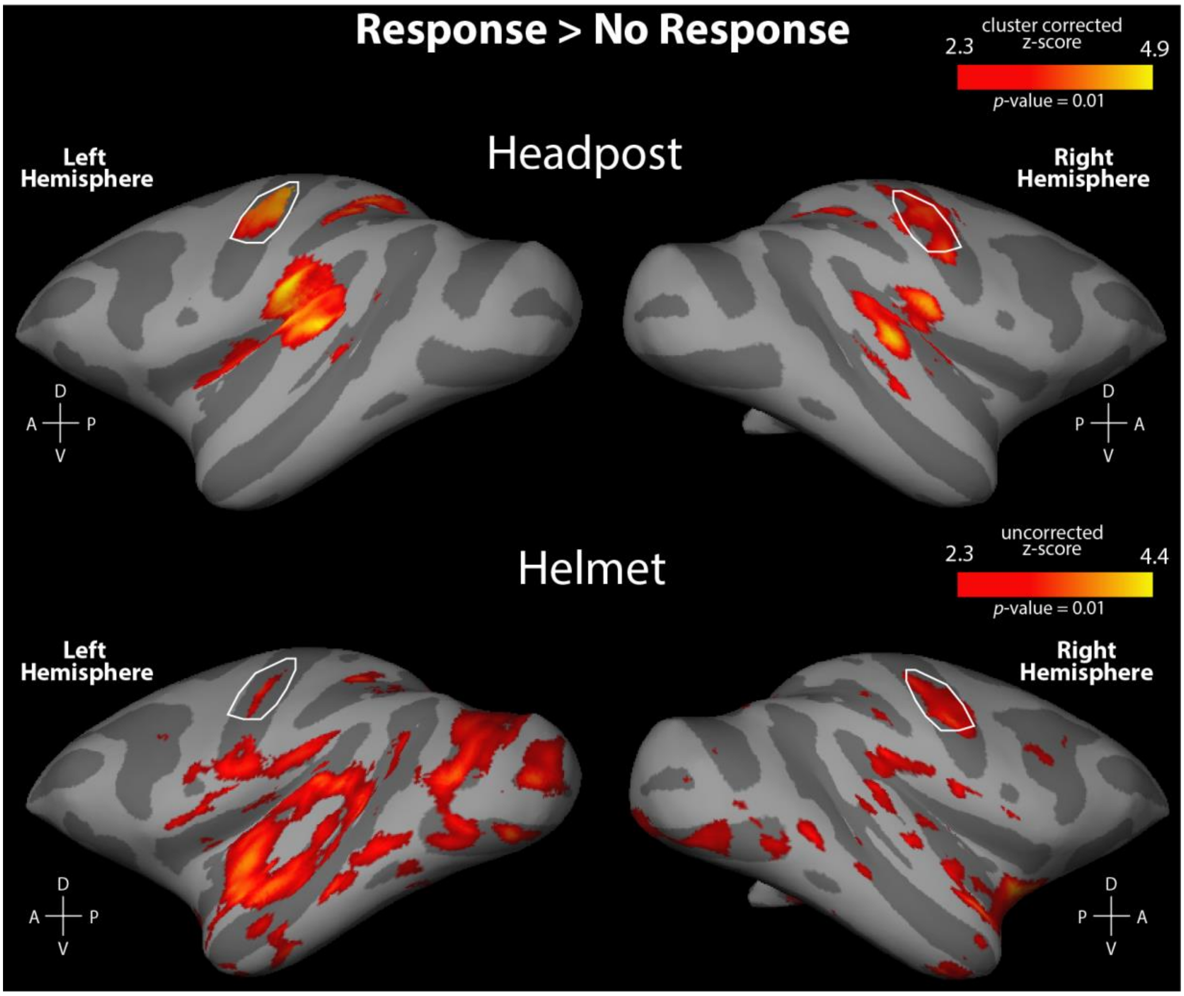


**Figure 4.5. Comparison of activity where a lever motor response was required.** Headpost data is from MK and MA, helmet data from MT and ME. Motor activity is apparent in both response conditions, however, activity with the helmet did not survive cluster correction. White outlines mark regions associated with arm and hand manipulation.

It was not possible to make a comparison of the strength of fMRI signal between the two methods as the animals in the two groups were presented with different stimuli (conspecific vocalisations recorded from different individuals), and the stimuli were presented using different types of headphones (MA and MK over ear headphones, MT and ME insert earphones).

To evaluate the effect of motion correction parameters on the data, a Pearson's correlation analysis was performed using data obtained from the same scanning sessions with no motion correction included in the analysis. Signal strength was determined using the mean value of the ten most active voxels in the mid-caudal regions of auditory cortex (AC). For each immobilisation method, no significant correlation was seen between the movement levels and the signal strength recorded (Helmet: $r = 0.25$, $p = 0.30$. Implant: $r = -0.14$, $p = 0.57$), however, when both datasets were combined, a significant positive relationship was revealed, suggesting that increasing levels of motion produce an increase in signal strength in MRI data ($r = 0.4$, $p = 0.01$).

In order to evaluate the effect of movement correction techniques on the signal strength in auditory cortex, a correlation analysis was again conducted to compare movement and signal strength in the same scanning sessions with motion correction techniques employed. For both methods of immobilisation, no correlation was seen between the levels of movement recorded during an individual scanning run and the signal strength observed in AC (Helmet: $r = 0.099$, $p = 0.679$, Implant: $r = 0.257$, $p = 0.275$), suggesting that movement did not influence the strength of the data which was obtained. While the correlation results suggest that levels of motion do impact on the signal strength, possibly due to the addition of noise from artefacts in the data, we see that this relationship can effectively be removed by using the readily available motion correction algorithms at the movement levels seen here (Figure 4.6).

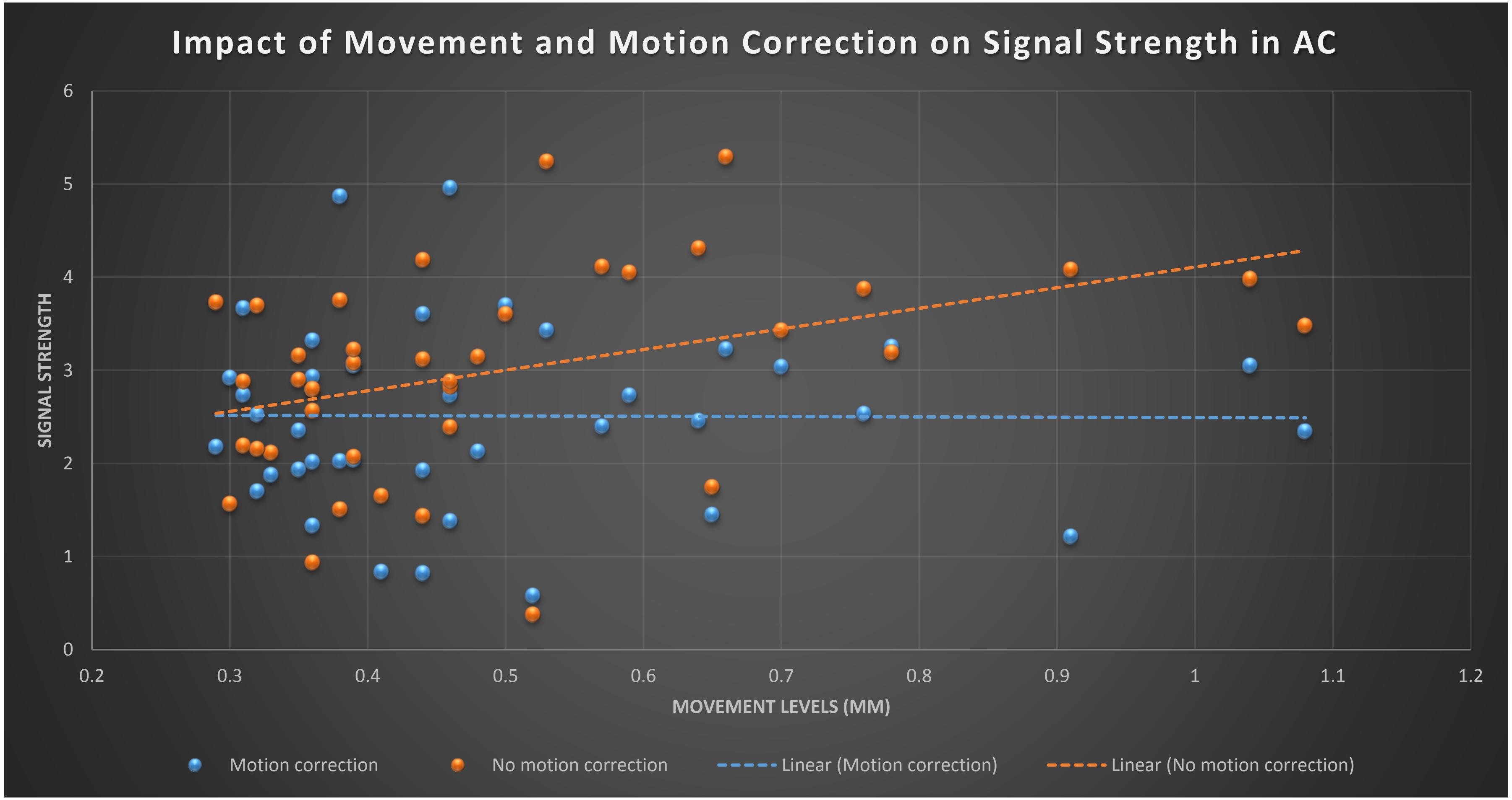


**Figure 4.6. Figure showing the effect of movement levels on the signal strength (average of the ten most active voxels in mid-caudal auditory cortex (AC)) with and without motion correction.** No significant correlation exists between movement levels recorded throughout the scanning run (100 sparse volumes) and the signal strength recorded for individual animals, or methods of immobilisation, however, a positive correlation is seen in data without motion correction which can be addressed by motion correction software.

### *4.3 fMRI Activity During Passive Stimulation (Single Animal Comparison)*

#### *Auditory Cortex Mapping*

As a measure of data quality, methods previously used for auditory cortex (AC) mapping (Petkov et al. 2006) were replicated with data from MK. The tone stimuli described above were used to functionally determine separate fields in AC in order to create an individual map of AC regions for MK. This was done using data collected when the animal was scanned with the helmet, and with the headpost, and the two datasets were compared. For mapping, fMRI activity based on the high and low frequency tones were used to delineate regions of AC which have been shown to specifically activate individual cortical fields (Figure 4.7). An auditory primary AC localiser was not used since this method is less robust and more variable than the tonotopic gradient responses shown (Petkov et. al., 2006).

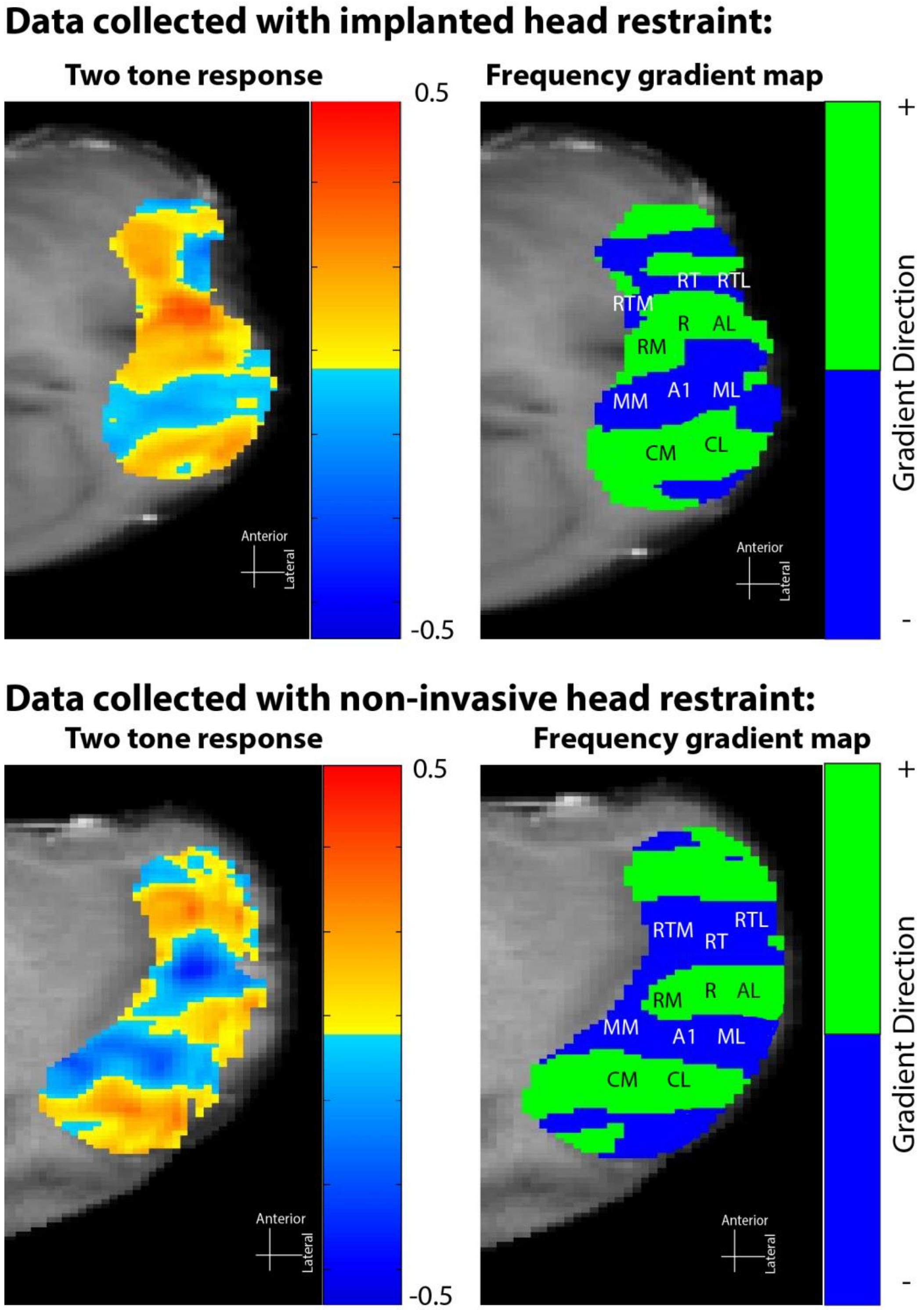


**Figure 4.7. Delineation of auditory cortex with tone stimuli in MK.** High and low frequency tones were used to functionally delineate auditory cortex (AC) regions based on their response to the stimuli (Petkov et al. 2006). Core and belt field labels are approximations based on the tonotopic responses.

To assess the variability in signal between the two methods, a region of activation was taken from core regions in AC which were associated with the high>low stimulus comparison and with the low>high stimulus comparison. The average z-values for the ROIs were taken from each lower level session to produce an average measure of signal strength across each of the sessions entered for higher level processing. A mixed 2x2 ANOVA revealed no significant interaction of the type of tone and the immobilisation method ($F_{1,28}$ = 1.62, $p$ = 0.214) and no significant effect of immobilisation method ($F_{1,28}$ = 1.78, $p$ = 0.192) Figure 4.8).

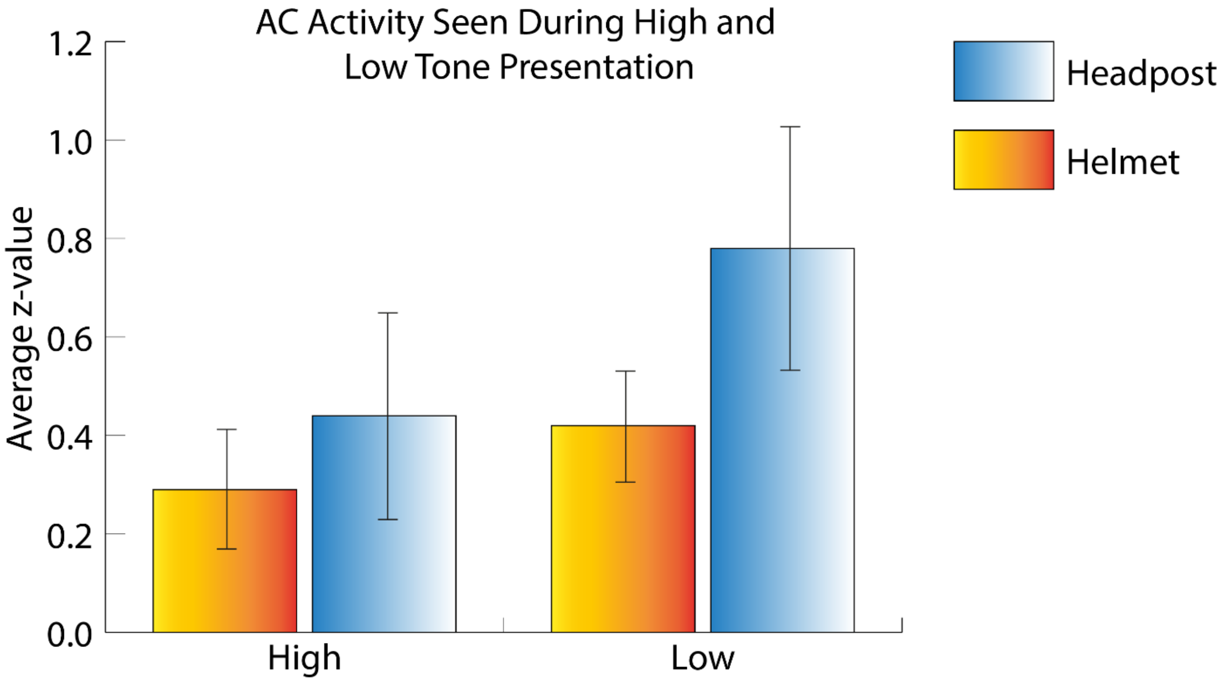


**Figure 4.8. Comparison of average z-values obtained in auditory core regions responding to high or low tones.** No significant differences in activation are seen in the tone comparisons. Maximum z-scores for headpost: high = 1.01, low = 1.52. For helmet: high = 1.94, low = 1.52.

***Band passed Noise***

Functional activity during scanning of MK shows similar patterns in the data from both types of immobilisation, although there are more areas of activity seen for the helmet (Figure 4.9). This could possibly be explained by movement artefacts seen in the helmet data which is not seen with the implant, or it could be due to differences in the placement of the surface coil during scanning. Helmet attachment requires some remaining plastic which forms flanges around the outside in order to allow the two halves to be held together, and to fix the system to the chair. This means that the coils may be up to 2cm further from the animal’s head during these sessions than for the sessions recorded with the implant.

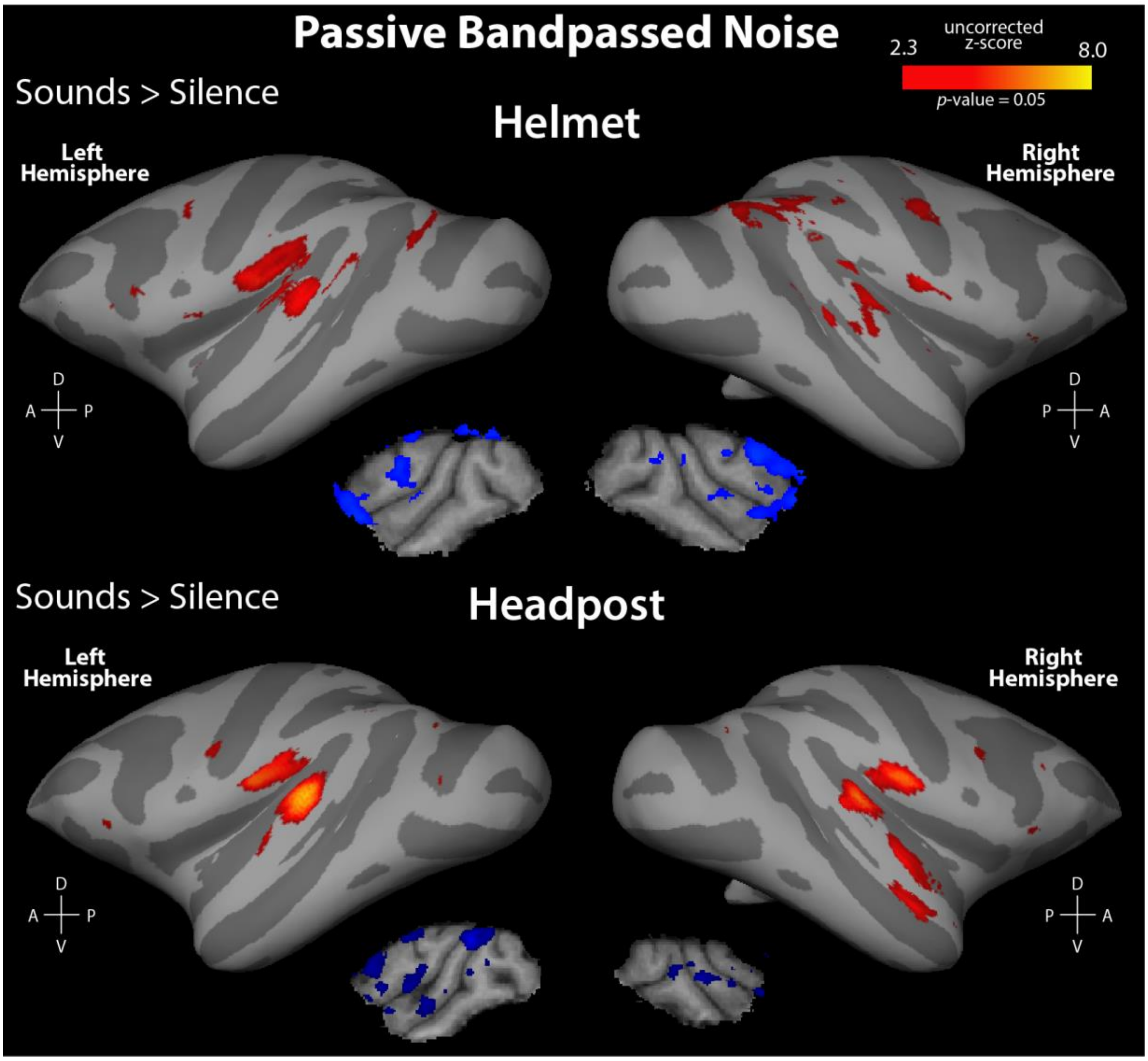


**Figure 4.9. Activity seen during scanning with both types of immobilisation.** Similar patterns of activity in left auditory cortex (AC) are seen, however, stronger clusters are revealed with the implant. Activity projected onto coronal slices to determine true level of somatosensory involvement.

Region of interest (ROI) analysis was conducted on the data in the same way as described previously. The same regions are seen to be active in the implant and both helmet datasets. A mixed 2x2 ANOVA revealed no significant interaction between the immobilisation method and the signal recorded in AC ($F_{2,50} = 2.68$, $p = 0.078$), and no significant effects of immobilisation method ($F_{2,50} = 3.08$, $p = 0.086$) or the strength of the signal in AC ($F_{2,50} = 2.22$, $p = 0.119$). Post-hoc pairwise analysis did however reveal a significant difference between the immobilisation methods in the low > high condition ($p = 0.006$) (Figure 4.10).

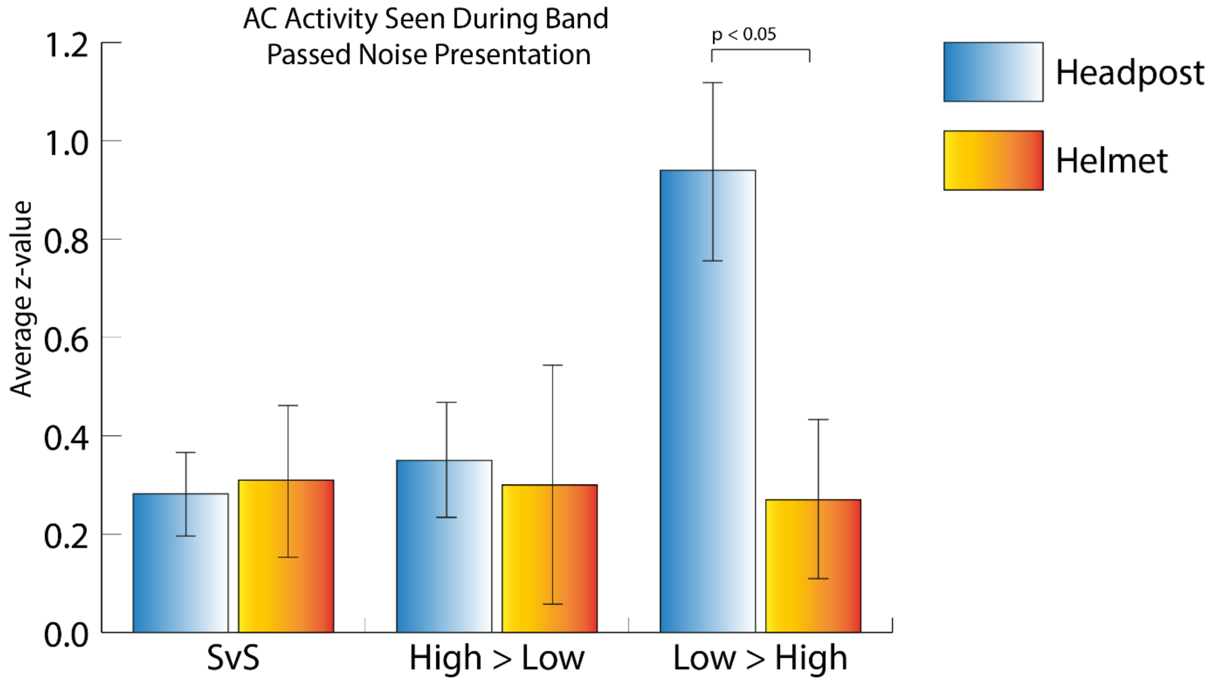


**Figure 4.10. Comparison of average z-values obtained in core regions of auditory cortex (AC) with MK.** Significant differences in MK's data is seen in the low > high comparison. Maximum z-scores for headpost: sound > silence = 2.04, high > low = 2.07, low > high = 4.16. Helmet: sound > silence = 1.68, high > low = 1.59, low > high = 2.47.

As with the task comparison, a Pearson's correlation analysis was performed to assess any impact of movement on the strength of activity seen in AC. Again, no significant correlation existed for either immobilisation method (Helmet: $r$ = -0.215, $p$ = 0.578. Implant: $r$ = -0.181, $p$ = 0.641), suggesting that movement levels did not impact on signal strength seen in AC sufficiently to prevent motion correction algorithms from correcting the data. However, the trend line suggests that there may be some detrimental effect with larger movement, possibly due to more signal being attributed to movement by the software (Figure 4.11).

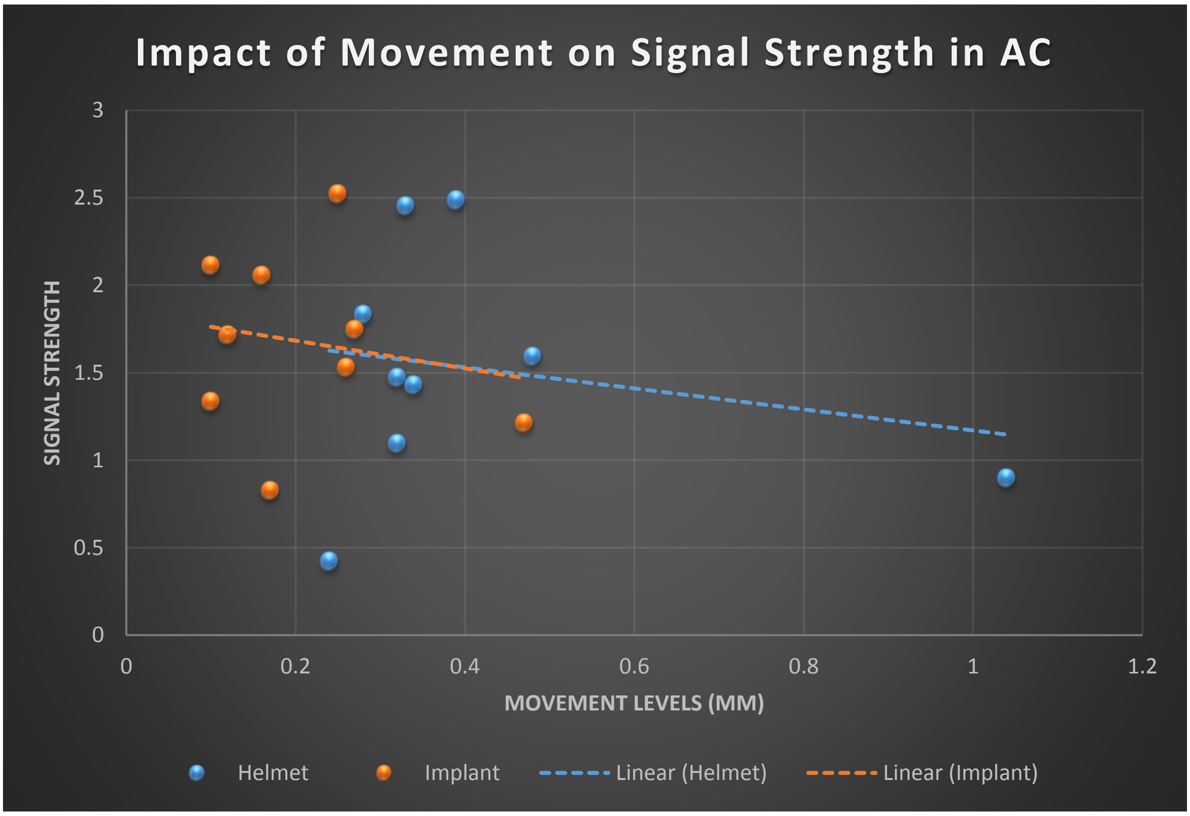


**Figure 4.11. The effect of movement levels on the signal strength (average of the ten most active voxels in mid-caudal auditory cortex (AC).** As with the lever press task, no significant correlation is seen between levels of movement recorded during scanning runs and the strength of signal recorded in AC.

Unlike the experiment involving scanning during task performance, for the passive scanning sessions we used a four channel surface coil setup which involves placing the coil as close to the head as possible. As mentioned previously, the flanges on the helmet meant that the distance between the coils and the animal's head was up to 2cm larger with the helmet than with the implant. To assess the impact of the distance from the surface coils to the animal's head, we calculated the signal to noise ratio (SNR) during scanning with both methods. This revealed a significant difference in the SNR between the two methods (paired-samples t-test $t_{6,9.5} = -5.08$, $p = 0.002$) with an average SNR of 95.94 for the sessions recorded with the helmet, and 134.41 for the equivalent number of sessions recorded with the implant. This suggests that the extra distance caused by the plastic impacted on the signal strength which was gained with the helmet, which is something that will be addressed before further experiments with this system.

## 5. Discussion

Comparison of the two methods of head immobilisation revealed that the non-invasive method can reduce head movement to the levels which can be achieved with an implant, which is revealed in the movement data from MT compared with the implanted animals. Here it is shown that, although movement levels with the NHIS are more variable, (as with ME) for passive auditory stimulation and a behavioural task requiring motor responses during scanning, in more experienced animals, on average movement rarely exceeded 1mm. From experience, 1mm is the point at which motion correction algorithms are unable to correct for movement artefacts in the data. In fact, fMRI data in human clinical subjects with movement disorders can generally be rescued with movements of up to 2mm (Wylie et al. 2014). These levels of movement in monkeys, however, would be difficult to correct for due to the smaller brain and distortions of the magnetic field homogeneity at higher field strengths (here 4.7 Tesla). Further, while data with the NHIS system can be noisier, similar patterns of activation are seen in both methods. This is especially evident in the band passed noise passive condition.

The tonotopic maps produced with the tone data from MK show activity which can be used to delineate the different fields in AC. However, the anatomy and maps show variability between the two methods. As mentioned previously, the helmet did not hold the animal's head in an expected orientation, therefore, the slices taken with the two methods are different in terms of angle. This is very likely the cause of the variability we see.

In terms of fMRI signal strength, few significant differences between the two methods are seen. However, where they are present, signal strength is greater with the implant, e.g., the low>high band passed noise presentations, and motor cortex activity in the lever press/release task. Passive auditory data can be affected by the animal's affective state, alertness and interest in the sounds, which may be one explanation for the differences seen in the passive datasets. Additionally, the flanges of the helmet meant that the surface coils were placed up to 2cm further from the animal's head in the passive condition, possibly decrementing the signal strength. The inversion of the lever response between the two groups of animals may have contributed to the differences seen in motor cortex. Correlation analysis of both datasets suggest that levels of movement do not significantly impact on the strength of the data acquired after motion correction has been performed, therefore the differences seen are unlikely to be due to differing levels of movement, and may depend more heavily on the factors mentioned.

The variability in movement levels with ME may be due to individual differences in temperament, or due to a poorer fit of the helmet system, which could be improved with better impression techniques, such as 3D imaging from CT scans. One possible contributing factor for the reduction in movement in MT between early and later scanning sessions may be down to improvement of the mould procedures gained through experience, and the implementation of the methods described in *Chapter3*, section 2.2, where alginate is used to refine the fit of an existing helmet. However, if this was the case, it was clearly not as successful for ME.

Additional improvements to the data could be made using prospective movement correction methods, such as those which track the movement of the head and adjust the magnetic field homogeneity in real time (Brown et. al., 2010) and those currently in development for use with fMRI data collection (Todd et. al., 2015; Maxim et. al., 2016). These techniques were not available to us for this project, nor have they yet been implemented for non-human subjects. However, this may be an avenue for further exploration once these methods are perfected for human subjects.

Another approach could be to explicitly train the animals to remain still. This has been accomplished in dogs (*canis familiaris*) (Berns et al. 2012) providing successful results. With non-human primates, previous studies have incorporated a jaw sensor to detect movement when the animal receives a juice reward (Keliris et al. 2007). This can be used to improve movement in the scanner by aborting trials where the sensor detects jaw motion, encouraging the animal to drink without moving the jaw. However, some animals can find this frustrating leading to a negative impact on performance during scanning. A similar system could be used in conjunction with the helmet, aborting trials when a sensor detects head movement. This may work well, as it was observed that the animals generally returned to the original position after head movement during scanning, so one movement would not disrupt the initial scanning setup for trials after the animal had moved. This would possibly, however, only work for sparse imaging paradigms.

## 6. Conclusion

Although the MRI-based movement with the helmet system can be comparable in some animals, or higher than with a surgically implanted head post, data from three animals shows that the helmet system can produce useable fMRI data, and that movement levels do not exceed the capabilities of movement correction software. However, as is seen with ME, in some animals the level of movement control attainable with surgical implants can be more difficult to achieve with the non-invasive approach. The system does, however, have the versatility to allow further techniques to be tested, such as EEG data collection, and this method of immobilisation was used for the collection of fMRI data in MT and ME for the subsequent experiments detailed in *Chapters 5* and *6*.

# Chapter 5 : Behavioural Training and Testing of Spatial Discrimination under Different Stimulus Conditions

This chapter contains data sets gathered for the publication **“Functional imaging of audio-visual selective attention in monkeys and humans: How do lapses in monkey performance affect cross-species correspondences?”** Cerebral Cortex 2017.

Teemu Rinne, Ross S. Muers*, Emma Salo*, **Heather Slater*** & Christopher I. Petkov

***These authors contributed equally***

The project which lead to the above publication, and provided the initial observations which lead to the hypothesis for the project detailed in this chapter, was initiated in June, 2011, in collaboration with Helsinki University. Initial training of MA and MK was conducted by the author and Emma Salo, however from May 2012 both animals were trained by the author with regular input from Teemu Rinne, Chris Petkov and Emma Salo. Ross Muers then took over with training of MK in May of 2013 once he joined the lab, at which point we initiated fMRI data collection. Data collection completed in September of 2014.

Two of the animals (MT and ME) were trained by the author with the NHIS described in previous chapters, and data from this task was included in the publication **“Individually Customisable Non-Invasive Head Immobilisation System for Non-Human Primates with an Option for Voluntary Engagement”** Journal of Neuroscience Methods, 2016.

**Heather Slater**, Alice E. Milne, Benjamin Wilson, Ross S. Muers, Fabien Balezeau, David Hunter, Alexander Thiele, Tim Griffiths, & Christopher I. Petkov.

## 1. Abstract

This project was initiated on identification of behavioural lateralisation during training of animals to perform an auditory spatial change detection task. We trained four macaque monkeys on an auditory spatial localisation task using a conspecific coo call (Coo) as the stimulus. The monkeys were presented with repeated presentation of the Coo, which either changed in spatial location on the second presentation (target: virtual acoustic space change from -90° to +90° azimuth, or vice versa) or repeated in the same location (non-target: stays at -90° or +90°). The monkeys were rewarded for making a lever

response to targets and for withholding a response to non-targets. We found early in training that performance was better for stimuli presented in left auditory space. However, this bias tended to normalise later in training. One of the animals was trained with a phase scrambled coo sound (sCoo) and did not show a behavioural bias towards either side of auditory space. This suggests that the initial left-space bias was not due to processing of spatial sound features, as such, but resulted from an interaction between the spatial and communication features present in the signals.

## 2. Introduction

Conspecific vocalisations have been shown in monkeys to elicit responses in auditory cortex (AC) predominantly in the left hemisphere (Poremba et al. 2004). However, although in humans spatial processing is associated more so with the right hemisphere (Flöel et al. 2005; Badzakova-Trajkov et al. 2010), studies investigating spatial processing in the macaque brain have failed to identify topographical representation, or lateralisation for this function in auditory regions (Ortiz-Rios et al. 2017). Of further interest is the finding that in humans, the superior temporal cortex region, which supports spatial processing in the right hemisphere, is topographically similar to regions associated with communication in the left (Binder 2000). Additionally, left hemisphere regions associated with communication have been shown to be capable of spatial processing on a sub-dominant level (Suchan and Karnath 2011). This has led some to postulate that spatial processing may once have been a bilateral process, but became lateralised to the right hemisphere once greater cortical representation of communication became necessary to support our developing linguistic abilities (Karnath et al. 2001, Oleksiak et al. 2011). In fact, where lateralisation is seen in macaques for spatial processing, many studies involving lesions or split brain paradigms have shown left lateralisation rather than right, at least in the visual system (Oleksiak et al. 2011).

As discussed in *Chapter 1*, in humans, a right ear advantage has been identified for dichotic listening tasks dependent on processing of aspects of communication (Kimura 1961). Additionally, in macaques, a rightward turning bias has been shown when the animals are passively presented with conspecific vocalisations (Hauser et al. 1998), with animals turning to the right in the presence of the vocalisation more often than turning to the left. As the task presented here involved the detection of a change in spatial location of the second of two conspecific vocalisations, if behavioural lateralisation was seen we may expect that behaviour would be lateralised to the right. However, here the task dependent feature was the spatial properties of the presented sound, rather than the communicative content. In humans a left spatial bias is often seen in tasks which require spatial discrimination which is attributed to the lateralisation of spatial processing (Bellgrove et al. 2004). As this is not seen in macaques, lateralised behaviour would not be expected with a spatial task. Yet, we did identify a left behavioural bias for this task. The resulting hypothesis was that the vocal nature of the stimuli had influenced the animals' performance when the sounds originated on the right side of space, and that in this case, the mechanisms which produce a right ear advantage for communication related tasks would instead prove to be a hindrance to successful performance on this task when the originating direction was to the right.

The aim of the original project was to train two monkeys, MK and MA, to perform an audio-visual spatial discrimination task, which lead to the publication above. Here the effects of audio-visual selective attention were investigated when the animals were asked to attend to one or the other stimulus set in the presence of the opposite modality. As natural sounds and conspecific vocalisations are known to elicit greater levels of attention (Petkov et al. 2008), we used recordings of macaque coos from an unfamiliar individual as the auditory stimuli. We expected that this would assist the animals in learning the auditory task as the stimuli would be more salient than an artificial sound, and would attract the attention of the animals more effectively. However, although the animals were able to learn the task with relative ease when presented with audio-visual stimuli, once the visual stimuli were removed performance dropped to chance levels. Furthermore, when performance with only the auditory task did improve, the behavioural lateralisation effects became evident, with accuracy in performance when the stimuli originated on the left side of auditory space being greater than performance with the right.

To investigate this further, we trained two animals to perform a similar task using Coos and scrambled coo sounds (sCoo) as stimuli to attempt to determine the effect of conspecific over the non-natural sCoo on performance of an auditory spatial discrimination task. We further hypothesised that the non-communicative stimuli would improve the accuracy of task performance.

## 3. Methods

Two monkeys (MK and MA) were trained to selectively respond to either visual or auditory stimuli, therefore training of these animals began with sound presentations in conjunction with a visual stimulus. Two further animals (MT and ME) were trained using the non-invasive head immobilisation system described in previous chapters with only the auditory task. Here, methods describing the training process for MK and MA are detailed, followed by the task for ME and MT. Training of MK and MA was performed by the author and Emma Salo. ME and MT were trained by the author.

### *3.1 Audio-visual Task*

***Stimuli:***

The conspecific auditory stimulus was a vocalisation (Coo) recorded from a male macaque unfamiliar to the four individuals tested. The coo vocalisation was 400ms in duration and sound onset/offset was shaped by 8ms onset and offset cosine amplitude ramps. Left and right virtual-acoustic space versions of the stimuli were created for headphone presentation by playing the sounds from a loudspeaker (Creative Inspire T10; distance 1m, ±90° in azimuth; 65dB SPL LAeq) and recording the sound using in-ear microphones (Knowles Electronics) in both ears. During the sound recordings, the monkeys were seated in a primate chair with their head immobilised. During the task, the two spatialized sounds were presented as discrete sounds to the left or right side of virtual acoustic space ($\pm 90^{o}$ in azimuth) left-left, right-right, left-right pairs (200ms inter-stimulus interval). The visual stimulus was a picture of an unfamiliar conspecific face (presented for a duration of 400ms, subtended 5° visual angle). The background luminance of the screen was 81.63cd/$m^2$ and the Michelson contrast of the face image was 0.054. The picture was presented at the left or right of the screen, where the centre of the picture was offset by ±5° from the centre of the screen. As with the auditory stimuli, the pictures were presented as left-left, right-right and left-right pairs with a 200ms within-pair interval.

***Task:***

The task for MK and MA initially consisted of a visual and auditory modality. The animals were required to respond using a lever when the stimuli appeared in two separate locations (±$90^{o}$ in azimuth) and withhold a response when the stimuli appeared twice in the same location. Each trial consisted of two stimulus presentations, after which the animals had a window of 1500ms in which to respond and a feedback period of 4000ms. The animals were given a reward if they correctly detected the spatial location change indicated by a lever press (hit) or refrained from pressing the lever when the sound did not change in spatial location (correct rejection). No reward was delivered and a time delay was introduced when a false alarm (the animal pressed the lever when the sounds did not change in spatial location) or a miss (the animal missed the change in spatial location and did not press the lever) occurred. Performance on the task was measured by calculating *d'* (described in *Chapter 2*, section 5). In the first condition, the animals were rewarded for correct responses to the auditory stimuli while visual stimuli appeared in consistent or inconsistent locations to the sounds. In the second condition the animals were rewarded for correctly responding to the visual stimuli while the auditory stimuli were presented in either consistent or inconsistent locations to the sounds (Figure 5.1).

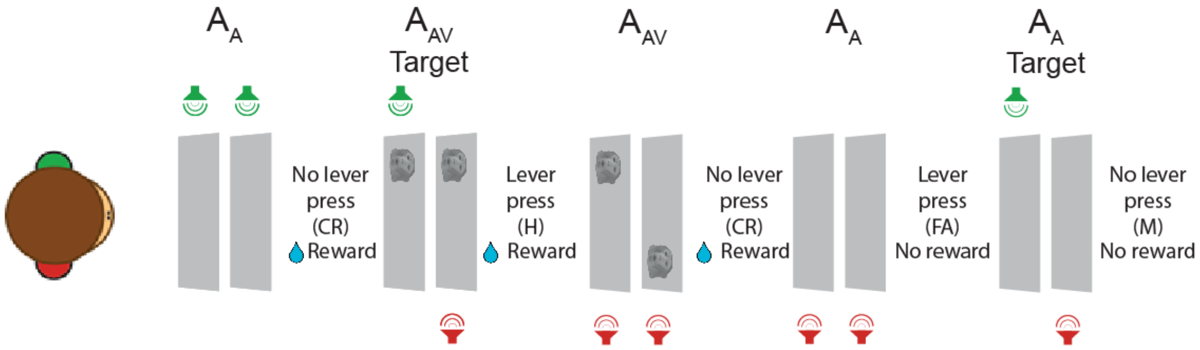


**Figure 5.1. Audio-visual task**. During the “attend auditory” condition (shown) MK and MA were rewarded for correct responses to the auditory stimuli regardless of the position of the visual stimuli. In the “attend visual” condition, the animals were rewarded for correct responses to the visual stimuli. $A_A$ = Auditory only, $A_{AV}$ = Audio-visual attend auditory, CR = correct rejection, H = hit, FA = false alarm, M = miss.

***Behavioural Training:***

Following lab habituation (described in *Chapter 2*, section 3), MK and MA were introduced to the audio-visual stimuli: an image of a conspecific face appearing to the left and then right of the screen with a Coo in the corresponding location from a speaker to the side of the monitor (a "target" condition). Speakers were used during initial training as the animals' head was not immobilised at this point in training, however, the sounds were presented via headphones, as described earlier, once head immobilisation training (with the implanted headpost) was complete. At this point the animals had to wait until after the stimulus presentation before pressing the lever and a short time out was given if they pressed before the end of the presentation. Once performance was above 70%, the "non-target" trials were introduced which required the animals to refrain from making a response. The non-target trials consisted of two presentations of the audio-visual stimuli in the same location. If the animal made a response during the 4s response window following the second of the two stimuli, a red screen was presented and a timeout of 3s was given. Again, when performance was regularly at 70% the animals were moved to the next stage, at which point the stimuli could originate in either direction (left-right, right-left targets (LOT or ROT), left-left, right-right non-targets (LONT or RONT)). The number of training sessions for this period of training was 81 for MK and 37 for MA. Training steps for the audio-visual task are summarised in Table 5.1.

| **Goal: Perform task with audio-visual stimuli** | | | |
|---|---|---|---|
| **Training Step** | **Monkey** | **Successful?** | **No. sessions** |
| Lever press for reward | MK | Yes | 22 |
| | MA | Yes | 9 |
| Respond to LOT | MK | Yes | 20 |
| | MA | Yes | 10 |
| Respond to LOT, withhold to LONT | MK | Yes | 20 |
| | MA | Yes | 6 |
| Respond to RO stimuli | MK | Yes | 4 |
| Respond to both Ts and both NTs | MK | Yes | 15 |
| | MA | Yes | 12 |
| Third location | MK | Yes | 2 |
| Respond correctly with only visual stimuli | MK | Yes | 4 |

**Table 5.1. Summary of training steps for audio-visual location change task for MK and MA.** The animals were not trained in tandem, with MK being trained several months before MA. Therefore some differences exist in their training summaries. RO = right originating.

As the final aim was for the two animals to perform the task in the visual and auditory modality independently, the auditory modality was then removed from MK's training. This significantly improved his performance (Bonferroni corrected $p < 0.001$), however since the visual only task followed the audio-visual version, performance would be expected to improve. The visual modality was then replaced with the auditory and his performance took a significant dip (Bonferroni corrected $p = 0.002$), dropping to chance levels (Figure 5.2). This was also seen for MA ($t_{40,\ 3.05} = 6$, $p < 0.001$), indicating that the animals had been relying on the visual stimuli for performance of the task and had not recognised the additional information provided by the auditory stimuli. Attempting to phase out the visual stimuli to encourage the animals to perform the task with the auditory only stimuli was unsuccessful, and therefore further training involved only the auditory stimuli.

Initially, only the left originating (LO) sounds were presented (LOT, LONT) in order to simplify the task. This worked well for MA who began performing well (above bootstrap *d'*) within 7 days, while MK did not improve. We tried several methods to address MK's performance with varying levels of success (Figure 5.2). Remedial steps included the introduction of cue periods, where ten target trials are presented followed by ten non-target trials before the full task proceeded in an attempt to emphasise the correct responses to the trials before moving on. This was ineffective (difference between the LO step and cue period step, Bonferroni corrected $p = 1.0$). We then changed the coo calls so that the left and the right stimuli were produced by different individuals. This did work and his performance improved (difference between cue period step and different coo step, Bonferroni corrected $p = 0.001$), however, the task was now identification of identity change rather than change in spatial location, which was not the final goal for task performance.

Both animals were then introduced to the right originating (RO) stimuli. For MK this involved the use of cue periods as described earlier with LO stimuli still present during the main task. MA was introduced to only RO stimuli in isolation as his performance had been more reliable in past conditions. At this point the performance of both animals diminished (significant drop in performance between this step and the previous step: MK, Bonferroni corrected p = 0.004. MA, Bonferroni corrected $p < 0.001$). Cue periods were then introduced for MA with little success (Bonferroni corrected $p = 1.0$). We then added a parameter which would only allow the animals to progress following a correct response. On an incorrect response the same trial would be repeated until the correct response was given, following which, the next trial would change. By doing this the hope was that the correct response would be highlighted to the animal before he could progress. Unfortunately this resulted in the animals adopting a response optimisation strategy and they would persist with one response until an incorrect trial, and then change response on the next trial. This lead to data which was difficult to interpret statistically.

Several further strategies were used. With MK, we reverted to the stimuli originating only on the left until performance improved, in order to encourage him to continue working, and prevent him from becoming frustrated with the task (significant improvement, Bonferroni corrected $p < 0.001$). We then changed the non-target stimulus so that it originated on the right (LOT, RONT). At this point we also reverted to using coo calls recorded from the same individual for both stimulus directions in order to move back towards identification of the spatial change rather than identity change. MK's performance improved, although not significantly. With MA further strategies were used to attempt to encourage him to perform with both the RO and LO stimuli. One of these strategies was to present a block of 100 trials originating only on the left, then a block originating on the right, to attempt to make the correct responses explicit before continuing. This was then followed by a mixed block. The initial direction of the first block was altered over training days. Performance improved (Bonferroni corrected $p = 0.001$) and MA moved on to performing with targets and non-targets originating in both directions. Initially he appeared to pick up the task but closer inspection revealed that he was mainly relying on the LO stimuli, and performance on the right remained at chance levels (below bootstrap *d'*, *Chapter 2*, section 5). Further training involved changing the proportion of LOTs and ROTs between runs of 100 trials to attempt to encourage him to work with both. His performance seemed to plateau, so the same strategy as was used with MK was employed, and he was trained with the LOT and RONT. At this point the second non-target was added for both animals. Both picked up the task, but further efforts to incorporate the ROT were unsuccessful. We therefore proceeded to train the animals for fMRI recording using the LOT and both non-targets. The total number of sessions from initial training to this stage was 390 for MK and 318 for MA.

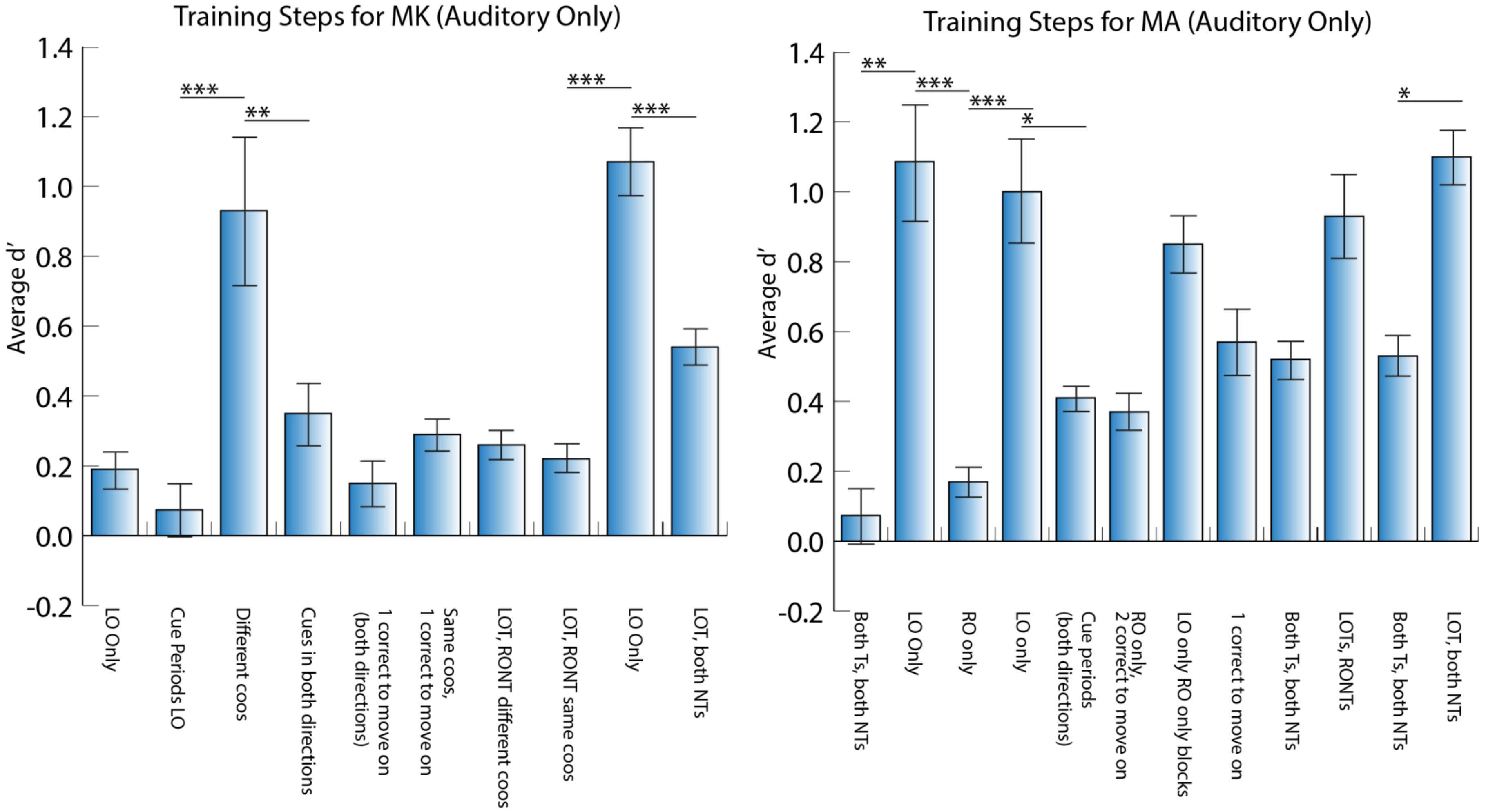


**Figure 5.2. Summary of performance measured by average *d'* for each session during auditory only training steps for MK and MA.** Performance for each animal on each of the auditory only training steps following audio-visual training. 'Different coos' indicates that the left and right coo were recorded from different individuals. T = target, NT = non-target, LO = left originating, RO = right originating, LOT/ROT left/right originating target, LONT/RONT left/right originating non-target. *p<0.001, **p=0.001, ***p<0.05

### *3.2 Auditory Detection Task*

Two further animals, MT and ME were trained on the auditory only task with target and non-target conditions originating on either side. One theory as to why MK and MA had been unsuccessful in performing the task with RO stimuli was that during initial training, the LO stimuli had been overemphasised. Therefore the animals struggled to generalise to the RO direction. As a counterbalance, with MT and ME balanced directional training was emphasised as far as possible, and when not possible the RO stimuli were emphasised over the left.

***Stimuli:***

As with MK and MA, a coo vocalisation (Coo) was used as stimulus for MT and ME in one comparison, and for the second, a coo vocalisation from a different individual was used which had been morphed into a noise bursts by scrambling the phase information, but preserving the spectral components to control for acoustic features (sCoo). This stimulus was 360ms in duration and virtual acoustic versions of the sounds were created using VisiSonics RealSpace 3D software, which used measurements of the animal's head to produce the sounds based the head related transfer functions (HTRF).

***Task:***

MT and ME were trained to perform the task with only auditory stimuli. Therefore the visual aspect was not part of their task (Figure 5.3). Additionally, whereas with MK and MA we had been unsuccessful in training them to perform the auditory task with the ROT this was not the case for MT and ME and training with these two animals was inclusive of the ROT. As with MK and MA, sounds were presented from two speakers placed on either side of a computer monitor in the initial training stages and the monkeys were trained to hold a touch lever inside the chair and release the lever whenever they detected that the second of two sounds presented in sequence was in a different spatial location in relation to the first sound (spatial location change ±90° in azimuth). The animals were given a reward if they correctly detected the spatial location change indicated by a lever release (hit) or refrained from releasing the lever when the sound did not change in spatial location (correct rejection). No reward was delivered and a time delay was introduced when a false alarm (the animal released the lever when the sounds did not change in spatial location) or a miss (the animal missed the change in spatial location and did not release the lever) occurred. Performance on the task was measured by calculating *d'* (described in *Chapter 2,* section 5).

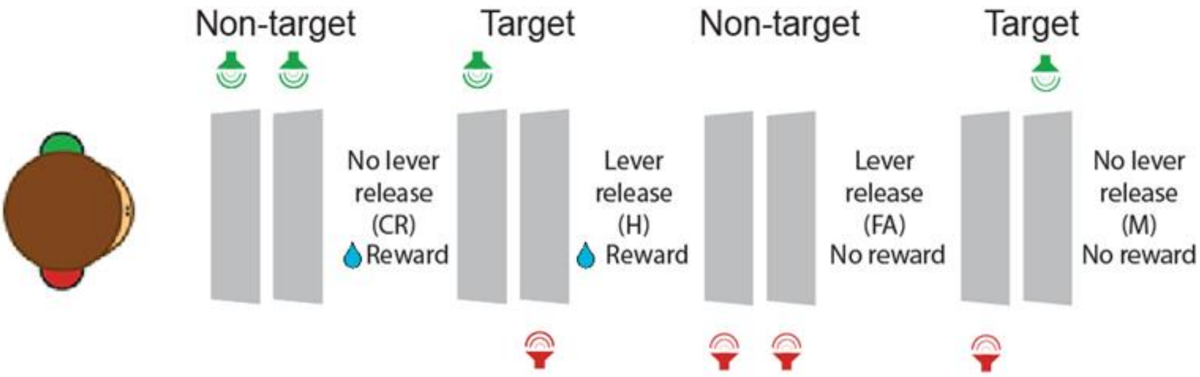


**Figure 5.3. Auditory only task.** Animals MT and ME are rewarded for a lever release to a target condition and for maintaining lever contact following a non-target condition.

***Behavioural Training:***

First, the left and right originating Coo target pairs were introduced for ME and he was required to release the lever following the second of the two sounds. Once good performance was established (performance was greater than 70%, which was achieved after 4 training sessions) both of the non-target pairs were introduced in order to balance the direction of the initial stimulus presentation. This had a negative impact on performance (Figure 5.4). Following this, approaches which had been employed with MK and MA were used for ME. Initially the right and left originating stimuli were presented in separate alternating blocks, e.g. first block of 100 RO only, second block LO only. This was unsuccessful in improving ME's performance (Bonferroni corrected $p = 1.0$). Location change was then restricted to target pairs; the next non-target would originate where the previous target terminated to attempt to make the spatial change more salient. This was also unsuccessful (Bonferroni corrected $p = 1.0$). A number of non-target trials were then presented prior to the target condition. Between 2 and 5 Coo sounds were presented in one location prior to the location change, at which point ME was required to make a response. Again, this aimed to emphasise the location change, but again this was unsuccessful (Bonferroni corrected $p = 1.0$). At this point a parameter was implemented which allowed the task to proceed only after a correct response as with MK and MA, which was again unsuccessful (Bonferroni corrected $p = 1.0$).

As MK and MA had managed to pick up the task when only LO stimuli were presented, as a counterbalancing measure with ME only the RO stimuli were presented. Unfortunately this was not successful for ME (Bonferroni corrected $p = 1.0$), but was an interesting observation as the only aspect which was different to that of the task for MK and MA was the direction of the stimulus. An additional success from MK and MA's training was the task involving the LOT and RONT. This was again unsuccessful for ME (Bonferroni corrected $p = 1.0$). Finally, only the LOT and LONT were presented, at which point ME picked up the task within 10 sessions (Bonferroni corrected $p < 0.001$) (Figure 5.5).

Following this, the RONT was implemented and his performance continued to improve, although not significantly. Next, the LOT was removed and replaced with the

ROT with both non-targets. Performance continued to improve. Finally, the LOT was re-introduced and ME was able to work on the full task. The number of training sessions from initial training to this stage for ME was 128.

In order to investigate the possible impact of the vocalisation stimulus on the left originating performance, MT was trained with the sCoo as a counterbalance to ME, MK and MA. MT was trained initially with RO stimuli as initial presentation of the full task had proven too large of a step for ME. MT was able to pick up the lever release to the ROT within 8 days, after which the RONT was introduced. Again, MT picked this up within 8 days. The RONT was then replaced with the LONT which did not hinder performance, and this was followed by introduction of the second non-target. After this, the target was changed from right to left originating and performance deteriorated (Bonferroni corrected $p = 0.003$). As a remedial step, MT was trained with LO only stimuli (which produced a significant improvement Bonferroni corrected $p = 0.006$) followed by changing the non-target to RO. Next, the LOT was reintroduced with no hindrance to performance. Finally, the second target was applied and MT continued to work well with both targets and both non-targets. The number of training sessions from initial training to this stage was 82.

To directly compare performance on the task with the different stimulus conditions, once MT and ME had sufficiently learned the task, the stimuli were switched, therefore ME performed with the sCoo bursts and MT with the conspecific sounds. Neither animal was hindered in performing the task with the alternate stimuli.

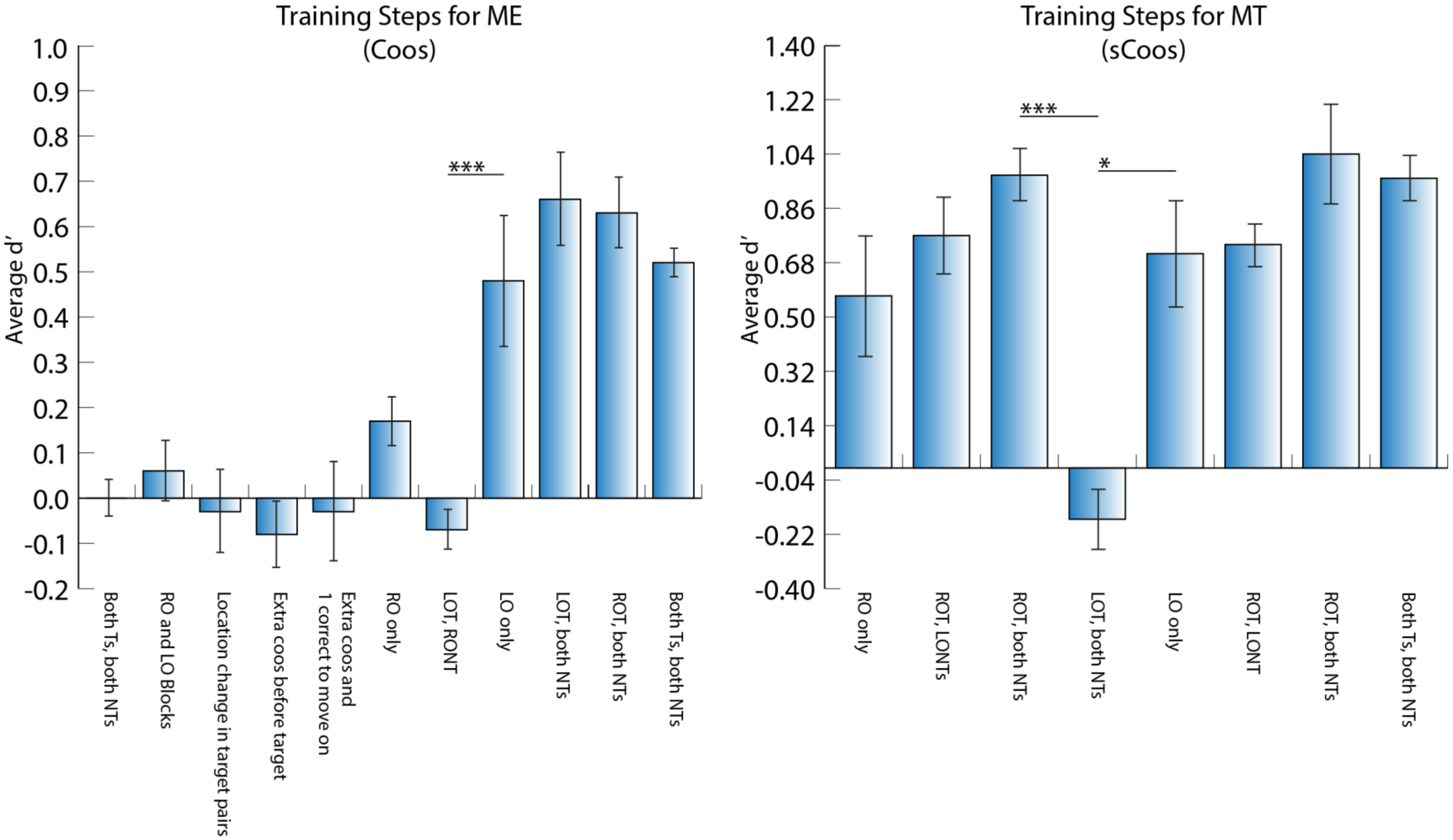


**Figure 5.4. Summary of performance measured by average *d'* for each session during training steps for ME (left, with Coos) and MT (right, with sCoos).** MT seemed to pick up each step well, except for the change of target direction initially. With ME the focus was balancing the initial direction of training but he was unable to pick up the task until left originating (LO) only stimuli were presented. MT was also trained with right originating (RO) stimuli to begin with. T = target, NT = non-target, LOT/ROT left/right originating target, LONT/RONT left/right originating non-target. *p<0.001, **p=0.001, ***p<0.05.

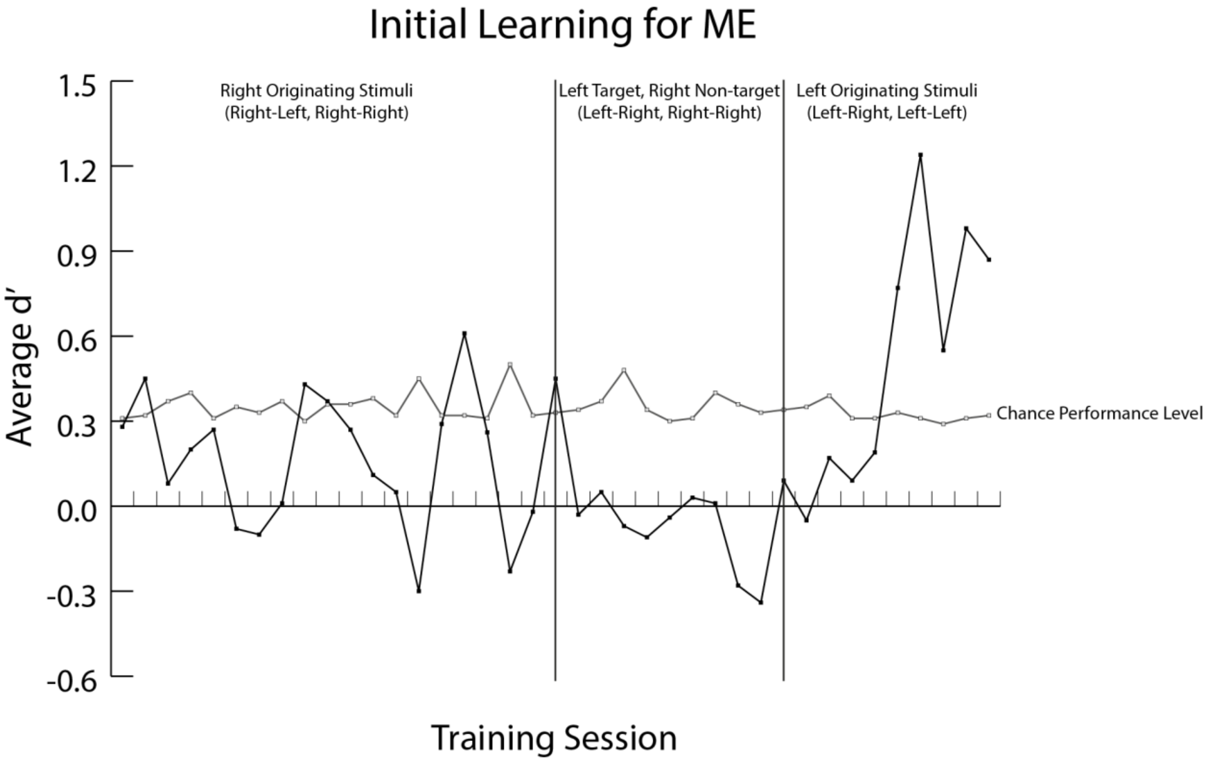


**Figure 5.5. Average performance per session for ME on the task using different training approaches.** Various strategies were used to attempt to assist ME with learning the task with right originating (RO) stimuli (only 2 shown here). However, we only saw consistent performance at above chance levels when he was presented only with the left originating (LO) stimuli.

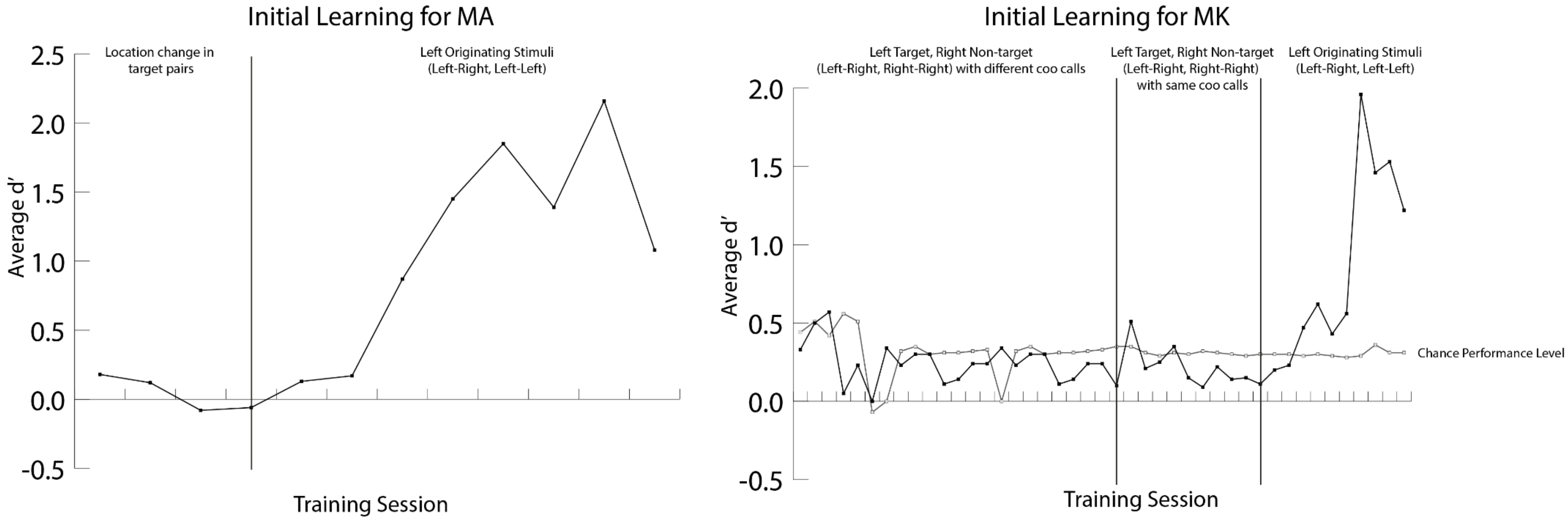


**Figure 5.6. Average performance per session for MA (left) and MK (right) on task using different training approaches.** A similar pattern is seen with MA and MK as with ME, despite differing training strategies. Chance performance levels were not calculated for MA at this point in his training, and data prior to that shown involved audio-visual trials intermingled. Here, data is taken from the period following audio-visual training, when the task was auditory only.

## 4. Results

Although MK and MA were initially trained with an audio-visual task, the aim of the current experiment was to investigate the animals' performance on the auditory task, as this was the modality which had produced the lateralised behavioural effect. Therefore, only auditory task data from MK and MA is included in further analyses.

### *4.1 Direction and Type of Stimulus*

We investigated differences in early task performance (initial 30 runs of 100 trials for each animal) in terms of the direction of initial stimulus presentation. As a counterbalancing measure, MT and ME began training with RO stimuli only in contrast to MK and MA whose training began with LO sounds. As Levene's test of homogeneity was significant, indicating that the data was not normally distributed, non-parametric tests were used for this comparison. An independent samples Kruskal-Wallace test revealed no differences in performance with LO stimuli across all four animals, three being trained with the Coo stimuli and one with the sCoo stimuli ($H_3 = 3.55$, $p = 0.314$, Figure 5.7). However, with RO stimuli, the same analysis revealed a significant difference in performance across animals ($H_3 = 24.4$, $p < 0.001$). Post-hoc Mann-Whitney U tests revealed that this was due to MT (working with sCoos) performing significantly better than each of the animals trained with Coos, (Mann-Whitney U test comparing each monkey to MT: MK $U = 29$, $p < 0.001$, MA $U = 170$, $p = 0.001$, ME $U = 198$, $p = 0.006$) possibly indicating that the nature of the stimulus used had not affected his ability to pick up the task with the RO stimuli. Additionally, Mann-Whitney tests revealed better performance with LO stimuli compared to RO stimuli for the animals working with the Coo stimulus ($U = -4.909$, $p < 0.001$), but no difference was seen for MT working with the sCoos ($U = 0.065$, $p = 0.948$).

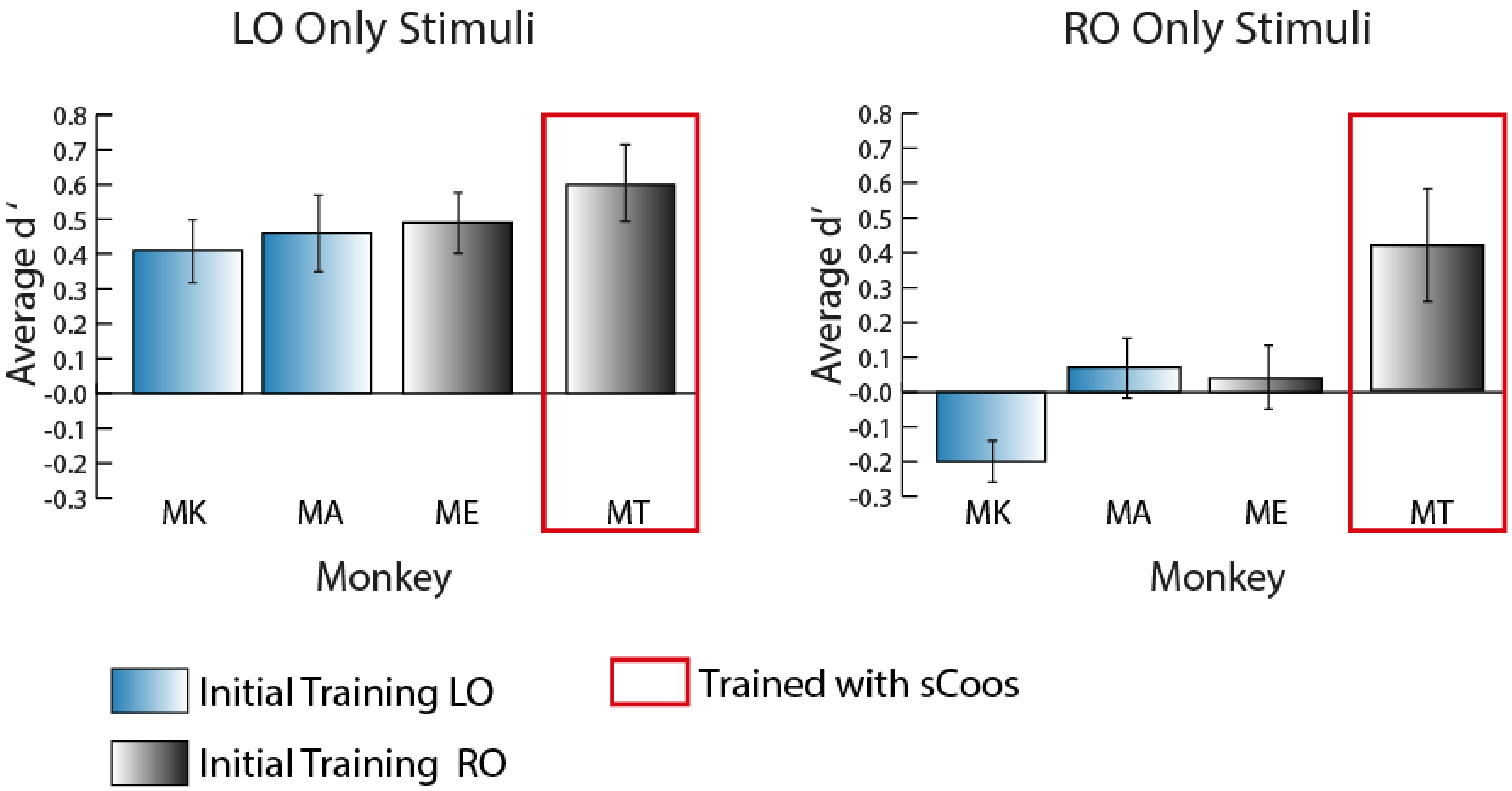


**Figure 5.7. Comparison of performance measured by *d'* per run with right and left originating stimuli.** First 30 runs with each stimulus direction is shown for each animal. All animals trained with the Coo stimuli show significantly better performance with left originating (LO) sounds. This is not the case for MT who was trained with sCoo stimuli.

### *4.2 Early versus Later Task Performance*

To explore the effect of training on performance with the left and right originating stimuli, data from MT and ME in earlier stages of training were compared against competent full task performance. More data was available for the earlier stages of training for ME and MT with RO stimuli than was included in the comparison with all four animals. Therefore more data was added to this comparison. As intermediate steps were used to train the animals, a linear progression of learning was not available and so the data sets were grouped into two time points in their training: ‘early’ and ‘later’. These were then used to compare performance during early learning stages and once they were able to perform the full task reliably. The early condition consisted of average *d′* values from training sessions where the animal was presented with LO only or RO only stimuli in separate sessions. The later condition consisted of data from the animals when they were performing the full task and the data for each direction were split to form the left and right conditions for analysis. MK and MA were unable to learn the task with all combinations of stimuli, progressing with only LONT, RONT and LOT presentations, therefore they are not present in some of the comparisons.

A 3 way ANOVA with monkey (MT or ME), time point (early or later) and direction (LO or RO) as factors showed no significant main effect ($F_{1,773} = 0.48$, $p = 0.49$). However, significant interactions of monkey by time point ($F_{1,773} = 9.21$, $p < 0.001$) and direction by time point ($F_{1,773} = 74.46$, $p < 0.002$) were seen. Post-hoc pairwise comparison of early with later time points showed an expected improvement in task performance for MT in left and right directions (left; $p < 0.001$, right; $p < 0.001$), with the RO preference remaining significant across both conditions (early; $p = 0.024$, later; $p = 0.001$). However, ME showed no significant improvement in performance with LO stimuli, but improvement was significant for the RO stimuli (left; $p = 0.57$, right; $p < 0.001$). When performing the full task, ME showed no significant difference between the left and right stimuli ($p = 0.26$), suggesting that performance on the RO stimuli had improved to match the level of performance on the left, but there was no change with LO stimuli (Figure 5.8).

MK and MA progressed to the next stage of the study without learning to perform the task with ROTs, therefore it was not possible to use *d′* as a comparable measure.

However, taking good performance based only on non-target stimuli, i.e. correct rejections, this pattern was also seen with behavioural data from MA (Mann-Whitney test early vs later Left $U = 1.56$ $p = 0.119$, right $U = 5.95$, $p < 0.001$. Left v right early $U = -4.09$, $p < 0.001$, later $U = 3.94$, $p < 0.001$, Figure 5.8). Performance seems to improve for the sCoo condition in both directions, however for the Coo condition, performance only improves on the right.

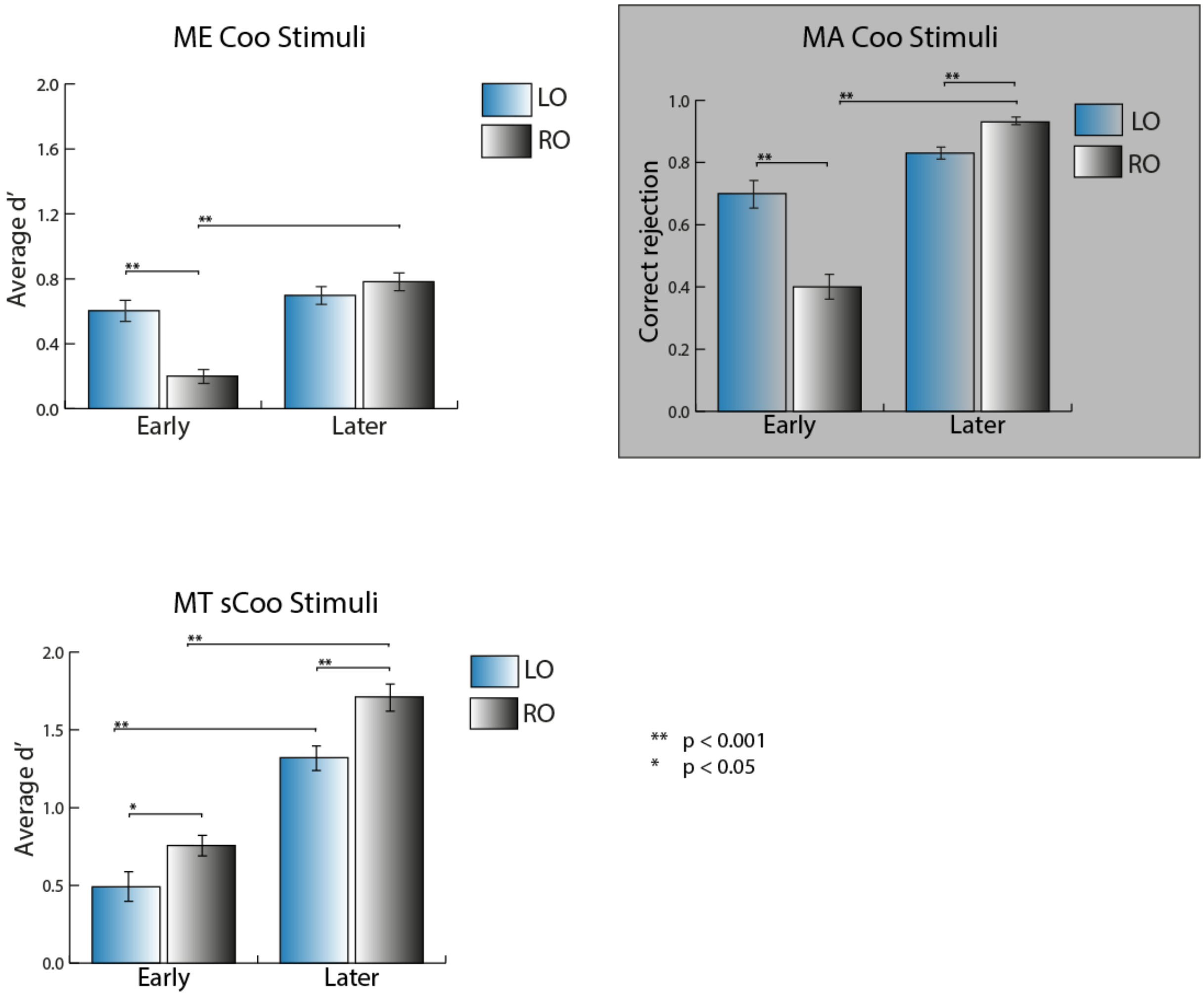


**Figure 5.8. Comparing potential biases during the first stages of learning (early) and after performance had reached a stable level (later).** ME shows significant improvement with RO stimuli but performance on the left remains similar. MT improves with both directions but shows significant RO preference at both time points. MA shows a similar pattern to that seen with ME, however the behavioural measure here uses only non-targets (left-left, right-right).

### *4.3 Effects of Stimulus change*

Once the animals had learned to perform the task to a reasonable standard (reliably performing above bootstrap level, 95% point in null *d′* distribution, *Chapter2*, section 5), the Coo and sCoo stimuli were switched to compare performance of the individual animals with the different sounds (Figure 5.9). For MT, a 3 way ANOVA of monkey by stimulus by direction revealed a significant main effect ($F_{1,232} = 14.94$, $p < 0.001$). Post-hoc analysis for MT showed a significant difference for RO stimuli ($p < 0.001$), with better performance with the Coo sounds. For ME the difference lay with the LO sounds ($p < 0.001$), with the Coo sounds again producing better performance, and the significant effect of direction was brought about by better performance on the sCoo sounds when they originated on the right ($p < 0.001$, Figure 5.9).

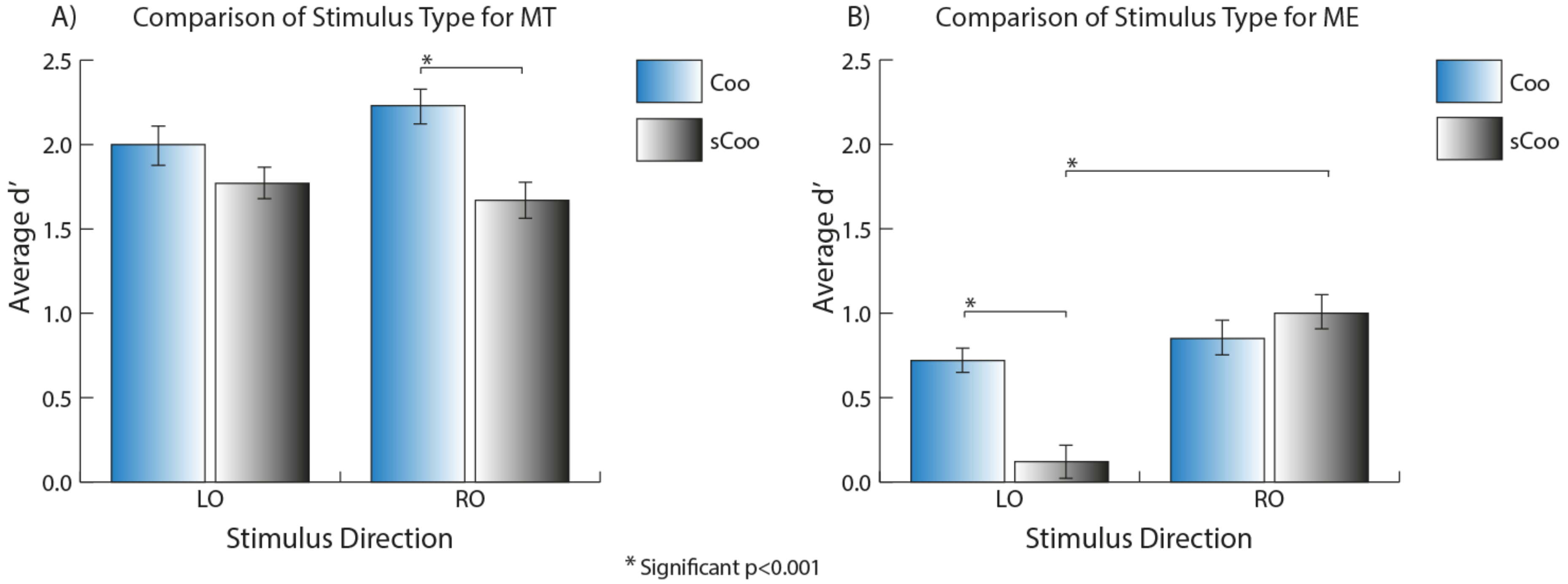


**Figure 5.9. Performance measured by average *d′* per session of each animal with each stimulus type.** Data was taken from 30 runs before stimulus change and 30 runs after stimulus change for both animals. MT (left) changed from sCoo to Coo and ME (right) changed from Coo to sCoo. LO/RO = right/left originating.

### *4.4 Comparison of Performance with both Types of Stimuli Following Further Training*

Once good performance was established for both animals with both types of stimuli (reliably performing at above bootstrap *d'*), it was possible to assess which of the two stimulus types were preferred in terms of performance. A 3 way ANOVA with the factors monkey, stimulus and direction showed significant main effects ($F_{1,232} = 4.80$, $p = 0.03$). Post-hoc pairwise comparisons revealed a significant difference for MT LO and with RO stimuli (LO: $p = 0.048$, RO: $p = 0.001$), with better performance with the Coo sound in both cases. For ME, better performance was seen on the right with the sCoo ($p = 0.017$). Differences were also seen for both animals in the direction of preference for good performance. MT performed significantly better with LO sCoos than right ($p = 0.01$), and ME performed better on the left with both types of stimuli (Coo: $p < 0.001$, sCoo: $p < 0.001$) (Figure 5.10).

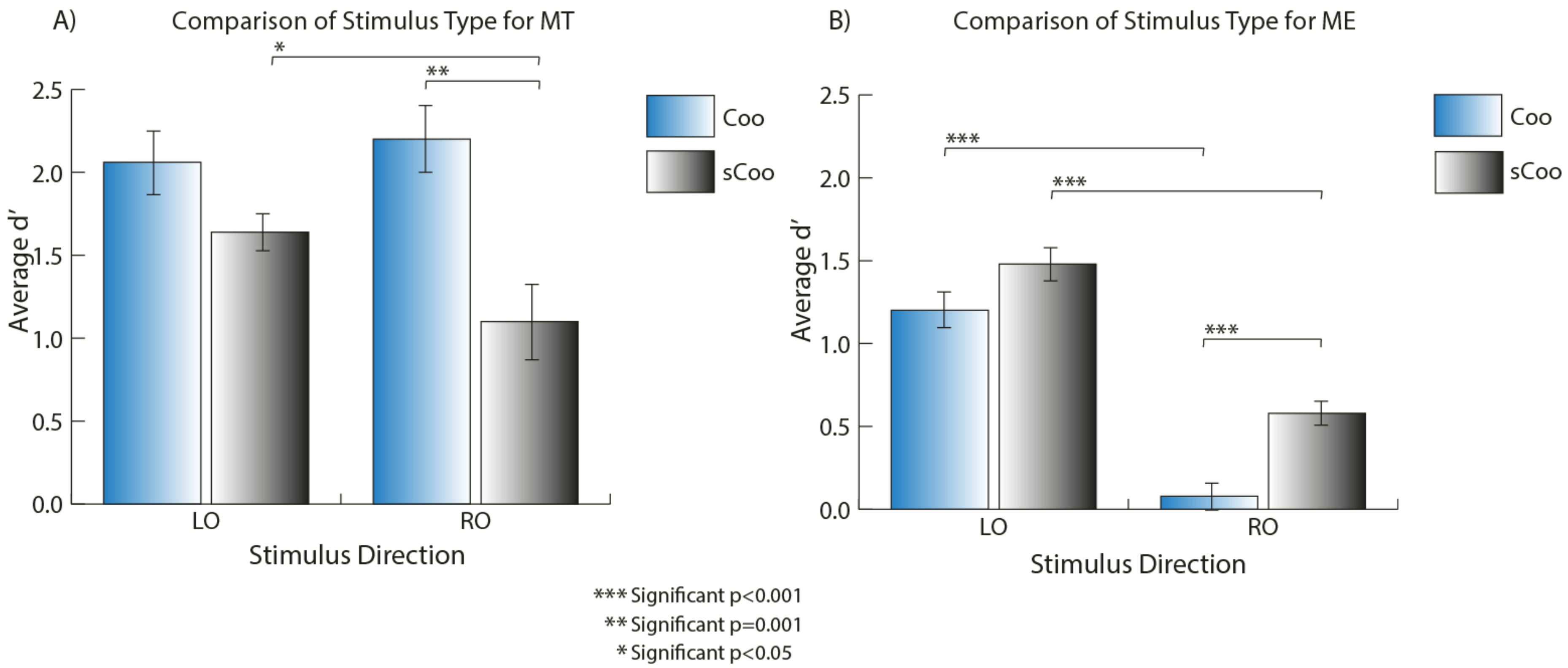


**Figure 5.10. Performance of each animal with each type of stimulus after further training.** 30 runs from each condition after stable performance was established (consistently breaching bootstrap for overall *d′*).

Additionally, MT's performance through all of the conditions was consistently better than ME for the Coo condition. Further, MK and MA never managed to pick up the task with targets originating in both directions and progressed to fMRI data collection performing with only targets originating from the left and non-targets originating on either left or right. This may suggest a long term detriment in performance of the task when learning is accomplished with conspecific coo sounds, but that the same sounds are able to boost performance when the task is learned initially with non-communication sounds.

### *4.5 Performance over Individual Sessions*

Reduced alertness during performance on an auditory spatial identification task has been shown to produce leftward decline in performance (Manly et al. 2005; Bareham et al. 2014). This is synonymous with clinical findings showing that right hemisphere stroke can produce similar effects, more so than left hemisphere damage (Bowen et al. 1999), although in some cases increased attention can overcome some of these impairments (George et al. 1999). One theory on the reasoning for this effect considers the two hemispheres to be in a state of dynamic competition, resulting in biases of attention to the contralateral hemifield space (Kinsbourne 1970). Additionally, sustained attention and spatial attention are thought to be closely related processes, and predominantly associated with the right hemisphere in humans (Robertson et al. 1997). Although differences in maintaining attention have been shown between humans and macaques (Rinne et al. 2017), closer scrutiny of the direction of lapses in performance may hint towards similar effects of lateralised sustained attention processes in the monkeys.

During a lab training session, the animals would complete between 4 and 7 runs of 100 trials, dependent on motivation. Over the course of a session performance could vary between each run as the animals became satiated with the fluid reward and/or lost interest in the task. To explore the possibility that there would be a more pronounced leftward decline in performance for the animals, performance was compared across training runs 1 to 5 within the same session (less data was available for runs 6 and above since the animals did not reliably perform this many runs in a session). A one-way ANOVA of *d′* by run number (1 to 5) showed no significant decline in performance over the course of a session for MT (Coo LO: $F_{5,42}$ = 1.57, $p$ = 0.193. RO: $F_{5,42}$ = 0.509, $p$ = 0.786. sCoo LO: $F_{4,42}$ = 0.491, $p$ = 0.742. RO: $F_{4,42}$ = 0.373, $p$ = 0.827). However, for ME, although no significant change in performance was seen for the Coo stimuli (LO: $F_{4,43}$ = 0.289, $p$ = 0.884. RO: $F_{4,43}$ = 1.046, $p$ = 0.396) a significant effect of direction was seen for the sCoo ($F_{5,50}$ = 3.103, $p$ = 0.017) with greater decline on left originating stimuli, but no difference with RO sCoo ($F_{5,50}$ = 0.471, $p$ = 0.796). Post-hoc Bonferroni-adjusted analysis revealed that the significant differences lay specifically with the third run in relation to the first ($p$ = 0.041) and second ($p$ = 0.025, Figure 5.11). This may suggest that it was more effortful for ME to maintain attention when using the sCoo sounds, resulting in the leftward decline in performance.

**Figure 5.11. Performance over training session on left originating sCoo stimuli for ME.** A significant drop in performance with left originating (LO) stimuli is seen during the third run of the session, however performance improves slightly (but not significantly) after this run. No significant variation is seen for the right originating (RO) stimuli.

## 5. Discussion

There exists a clear difference in learning of the task with RO stimuli when the animals are initially trained with conspecific coo sounds. Performance with LO stimuli was unaffected by type of stimulus, but performance with RO stimuli was disrupted when conspecific stimuli were used. This effect was especially evident in data from ME, whose performance remained at chance levels until the stimuli were switched to LO only (Figure 5.5). This was also seen in the data from MK and MA (Figure 5.6). Not only does this indicate that the LO preference was not due to training bias, but also that some feature of the RO stimulus was present which hindered the animals, which was not present for the LO stimulus. The fact that MT did not encounter the same difficulties may indicate that this was due to the communicative nature of the stimuli (Figure 5.7), although it is not possible to rule out individual differences as only one animal was trained with the sCoo first. These results are contrary to the right ear advantage for speech sounds in humans involving processing of verbal material. This does not appear to be the case when the stimuli were sCoo sounds. Although there is little difference in the two conditions for the LO stimuli, early performance with RO stimuli is significantly better when the stimulus is non-communicative in nature and in fact in some cases seems to be the preferred direction for good task performance. This finding may indicate prioritisation of the communicative information in left auditory cortex (AC) regions, resulting in confusion over interpretation of the task related spatial properties of the stimuli.

As mentioned previously, in humans, spatial processing abilities have been associated with the right hemisphere. However, in macaques there is little evidence for this, and in some cases lateralisation for spatial processing seems to be more associated with the left hemisphere (Oleksiak et al. 2011). It may be that when the stimulus is non-communicative in nature, left hemisphere regions are able to perform at least as well, if not better than the right. However, when the stimulus is a communication sound, the salience of this property confuses what may be a less salient aspect, which in this case was the change in spatial location. Additionally, this may indicate a difference in the attentional demands of the animals. Communication sounds are known to elicit bottom-up or involuntary attentional processing (Rinne et al. 1999), whereas, the volitional attribution of attention is a top-down process (Massoudi et al. 2013). As bottom-up processing occurs on a faster timescale (Buschman and Miller 2007), the salience of the communication aspect may outweigh the task demands requiring top-down attention.

As the animals progressed with their respective tasks their performance improved in different ways. MT's performance improved with both directions, although a right originating preference was present. However, interestingly, performance on the left for ME remained similar over the course of his training, while the right performance improved and became comparable with the left. Therefore good task performance with LO stimuli was acquired relatively early in training of ME and improvements were seen on the right only after practice. This could point to a change in the representation of the sound in AC once learning had taken place and the salient property of the stimulus was categorised as spatial rather than communicative. Several studies have investigated and established a cognitive difference in processing depending on whether a perceptual (involuntary attention) or decisional (voluntary attention) process is necessary (Maddox et.al., 2000, 2001, 2002), with perception involving lower level processing from posterior brain structures (bottom-up), and decision depending on higher level processing mediated by anterior structures (top-down). It is known from such research that learning has a greater influence on decisional processing than it does perceptual (Maddox et. al, 2002), which, when applied to these results, may support the idea of left auditory areas preferentially categorising the communication sounds as the salient feature, which wasn't the case with the sCoo sounds, thereby inhibiting performance on RO stimuli.

On swapping of the stimulus types, MT seemed unhindered in performance with either stimulus, and in fact performance was better for the Coo sounds despite his initially being trained with the sCoos. However, ME was less able to perform the task with the left originating sCoo stimuli straight away and additionally, was less able to maintain performance on this aspect over the course of the training session. Based on research in humans showing right hemisphere preference for spatial tasks, one may expect that left originating stimuli would produce better performance if this property is also present in macaques. This, however, was not the case. This could point to an influence of the communication aspect on ME's performance, which, once removed, he was no longer able to rely on. Alternatively, it may be a reflection of the sustained attention effects seen in humans, whereby poor performance on a sustained attention task is associated with attenuation of a leftwards spatial bias (Bellgrove et al. 2004; Manly et al. 2005). This effect is thought to be related to a right hemispheric dominance for sustained attention in parietal regions (Malhotra et al. 2009) which in lesion studies have been shown to mediate reciprocal balance between the hemispheres (Kinsbourne 1993).

Relatedly, in some cases, task difficulty has been shown to affect lateralised behaviour, and the difficulty of a given task can produce inconsistent lateralisation effects even within the same species (Fitch and Braccini 2013). Additionally, asymmetries have been noted in relation to visual task difficulty, with easier tasks engaging right hemisphere regions, while more difficult tasks produce bilateral activity (Helton et al. 2010), possibly due to the need for greater interhemispheric transfer. Some studies have shown left hemisphere preference in dichotic listening tasks (Mondor and Bryden 1992), with participants in this study showing a behavioural bias to the left ear when task difficulty was minimal, which was transferred to the right when task difficulty was increased. Since the performance of ME never reached a level similar to that of MT, it is possible that ME simply found the task more difficult with either set of stimuli, as evidenced by his lower performance level in the task in the full task comparisons (Figure 5.9 Figure 5.10). However, the fact that MK and MA also struggled to reach adequate performance, specifically when the ROT was incorporated, suggests that it is not an individual difference in this animal’s cognitive abilities, but may be an effect of the type of stimulus used during initial task training.

In humans, reduced alertness during performance on a spatial identification task usually shows a leftward decline in performance in visual and auditory tasks (Bellgrove et al. 2004; Pérez et al. 2009; Bareham et al. 2014). Accordingly, we may expect that a similar effect would be seen for both animals since they are also performing a spatial attention task. Over the course of a training session ME showed significant decline in performance on the left originating sCoo stimuli over the right, but no significant differences were seen for the Coo stimuli. Additionally, no significant effect was seen with MT. This effect could be a result of the stimulus used, or it could reflect differences in the capacity of the two animals to sustain attention.

After extensive practise with both types of stimuli, a preference for the Coo stimuli continued in MT, and his performance with the RO sCoo sounds diminished. This could be due to a range of factors. It is possible that the conspecific sounds were better able to capture the attention of the animal, were more salient to the animal, or it could reflect a greater level of activation in areas associated with communication which contribute to performance. AC region AL has been shown to respond to different types of conspecific vocalisation (Tian et al. 2001) and to be involved with perceptual decision making (Tsunada et al. 2016). Since MT had already learned that he was performing a spatial task without needing to interpret the relevance of the conspecific sounds, the further addition of a communicative aspect may have served as a boost to his ability to perform well, whereas with ME, the initial hindrance to learning the task with the conspecific sounds seems to have prevailed throughout his training with either type of stimulus.

Of additional note was the observation that ME showed a preference for the left hand during motor tasks, whereas MT seemed to prefer the right. As handedness has been shown to be an indicator of differing levels of lateralisation between human individuals, it may also hold for the animals, which may have contributed to the individual differences. Unfortunately it is not possible to test degrees of laterality in the animals in the same way as humans.

An additional contributing factor may be due to the effects of plasticity within AC. Several studies have demonstrated tuning of AC when stimuli are paired with a reward or context (Weinberger 1997; Ohl and Scheich 2005; Niwa et al. 2012; Fritz et al. 2003; Fritz 2005; Atiani et al. 2009), and spatial sensitivity in auditory cortex is known to sharpen during task performance (Lee and Middlebrooks 2011). Additionally, after time, any semantic context which the communication signals once contained may have become satiated (Lambert and Jakobovits 1960), in which case the animals would appreciate the sounds differently after extensive training.

The differences in timescale and training outcomes between ME and MT when compared to MA and MK may be related to the training methods used. During initial training stages, MA and MK were exposed to the sounds in combination with the visual stimuli. At first they were performing the task with no form of head immobilisation, as the surgical implantation of the headpost needed for immobilisation necessitates a recovery time before it can be used. Therefore the spatial location change of the sounds may not have been as apparent as they were able to turn their heads during task performance. However, with ME and MT, they were exposed to the task in conjunction with head immobilisation habituation training Therefore, although in the first stages their heads were not immobilised, the presence of the facemask encouraged them to face in the correct direction in order to receive the reward. However with these two animals the auditory stimuli were the only cues which they were given to inform their responses, therefore the importance of the sounds was emphasised from the beginning of their training.

## 6. Conclusion

Initial performance on the spatial task detailed here seems to be affected by the type of stimulus used, and the direction of presentation during initial training. Three animals trained initially with communicative sounds were hindered in their performance of the task when sounds originated on the right. This was not seen with the animal trained with non-communicative stimuli. This has implications for the possible functional mechanisms underlying this effect. Left hemisphere preference for communicative sounds may have overridden detection of the spatial location from which the sound originated, hindering performance with the right originating stimuli. This was the hypothesis which was further developed to explore the effect in terms of functional lateralisation in AC with fMRI. This is presented in the following chapter.

# Chapter 6 : Functional Lateralisation of Macaque Auditory Cortex in Response to Spatial Attention Task

This chapter contains alternative analysis of audio-visual data sets gathered by the author and Ross S. Muers, which were included in the publication **"Functional imaging of audio-visual selective attention in monkeys and humans: How do lapses in monkey performance affect cross-species correspondences?"** Cerebral Cortex 2017.

Teemu Rinne, Ross S. Muers*, Emma Salo*, **Heather Slater*** & Christopher I. Petkov

***These authors contributed equally***

As detailed in Chapter 5, fMRI data collection leading to the above publication was completed by the author and Ross Muers. Data for that project was analysed by the author, Ross Muers, Teemu Rinne and Chris Petkov, and the manuscript was written by Teemu Rinne and Chris Petkov, with input from Ross and the author. All data collection and analysis for the project detailed in this chapter was completed by the author.

## 1. Abstract

Human neuroimaging studies have established hemispheric lateralisation for certain functions. However, lateralisation in non-human primates seems less clear. Prior research involving auditory functions in the primate brain has produced variable results and has focused mainly on passive stimulation. To understand the effect of attention on lateralised processes, we trained four rhesus macaques to perform an active auditory spatial discrimination task using either a conspecific coo vocalisations (Coo) or a phase scrambled coo (sCoo). As seen in the previous chapter, behavioural results indicated a directional bias during the task with Coos, with the animals performing the task with relative ease when the Coo initially appeared on the left, but initial performance being hindered when the Coo first appeared on the right. No bias was observed with an animal initially trained with the sCoo.

Here, attention effects on hemispheric laterality were explored using fMRI with the trained animals and, as a point of reference, a naïve animal (MD) who was passively presented with the task stimuli. Presenting the Coo task components which were more challenging for the trained animals to learn produced significantly greater right lateralisation in the naïve animal than in the trained animals. Further, the results revealed differences in hemispheric lateralisation during good and poor task performance, with

stronger right hemisphere activity associated with poor performance with the Coo stimuli and left lateralised or bilateral activity associated with good performance with the sCoo stimuli. Further, animals required to perform an audio-visual task which required the auditory stimuli to be ignored produced left lateralisation. Taken together, the results indicate that behavioural biases can result from perceptual interactions between spatial and communication feature aspects. Moreover, attention during active tasks affects hemispheric lateralisation in the primate brain.

## 2. Introduction

Many studies have shown that auditory stimuli passively presented to one ear produces activity in the contralateral auditory cortex (AC) (Schönwiesner et al. 2007; Carrasco et al. 2013; Gutschalk and Steinmann 2015). Therefore, for uni-directional (presented on one side of space) passive presentations we may expect that there would be a lateralised effect in the passively stimulated animal. However, different methods have produced more variable results. When stimuli are presented binaurally from one or another direction, i.e., when the location is virtually manipulated using inter-aural time differences (ITD) and/or inter-aural level differences (ILD), different effects are seen. For instance, a recent study showed that in macaques, pure tone sounds with ITD properties removed produce greater activation in the right hemisphere for leftward sounds, and rightward sounds show bilateral activity in AC (Ortiz-Rios et al. 2017). This is hypothesised to be due to a suppressive effect of the left hemisphere over the right, which is only present when the sounds contain spatial cues with ITDs. Another study found similar results when comparing monaural (sounds presented to one ear or the other separately) versus binaurally presented stimuli (Woldorff et al. 1999a), although here no contralaterality was seen for the binaurally presented sounds.

Conspecific vocalisations have been shown to elicit left hemisphere preference in auditory regions (Hauser and Andersson 1994; Gannon et al. 2008; Joly et al. 2012; Heffner and Heffner 1984; Poremba et al. 2004; Carrasco et al. 2013), however, other studies have found this not to be the case (Gil-da-Costa and Hauser 2006; Petkov et al. 2008; Ortiz-Rios et al. 2015). The variability seen in the previous studies may stem from a lack of an active task, therefore lack of attentional control means that it is not possible to ascertain how much attention was being allocated to the sounds during presentation.

In humans, the planum temporale has been implicated in processing of vocal stimuli perceived as “outside of the head” (binaurally presented) more so in the left hemisphere (Hunter et al. 2003). Further, verbal sounds presented in space have been shown to activate left hemisphere posterior auditory regions, regions allocated to the “where” pathway of auditory processing (Mathiak et al. 2006). Although macaques have been shown to lack this structure, Tpt, along with CL and CM, are thought to be homologous regions (Poirier et al. 2017), and there is some evidence of CL neurons responding to species specific vocalisations presented in space (Tian et al. 2001). This indicates that this area could be a point of convergence for the ‘what’ and ‘where’ streams.

Another aspect which may affect levels of lateralisation in AC is attention. Information on the attentional effect of AC lateralisation is predominantly investigated in humans using dichotic listening paradigms. This involves the presentation of different sounds to each ear, and the participant is asked to report on the information presented to one ear over the other. These studies have revealed a bias in performance of tasks involving verbal stimuli when presented to the right ear, and effect known as the right ear advantage (REA) (Kimura 1961). The effect is believed to be due to the more direct anatomical connection of the right ear to the contralateral auditory regions, and more successful suppression of the ipsilateral AC. These experiments have shown that directed attention elicits activation in the contralateral hemisphere to the direction of attention (Alho et al. 1999) and are generally based on monaural presentation of auditory stimuli where the participant is explicitly cued to the direction of presentation. However, when attention is directed to the detection of the spatial location of a given stimulus and not previously cued to one direction, we may see a different effect on lateralisation of AC, especially in non-human primates, which may better our understanding of hemispheric preferences for spatial processing. Lack of control over attention may be one of the reasons why there has been variability in previous findings regarding lateralisation induced by vocalisations in non-human primates, and this is something which the work presented in this thesis hoped to address. In addition, requiring the animals to make a decision based on the auditory stimuli presented may also affect processing streams in AC. Decisions made based on the properties of a sound have been associated with AC region AL, which forms part of the ventral stream (Tsunada et al. 2016).

The stimuli used in this project (see Chapter 5, section 3.2 for full details) were binaural presentations of both types of stimuli. Therefore, during unidirectional presentations of the sCoos with the naïve animal, we may expect to see contralateral responses to the sounds. However, with the Coo sounds, we may see more left hemisphere activity regardless of the direction of presentation. By scanning a naïve animal with the stimuli used during the active task, the aim was to ascertain a baseline of functional responses to the stimuli when the animal was not specifically asked to attend to the sounds. By doing so, a comparison was made relating to the passive presentation in space of the two types of stimuli. Further, the effects of attention on this baseline activity could be evaluated by comparing data from this animal with ME and MT. Finally, MK and MA performed an audio-visual task in which the goal was to ignore the auditory stimuli distractor, and instead respond correctly to a visual task. Auditory activity during good and bad performance with the Coos as an auditory distractor was therefore compared, providing a further category of attentional attribution. Few studies have investigated the effects of attention on auditory lateralisation in non-human primates, therefore the aim here was to bridge this gap in our understanding of the evolution of lateralisation in auditory regions relating to an active task.

As shown in the previous chapter, behavioural lateralisation was seen during performance of a spatial location change task when the stimulus used for the task was a conspecific vocalisation. In order to investigate the functional properties of the observed effects, the animals were further trained to perform the task during fMRI. Task data from early performance of the animals suggested that there may be a performance bias towards the left originating stimuli, therefore the fMRI data was analysed in terms of lateralisation. At the time of scanning, no behavioural lateralisation effects were evident for MT, however the left originating bias returned for ME with the Coo stimuli, specifically during scanning sessions which met the minimum performance levels required for inclusion in the fMRI analyses (d’ was greater than the bootstrapped equivalent).

Based on the biases seen in the behavioural data, the hypothesis was that attention would affect the functional lateralisation present in auditory regions for the Coo stimuli, which would be seen on comparison of a passively stimulated animal and trained animals. Further, performance on the task would show differences in the lateralisation of AC when the animals were performing well compared to poor performance. Finally, lateralisation of AC would return for the Coo stimulus presentation when the animals are required to ignore the auditory cues.

## 3. Methods (fMRI)

For full task training and stimulus details, see *Chapter 5*, section 2. Monkeys MT and ME proceeded to scanning using the NHIS as described previously. They were scanned by the author with the auditory only task, initially with sCoo stimuli (for scanner habituation training see *Chapter 2*, section 6). This was due to the fact that, while MK and MA were performing a slightly different task, the sCoo task was of greater importance with MT and ME as a counterbalanced comparison to the Coo stimuli. The auditory stimuli were delivered using MRI-compatible insert earphones. The Coo and sCoo sounds were presented with ~5Db RMS ILD difference between the two channels to simulate the direction of the sound (calibrated with an NTI Audio XL2 sound level meter).

### Audio-visual Task:

Monkeys MK and MA were scanned by the author and Ross S. Muers while performing an audio-visual task. During scanning while the animals were performing the full audio-visual task, stimuli were presented during auditory (A) and visual (V) tasks in auditory only (Aa), visual only (Vv) or audio-visual (AVa, AVv) trials. In the bimodal stimulation trials, the first part of the visual stimulus pair was always presented 300ms after the onset of the first part of the auditory stimulus pair, to avoid direct masking or competition between simultaneously presented auditory and visual stimuli. In $A_A$ and $V_V$ trials, the stimuli in the opposite modality were omitted. In these trials the timing of the unimodal stimuli was identical to the stimuli of that modality in the bimodal trials. In bimodal trials, the auditory and visual pairs were randomly combined (i.e., the different auditory/visual combinations were as follows: $A_{LR}V_{LR}$, $A_{LL}V_{LL}$, $A_{RR}V_{RR}$, $A_{LR}V_{LL}$, $A_{LR}V_{RR}$, $A_{LL}V_{LR}$, $A_{LL}V_{RR}$, and $A_{RR}V_{LL}$. To help to cue the monkeys to the attended modality during bimodal trials, most (60%) of the non-silent trials were unimodal. 20% of all trials were silent.

During the first phase of scanning MK and MA were required to perform the task in the auditory modality while ignoring visual stimuli presented congruently or incongruently to the auditory stimuli. The second phase required them to perform the task in the visual modality while the Coo stimuli were presented congruently or incongruently to the visual stimuli (see Figure 5.1). During each scanning run, 40% of the trials presented were unimodal to serve as a reminder to the animals as to which modality they were required to respond. Attentional effects of the full task were published in Cerebral Cortex (Rinne et al. 2017), however, here focus is placed on the hemispheric effects of the stimuli, initially with the auditory only data from MT and ME, followed by comparisons during the attend visual task with MK and MA when the auditory stimuli were actively ignored.

**Passive Stimulus Presentation:**

Monkey MD was already trained to perform a fixation task and habituated to scanning using an implanted headpost. During scanning runs he was required to fixate for 3s before hearing the stimuli which were played in the same manner as for the previous animals. Following this, the scanning volume was acquired (for fMRI methods see *Chapter 2,* section 7) and a fluid reward and green screen was presented following adequate fixation. Poor performance resulted in a red screen and no reward. Behavioural performance on the fixation task was not used as a pre-requisite for inclusion of scanning trials in data analysis and all scanning trials were used.

**Data Analysis:**

Data analysis for fMRI was carried out as described in *Chapter 2*, section 7 with the following explanatory variables (EVs) incorporated into the GLM. For the passive presentations for MD, the EVs were defined as left originating non-target (LONT), right originating non-target (RONT), right originating target (ROT) and left originating target

(LOT). For the auditory task for ME and MT, EVs were defined as left hit, left correct rejection (CR), left miss, left false alarm (FA), right hit, right CR, right miss and right FA. For the audio-visual (auditory distractor) task, EVs were defined as left consistent hit, left consistent CR, left consistent miss, left consistent FA, left inconsistent hit, left inconsistent CR, left inconsistent miss, left inconsistent FA, right consistent CR, right consistent FA, right inconsistent CR, right inconsistent FA and unimodal trials.

For fMRI data analysis, tonotopically determined auditory cortex (AC) regions of interest (ROIs) were grouped into 4 key areas: caudal regions (CL, CM, and CPB), mid-caudal regions (A1, MM and ML), mid-rostral regions (AL, R, and RM) and rostral regions (RT, RTL, RTM and RPB) for analysis (Figure 6.1).

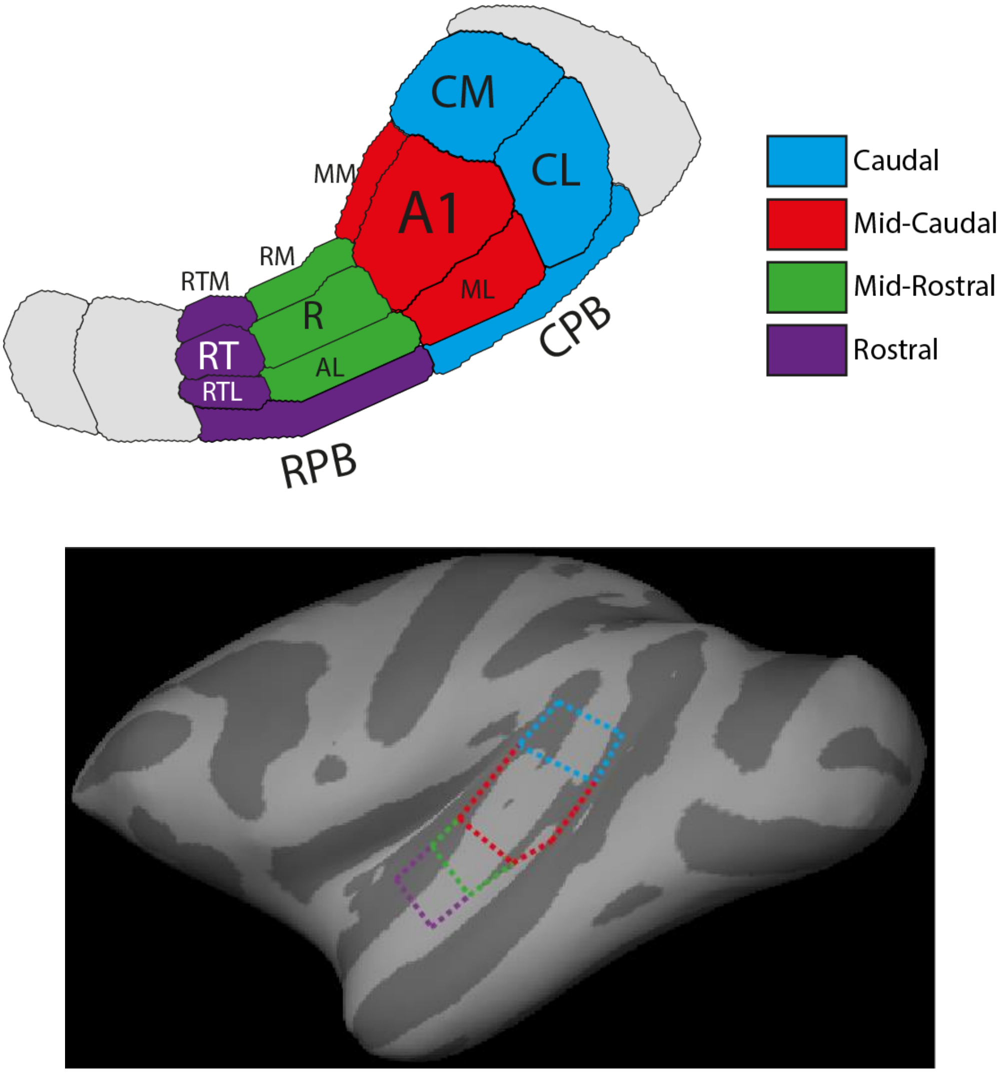


**Figure 6.1. Regions of Interest (ROIs) used for functional analysis.** Above, auditory regions were grouped into four categories for analysis based on the “what” and “where” streams of processing (Rauschecker and Tian 2000). Caudal regions are associated with the processing of a sound location, whereas rostral regions are associated with the identification of a sound. Further details are provided in *Chapter 1*, section 3.3. Below, approximate regions projected to cortical surface (based on tonotopy data from MA).

Lateralisation was measured using lateralisation index (LI). Initially, the z-value of the most active voxel in a cluster of activity was identified in each ROI (maxZ) and the average of these values over the included scanning runs was taken. This value was calculated for each hemisphere and the LI was then calculated as follows:

$$LI = \frac{LHz - RHz}{LHz + RHz}$$

where LH refers to left hemisphere ROI and RH refers to right hemisphere ROI. A LI of greater than +/- 0.2 is generally accepted to be the threshold at which activity is considered to be lateralised, with 0.2 being left lateralised, and -0.2 being right lateralised (Springer et al. 1999; Deblaere et al. 2004; Seghier 2008). However, the LI data was also compared to 0 as an additional measure of significance.

An additional method of analysis was also performed. The laterality index toolbox in SPM (Wilke and Lidzba 2007) computes LI as a function of the statistical threshold (t-score) using the t-stat maps produced for each comparison. Activity is measured across the whole of the specified ROI (specified by masks of the AC of the individual animal based on functionally determined AC maps). This analysis uses a minimum level of activity, ensuring that the measured laterality is not based on a small number of highly active voxels. Within the toolbox, the following parameters were specified: inclusive masks based on caudal, mid-caudal, mid-rostral and rostral regions, exclusive mask of +/-5mm mid-saggital, default bootstrapping parameters, minimum cluster size of 5 voxels. This yields a mean and weighted mean laterality value between 1 and -1, as well as a standard error value of the activity across the region. This method is prominent when investigating lateralisation in fMRI data, and is used in a number of publications (Badzakova-Trajkov et al. 2010; Powell et al. 2012; Joly et al. 2012; Cai et al. 2013; Mellet et al. 2014; Ortiz-Rios et al. 2015; Pernet et al. 2015; Bradshaw et al. 2017, for example). Due to the fact that a minimum level of activity is required for this analysis, and the fact that the ROIs are quite small, only the group analyses for each comparison yielded meaningful data, therefore the data presented from the toolbox uses only the group analyses. Additional full brain analyses were also carried out, which are reported in the tables presented.

Task components were split for analyses into the following categories: LONT, RONT, LOT, ROT, left originating (LO) stimuli (LOT and LNOT presentations), right originating (RO) stimuli (ROT and RONT presentations) and full task (all task components).

## 4. Results

### *4.1 Behavioural Performance during fMRI*

For scanning runs included in fMRI analysis, a 3 way ANOVA with the conditions monkey, stimulus and direction showed a significant main effect ($F_{1,80} = 5.79$, $p = 0.018$). Pairwise comparisons of monkey showed a significant difference in the performance of the two animals ($p < 0.001$), and a significant difference in the animals' performance with the two types of stimuli ($p < 0.001$). Further, for MT there was no significant difference in his performance with the left and right sCoo sounds ($p = 0.433$), however performance was significantly better with right originating Coos ($p = 0.026$). Additionally he performed significantly better with both directions of Coo sounds than with the same direction of sCoo sound ($p < 0.001$). ME also showed no significant differences in the sCoo condition ($p = 0.217$), but showed a bias for LO Coos ($p = 0.003$) (Figure 6.2).

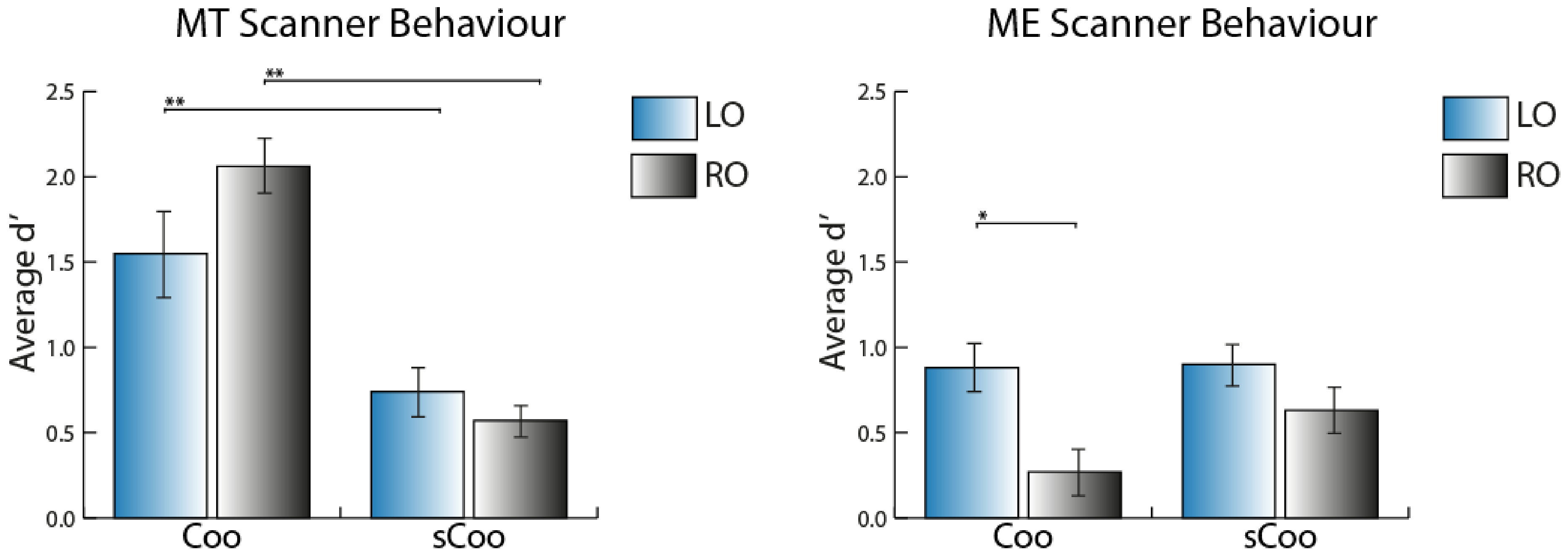


**Figure 6.2. Task performance of MT and ME in the scanner.** MT performs the task significantly better with the Coo, and a left originating (LO) preference returned for ME, specifically during scanning runs where performance breached bootstrap *d′*. RO = right originating **p = 0.01, *p = 0.03.

### *4.2 Effect of Stimulus Type on Lateralisation*

As sound presentation to one ear is known to activate AC in the contralateral hemisphere, to better understand the effect of the stimuli on lateralisation in the naïve animal, the differences in LI calculated with the maxZ analysis seen when uni-directional stimuli were presented (either left-left or right-right presentations) were assessed. None of the activity seen survived cluster analysis, therefore, in contrast to analysis of the data from the trained animals, LI analysis was based on the most active voxel in the ROI. A 3 way ANOVA with the factors region, direction and stimulus showed no significant interactions. Additionally, none of the LIs were significantly different from 0 ($F_{3,176} = 0.64$, $p = 0.979$). (Figure 6.3).

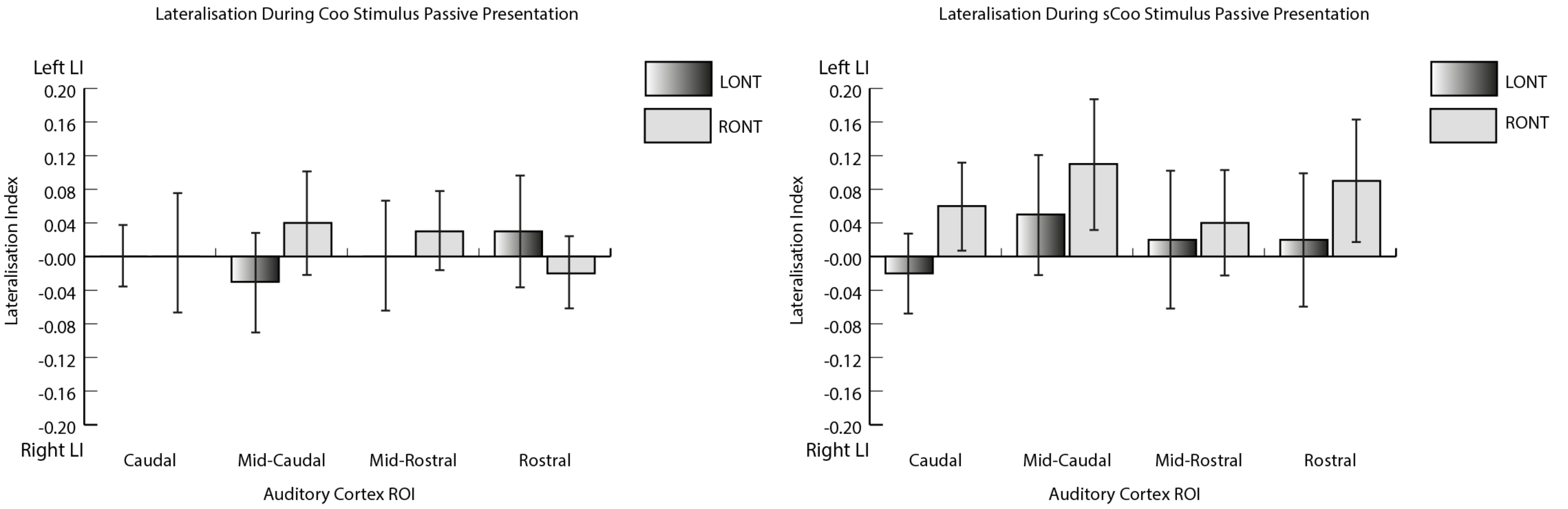


**Figure 6.3. Lateralisation Index (LI) in each ROI during passive unidirectional stimulus presentation (MD).** No significant differences in lateralisation index (LI) are seen on stimulus comparison in either direction, and none of the LIs were significantly different to zero. Error bars show standard error of the maximum voxel based LIs across sessions. LONT/RONT = left/right originating non-target.

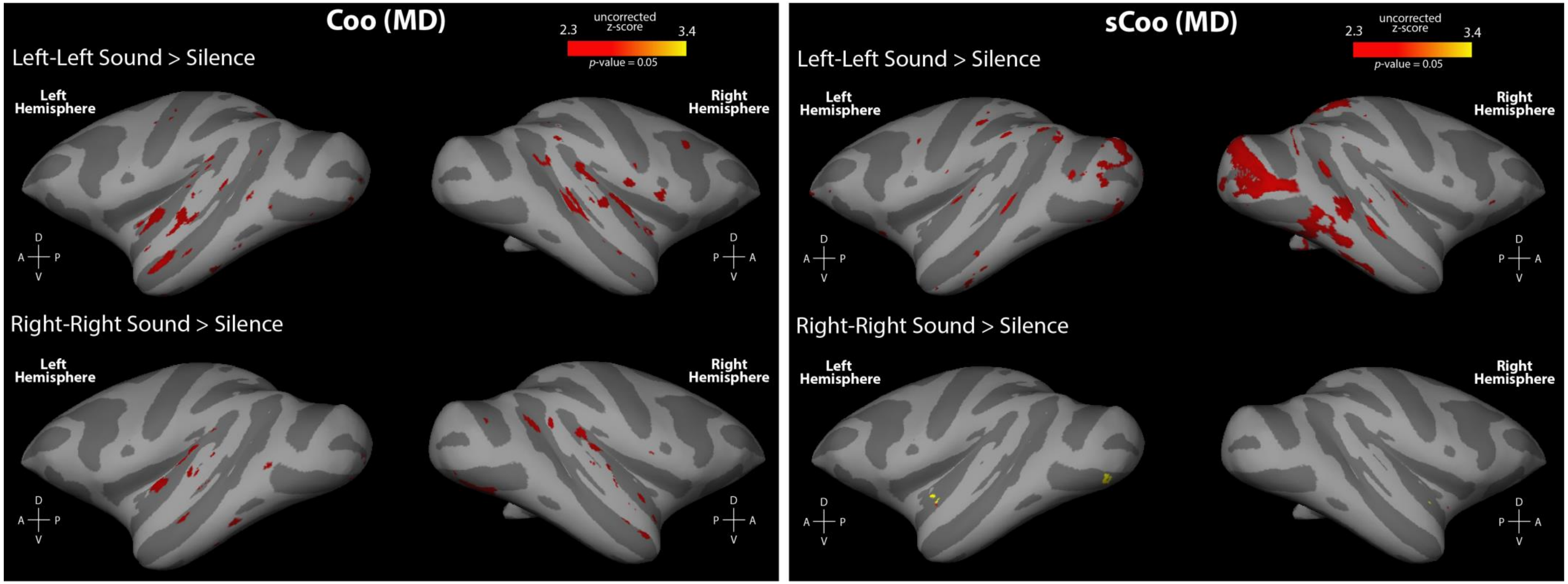


**Figure 6.4. Surface projections of activity seen during unidirectional stimulus presentation for the passive animal (MD).** Bilateral activity is seen for the left unidirectional Coo presentations (left top), as well as left hemisphere ventral activity. The sCoo condition elicits mainly contralateral activity (right). None of the activity survived cluster correction.

For the analysis with the lateralisation toolbox, different effects are seen. With the Coo stimulus comparison, uni-directional left and the right originating presentations elicit contralateral activity in most regions. Contralateral activity is also recorded for the left originating sCoo condition, however there was not enough data present for the right originating comparison to obtain an LI value (

Table **6.1**). This appears to more closely corroborate the surface projections for this comparison (Figure 6.4).

| Coo | | | | | sCoo | | | |
|---|---|---|---|---|---|---|---|---|
| | Caudal | Mid-Caudal | Mid-Rostral | Rostral | Caudal | Mid-Caudal | Mid-Rostral | Rostral |
| **LONT** | -0.14 | -0.29 | -0.26 | -0.31 | 0.08 | -0.02 | -0.28 | -0.14 |
| **RONT** | -0.05 | 0.35 | 0.18 | 0.29 | Too | few | active | voxels |
| **LOT** | 0.18 | 0.16 | 0.33 | 0.19 | -0.33 | 0.04 | -0.28 | -0.05 |
| **ROT** | -0.33 | -0.54 | -0.36 | -0.47 | 0.31 | 0.39 | 0.32 | 0.38 |
| **LO** | 0.05 | -0.32 | 0.07 | 0.07 | -0.14 | -0.21 | 0.42 | 0.56 |
| **RO** | -0.02 | -0.41 | -0.03 | -0.03 | 0.26 | 0.21 | 0.15 | -0.08 |
| **Full** | -0.02 | -0.41 | 0.04 | 0.04 | 0.26 | 0.13 | -0.08 | 0.04 |

**Table 6.1. Weighted mean Lateralisation Index (LI) seen in each region of interest (ROI) during passive stimulus presentation (MD).** LI measured using LI-toolbox. LIs show significant lateralisation (LI > -0.2) contralaterally to the direction of stimulus presentation for both types of stimuli. Too few active voxels were present in the right originating sCoo comparison. Data shown is based on the group analysis rather than the mean of individual sessions.

For the maxZ analysis with the trained animals, LI values for each task component were calculated and entered into a 4 way ANOVA with the variables monkey, direction, stimulus and region. No significant main effect was evident ($F_{3,288} = 0.783$, $p = 0.505$). Individual ROIs were then split and pairwise comparisons revealed that a significant difference was evident for ME in rostral regions when comparing the stimuli in both the left and right direction (LONT: $p = 0.016$, RONT: $p = 0.002$). No significant differences were seen on comparison of the direction within the stimulus condition. For MT, the LI for LONT Coo was significantly different from zero in mid-rostral ($p = 0.037$) and rostral ($p = 0.012$) regions. The LI for the RONT was significantly different from zero in rostral regions ($p = 0.001$). For the sCoo LIs significantly different from zero were seen for the LONT in caudal ($p = 0.002$) and mid-rostral ($p < 0.001$) regions. For the RONT significant differences from 0 were seen in mid-caudal ($p = 0.025$), mid rostral ($p = 0.037$) and rostral regions ($p = 0.002$). For ME significant differences from zero were seen only in the rostral region for the sCoo with both the LONT ($p = 0.031$) and the RONT ($p < 0.001$).

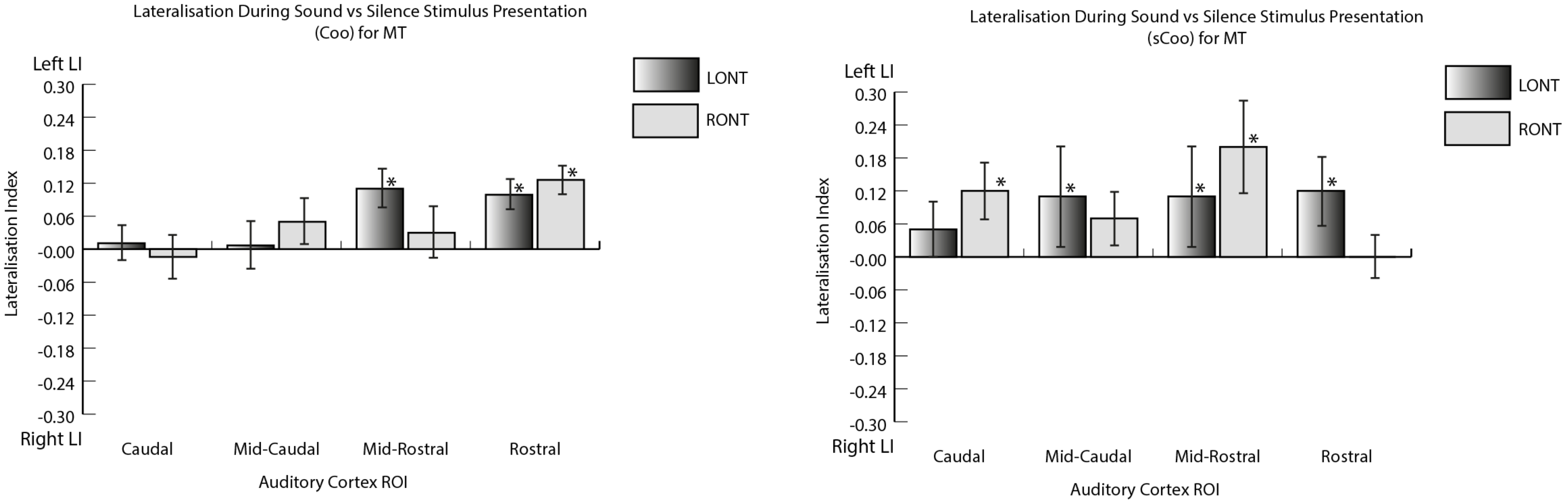


**Figure 6.5. Uni-directional stimulus presentations for MT.** No significant differences in lateralisation index (LI) were seen between the stimulus types in any of the regions of interest (ROIs). LIs which are significantly different from 0 are marked with an asterisk. Error bars show standard error of the maximum voxel based LIs across sessions. * = significantly different to 0.

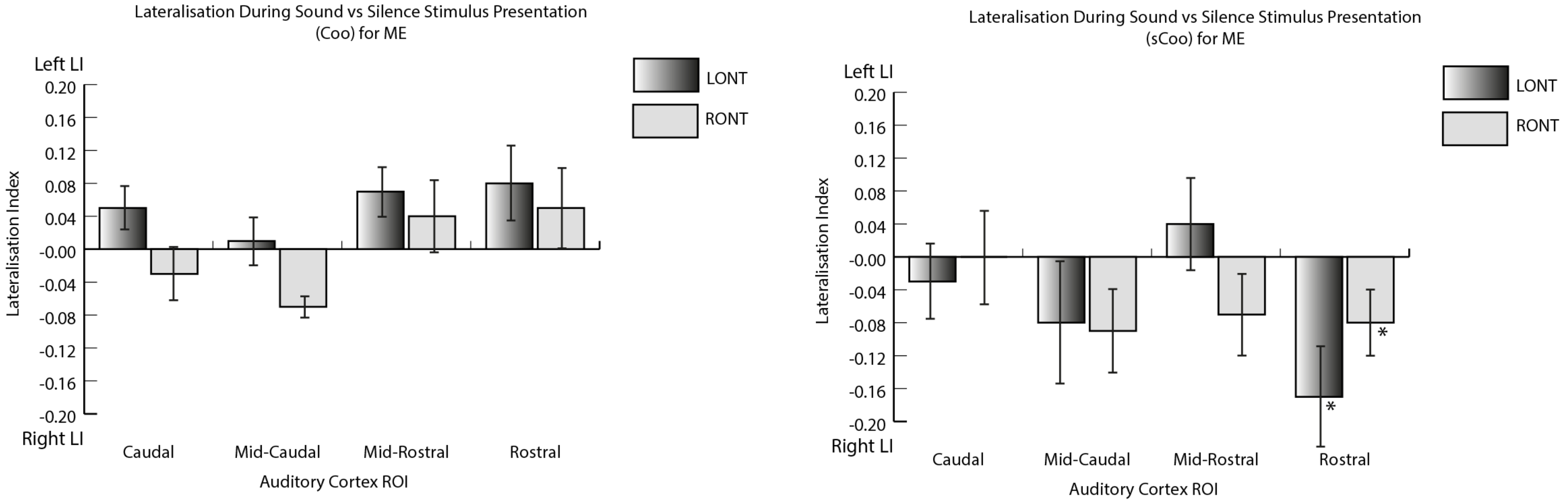


**Figure 6.6. Uni-directional stimulus presentation for ME.** Significant differences in lateralisation index (LI) are seen in rostral regions for both directions of stimulus presentation. LIs which are significantly different from 0 are marked with an asterisk. Error bars show standard error of the maximum voxel based LIs across sessions.

Analyses using the SPM LI-toolbox show that, for MT, during presentation of the Coo stimuli, for the LONT, both caudal and rostral regions are right lateralised, with mid regions being more bilateral. For the RONT, all regions are right lateralised except for the rostral regions. This is in contrast to the data from the naïve animal, where contralateral activity was shown during both directions of stimulus presentation. For ME, this result is less clear, and activity is mostly bilaterally distributed for both directions of Coo presentation, whereas the sCoo produces rightwards LI during both the left and right presentation (

Table **6.1**).

Finally, whole brain analysis for the trained animals revealed greater activity in areas right RTL and AL, left ML and both the inferior and superior colliculus for the Coo condition, which were not present in the sCoo condition. Left RT and right CPB were present for the sCoo but not the Coo stimuli (Table 6.3 Table 6.4).

**MT**

| **Coo** | | | | | **sCoo** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** |
| **LONT** | -0.36 | 0.05 | 0.1 | -0.26 | -0.24 | 0.09 | 0.24 | 0.06 |
| **RONT** | -0.54 | -0.25 | -0.23 | -0.09 | 0.14 | 0.18 | 0.4 | 0.14 |
| **LOT** | -0.41 | -0.19 | 0.1 | -0.1 | -0.36 | 0.1 | -0.11 | -0.14 |
| **ROT** | -0.61 | -0.13 | 0.06 | -0.37 | 0.12 | 0.22 | 0.11 | -0.13 |
| **LO** | -0.45 | -0.08 | 0.05 | -0.37 | -0.32 | 0.08 | 0.08 | 0 |
| **RO** | -0.54 | -0.09 | -0.18 | -0.31 | 0.27 | 0.27 | 0.28 | 0.02 |
| **Full** | -0.56 | 0.12 | -0.05 | -0.4 | 0.06 | 0.33 | 0.22 | 0.13 |

**ME**

| **Coo** | | | | | **sCoo** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** |
| **LONT** | 0.09 | -0.06 | 0.17 | 0.06 | -0.56 | -0.63 | -0.43 | -0.64 |
| **RONT** | -0.11 | -0.26 | 0.11 | -0.04 | -0.35 | -0.28 | -0.18 | -0.36 |
| **LOT** | 0.15 | 0.01 | 0.09 | 0.02 | -0.34 | -0.48 | -0.45 | -0.73 |
| **ROT** | 0.25 | -0.02 | -0.12 | 0 | 0.1 | 0.12 | 0.09 | -0.19 |
| **LO** | 0.13 | 0 | 0.2 | 0.06 | -0.44 | -0.55 | -0.34 | -0.65 |
| **RO** | 0.12 | -0.11 | -0.04 | 0 | -0.09 | -0.09 | -0.03 | -0.3 |
| **Full** | 0.12 | -0.05 | 0.1 | 0.05 | -0.28 | -0.35 | -0.17 | -0.52 |

**Table 6.2. Weighted mean lateralisation indices (LI) calculated using LI-toolbox for trained animals.** For MT (top) activity is mostly bilateral for the coo stimuli, except for caudal and rostral regions which often show right laterality. A more contra-lateral distribution is seen with the sCoos. For ME, mostly bilateral activity is seen with the coos, and the sCoos are associated with right laterality, more so during contralateral sound presentation.

| | | Co-ordinates | | | | |
|---|---|---|---|---|---|---|
| **Active Region** | **z-stat** | **x** | **y** | **z** | **Number of Active Voxels** | **% of area active** |
| STS fundus area | 4.06 | -17.5 | 14.5 | 5 | 787 | 4 |
| STS fundus/dorsal bank area | 3.09 | 16 | 23.5 | 5 | 808 | 1 |
| Intraparietal sulcus associated area in the superior temporal sulcus | 4.38 | 19.5 | 10 | 9 | 395 | 37 |
| Auditory region, RTL | 4.04 | 27 | 21.5 | 10.5 | 32 | 7 |
| Auditory region, AL | 4.40 | 29.5 | 17.5 | 12.5 | 775 | 16 |
| Inferior Colliculus | 5.49 | -3.5 | 2.5 | 13 | 916 | 58 |
| Superior Colliculus | 4.92 | -3.5 | 3 | 13.5 | 915 | 46 |
| Floor of superior temporal area | 4.58 | 21.5 | 4.5 | 14 | 323 | 34 |
| Auditory (koniocortex) | 4.79 | 25.5 | 13.5 | 15.5 | 8 | 39 |
| Temporal parietooccipital associated area in sts | 4.65 | -23.5 | 6 | 20 | 39 | 23 |
| Auditory region ML | 4.29 | -22 | 6.5 | 21 | 563 | 29 |
| Medial superior temporal area | 3.47 | 17 | -2.5 | 28 | 804 | 4 |

**Table 6.3. Active regions during Coo presentation in trained animals MT and ME.**

| Active Region | z-stat | Co-ordinates | | | Number of Active Voxels | % of area active |
|---|---|---|---|---|---|---|
| | | x | y | z | | |
| Superior temporal sulcus dorsal bank | 4.12 | -25 | 21.5 | 4 | 31 | 68 |
| Superior temporal sulcus area, rostral part | 4.08 | -25 | 22 | 4 | 32 | 83 |
| Rostrotemporal pole | 3.67 | -20 | 26 | 7.5 | 808 | 53 |
| Auditory region RT | 4.73 | -22 | 24 | 9 | 33 | 94 |
| STS fundus/dorsal bank area | 3.78 | -20.5 | 7.5 | 12.5 | 395 | 68 |
| Auditory region CPB | 3.54 | 26.5 | 7 | 16 | 787 | 45 |
| STS dorsal bank area | 4.99 | 19 | 5 | 16.5 | 39 | 90 |
| area PG associated region of the superior temporal sulcus | 4.90 | 17.5 | 4.5 | 17.5 | 622 | 94 |
| auditory (higher level - lateral) CPB | 3.78 | -26 | 7 | 19.5 | 564 | 87 |
| para-auditory area, caudal part | 3.93 | -23 | 5.5 | 21 | 563 | 99 |

**Table 6.4. Active regions during sCoo presentation in trained animals MT and ME.**

### *4.3 Performance Effects on Lateralisation*

In order to ascertain the relationship between lateralisation and performance, the FSL GLM z-score values were used for comparisons of good and bad performance, defined by hits and CRs for good performance, and misses and FAs for poor performance. These values were then used to calculate LIs for performance effects. An ANOVA comparing monkey, stimulus and performance revealed no significant effect in caudal ($F_{1,77} = 0.661$, $p = 0.419$), mid-caudal ($F_{1,77} = 0.137$, $p = 0.713$) regions, but significant effects in mid-rostral ($F_{1,77} = 4.54$, $p = 0.036$) and rostral ($F_{1,77} = 11.12$, $p = 0.001$) regions. A significant effect of monkey was also seen in all but caudal regions (caudal $p = 0.419$, mid-caudal $p = 0.020$, mid-rostral $p = 0.002$ and rostral $p = 0.001$). Pairwise comparisons of performance for each animal showed significant differences for the Coo stimuli with MT in caudal ($p = 0.027$) and rostral ($p = 0.043$) regions, and for the sCoo significant effects were seen in mid-rostral ($p = 0.005$) and rostral ($p < 0.001$) regions, with poor performance associated more heavily with right lateralisation. For ME significant effects with the Coo stimuli were seen in mid-rostral ($p = 0.015$) and rostral ($p = 0.007$) regions, with poor performance again associated with rightward LIs. For the sCoo no significant differences were seen. Additionally, significant differences were seen between the two stimuli only for MT during poor performance in caudal ($p = 0.028$) and rostral ($p < 0.001$) regions, with the Coo producing more leftward lateralisation.

Performance for each of the animals was also compared with zero to ascertain significant divergences of lateralisation in each direction. Asterisks on the bars in Figure 6.7 show significant variation from 0 at a minimum of $p < 0.05$.

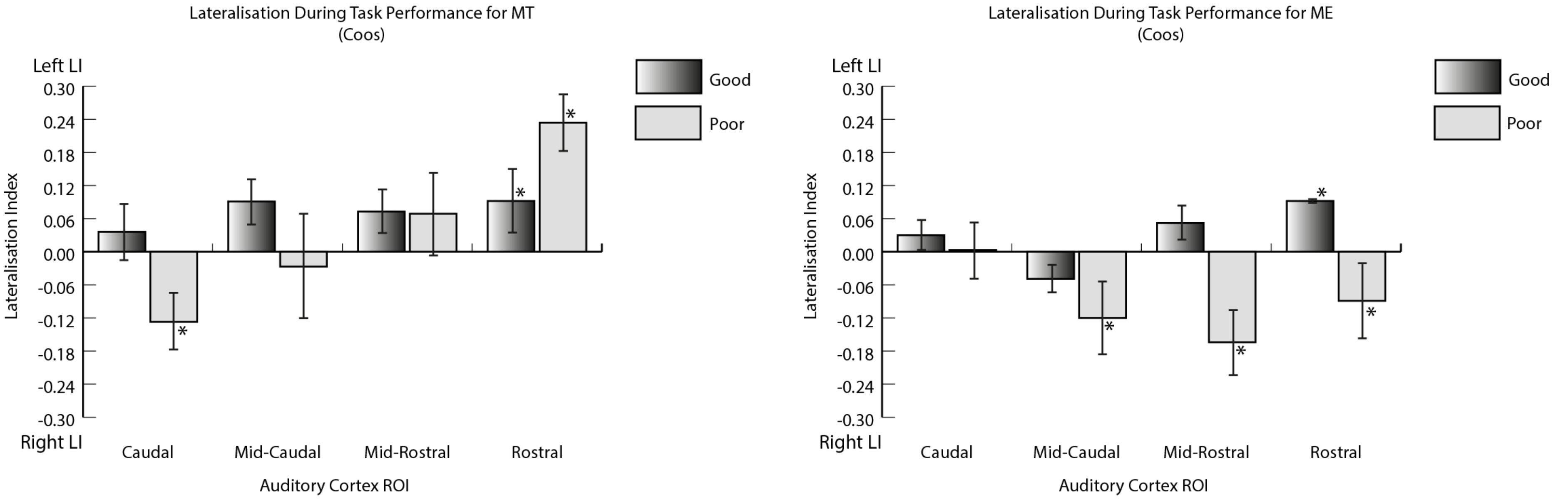


**Figure 6.7. Maximum z-score (maxZ) based comparison of good versus poor performance for MT (left) and ME (right) when performing with Coos.** More left lateralisation is seen is rostral regions for MT during poor performance, and right lateralisation is associated with poor performance for ME. Error bars show standard error of the maximum voxel based LIs across sessions.

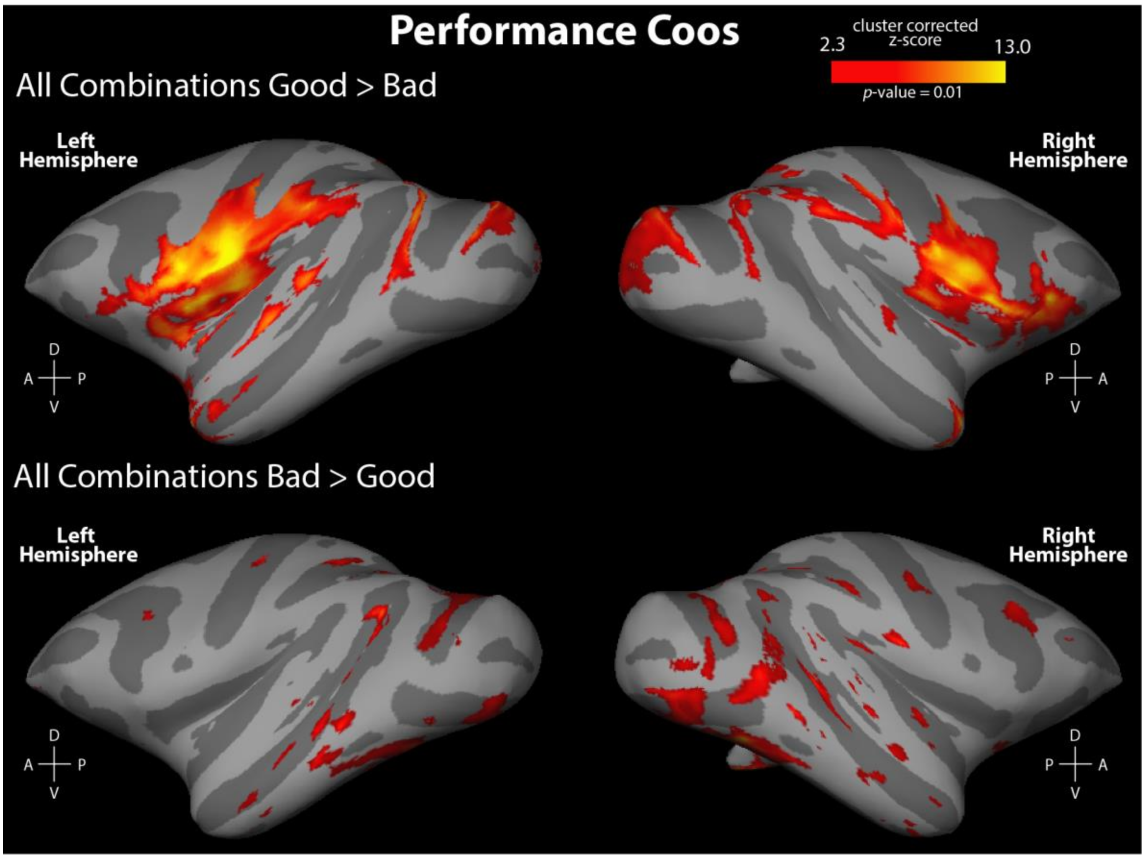


**Figure 6.8. Regions preferentially activated either by good (above) or bad (below) performance.** Surface projections of data from MT and ME combined. Activity in lower figures is uncorrected and sub-threshold.

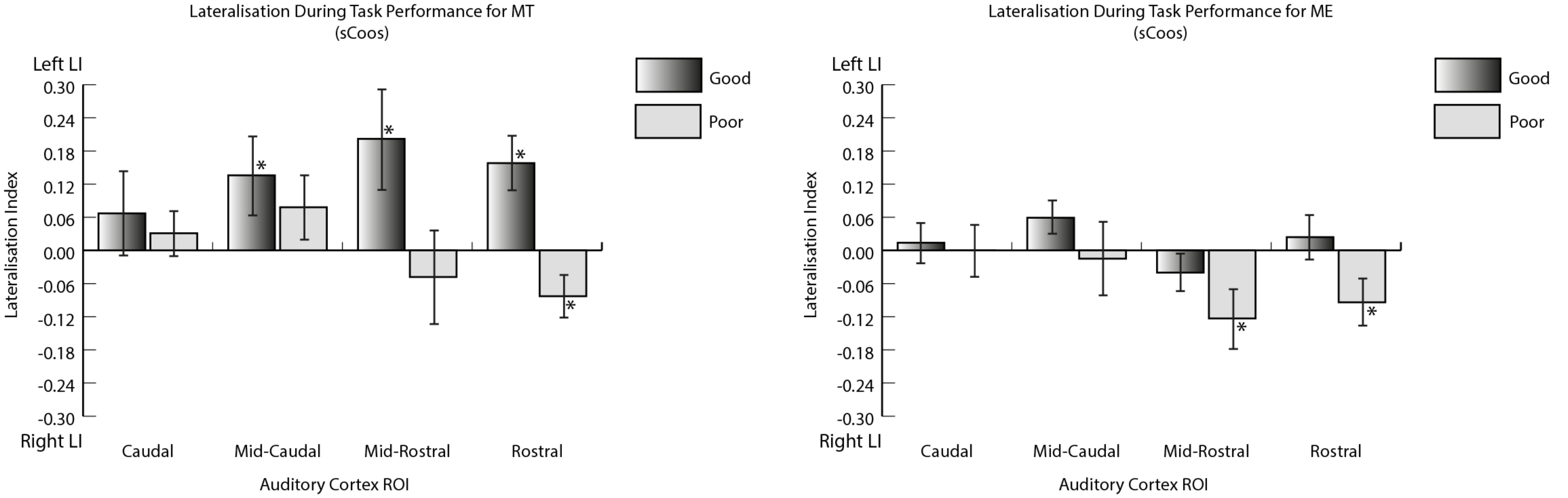


**Figure 6.9. Maximum voxel based comparison of good versus poor performance for MT (left) and ME (right) when performing with sCoos.** More left lateralisation is seen for MT during good performance, and right lateralisation is associated with poor performance for ME. Error bars show standard error of the maximum voxel based LIs across sessions.

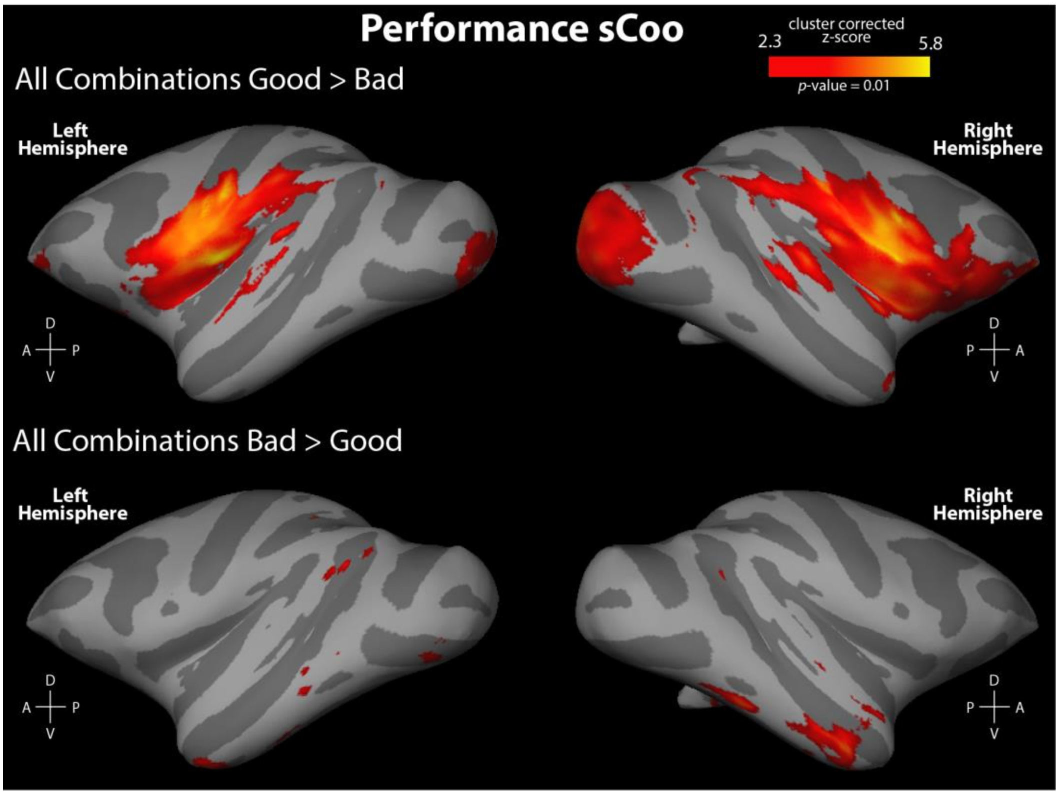


**Figure 6.10. Surface projections of activity related to performance.** Surface projections from ME and MT combined. Activity in lower figures is uncorrected and sub-threshold.

For the data entered into the LI toolbox, functional analysis of good performance and poor performance versus silence was performed before entering the data from the group analysis into the toolbox. For both of the animals the data show that during poor performance on the task with the Coo stimuli, activity is mainly right lateralised, whereas good performance produces more bilateral LIs. For the sCoo stimuli, again right lateralisation is seen during poor performance for ME, and good performance is associated with left lateralisation in MT (Table 6.5). These results may indicate that good performance on the spatial task is more dependent on bilateral processing, and when lateralisation is strongly to the right, performance deteriorates. Although this seems to also be true for the sCoos with ME, it may be that the communicative aspect of the Coo stimuli disrupts the bilaterality of activity needed for good performance.

**MT**

| Coo | | | | | sCoo | | | |
|---|---|---|---|---|---|---|---|---|
| | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** |
| **Good Perf** | -0.18 | 0.04 | 0 | -0.3 | 0.02 | 0.34 | 0.23 | 0.1 |
| **Poor Perf** | -0.89 | -0.41 | -0.24 | -0.46 | -0.14 | 0.29 | 0.11 | 0.03 |
| **LO Good Perf** | -0.33 | 0 | 0.04 | -0.38 | 0.05 | 0.29 | 0.32 | 0.06 |
| **LO Bad Perf** | -0.65 | -0.26 | 0.19 | -0.44 | 0.1 | 0.36 | 0.08 | -0.26 |
| **RO Good Perf** | -0.07 | 0.11 | 0.01 | -0.3 | -0.02 | 0.32 | 0.25 | 0.12 |
| **RO Bad Perf** | -0.81 | -0.54 | -0.47 | -0.37 | -0.51 | 0.09 | 0.02 | 0.07 |

**ME**

| Coo | | | | | sCoo | | | |
|---|---|---|---|---|---|---|---|---|
| | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** |
| **Good Perf** | 0.08 | 0.1 | -0.05 | -0.03 | 0.07 | -0.39 | 0.02 | -0.1 |
| **Poor Perf** | -0.62 | -0.72 | -0.69 | -0.68 | -0.37 | -0.49 | -0.39 | -0.53 |
| **LO Good Perf** | -0.02 | 0.16 | 0 | 0.12 | -0.03 | -0.49 | -0.11 | -0.1 |
| **LO Bad Perf** | -0.64 | -0.69 | -0.72 | -0.72 | -0.37 | -0.73 | -0.69 | -0.68 |
| **RO Good Perf** | 0.18 | 0.07 | -0.13 | -0.12 | 0.1 | -0.31 | 0.04 | -0.08 |
| **RO Bad Perf** | -0.55 | -0.73 | -0.77 | -0.65 | -0.34 | -0.32 | -0.14 | -0.22 |

**Table 6.5. Comparison of good versus poor performance for MT (above) and ME (below) analysed with LI-toolbox.** More right lateralisation is seen for both animals during poor performance with the Coos, and for ME also with the sCoos. For MT left lateralisation is associated with good performance with the sCoos regardless of the originating direction of the stimuli.

### *4.4 Audio-visual Task*

MK and MA performed the task in a similar way to MT and ME, however, their task also contained an audio-visual component. In the first instance they were required to attend to the auditory stimuli while the visual stimuli were presented either congruently or incongruently. Following this, the task was switched and they were trained to respond to the visual stimuli while ignoring the auditory (full details in *Chapter 5, section 2.1*). This therefore allowed us to investigate the effects of suppressed auditory attention on the functional response to the sounds.

To investigate the differences in lateralisation seen when the animals were asked to attend to the auditory stimuli compared to when they were asked to attend to the visual stimuli, a four way ANOVA with the factors monkey, task, region and performance was conducted using the maxZ LIs. This revealed significant interactions of task and performance ($F_{1,408} = 11.68$, $p = 0.001$), monkey and region ($F_{3,408} = 3.34$, $p = 0.019$) and monkey and performance ($F_{1,408} = 16.11$, $p < 0.001$). Pairwise comparisons revealed that significant differences in lateralisation between the two conditions was seen in mid-caudal ($p = 0.002$) and mid-rostral ($p = 0.018$) regions.

To investigate the effect of the auditory stimuli on performance of the visual task, activity present during good and poor performance of the task was compared. Left lateralisation in caudal ($F_{1,57} = 10.45$, $p = 0.002$) and mid-caudal ($F_{1,57} = 4.277$, $p = 0.043$) regions of AC is seen during good performance on the task in comparison with poor performance (Figure 6.13). In contrast, when the animals were performing well on the auditory task with visual distraction, left caudal regions were more heavily associated with poor performance ($F_{1,51} = 7.07$, $p = 0.011$). Lateralisation seen during both types of performance was also compared to 0 with Bonferroni post-hoc analyses. Asterisks in Figure 6.14 show lateralisation significantly different to 0. In all cases shown, $p < 0.005$, except for rostral regions in good performance with the AVv task, where significance was $p = 0.029$.

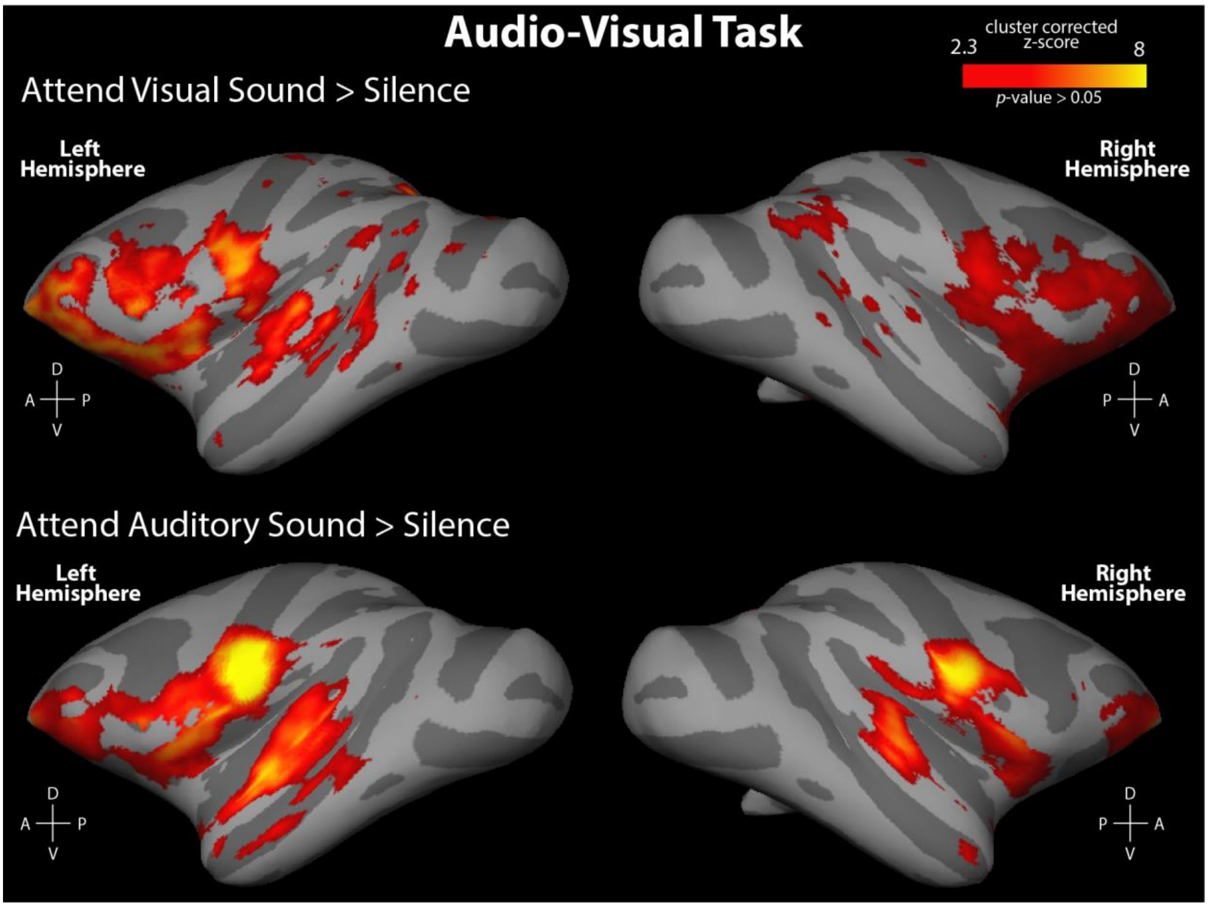


**Figure 6.11. Surface projection showing activity from MK and MA during sound versus silence.** Activity in auditory cortex is stronger in the left hemisphere during both tasks, but more so in the attend visual task.

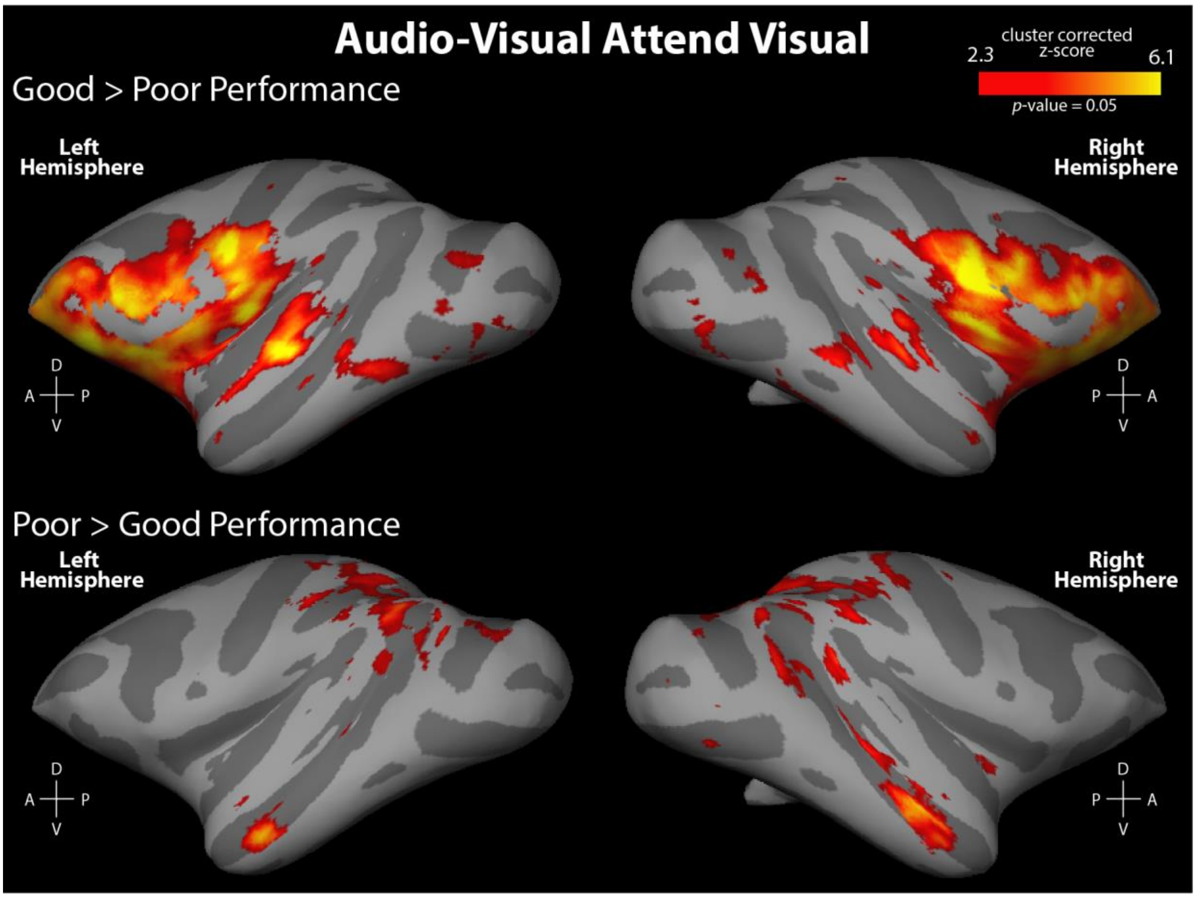


**Figure 6.12. Surface projections of activity during good and poor performance on the attend-visual task for MK and MA.** Left lateralisation is seen in the good performance comparison which is not seen during poor performance. Additionally, ventral ("what") regions are highlighted bilaterally during poor performance.

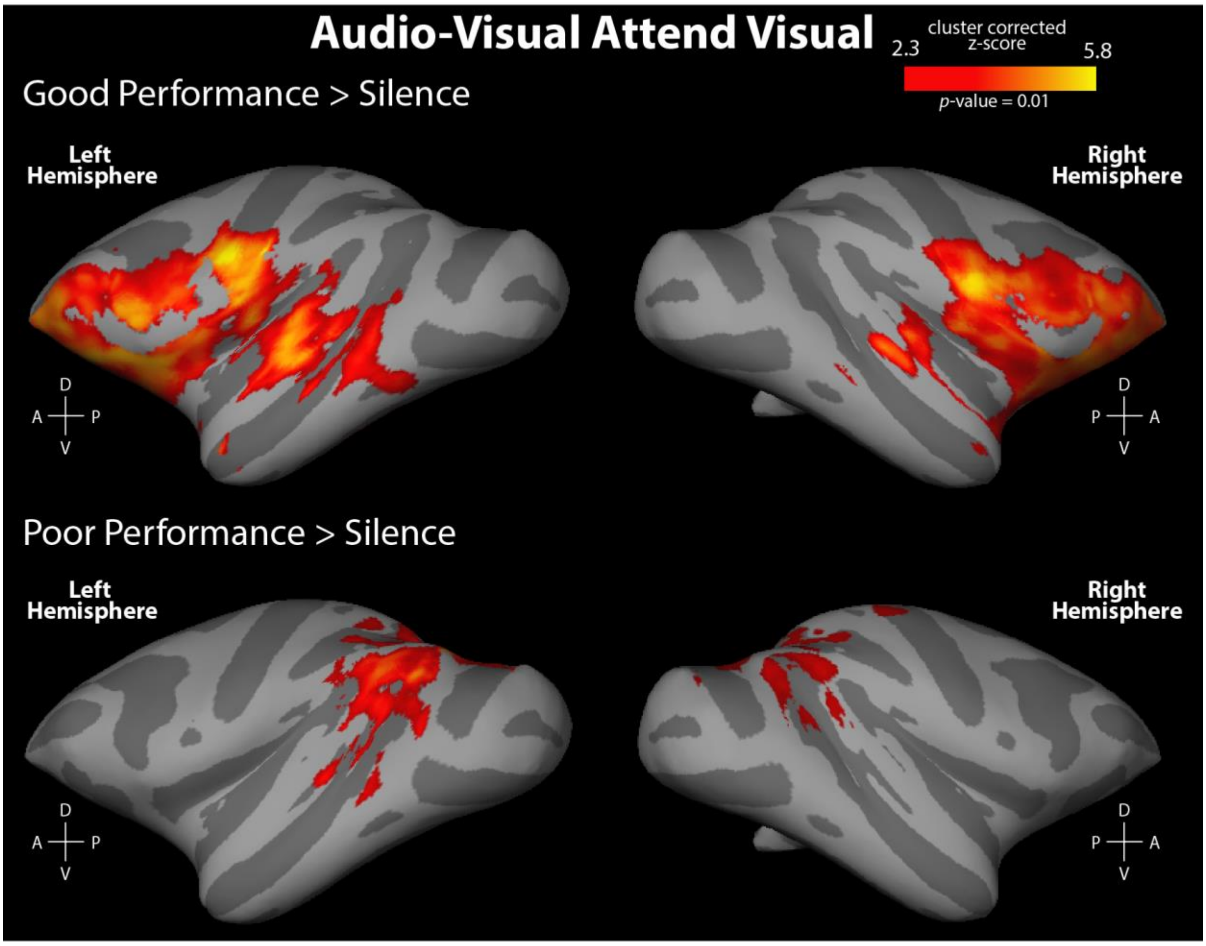


**Figure 6.13. Good and poor performance versus silence.** Ventral activity seen in the poor > good performance comparison in Figure 6.12 is not evident on comparison of poor performance versus baseline. Therefore activity during the previous comparison may be related to suppression of activity in ventral regions during good performance rather than activity during poor performance.

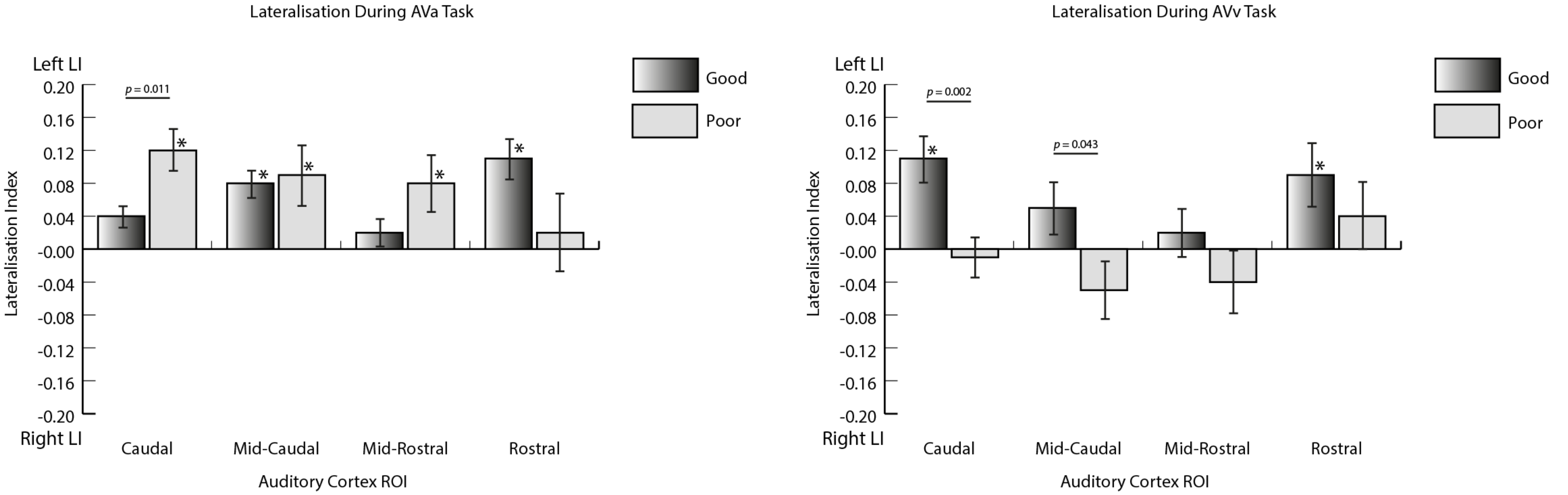


**Figure 6.14. Lateralisation indices for good and poor performance.** Left lateralisation is significantly stronger during poor performance on the AVa task than during good performance in caudal regions. In contrast, left lateralisation is associated more with good performance for the AVv task. Asterisks indicate LI is significantly different to 0. (AVa = audio-visual attend auditory, AVv = audio-visual attend visual).

As for the previous analyses, data was also analysed using the SPM LI-toolbox. For the sound versus silence comparison, we see that left lateralisation is associated with the auditory stimuli when the animals are not thought to be attending to the sounds (Table 6.6).

| **AVv** | | | | |
|---|---|---|---|---|
| | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** |
| **SvS** | 0.08 | 0.49 | 0.41 | -0.08 |
| **Good Perf** | 0.41 | 0.66 | 0.4 | -0.26 |
| **Bad Perf** | 0.09 | -0.18 | 0.12 | 0.1 |
| **AVa** | | | | |
| | **Caudal** | **Mid-Caudal** | **Mid-Rostral** | **Rostral** |
| **SvS** | 0.62 | 0.75 | 0.45 | 0.23 |
| **Good Perf** | -0.08 | 0.36 | 0.11 | -0.15 |
| **Bad Perf** | 0 | 0.18 | 0.14 | 0.18 |

**Table 6.6. Lateralisation indices for good versus poor performance with auditory and visual tasks.** Left lateralisation is evident during good performance with the visual and auditory tasks. AVv = Audio-visual attend visual, AVa = audio-visual attend auditory.

Further comparison with directional components of the task was not possible as there was insufficient data with the animals performing poorly on individual directional components. Additionally, these two animals were not trained to perform the task with RO targets, therefore balanced directional comparison was not possible. Good performance on the visual task with the auditory distractor is heavily associated with left auditory cortex activity. Mid-rostral auditory regions are associated with poor performance.

## 5. Discussion

Natural sounds have been shown to elicit greater neuronal responses in auditory core region A1 under attentive states than clicks or tones (Fritz et al. 2007), however, the results seen here are less clear. Additionally, in humans, speech sounds have been shown to elicit greater responses in AC overall in both hemispheres (Belin et al. 2002). This is seen in MD in the naïve condition, however is not a statistically robust effect. In the trained animals, this property may have served as a boost to their ability to perform the task once they had learned that the task dependent feature of the stimuli was the spatial cues. However, performance of ME never matched that of MT for the Coo stimulus condition. It may be possible that the initial hindrance to learning the task with the conspecific sounds prevailed throughout his training with both types of stimuli. This is supported by the fact that his performance never matched that of MT, and is further supported by the fact that MK and MA never achieved adequate performance with the ROT task component. However, as only one animal was trained with the sCoo stimuli initially, this can only serve as speculation.

Based on the findings from the behavioural data, for analysis of the fMRI results the following questions were addressed:

1. How does the functional response to the different stimuli differ in terms of AC laterality on initial presentation (naïve response)?

2. How does the functional response to the different stimuli differ in terms of AC laterality after training?

3. How do these responses differ when the animals are performing well versus performing poorly on the task?

4. How do functional responses differ when the animals are asked to attend to the auditory or the visual stimuli in the presence of the opposite modality?

***How does the functional response differ between stimuli on initial presentation?***

In this condition the maxZ analysis produces variable results, although in the sCoo condition the RONT does produce stronger left LIs than the LONT. Error bars however, reveal a large amount of variability across the sessions included in the analysis, indicating that this comparison may benefit from further data. However, with the LI toolbox, contralateral activity is seen for the Coo non-target presentations, and in the LONT sCoo presentation. Too few voxels were active in the RONT comparison to generate LI with the toolbox, again indicating that further data may be useful. Activity in the surface projections for the LONT show left hemisphere ventral activity for the Coos, which is not seen for the RONT or for the sCoo stimuli. Little activity is seen for the sCoo stimuli (Figure

6.4). Full brain analysis also highlighted activity in RPB for the Coo condition and CPB for the sCoo condition, suggesting that the communicative quality of the Coo stimuli was a salient feature, and the spatial aspect of the sCoo stimuli was more obvious, and was processed preferentially by anterior left hemisphere regions, as may be expected. Activity was also present in CM and the inferior colliculus in both conditions, which may suggest that the spatial property was processed with both types of stimuli.

***How does the functional response differ between stimuli after training?***

In the trained animals, lateralisation differs in the data from the two animals. For MT, mostly leftward LIs are associated with both the left and right presentations of both types of stimuli, however this is mainly significantly different from 0 in the sCoo condition. This result differs from the values obtained with the LI toolbox, as with this method right laterality values are seen for both directions of coo presentation. However, for the sCoo the results are in agreement with the maxZ analysis. For ME, lateralisation seen during the Coo presentation is mostly bilateral and this is corroborated by the LI toolbox. For the sCoo, rightward lateralisation is seen for both directions of presentation, however for the maxZ, only rostral regions show significant difference to 0. The LI toolbox additionally verifies these results although stronger effects are seen.

When investigating the effects of training on levels of lateralisation elicited by the stimuli, results from the LI toolbox suggest that training balances the right hemisphere preference for the LONT, and inverts the left hemisphere preference for the RONT. The naïve animal would ideally represent how the animals reacted to the stimuli pre-training. This does not seem to be the case for the sCoo, however no naïve LI is available for the RONT sCoo.

These findings may indicate preferential processing in the left hemisphere, with priority placed on the communication aspect of the stimuli resulting in confusion over the task relevant (spatial) properties of the task during early training stages. When the stimulus is non-communicative in nature, performance on the right is at least as good, if not better than the left. In humans, speech sounds are processed by the left hemisphere on a pre-attentive timescale (Rinne et al. 1999). This result suggests that this may also be the case for the macaques.

### *How do the responses differ when the animals are performing well versus performing poorly on the task?*

A striking difference is seen in the data when good and poor task performance for the Coo stimuli are compared which is seen in both animals, and for both types of analyses. With either type of stimulus, when activity is more strongly associated with the right hemisphere, poor performance is seen more frequently, and good performance is more heavily associated with bilateral or left lateralisation. This is contrary to what would be expected if spatial processing was associated more heavily with the right hemisphere as is suggested in humans, and shows that bilateral or left lateralisation is important for good performance, which is also shown in performance results for the sCoo stimulus. Of additional note is the fact that, in the naïve animal, with the LI toolbox analysis, the ROT elicited rightward lateralisation to the Coo stimuli, and leftwards lateralisation in the sCoo stimuli (Table **6.1**). Right laterality was then associated with poor performance when the animals had been trained to perform the task. This may be an indication as to why this aspect of the task was most difficult for ME to learn, and was never achieved with MK and MA, producing the initial behavioural bias to the left side of auditory space.

### *Audio-visual task*

The number of comparisons which could be performed with the audio-visual data was limited due to several factors. The animals were presented with unimodal stimuli for 60% of the trials to ensure that they were reminded of the task dependent modality. Additionally, the animals were very good at the visual task and in many cases did not make incorrect responses often enough to provide data for individual directional comparison. This experiment was not designed for the type of analysis which would have been ideal for these comparisons, but was taken from another experiment (Rinne et al. 2017).

However, for the analyses which were available, some interesting effects are seen. When the animals were performing the AVa task, leftward LIs were noted during sound versus silence, and both good and poor behaviour both with the maxZ and LI toolbox analyses. This may suggest that left laterality was generally associated with the stimuli rather than performance. However, with the AVv task, leftward laterality was seen on comparison of sound versus silence, and during good performance, but poor performance was associated with more bilateral activity. As MK and MA were not trained to perform the task with the ROT, for them, the RONT would have been the only condition in which the first auditory presentation was on the right. This may, therefore, have cued a greater attentional response to the right side of auditory space, and so produced left lateralisation. Additionally, the animals were very good at the AVv task, therefore good performance may have been represented more strongly in the analysis. This may be why left lateralisation is seen during sound versus silence and good performance in the AVv task, due to underrepresentation of poor performance activity.

With the AVv task, the increased level of activity in the left hemisphere may reflect a return to a passive-like state when the animals are not asked to respond to the auditory modality. We may, therefore, be seeing an effect of the functional response to the communication aspect rather than the spatial aspect seen during the task. When comparing the activity seen in surface projections we see that when the activity associated with good performance is removed, poor performance is associated with activity in ventral regions, possibly suggesting distraction by these regions. However, during both good and poor performance in relation to silence, this activity is not seen during poor performance, and is seen minimally during good performance. This may therefore represent active suppression of these areas during good performance, limiting the distraction of the auditory cues.

The differences in types of head immobilisation used for the animals is unlikely to have contributed to the differences in the results seen, rather, the task requirements are likely to have caused this. Less data was available for the animals using the NHIS due to some development issues; however, the results we see are robust in both of the animals for the performance comparisons.

## 6. Conclusion

These results suggest that the identification of changes in spatial location of auditory stimuli depend on bilateral or left hemisphere processing. In contrast to human literature, strong right lateralisation during task performance is heavily associated with poor performance with both types of stimuli. This may indicate that the communicative aspect of the coo vocalisations disrupts the bilateral activity required to perform this task, and may suggest an evolutionary advantage to the lateralisation of AC, as left regions are able to prioritise communication aspects without interference from spatial aspects. This may relate to the findings of Ortiz-Rios et al. 2017 which suggest that right hemisphere AC is suppressed by left hemisphere regions during binaural sound presentation. It may be that if this suppression is interrupted by the prioritisation of vocal information, the bilateral activity required for good task performance is disrupted. This would support the idea that the overcrowding hypothesis for the evolutionary development of lateralised processing (Cai et al. 2013) also holds for auditory processing. Following this, it may be possible to further investigate the early evolutionary processes which lead to lateralisation of human auditory functions, and the prioritisation of the processing of communication in the left hemisphere.

# Chapter 7 : General Discussion

## 1. Non-Invasive Head Immobilisation System (NHIS)

This effort resulted in a system that has considerable flexibility in how it can be used. The system incorporates preferable features which exist in previous non-invasive head immobilisation developments and combines these features to form one comprehensive approach. It has an option for automated voluntary engagement with the facemask, as an initial step towards the animals habituating to full head immobilisation. It has been comprehensively evaluated with data from several animals, against the specified criteria within the context of documented behavioural habituation and training steps. With one animal, data was obtained which directly compared the NHIS with the animal's own surgically implanted head post, which shows encouraging results. The findings, indicate that the NHIS can provide a feasible approach for non-invasive head immobilisation that is not overly time consuming to implement and as a relatively low cost alternative to surgically implanted options where direct access to the brain is not required.

### Benefits of the System

The NHIS has proven to be a success in terms of behavioural and fMRI data collection, and expressions of interest have been received from both within Newcastle University and further afield, and we have had supportive comments from authors cited in the 2016 paper. In particular, a newly established primate lab has expressed interest in using the system, and we are currently supporting researchers in this facility to implement this option.

The system has provided a positive impact on the welfare of the animals in our lab. Four out of five of the animals who have used the NHIS have taken to it well and produced results comparable to those from implanted animals. Two monkeys (MT and MW) have been trained to perform tasks which require head immobilisation without being implanted, and one animal has not been re-implanted after the existing surgical implant failed (ME). One of the animals was later re-implanted as an animal was required for electrophysiology research and the helmet system had not yet been sufficiently tested to recommend it for use with invasive procedures (MC).

Data presented in *Chapters 3* and *4* have also shown that there are ongoing benefits in terms of the health of the animal. Tissue around an implant is unable to heal properly with a foreign object present, although this may be improved with the use of more biocompatible materials, and the use of coated implants which reduce the size of the wound margin. However, many animals experience bleeding or infections around implant margins. Treatment for this can involve sedation for cleaning and cauterisation of granulated tissue, which then requires some mild form of pain relief. Additionally, when an animal is first implanted, the change in appearance can impact on the social dynamics in pair or group housed animals, resulting in changes in dominance or lack of engagement from conspecifics. These procedures can be avoided or delayed until a later point where more invasive methods are required by using a non-invasive restraint. While sedations for moulds are still required, these are often far less frequent and do not require pain relief or discomfort to the animal.

The NHIS also has animal welfare benefits which may be unexpected. For example, data presented in *Chapter 4* shows that movement levels during fMRI recording are reduced following greater levels of habituation to the scanner and scanning process. This may force researchers to allow the animal more time to acclimatise to procedures, rather than going at a pace with which the animal may not be completely comfortable. However, the flip side of this is that more sessions may be needed to acquire the same amount of data, which may have more of a cumulative impact on the animal, and may have financial implications to the facility, which may act as a deterrent to the implementation of this option.

For the lateralisation experiments, only one animal would have been available for collection of both behavioural data and fMRI data had the NHIS not been available. As ME had lost his implant, re-implanting him for behavioural and fMRI data collection would have been potentially dangerous. Additionally, while initial training of MK and MA was conducted without any form of head immobilisation, it was possible to immobilise ME and MT while they were performing the task during habituation to the full restraint. MK and MA were not implanted when training began, and post implant surgery, could not be immobilised for at least 6 week prior to immobilisation training. This may have impacted on their initial performance of the task as head turns during stimulus presentation may have made it more difficult for them to appreciate the location change, whereas voluntary engagement training with ME and MT meant that task training could begin with the head immobilised, at least temporarily, almost immediately.

**Limitations of the NHIS**

There are several instances where additional developments could further increase the efficacy of the NHIS. Issues arise when animals acquire injuries to the face or head during fights between pair or group housed animals, for example, which occurred with three of the animals trained to use the system. In this case the animal needs ample time for the wound to completely heal before he or she can return to work, where an animal with an implant may require less time. This could arguably have a positive impact on animal welfare, as they are given extra time to recover, however, through experience we know that monkeys can become bored and restless when not working regularly, increasing the likelihood of fights with conspecifics or the development of stereotypic behaviours (abnormal behaviours which develop in response to boredom or stress in captive animals). A similar situation would apply with females who experience seasonal swellings to the face. These would need to be closely monitored to ensure that no excess pressure is being placed on the affected areas, although the versatility of the system does mean that holes can be made to accommodate either seasonal swellings or facial wounds.

Another issue which has proven challenging to overcome is present during the mould procedure. Very often only one half of the restraint is produced at a time, with the animal's head being placed in the other half in order to acquire the second piece. Where this involves placement of the head into the back piece to acquire the front, quite often the animal's head can tilt back or to one side within the back piece which is difficult to identify during the mould procedure. This can result in a facemask which is tilted, or otherwise skewed in some way. Additionally, difficulties have arisen when the NHIS holds the head in an unexpected position. Following initial fMRI data collection, it was discovered that the helmet held the head at a slight tilted angle in some of the animals which was not obvious on visual inspection. This meant that, during scanning, the two auditory cortices (AC) were not aligned along the same scanning slice and this needed to be corrected for. Once this was identified the problem was easily rectified by aligning the scanning volumes to the angle of the head, and taking extra slices to ensure full coverage.

However, it did result in some initial datasets being unusable, and extra problems with producing the tonotopic maps, as the left and right AC were located in different slices.

We aimed to produce more accurate head models by performing structural MRI to create a 3D printed model. However, the vertical bore scanner at the facility in Newcastle University, which is designed to scan behaving monkeys in a more natural sitting position, means that scanning anaesthetised monkeys, especially larger animals, is challenging and is associated with a greater risk of complications. Most of the scans which were acquired did not adequately capture the lower part of the head due to the position of the animal in the primate chair, and masks produced from these models were not suitable for use. However, if a CT scanner was available, the 3D imaging method may prove more useful, and a more standardised method of head model production would help to avoid the problems described, and possibly improve the fit of the completed NHIS.

An additional limitation of the system for use with the lateralisation experiments was that the method differed from that used with MA and MK. Therefore this needs to be considered as a factor when comparing the results. As the task for the two monkeys with implants differed slightly from the animals using the helmet, it is not possible to make direct comparisons based only on the method of head immobilisation used, but this is a potential additional variable. Additionally, because development of the system was ongoing while the lateralisation related fMRI data was being collected, some of the initial data needed to be discarded.

### *Further Developments*

The data from the eye tracking experiment has been added to since publication of the results. In the first instance we had data from only one animal (MC), which, although we showed that significantly less eye movement was seen during the fixation period with both methods, significantly more eye movement was seen overall with the non-invasive method when compared to the implant. It is not possible to tell from the eye tracking data whether this was due to movement of the head in the helmet, or due to poor fixation performance of the animal. It has since been identified that this monkey is experiencing sight difficulties and his current performance on a simpler fixation task with implanted head immobilisation is very poor. Conversely, the two animals who are now being trained on the same task are performing well, and data looks promising, although could not be properly analysed in time to incorporate into this report.

The system has the potential for further development for other techniques, and its use in combination with new prospective movement correction during fMRI may further improve its applicability for this method. Further, EEG, PET and various other imaging techniques where direct access to the brain is not necessary could benefit from the application of this method.

### ***Conclusion***

It does seem reasonable that the introduction of any new method would require further refinement after initial development. Implanted head restraints have benefited from a number of years of experience and improvements, which are still ongoing. It is entirely possible that other groups taking up this system as a preferred option for head immobilisation can improve upon it and adapt it for their purposes in the same way as improvements to the implanted head restraint are being developed, especially by those who may have access to more sophisticated equipment, and who are more experienced with various neuroimaging techniques.

The fact that there are a variety of non-invasive methods available hopefully suggests that this is a subject which several groups using primates are interested in becoming involved with. With additional input from groups using a variety of methods, it may be possible to use this system, which amalgamate the positive features of each of the available systems into one, as a base for further development. Several groups working to refine the same method would be likely to produce better results than each working on developing their own method.

Here we have seen that it is possible to achieve behavioural training, eye-tracking data and fMRI with this non-invasive approach, and that the data can be comparable to the implant techniques. We also see that the data is of a good standard, and this method has produced much of the data for the scientific aims of this report.

## 2. Lateralisation in Macaque Auditory Cortex

While the behavioural lateralisation of task performance was unexpected, the effect was robust, and replicated across three animals. Based on the literature presented, there are some similarities between the results shown here and those seen in humans. Previously, a right ear behavioural bias has been documented in humans for tasks dependent on processing of communication, and in monkeys, a right ear preference for communication calls has been seen (Hauser and Andersson 1994). However, data presented here suggest that task performance is hindered, specifically on the right, when the monkeys are performing a spatial task when the stimulus is a communication sound. This may point to prioritisation of the communicative aspect of the sound over the spatial location properties.

The leftwards bias in performance of spatial tasks in humans is well documented, and is often attributed to functional lateralisation of human spatial abilities to the right hemisphere (Bellgrove et al. 2004). However, in macaques, functional lateralisation for spatial processing has not been shown (Ortiz-Rios et al. 2017). The results seen in this thesis with the macaques therefore raise some interesting questions. The fact that, in contrast to the animals working with the Coo stimuli, the animal working with the sCoo sounds showed no preference at first, then a right originating (RO) preference at a later time point, may suggest bilateral or left hemisphere superiority for spatial discrimination. Further, for ME with the Coo stimuli, the left behavioural bias was balanced over time and on switching to the sCoo stimuli, became the direction of less reliable performance. Additionally the data shows that, although performance with RO stimuli improves over time, performance with the left originating (LO) stimuli remains constant. This is pattern is also seen in data from MA. This could therefore suggest that, once the initial distraction of the communicative aspect of the stimulus was reconciled, and the animals had learned to allocate attention to the spatial properties of the stimuli, left hemisphere regions were able to improve performance, whereas the right remained the same. This may relate to the bottom-up and top-down theories for allocation of attention. Initially, when the animals are presented with the stimuli, bottom-up attentional capture would be the main influential factor, as the animals have no context for the sounds they are hearing. If, as previously mentioned, top-down attentional processing is dependent on feedback from bottom-up processing (Theeuwes 2010), it could be that the task related spatial properties of the sounds took longer to place in context when the more salient communicative aspect was present, especially in left hemisphere regions, which are more keenly tuned to communicative processing.

However, alternative effects may have played a part which were not accounted for by the analyses conducted. For example, a recent study revealed strong right lateralisation in auditory cortex when animals were cued to expect a greater reward as compared to a smaller reward (Wikman et. al., in prep). Although the authors do not discuss the results in terms of laterality, or speculate as to the possible reason for the lateralised effects, the right lateralisation seen is noted. Other influential factors may relate to the nature of the sounds used. As the Coo sound is something which is very familiar to the animals, and something which they themselves produce, it may be possible that the sound elicited a different emotive response from the animals than the sCoo. Differences in lateralisation depending on emotive state have been identified in humans, however this is mostly related to frontal cortex activity rather than auditory regions (Harmon-Jones et al. 2010). Different call types have been shown to elicit differences in lateralisation produced dependent on the function of the call type (Taglialatela et al. 2009), however this was shown in chimpanzees and the author notes that the results presented may indicate that macaques and chimpanzees perceive and process conspecific vocalisations in different ways.

One interesting, but anecdotal effect seen with ME was that the left behavioural bias returned during the task with the Coo stimulus when the individual producing the vocalisation was changed. This effect lasted for only two training sessions before performance returned to normal levels, therefore it was not possible to collect a robust dataset on this observation. Of additional note is that the LO preference returned for ME with the Coo stimuli when he was performing the task in the scanner, but only for sessions which met the performance criteria for inclusion in fMRI analysis. Therefore, when he was performing the task to a higher standard, there was a preference towards the left. This may reflect lateralisation of sustained attention in the presence of the additional distraction of the scanner noises and environment. In humans, increased task difficulty has been associated with a bias of attention towards the right ear, specifically for verbal cues (Mondor and Bryden 1992). This may indicate that ME found the task more difficult in the scanner, which lab behavioural data supported. However this effect is not seen with MT.

Other factors may also have played a part. As the animals had to learn the task implicitly, it is not possible to say exactly what cues they were using to elicit a correct response. As we saw with training of MK and MA, it was difficult to know what they were responding to and how to guide them towards responding in the desired way, especially as they seemed to find the task very challenging. For the auditory task, MK and MA performed the task with only the LO target, but with both non-targets. Therefore it is possible that the responses they produced were due to monitoring one side of space and responding according to the number of sounds which appeared in that space. As ME and MT were trained to perform the task with both types of target, this is presumably a tactic which would not have worked for them, at least in the long term.

To attempt to place the functional activity elicited by the two types of stimuli into the context of attentional effects, when exploring the behavioural effects in terms of functional activity we began by analysing data from a naïve animal. For this animal, as a baseline, the uni-directional or non-target sCoo sounds would be expected to produce contralateral activity in line with previous literature. This result is seen for the left originating non-target (LONT) when using data from the lateralisation index (LI) toolbox, however, too little data was present to produce a comparison for the right originating non-target (RONT). For the Coo sounds, clear contralateral activity is seen for the LONT and the RONT. With the analysis based on the maximum z scores (maxZ), for the sCoo contralateral activity is seen for the RONT, however the LONT produces more bilateral results, and for the Coo sound the results are predominantly bilateral. For this analysis none of the data produced an LI significantly different to zero. This may be due to the lack of power generated from data from only one animal. Additionally, in contrast to the other comparisons performed, for the naïve animal the maxZ LI values were based on un-corrected data. This may have introduced further noise to the dataset which could account for the variability in the results. However, it could also be argued that the uncorrected data is not biased by the cluster correction before sampling, therefore is a legitimate way of performing the analysis.

The sound versus silence fMRI data from the trained animals show varied results. Here, with the LI toolbox activity seen for the Coo sounds is right or bilaterally distributed for MT, with consistent right lateralisation in caudal regions. However, little lateralisation is seen for ME. For the maxZ analysis, left lateralisation is seen in rostral regions for MT and no LIs significantly different to zero are seen for ME. In relation to the behavioural data, this may support the hypothesis that the communication property of the stimulus hinders initial performance, as the right Coo primes left auditory cortex (AC) for communication before the left Coo is presented. This may be the reason for the initial confusion. Then, as the animals learn to attend to the spatial properties, the activity becomes less left lateralised. However, as the effects are seen only in one animal, and in only one of the types of analysis, this cannot be fully supported by the data shown. For the sCoo stimuli, LI toolbox results show left laterality for MT when presented with RO stimuli, and predominantly right lateralisation for ME across all conditions. This is supported by the maxZ analysis for ME, however, MT shows left laterality for both right and left originating sounds.

More interesting results are found when performance effects for the auditory task are compared. For the Coo condition, LI toolbox data from both ME and MT reveals that more rightward lateralisation is seen during poor performance on the full task, specifically in more caudal regions, and bilateral activity is more associated with good performance. However, with the sCoos in MT, good performance is associated more with left hemisphere activity more broadly, while for ME a similar result is seen as for the Coo sounds. This is also supported by the maxZ results. This hints towards left hemisphere specialisation for spatial processing, but could also point to a need for a suppressive effect on the right hemisphere regions, which, when not achieved, results in poor performance. A suppressive effect on the right hemisphere has been shown to be altered by the presentation of binaural over monaurally presented stimuli in passive animals (Ortiz-Rios et al. 2017), and this effect may suggest that this is necessary for adequate spatial processing, which may be more challenging with binaural over monaurally presented sounds. This may also raise the possibility that the differences in the difficulty of perceiving the location of the binaural and monaural sounds is the feature which drives the suppressive response, rather than the presence of the inter-aural time difference or inter-aural level difference properties of the sounds. Additionally, a right lateralised network has been implicated in involuntary allocation of attention to a given stimulus during change detection tasks (Downar et al. 2000). When comparing effects between the naïve and trained animals, LI toolbox results indicate that the right originating target (ROT) produces greater levels of right lateralisation for the Coo stimuli, which is balanced in medial regions in the trained animals. This is of interest since the animals trained with the Coo stimuli found this aspect of the task most difficult to learn, and two of the animals were unable to perform the task with this component. This may support the idea that greater involvement of the right hemisphere regions hinders performance on the task and more bilateral processing is needed for adequate performance.

The effects of the visual task with auditory distractor provided an additional level of attentional control. Although these animals were trained initially to perform the task with the auditory stimuli, performance on the visual task in the presence of the Coo distractor gives a comparison during which the animals were least likely to be attending to the sounds. In this comparison we see that the left hemisphere activity returns, but is more heavily associated with good performance on the visual task. It is not possible to say for sure what information the animals were responding to in the audio-visual task, however, since the left hemisphere activity seen during good task performance is also prevalent during good performance with the inconsistent auditory trials, it seems reasonable to suggest that the animals were not using the consistent auditory cues to boost their performance, but instead this may indicate that the presentation of the communication sounds in space activated caudal regions in line with previous research (Mathiak et al. 2006) more so in the left AC. The presence of left lateralisation during both good and poor performance with the auditory trials could indicate that this result was independent of the level of performance, again hinting towards left hemispheric preference for the communicative properties of the stimuli being prioritised over the spatial aspect when the animals were initially presented with the task. However, it is unclear as to why this would be associated with good performance on the visual task, although a possible reason for this could be that the animals produced far more correct responses during this task than incorrect responses, therefore poor performance may be underrepresented in the data. Additionally, the presence of ventral activity in the poor versus good comparison, although initially may be interpreted as an effect of increased levels of distraction, with the Coo activating rostral “what” regions, is not evident when poor performance is compared to silence. Therefore the activity is likely to be related to active suppression of these regions during good performance.

***Further Possible Experiments***

An interesting experiment which could possibly help to answer some of the questions raised by the results seen here would be to recruit human participants to perform a similar task. If the effects seen here are at least in part due to the animals misinterpreting the task relevant aspect of the Coo stimuli, asking humans to perform the task with no prior instructions using a human vocalisation may produce different effects than the same condition with a phase scrambled human vocalisation. However, it may be difficult to control for task difficulty effects between the two species. Humans were tested in Helsinki as part of the project with the audio-visual task, performing a similar task to MA and MK. As expected, the humans were able to perform the task with ease, and behavioural results were mostly at ceiling levels (Rinne et al. 2017). Therefore, with humans performing a similar task, we may miss behavioural and, if scanned, functional effects seen with the animals which may be attributable to effects of task difficulty, sustainability of attention and cognitive load.

Another experimental protocol which would be interesting is to scan the animals prior to training, to ascertain how functional representations of the sounds change as the animal is learning the task. This would have been difficult to accomplish here as training and habituation to the scanner was performed at the same time, and both are lengthy processes. Accomplishing both of these things in isolation would add months, if not years to the project unless the animal had been habituated to the scanner before the study began.

Finally, as we see a return to left hemispheric lateralisation in the visual task in the presence of an auditory distractor, it would be interesting to see how these effects would compare to those which may be seen in the presence of the sCoo as an auditory distractor rather than the Coo. This would help to tease apart the effects seen here which could be due to spatial or communicative processing, or a combination of the two.

### *Limitations of the Study*

Animal numbers for the task and passive comparison was a hindrance to interpretation of the data. Ideally, 2 animals would be tested on each protocol. Naïve data did not show the expected pattern of activity, nor did the activity which we did see survive more conservative thresholding. It is therefore likely that this dataset was underpowered, which could be addressed by collecting data in a second animal.

During initial training, ideally four animals would be trained with each type of stimulus, and two of each of the animals in each stimulus condition would initially be presented with left originating stimuli and the others with right originating stimuli during early training. This would establish that the effects seen were not simply an individual difference in one animal, but would transfer across animals in the same way as we see between ME and MA. ME, MK and MA all showed relatively poor performance on the auditory task throughout training, which was the reason for implementation of the bootstrap *d′* as a measure of chance performance, rather than using the commonly used threshold of a *d′* of 1 (Green and Swets, 1966). This held in the case of ME when the stimuli were changed to the sCoos. As MT did not have this initial problem, we could infer that his performance was unhindered by the communication aspect, whereas the presence of the communication aspect of the Coo stimulus hindered the other three animals in terms of learning the full task. Unfortunately, because only one animal was initially trained with the sCoo stimulus, it is not possible to conclude that this was an effect of the stimulus and not of some individual effect on the animal's performance. Additionally, when MT was first introduced to the left target, his performance declined. It would be interesting to know if we see an opposite behavioural lateralisation effect if the animal trained with the sCoo is initially trained with the LO stimuli.

Several other instances also occur in the data where significant results are seen only in one animal. This could be due to a range of factors, such as individual differences in the cognitive abilities, temperament or emotional state of the animals, differences in initial training or differences in the strategies which the animals may have learned, which were not evident during testing. Further, had MK and MA been able to perform the task with the RO target stimulus, data from those two animals would have been useful to support some of the comparisons for later performance effects, and further comparisons with the audio-visual data would have been possible. The data which was collected was insufficient to perform more in depth analysis to probe the contribution of the different stimulus directions to the results seen. Nonetheless, the data which has been presented has revealed some interesting effects.

The use of LI does not seem to be a robust measure when applied to fMRI data, which we especially see in the animals. In human literature, LI can vary widely between subjects (Wilke and Lidzba 2007) which can be counteracted by recruiting more participants. However, as mentioned earlier, research involving non-human primates is far more restrictive in terms of the number of animals which can be used for a study, and additional statistical power is often achieved by scanning the same animal more often, which in itself is a limitation due to the fact that the animals can overlearn the task. A large amount of variability was present in the animal data and this was indeed difficult to address. The data presented here analysed with the maxZ method in fact rarely breached what in most of the literature is regarded as the threshold for lateralisation: ±0.2. However, when analysing the data in terms of significance of the difference between LI for one group comparison or another, or analysing the significance of the result as compared to zero, interesting results appear. This may suggest that an arbitrary cut-off is not sensitive enough to detect more subtle effects, especially in smaller populations, but comparison of the strength of lateralisation seen between two conditions, or computing the significance of the LI values compared to zero may be a viable option, and may give a better indication of effects.

A drawback of the maxZ analysis is the fact that the data analysis is based on the z-value of the most active voxel in a cluster of activity in the region of interest (ROI). This measure was used as it is not susceptible to outliers in the same way that the mean z-score of the voxels in that region may be. However, this may not be a good measure of the activity of the region since this one voxel may itself be an outlier. Additionally, the fact that the ROIs spanned up to 4 auditory fields means that the maximum z-value recorded for the two hemispheres from the same comparison could have originated in a different field in the left hemisphere than in the right. This may account for the levels of variability seen in the same animals’ data across scanning runs, and for the fact that the LI data does not always reflect the surface projected data.

Use of the LI toolbox may be a more robust and widely used method, but it also has advantages and disadvantages. The toolbox uses the t-stat measure of activity based on the whole of the masked area. It can therefore account for differences in the data across several regions, and is not susceptible to biases caused by larger activity of one or two voxels. However, the toolbox is designed for use with larger regions of functional data than were being investigated here, and in some cases the whole of the hemisphere of a human brain can be used. Macaque brains are considerably smaller, and looking at the specific auditory regions meant that in some cases there was an insufficient number of active voxels to generate a LI value. Because of this, only the overall group analysis of the fMRI data produced meaningful results, meaning that variability in signal between individual scanning sessions could not be addressed. This was, however, possible with the maxZ analysis, and in some cases revealed a large amount of variability between sessions.

It is encouraging that for the performance related comparisons, the two types of data analysis corroborated, however it is of concern that for the sound versus silence analyses this was not always the case. This may be a result of the small amount of data available in the naïve comparison, and it is possible that the addition of more data would improve the agreement between the two methods. There was considerable agreement between the two methods in regard to the audio-visual data, which is the comparison which had the largest amount of data included. However, it is difficult to speculate on which method of data analysis produced more reliable results. Further methods of data analysis are now being explored, including one method where the average z-score of the 10 most active voxels is taken for each session.

### ***Conclusion***

Behavioural data provided here robustly shows a performance bias in task performance with communication sounds. However, fMRI data is more variable. The effects which are seen do seem to suggest that the aspect of spatial processing investigated here in the macaque AC is more bilaterally distributed, if not more left lateralised, than in humans, at least under attentive conditions. The results presented may therefore fall in line with data in humans which suggests that the functional overcrowding of one hemisphere lead to the lateralisation of functions competing for cortical representation. It may be evident that the segregation of language and spatial processing to opposite hemispheres provided an evolutionary advantage to our cognitive processing, especially as linguistic abilities became more prevalent in our species. This notion is supported by findings in humans that the left hemisphere is capable of spatial processing to a lesser extent than the right in left language dominant individuals (Suchan and Karnath 2011), however, the debate surrounding the idea that humans with greater degrees of lateralised segregation of the two functions perform better on cognitive tasks (Powell et al. 2012) may not be seen in macaques, however, in this experiment there was no communication based task. Separation of the spatial and communicative abilities in AC may however, have proven an evolutionary advantage which prevents the two aspects from competing when they are delivered in the same sound.

Taken together, the behavioural and functional results presented here may support the idea that smaller degrees of lateralisation is beneficial to performance on certain tasks, as we see that strong lateralisation is detrimental to performance. The results may also suggest that levels of lateralised activity in response to auditory stimuli can be controlled by attentional processes, preventing hindrance to task performance. This has implications for the study of lateralisation in human and non-human primates, and indicates that attention may need to be controlled during exposure, otherwise it is not possible to identify what aspects of the stimuli the animals may or may not be attending to. It is not possible to say what the naïve animal was attending to, which may be why results for this comparison are so variable, however, when it is more likely that the animals are not attending to the sounds, as in the AVv experiment, strong left lateralised activity is seen.

Small sample size during this study was an issue, however, as previously mentioned, this is a common theme in research with non-human primates. Although the two trained animals did not always show similar results, there are similarities in some comparisons which could be boosted by the addition of further animals. A third animal may have been useful had the results shown agreement with data from one of the animals included here, as, while MK and MA performed a similar version of the task, they never encountered the sCoo sound, which may have altered the way in which MT and ME appreciated the task.

Although less data was available for the animals scanned with the NHIS, this was not due to the method in itself. Development of a new method has the potential to cause issues and some data was lost from the project because of these issues. However, once this was resolved, good results were obtained, although time constraints prevented collection of further datasets. The use of the NHIS during this experiment shows that fMRI experiments can be conducted without the need for invasive implanted head immobilisation options, and where access to the brain is unnecessary, data can be collected non-invasively.

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# Appendix: Other work published or presented by the author

## 1. Journal articles

Rinne T., Muers R.S.*, Salo E.*, **Slater H.*** & Petkov C. I. (2017). Functional imaging of audio-visual selective attention in monkeys and humans: How do lapses in monkey performance affect cross-species correspondences? *Cerebral Cortex*. 27(6) 3471-3484.

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**Slater H.**, Milne A. E., Wilson B., Muers R.S., Balezeau F., Hunter D., Thiele A., Griffiths T., & Petkov C.I. (2016). Individually Customisable Non-Invasive Head Immobilisation System for Non-Human Primates with an Option for Voluntary Engagement. *Journal of Neuroscience Methods.* 269 46-60.

Wilson B., **Slater H**., Kikuchi Y., Milne A. E., Marslen-Wilson W. D., Smith K. & Petkov C. I. (2013). Auditory artificial grammar learning in macaque and marmoset monkeys. *Journal of Neuroscience*, 33 (48), 18825-18835.

## 2. Publications in Preparation

**Heather Slater**, Fabien Balezeau, Ross S. Muers, Alex Theile, Tim Griffiths and Christopher I. Petkov. Assessing the Quality of fMRI Data Obtained During Non-Invasive Head Immobilisation with Task Behaving Macaque Monkeys.

**Heather Slater**, Ross S. Muers, Emma Salo, Teemu Rinne and Christopher I. Petkov. Behavioural and Functional Asymmetries during an Auditory Attention ask in Rhesus Macaques.

## 3. Oral Presentations

**Heather Slater**, Fabien Balezeau, Ross S. Muers, Alexander Thiele, Timothy Griffiths and Christopher I. Petkov. Assessing the Quality of Non-Invasive fMRI with Non-Human Primates. NC3Rs Primate Welfare Conference, London 2017.

## 4. Conference Poster Presentations

**Heather Slater,** Ross S. Muers, Emma Salo, Teemu Rinne & Christopher Petkov. *Influence of Attention on Functional Lateralisation in Primate Auditory Cortex*. Presented at The British Psychological Society Cognitive Section Conference, Newcastle 2017.

**Heather Slater**, Ross S. Muers, Emma Salo, Teemu Rinne & Christopher I. Petkov. *Evolutionary Roots of Brain Lateralisation in the Primate Brain*. Presented at North Sea Laterality Conference, Groningen 2016.

**Heather Slater***, Emma Salo*, Ross Muers*, Teemu Rinne & Christopher I. Petkov. *Task-dependent modulations of the fMRI BOLD response in monkey auditory cortex*. Presented at Auditory Cortex Conference, Magdeburg 2014. ***authors contributed equally.***

**Heather Slater**, Olivier Joly, Ross S. Muers, Alex Theile, Tim Griffiths and Christopher I. Petkov. *Individually Customisable, Non-Invasive Head Immobilisation for Primates with the Option for Voluntary Engagement*. Presented at NC3Rs Primate Welfare Meeting, London 2013.

**Heather Slater**, Alice Milne, and Christopher I. Petkov, *Approaches to improve animal welfare in the laboratory.* Presented at Leipzig Newcastle Bi-national PhD Conference ("From signal processing to behaviour"). 2011. Presented at Max-Planck Institute for Cognitive and Brain Sciences, Leipzig, Germany.