2011

FUNCTIONAL VALUE AND SPATIAL ATTENTION SIGNALS OCCUPY TOPOGRAPHICAL HUBS IN THE MACAQUE FRONTO-CINGULATE CORTEX

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(Spine Title: Value and Spatial Attention in Macaque Fronto-Cingulate Cortex)

(Thesis format: Monograph)

by

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Graduate Program in Neuroscience

A thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Science

School of Graduate and Postdoctoral Studies

The University of Western Ontario,

London, Ontario, Canada

THE UNIVERISTY OF WESTERN ONTARIO SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

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Entitled:

Functional value and spatial attention signals occupy topographical hubs in the macaque fronto-cingulate cortex

Is accepted in partial fulfillment of the requirements for the degree of Master of Science

Date June 13th 2011

Chair of the Thesis Examination Board

Abstract

For behaviourally relevant choices to be made a variety of bottom-up sensory cues from the environment and top-down signals from the brain must integrate in a context dependent manner in order to optimize behaviour. Of these signals the location and value of potential stimuli are of critical importance. We tested the hypothesis that the neural correlates of these signals are present and integrate in cortical hubs across the fronto-cingulate axis. Furthermore we postulated that failure of this recruitment of information would result in suboptimal performance (errors). We recorded from 811 single cells across the fronto-cingulate cortex in areas, 24a/b/c, 32, 10, 8, 8b, 9 and 46 from two male rhesus macaques. Using a variant of a well-established paradigm eliciting specific rule guided behaviour we were able to independently analyze and map the neural correlates of spatial attention and reward value. We discovered four functional clusters that convey attentional rule signals in the lateral prefrontal cortex (lPFC) and anterior cingulate cortex (ACC). We also found that clusters of neurons coding value intersected with spatial attention signals in the ACC when value was high and in the lPFC when value of the cued target was low. Absence of neural activity in these clusters during periods of shifting attention was associated with errors. Therefore we concluded that reward value-expectancy and spatial attention selection signals, which are part of a larger cognitive control network, exist and are integrated in confined functional hubs of the fronto-cingulate cortex.

Key Words: Attention, reward, value, macaque, prefrontal cortex, anterior cingulate cortex, single cell recording, brain mapping.

Acknowledgements:

I would like to extend thanks to all the members of the Everling laboratory for their support and friendship throughout this project. To my supervisor, Dr. Thilo Womelsdorf, for his inspiration, guidance, and wealth of knowledge with respect to all aspects of this project from training, to data collection, analysis techniques, and writing of the final document. Your guidance, encouragement and confidence in me were truly what made this project the success that it was for me. Also to my supervisor, Dr. Stefan Everling, for his expertise in the surgical techniques and data recording along with his patience, understanding and continued support throughout the entirety of my time in his lab. To my Advisory Committee members, Dr. Brian Corneil and Dr. Ravi Menon for their continued insight and valuable feedback. A special thanks to my close colleague Michelle Bale for the long hours spent collecting data together and for the time she put in training the animals. To Brian Soper, Johanna Stuke and Darren Pitre for caring for the animals and ensuring their continued well-being.

Lastly this project would not have been a success without the continued support of my friends and family. To Natalie Roberts for her hours assisting with revisions of the final document and to my parents and sister for their love and continued encouragement throughout all the sages of my academic career. Without your support I would not be where I am today.

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List of Abbreviations

2D – two dimensional

ACC – anterior cingulate cortex

ACS – anterior cingulate sulcus

ANOVA – analysis of variance

ARC – arcuate sulcus

Contra – contralateral

CRT – cathode ray tube

CS- cingulate sulcus

dlPFC – dorso-lateral prefrontal cortex

lPFC – lateral prefrontal cortex

FEF – frontal eye field

Ipsi – ipsilateral

MRI – magnetic resonance imaging

MR – magnetic resonance

n – number (sample size)

NA – nucleus accumbens

OFC – orbital frontal cortex

PFC – prefrontal cortex

PS – principal sulcus

ROC – receiver operating characteristics

SN – substantia nigra

VS – ventral striatum

VTA – ventral tegmental area

List of Symbols

± – plus or minus

≥ – greater than or equal too

≤ – less than or equal too

α – learning rate

δ – prediction error

μm – micrometer(s)

cm – centimeter(s)

h – hours

Hz – hertz (cycles/second)

kg – kilogram(s)

MΩ – megaohm(s)

mm – millimeter(s)

mg – milligram(s)

ms – millisecond(s)

min – minutes

s – seconds

T – tesla

V_t – level of expectation of reward on the current trial

$V_t + 1$ – level of expectation of reward on the next trial

"Religion and science are the two wings upon which man's intelligence can soar into the heights, with which the human soul can progress. It is not possible to fly with one wing alone! Should a man try to fly with the wing of religion alone he would quickly fall into the quagmire of superstition, whilst on the other hand, with the wing of science alone he would also make no progress, but fall into the despairing slough of materialism."

Abdu'l-Baha, *Paris Talks,* p. 143

Dedication,

For my grandparents, Karamat-ullah and Homayoun Ghodrati, Saadat-ullah and Ruhiyyih Janemi. Although we have always been oceans apart your strength, wisdom and courage has always been a source of encouragement and inspiration for me. May your legacy live forever in these pages.

1.1 Introduction

For behaviourally optimal choices to be made one must be able to internally process a variety of incoming information from one's environment. From the sensory level through to higher order cognitive processing such as referencing previous experiences and response inhibition the brain assesses a multitude of variables resulting in case specific actions (Corbetta and Shulman, 2002). Evaluating one variable without the others could cause suboptimal decision-making and result in unfavourable behaviour in many cases. Two key higher order variables contributing towards decision-making are spatial attention, and value or reward processing, however these processes have chiefly been discussed as mutually exclusive neural mechanisms (Corbetta and Shulman, 2002; Corbetta et al. 2008; Haber and Knuston, 2010; Rushworth and Behrens, 2008).

Attention is the cognitive process of selectively concentrating on a particular aspect of the environment while ignoring other things (Anderson 2004; James 1890). Examples of attention include focusing on studying while in a noisy environment or listening to what someone is saying while ignoring other conversations in a room (the cocktail party effect). In 1890, William James, in his textbook Principles of Psychology remarked that attention, "... is the taking possession by the mind, in clear and vivid form, of one out of what seem several simultaneously possible objects or trains of thought. Focalization and concentration, of consciousness are of its essence. It implies withdrawal from some things in order to deal effectively with others, and is a condition which has a real opposite in the confused, dazed, scatterbrained state..."

Shifts in spatial attention occur when attention is directed to a certain area of space from one location to another. Shifts in spatial attention are made depending on the context of the situation the subject is in, and it is the context that determines the inherent value of the stimulus that the attention shift is being made towards (Gold and Shadlen, 2007). For example, the sound of a police siren coming from the radio of your car when you are driving may have a greater behavioural or autonomic effect than when you are sitting at home because in the context of driving the siren could have several implications that do not exist when you are at home. To visualize this concept more clearly one can picture a setting where you are driving down a busy street. In this scenario a multitude of variables must be considered to optimize behaviour, which in this case would be driving safely from the location of origin to the destination. Environmental cues direct spatial attention to the road and a successful voyage will ensue if these cues can be prioritized based on value. An accident may occur if attention is directed to stimuli that are suboptimal in this context. For example, if spatial attention was shifted from the conditions of the road to the ringing of the driver's mobile phone this could result in an accident (Strayer, 2003). Similarly, other stimuli that have inherent value in other contexts would be ranked as having lower value and would thus have less of an influence on where spatial attention is directed. A coin on the road or a physically attractive person would not demand an attention switch, as they are not highly valued in this scenario. These real world examples support the foundation on which we build the practical and scientific basis for this study outlined in the hypothesis.

1.2 Purpose and Hypothesis

The basis for this study was seeded in the notion that an overview of the literature with respect to reward and attention processing in the brain revealed that these two concepts (attention and reward) have typically been referred to as distinct and fundamentally different processes (Haber and Knuston, 2010; Corbetta and Shulman, 2002). However, a closer and perhaps more functional versus anatomical look at these concepts would show that although these two networks may be different in their anatomical architecture they must be fundamentally linked for behaviour to be favourable (Kable and Glimcher, 2009). Furthermore we were interested in the distinct functional roles of the cells in various fronto-cingulate areas within the framework of a frontal attention-reward network. Various authors have suggested overlap of the anatomical architecture of these networks in the ACC and PFC of primates (Haber and Knuston, 2010; Medalla and Barbas, 2009; Corbetta et al., 2008; Miller and Cohen, 2001; Kable and Glimcher, 2009) but there have been no direct attempts at producing a functional map at the single-cell level of the attention and reward signatures in the frontal cortex.

As the previous examples have shown, relevant decision-making dictating behaviour (e.g. attend to the road not my mobile phone) relies on the incorporation of value discrimination and the allotment of spatial attention towards environmental cues. This information is derived from environmental sources in two ways (Corbetta et al., 2002). The first is in a bottom-up manner; information processing that depends on the brain's interpretation of the environment directly from sensory input through to perceptual analysis without involving feedback

information from other cortical areas. The second way is by being internally generated through top-down processing which involves referencing of previous experiences, existing knowledge, expectations, and motivations regarding the specific context of the situation. These factors all work together to determine how scenarios are interpreted and dealt with (Corbetta and Shulman, 2002).

The utilization of associative rules relies on top-down integration of attention and reward information. This has led us to *hypothesize* that when attention shifts are internally generated towards a relevant target, functionally distinct nodes in the anterior cingulate cortex (ACC) and the prefrontal cortex (PFC) will convey and integrate information about 1) target stimulus location and 2) target stimulus value (reward). Furthermore, a failure of these processes on a cellular level will result in unwanted behaviour (errors).

The following will be a brief review of what is known about the attention and reward networks in the brain and how they structurally overlap via the ACC and the PFC. It also will provide evidence that these areas are uniquely poised to integrate functional information about the location and value of targets during periods of shifting attention.

1.3 Cortical Attention Networks

Attention in the brain has been conceptualized to be composed of three fundamental concepts that are, orientation, target detection and the maintenance of an alert state (Posner and Peterson, 1990). In orientation, the visual system orients attention to different areas of the visual field until a target event has been detected. Upon detection, the system must then prepare and sustain alertness to

process high priority signals from the environment (Posner and Peterson, 1990). These key concepts have lead to three main findings which are, 1) the attention system is anatomically separate from other neural processes such that attention can interact with other brain regions but maintains its own independence, 2) attention is carried out by a network of anatomical areas, does not have a single centre and is not a general function of the whole brain, 3) these areas involved in attention each have different functions which can be specified in cognitive terms (Posner and Peterson, 1990). With respect to attention this study is focused on its orientation in space. The following paragraphs will illustrate the anatomical organization of spatial attention networks in the brain.

Human neuroimaging studies have suggested that spatial attention can be viewed as being composed of two distinct networks, a dorsal goal-directed or 'maintenance' attention network and a ventral attentional orienting or 'shifting' network (Corbetta et al., 2008). These studies have shown that during sustained attention, for example when a subject is reading a book or writing an essay, brain regions that comprise the dorsal network are activated. These areas include the intra-parietal sulcus, superior parietal lobe, frontal eye fields (FEF) and visual regions of the occipital cortex (Corbetta et al., 2000, Corbetta et al., 2002). These areas show sustained activation during the attention period to the contralateral side of the visual field (Corbetta et al., 2002). Another hallmark of this network in addition to these activated regions is sustained deactivation of more ventral regions including the supramarginal gyrus and superior temporal gyrus as well as middle and inferior PFC (Corbetta et al., 2002). It is the transient activation of these ventral regions in addition to continued activity in the dorsal regions that

constitutes the organization of the ventral attentional orienting network (Corbetta et al., 2000). Also known as the attentional saliency network, the ventral attention regions are chiefly right hemisphere lateralized and become transiently active when there is a new salient target in the environment that trigger an attentional shift (Corbetta et al., 2000). Highlighted by human imaging, these two anatomical systems demonstrate the broadness of the attentional orienting circuitry within various brain regions and are fundamental to the understanding of attention processing in the brain.

Historically, studies indicated that some stimuli by factor of their physical attributes are more salient than others and demand switches in attention more so than background stimuli. For example, things that are moving, bright, metallic or bloody tend to cause an automatic shift in human attention (James, 1890). Behaviourally this applies, as in reality there are things that draw our attention irrespective of context (such as an unexpected alarm). Conversely, more recent evidence concludes that bottom-up sensory information is evaluated by top-down case specific encoding of the relevance of a stimulus (Corbetta et al., 2008). For example, typically one may not notice that one's spouse is wearing the colour red. However, if it were Valentine's day the context of wearing red causes a bottom-up shift in visuo-spatial attention to the red item based on a top-down referencing of the colour red and its association with Valentine's day. Both bottom-up and top-down attentional processing in the brain are of critical importance in order to make accurate switches in attention depending on the circumstances of the environment.

In summary to the introduction of the attention networks of the brain, it has been discussed that attention is composed of three key concepts, orienting, detecting and maintaining an alert state (Posner and Peterson, 1990). Additionally the spatial attention systems of the brain have been shown to be composed of a ventral "shifting" and dorsal "maintenance" attention networks (Corbetta et al., 2000) that are reliant on information from bottom-up and top-down processing (Corbetta et al., 2008). The areas of the frontal cortex that have been shown to sub serve attention processing in both humans and monkeys are the ACC areas 24 and 32 (Posner et al., 1988; Posner and Peterson, 1990; Rushworth and Behrens, 2008) and the PFC areas 46, 9 and 8 (Lebedev et al., 2004; Everling et al., 2002). As such, this study will investigate the roles of the ACC and PFC in covert shifts of spatial attention. The following two sections will discuss the function of each of these areas with respect to spatial attention processing in humans and primates and what is known of the neural correlates of attentional control in the primate brain.

1.4 Spatial Attention in the Anterior Cingulate Cortex

Within the framework of this project the ACC can be defined as those cortical regions on the medial wall of the hemisphere (area 24 and 32), including the cingulate sulcus, anterior to the arcuate sulcus (ARC) ventrally to where area 32 meets area 10, bordered inferiorly by areas 14 and 25 and superiorly by area 9. Figure 1 highlights this area according to the anatomical organization of Barbas and Zikopolous (2007).

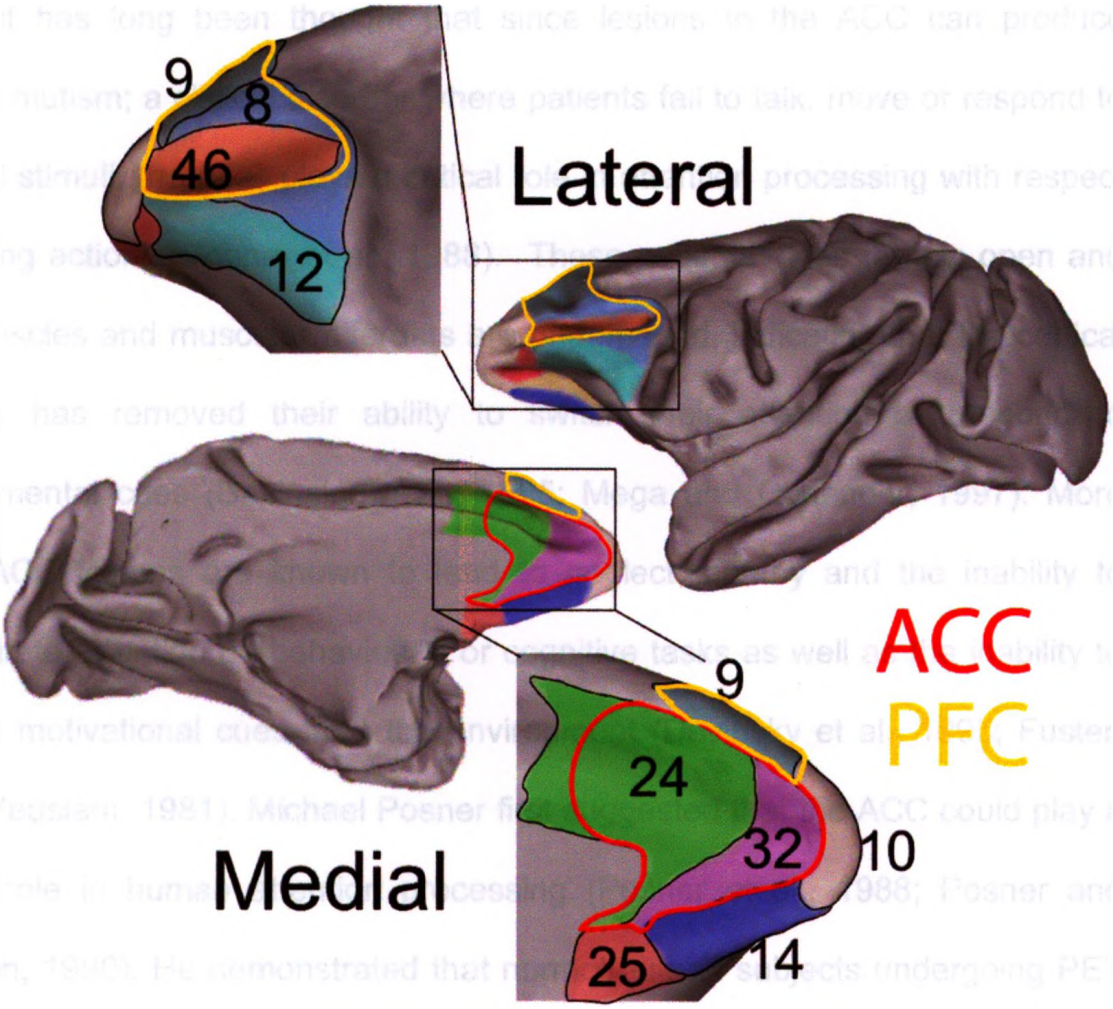

Figure 1. Anatomical organization of ACC and PFC as defined by this project. Yellow and red contours out line PFC and ACC respectively and the numbers indicate area subdivisions according to Barbas and Zikopolous (2007).

It has long been thought that since lesions in the ACC can produce akinetic mutism; a clinical disorder where patients fail to talk, move or respond to external stimuli, the ACC plays a critical role in attention processing with respect to making actions (Posner et al., 1988). These patients' eyes remain open and their muscles and muscular afferents are undamaged, indicating that the cortical damage has removed their ability to switch their attention to respond to environmental cues (Devinsky et al., 1995; Mega and Cohenour, 1997). More minor ACC lesions are known to lead to neglect, apathy and the inability to concentrate attention on behavioural or cognitive tasks as well as the inability to process motivational cues from the environment (Devinsky et al., 1995; Fuster, 2001; Meuslam, 1981). Michael Posner first suggested that the ACC could play a central role in human attention processing (Posner et al., 1988; Posner and Peterson, 1990). He demonstrated that normal human subjects undergoing PET and fMRI show marked activation of the ACC in tasks that demand sustained effort and concentrated attention (Posner et al., 1988). In view of these findings from the past two decades demonstrating functional correlates of attention in the ACC, primate single cell electrophysiology has yet to show spatial attention signals in the ACC.

When referring to ACC function a variety of roles including reinforcement-guided learning (Rushworth and Behrens, 2008), error detection and performance monitoring (Carter et al., 1998), reward processing (Kennerley and Wallis, 2009) and conflict monitoring (Botvinick et al., 2004) are considered, but spatial attention processing is not one of them. In fact, in a 2009 study Kennerley and Wallis, recording in the dorsal bank of the anterior cingulate sulcus (24c:

Petrides and Pandya, 2007; Saleem et al., 2008), indicated that they "found little evidence of spatial attention being encoded by cells of the ACC" in macaques (Kennerley and Wallis, 2009). As such the findings between human neuroimaging and monkey electrophysiology in this regard thus far has not been comparable. As indicated by Posner (1988) imaging experiments in normal human subjects have shown that there is strong activation of ACC regions during cognitively demanding tasks. Furthermore, more recent higher resolution fMRI studies have illustrated that microdomains of the ACC are involved in various functions including orientation towards attended stimuli (Wang et al., 2005). As our hypothesis indicates, it is the hope of this study to highlight spatial attention signals in cells of the macaque ACC. This finding would bridge the gap between human imaging studies and primate electrophysiology clarifying the exact contribution of the ACC in spatial attention processing.

It is known that the ACC has anatomical connections to areas of the brain implicated in attention processing in both humans and non-human primates (Corbetta et al. 2002). Additionally it has been suggested that the ACC plays a gating role between the ventral shift network and the dorsal maintenance network when attention needs to be shifted (Corbetta et al., 2008). By maintaining the inhibition of the ventral network when attention is focused the ACC can segregate the dorsal and ventral network activity and allow for transient shifts to occur in response to the environmental conditions (Corbetta et al., 2008). Also the ACC is thought to monitor conflict (Botvinick et al., 2004) and is connected to the limbic and reward circuitry (Haber and Kunston, 2010). Therefore it would be an ideal candidate to communicate conflicts between what attention is currently being

devoted and new stimuli that have entered the environment (Corbetta et al., 2008). Indeed this pattern of ACC activity would require cells that are sensitive to spatial locations and posits that further research should be done to show the existence of spatially selective signals in the ACC.

Additional support for the ACC's role in spatial attention comes from human patients with lesions of the ACC (area 24). These patients commonly exhibit some degree of spatial neglect and difficulties directing attention to discrete locations in visual space (Fuster, 2001). They have trouble attending to novel stimuli in expected locations or shifting their attention from one place to the other (Meuslam, 1981; Fuster 2001). These findings in combination with imaging work done on normal humans provide the framework for extrapolating these signals onto other primates. Anatomical models comparing human and macaque brains have suggested homology of the ACC in the two species (Wallis and Kennerley, 2010; Petrides and Pandya, 1999; Mansouri et al., 2009). It is on these grounds that we put forth our hypothesis that spatial attention signals will be present in the ACC of rhesus macaques.

1.5 Spatial Attention in the Prefrontal Cortex

Contrary to the uncertainty of homology for spatial attention signals in the ACC between humans and macaques, many studies from both human imaging (Corbetta et al., 2000) and primate electrophysiology (Asaad et al., 1998; Lebedev et al, 2004; Everling et al., 2002) have demonstrated spatial attention signals in the PFC of humans and macaques.

We define the macaque lPFC as those cortical areas according to the anatomical distinctions of Barbas and Zikopoulus (2007), anterior to the ARC, to the margin of area 10 and from the principal sulcus (PS) medially to the cingulate sulcus. This includes areas 9, 46, and 8 staying anterior enough to avoid any influence from the FEF or other motor areas (figure 1). The various anatomical associations of these and other regions of the PFC were illustrated in a computational study by Averbeck and Seo (2008). The authors drew on the multitude of anatomical tracer studies in macaques and applied statistical techniques to illustrate that the various PFC areas, although they have very dense interconnectivity, seem to be organized in a very structured manner with respect to their inputs. The authors demonstrate that the statistical weight of the input systems to each region was clustered into functional and anatomical regions. Areas 9, 46 and 8, from Barbas and Zikopolous (2007) were shown to receive sensory information from the temporal and parietal cortex. The authors conclude that this pattern of connectivity sub-serves the PFC's essential role in decision-making and that the anatomical inputs likely drive the function in these regions (Averbeck and Seo, 2008). The sensory input and interconnectivity to these regions in the PFC shown by Averbeck and Seo (2008) in combination with various executive functions in the PFC (Mansouri et al., 2009; Petrides, 2005) underline the importance of this region and its potential as a key player in attention processing.

With respect to executive control in the brain the PFC is known to play a critical role. It harbours a variety of cellular signals that correlate with functions including working memory (Petrides, 2005; Fuster, 2001; Mansouri et al., 2009),

object and location related activity (Asaad et al., 1998; Lebedev et al., 2004), the processing of reward stimulus associations (Watanabe, 1996; Leon and Shadlen, 1999) and in the formation of rules (Asaad et al., 1998). These signals have been implicated as neural correlates of learning and are attributed to the PFC (Asaad et al., 1998).

Spatial attention has been isolated as one of the key functions of the PFC by human imaging. Studies have shown distinctive patterns of activity in the PFC for subjects attending to various locations in the visual field (Corbetta et al., 2000). A study by Corbetta et al. (2000) showed the presence of a distinct network for sustained attention called the dorsal-stream network. In their fMRI study human subjects were shown an arrow directing them to shift their attention covertly (without moving their eyes) to a certain side of the view screen (Corbetta et al., 2000). Authors in the study were able to illustrate different patterns of activation for attention to various locations in space. The PFC was noted to show a distinct pattern of activity during reorientation of attention to targets in different areas of space (Corbetta et al., 2000).

As human imaging has implicated the PFC in attention processing, primate electrophysiology has also demonstrated various correlates of spatial attention in cells of the PFC. Asaad and colleagues (1998) provided evidence that neurons in the PFC were location specific in awake and behaving macaques. The authors showed that during a delayed conditional visuomotor task where monkeys had to match their response to the provided cue, cells in the PFC had a variety of responses including in some cases greater activity for location versus objects. The authors also demonstrated that these cells code for the rule of the stimulus-

reward association and implicated the cells in the PFC as the neural correlates of associative learning. Additionally the latency of responses in these cells decreased as the animal became more familiar with the task after several trials which showed that these cells were 'learning' the task and responding accordingly (Asaad et al., 1998). This study showed that the cells in prefrontal cortex could code spatial locations as well as associate those locations with rules dictating a behavioural response.

As Asaad and colleagues (1998) demonstrated the role of the PFC in identifying spatial locations and associative-rule making, subsequently, in 2004 Lebedev and colleagues were able to dissociate the roles of spatial attention and working memory. They demonstrated clear spatial attention signals in cells of the PFC independent of working memory. The authors trained macaques to fixate on a central fixation point then presented them with a stimulus they had to remember to the left or right of their fixation point. Next, the remembered stimulus disappeared (they had to maintain its location in working memory) and they were presented with a stimulus they had to covertly attend to above or below their fixation point. This stimulus would then change in brightness becoming either brighter or dimmer which would differentially cue the animal to respond by making a saccade to the remembered stimulus or to the attended stimulus. The authors recorded from cells in the dorso-lateral PFC (dlPFC) (areas 46, 8) and demonstrated that the majority of cells (61%) in this area are more responsive to attended locations than they are for remembered locations (16%) (Lebedev et al., 2004). They also reported some hybrid cells (23%) that responded to both remembered location and attended location. However, even among these hybrid

cells the authors found that 73% of them responded more to the attended than the remembered location where the latter 27% responded equally to both conditions. Thus it was concluded that the majority of the cells recorded in this area of the PFC were modulated by spatial attention rather than spatial working memory.

Lebedev and colleagues (2004) demonstrated the presence of attended target location signals in the PFC, however, of equal importance is the filtering of unattended signals. In 2002, Everling and colleagues highlight the importance of the PFC in filtering of distractor stimuli. Everling et al. (2002) provide and example where in the visual system a sense of blindness can be associated with unattended stimuli. Although light is entering the eye from these stimuli if they are present in the visual field, causing the activation of retinal ganglion afferents to the brain, filtering of these stimuli must occur in order to stop a neural overload of sensory information. Only those stimuli relevant to the current context of the environmental conditions should be processed and as such filtering must occur (Everling et al., 2002). The authors demonstrated that different cells in the PFC were highly tuned to both cue and non-cue targets in a spatially specific manner. In a task where monkeys had to covertly attend to a preferred or non-preferred location while being shown a series of distractor stimuli while awaiting a cue, some neurons of the PFC only fired higher when the target was presented. This implicates that the PFC can filter out non relevant stimuli in signaling the presence of target cues. Furthermore, the authors demonstrated that this activity was spatially tuned with the activity being higher in the preferred location than in the non-preferred location for both cued and distractor targets (Everling et al.,

2002). This suggests that the PFC has the ability to distinguish relevance in a context dependent manner and highlights its important role in attentional processing and cognitive control (Everling et al., 2002).

The PFC, with its various functionalities is also involved in processing spatial attention. Lesion studies in the lateral PFC corresponding to areas 9, 8, 46, 12 (Barbas and Zikopolous, 2007) of macaques have shown impaired spatial allocation of attention to cued locations particularly when the cued location is frequently changing over trials (Rossi et al., 2007; Rossi et al., 2009). As such the PFC has been suggested to be critical for flexibly switching attention between various stimuli depending on the context of the situation and the relevance of each stimulus (Rossi et al., 2007; Rossi et al., 2009). Furthermore these impairments in attention switching are thought to be long term, as other regions of the brain associated with attention cannot compensate for the loss of PFC function over time. This pattern of activity is compatible with large lesions in the human PFC that cause subjects to remain fixated on a stimulus or response for prolonged periods of time (Rossi et al., 2007). Additionally, Petrides (2005) reviews the effects of lesions to various areas of the PFC in macaques and humans. Area 8 is identified as being important in selecting between various visual stimuli, where areas 9 and 46 are implicated in monitoring the conditions of tasks and working memory (Petrides, 2005). Lesions isolated to area 46 have also been shown to uniquely result in the inability to support working memory for the application and maintenance of abstract rules in a Wisconsin card sorting task analogue (Buckley et al., 2009).

The various studies combining anatomical organization, electrophysiological functions in macaques, imagining in humans, and lesion studies have all demonstrated the important role of the PFC in spatial attention processing. It is the combination of these functional evidences in addition to the massive anatomical connectivity to the PFC that leads us to hypothesize that it plays a fundamental role in the attention and reward circuitry of the frontal cortex.

1.6 Cortical Reward Networks

Reward expectation and valuation are often the driving force behind the previously discussed top-down shifts in spatial attention (Kable and Glimcher, 2009). Reward expectation in the context of this study can be referred to as the value (high or low) of those stimuli that optimize behaviour. The physical reward, that is, the magnitude of the expected outcome, can vary between high and low and is determined by the identity of the attentional target. Similarly, expected value that can lead to the avoidance of suboptimal stimuli can be considered rewarding (Watanabe et al., 2006). In 1954 Olds and Milner conducted some of the earliest demonstrations of reward processing in the brain and its influence on behaviour. The authors demonstrated that rats would work for micro-stimulation of specific sites in their brain (Olds and Milner, 1954). The beginning of the 21st century saw a surge of data suggesting that the nucleus accumbens (NA) and ventral tegmental area (VTA) were the center of the reward circuitry via their dopaminergic neurons (Hikosaka et al., 2008). However, more recent studies have demonstrated that the striatal and midbrain components of reward processing are more extensive than previously thought including the entire

ventral striatum (VS) and dopamine neurons from the substantia nigra (SN) (Haber and Knuston, 2010). Additionally, it was described that the VS receives its main cortical inputs from the orbital frontal cortex (OFC), and ACC. The VS also projects axonal connections to the ventral pallidum and VTA/SN that in turn relay through the medial dorsal nucleus of the thalamus and then project to the lateral PFC (lPFC) (Haber and Knuston, 2010). This cortico-basal ganglia loop and a variety of areas including the hippocampus, amygdala, and specific brain stem structures are the areas that influence and regulate the reward circuitry (Haber and Knuston, 2010). The complex and widespread anatomical organization of the reward system points to the important role that valuation of stimuli must have on behavioural outcomes. Incentive based learning, goal directed behaviour, and appropriate responses to stimuli all depend on the ability to evaluate factors from the environment using both top-down and bottom-up processing (Haber and Knuston 2010). The main areas of the neocortex associated with reward processing are the ACC areas 24, 25 and 32, the OFC areas 11, 12, 13 and 14 (Barbas, 1992; Carmichael and Price, 1994; Fuster, 2001) as well as the PFC areas 46 and 9 (Watanabe, 1996; Wallis and Kennerley, 2010; Leon and Shadlen, 1999). Although complete homology is not conserved between macaque monkeys and humans there is general agreement on how these areas map across the cortex of both species (Mansouri et al., 2009; Petrides and Pandya, 1999).

The following two sections will focus on the roles of the ACC and the PFC and discuss their contribution to value processing in macaques and humans.

1.7 Reward in the Anterior Cingulate Cortex

The ACC (areas 24 and 32, figure 1.) is implicated as one of the main regions that play an important role in conflict monitoring (Botvinick et al., 2004; Carter et al., 1998) and stimulus valuation (Kennerley et al., 2009). Behaviourally these two functions would be active together when comparing similarly valued stimuli in order to distinguish which one is optimal. This allows variables such as cost vs. preference to be resolved through ACC function (Vogt et al., 2005; Paus, 2001). From the perspective of reinforcement-guided decision-making, which suggests that reinforcement from positive reward outcomes will influence future decisions, the ACC plays a key role. A model of this theory by Rushworth and Behrens (2008) suggests that optimal decisions will be made based on factors coded for in the ACC. These factors are: 1. reward expectation (V_t): what reward does the subject expect to receive on the current trial?; 2. prediction errors (δ): errors in outcome predictions versus experienced outcomes that can be positive or negative; positive having received unexpected reward and negative having not received an expected reward; and 3. the learning rate (α): which can vary depending on how familiar the situation is to the subject. In new environments the learning rate should be higher as the subject is faced with a high degree of variability but in familiar environments the learning rate should be lower as the subject knows what to expect and does not assume a variable environment. The sum of the expected reward and the product of the learning rate and prediction error will determine the expectations for subsequent trials and as such determine behavioural outcomes (Rushworth and Behrens, 2008).

$$V_t + 1 = V_t + \delta\alpha$$

For this theory to be quantified, studies have demonstrated different neuronal correlates of functional reward processing in the ACC. Across most species, the source of reward expectation and prediction error computations are thought to reside in the dopamine system, the axons of which project to the ACC (Rushworth and Behrens, 2008; Haber and Knuston, 2010). Studies in awake and behaving macaques have shown these value related signals in cells of the ACC. Fictive reward processing which is the value of a reward that could have been received, reward value expectancy signals, reward dependent error activity, reinforcement based prediction error signals and variable reward network signatures are examples of the reward related activities found in the ACC and will be described next.

In 2009, Hayden and colleagues illustrated that cells of the ACC respond to both reward value, during a pre-rewarded period, and fictive reward value. They used a behavioural task where the subjects (Rhesus Macaques) fixated on a central fixation point, and were then presented with a circular array of eight visual targets. After a 0.5s delay the subjects were then cued to make a saccade towards any target. Following another 0.5s delay period the value of the targets were revealed; at this time the subjects were made aware of the value of their choices. Seven of the eight targets were valued the same at a middle value and one was varied between high reward and no reward at random, the values were indicated by a colour (Hayden et al., 2009). To illustrate the reward activity the authors showed that cells in the ACC responded to the value of the reward during the post choice reveal period but before the reward was actually received. This

signal is analogous to a reward expectation following the choice of a target. Furthermore, the authors demonstrated that these cells were also sensitive to the amount of reward that would have been received if the subject had chosen the correct target, the fictive reward. By analyzing those trials where the subject chose one of the seven arbitrary targets and were then shown the value of the eighth potentially more rewarding target they demonstrated the ACC cells not only respond to actual reward values in an amplitude dependent manner but also that fictive reward signals were modulated by potential reward value, larger fictive reward eliciting a larger response in the cells (Hayden et al., 2009). Likewise in our experiment we will determine the influence of reward value during the pre-reward period and determine whether cells in the frontal cortex are modulated by value during periods of attentional shifts and reward expectancy before the outcome of the task is known.

The pattern of responses with respect to fictive reward signals is similar to the findings of Amiez et al. (2005) who demonstrated that ACC cells show error related activity incrementally with pre-determined reward value. The authors illustrated that the more weight or expected reward a trial has the more the ACC cells fire upon erroneous behaviour in a graded manner (high vs. low reward lost corresponding to a higher vs. lower response) (Amiez et al., 2005). Unlike the above mentioned study (Hayden et al., 2009), the macaque subjects in this task knew the reward value to come and their cells responded to an explicit loss and not a fictive reward. Additionally, although not discussed openly by the authors, the spike time histograms in their first figure (Amiez et al., 2005), upon visual inspection, suggest reward modulation in the pre-error period for the indicated

cell, a difference apparently present between high reward and low reward/no reward conditions. Although not discussed by Amiez et al. (2005) our results will show that indeed the ACC does harbor value related reward expectancy signals very early upon forced spatial attention switches in a task where reward amounts are predetermined and correct behaviour has no impact on the magnitude of the reward received.

In a more recent study Hayden et al. in 2011 showed that cells in the ACC respond with varying activity patterns during a post-reward period. The researchers demonstrated that some cells in the ACC respond to reward by increasing their spike rate and some by decreasing it. They also showed that the amplitude of this response was dependent on the amount of the reward be it high or low, with high reward having a greater absolute difference in change of cellular activity (spikes per second) than lower rewards. These effects were maintained in their population results irrespective of whether the cells increased or decreased their firing. The authors went on to show that the degree of reward response was also modulated by the ambiguity or "riskyness" of receiving a high vs. low reward in a task that involved some gambling on the part of the subjects (Rhesus Macaques). The authors concluded that this pattern of activity illustrates the integration of the reinforcement-guided learning in the ACC on one hand and its cognitive control functions on the other, as reward information and modulation of risk merge in one area (Hayden et al., 2011).

As mentioned earlier an important facet of reinforcement-guided decision-making is the ability to distinguish between and incorporate reward value, reward probability and reward prediction errors (Rushworth and Behrens, 2008). In 2007

Matsumoto and colleagues demonstrated that cells in the ACC encode positive and negative prediction errors at the time of trial outcome. A tight association between detecting errors in predictions and reinforcement from reward are critical for learning. These cells were shown to have preference for either positive or negative prediction error or were non-differential. Those cells that coded positive prediction error responded when the subject (Rhesus Macaques) was rewarded when they expected no reward. Their activity diminished in a graded manner on subsequent correct responses as the subject learned from reward reinforcement (Matsumoto et al., 2007). These cells did not, however, respond when the subject made an error. In error cases cells favouring negative prediction error were active. The coding of these variables in the brain is important for successful behaviour and cognitive control. Being able to determine, from negative or positive reward feedback, that you have made a mistake or have stumbled upon a new salient stimulus is of utmost importance for learning, direction of attention and optimization of actions.

A final example of pre-reward related activity in ACC cells was demonstrated by Kennerley and Wallis in 2009. These authors recorded from cells in the ACC and OFC searching for spatial signals as well as value signals. The authors determined that the OFC and the ACC have different roles in reward processing. They show that the ACC has strong reward value signals that are weak at first but then get stronger whereas the OFC shows earlier reward signals that are not as strong as those of the ACC. They suggested that the OFC may initially process incoming reward stimuli from sensory afferents and then pass the information on to the ACC, which can then integrate the information with signals

from limbic, and striatal structures also involved in reward processing (Kennerley and Wallis, 2009). Additionally they illustrated that cells in the ACC respond to reward values after the reward-indicating cue has been presented but before the actual reward has been given. The authors showed that the cells could increase their firing rate for high reward or low reward following the cue, or can activate in a value dependent manner only during the delay period between the reward and spatial attention cue (Kennerley and Wallis, 2009). Furthermore, Kennerley and Dahmubed in another 2009 publication demonstrated the ACC's ability to code the various aspects of reward processing. They indicated that the ACC multiplexed information about potential pay off, reward probability and the cost of reward in terms of time and effort all before the physical reward was experienced and in a value dependent manner (Kennerley et al., 2009). These are two studies that have looked at spatial attention and reward value together during a pre-reward period illustrating the cellular correlates of reward and attention before the administration of reward a method that is similar to the goal of this project.

The above studies have demonstrated that the ACC is a cortical area that harbours a variety of signals related to reward selectivity. Its cells can increase or decrease their firing rate with changes in received reward size, they respond to expected reward sizes (Amiez et al., 2005, Hayden et al., 2011), and fictive rewards (Hayden et al., 2009). They can also respond to loss of potential reward (Amiez et al., 2005) and signal errors in reward expectancy (Matsumoto et al., 2007). The above studies illustrate these functions on a single cell and population level but lesion studies in macaques have also demonstrated that ACC damage impairs the ability to use reward value to influence future behaviour as well as

limiting the influence of past reward history (Kennerley et al., 2006; Buckley et al., 2009). However, these lesions fail to completely abolish the subject's ability to make a correct response after an error which disputes the conflict monitoring and error detection theories of the ACC according to Buckley et al. (2009) who suggest that the ACC's role in monitoring outcomes is more an active referencing of the value of recent choice-outcomes during rule-based decision making. The importance of the ACC in producing selective reward signals that influence behaviour will be addressed in this study.

1.8 Reward in the Prefrontal Cortex

Unlike the ACC the role of reward processing was, until recently, one of the least documented of the various functions of the PFC. However, several recent studies highlighting functions of the PFC in reward and value processing have brought the spotlight back to this region of the brain among various researchers (Watanabe, 2007; Schultz, 2007; Wallis and Kennerley, 2010). Also known for its roles in working memory (Petrides, 2005), and spatial attention processing (Lebedev et al., 2004), the PFC has been shown to have connections to the valuation circuits of the brain (Haber and Knuston, 2010). Haber and Knuston, in their 2010 anatomical discussion of the reward circuitry, indicate that the dlPFC has direct and indirect connections to the VS and therefore suggest that it plays some role in the cortico-basal ganglia reward loop. As such this recent return to the PFC in reward processing matched with its demonstrated connectivity to the reward circuitry suggest that it could be an ideal candidate to house both reward and spatial attention signals as discussed earlier.

Since the mid 1990's, Watanabe and colleagues (1996) have published a series of studies showing that up to 50% of neurons recorded in the PFC of macaques code different aspects of reward value (Watanabe, 1996). Watanabe (1996) demonstrated that PFC cells are not only sensitive to the position of stimulus during the delay period of a spatial delayed-response task but also that their activity is modulated by the method through which the reward is given. In a task where macaques received either a food or liquid reward at different spatial locations following a delay, the authors showed that some cells fired variably during the delay period with respect to a change in reward (food vs. liquid) and not with respect to a change in space (Watanabe, 1996). This study indicates that PFC cells are involved in the expectancy of specific rewards. It also shows that these cells are involved in dictating behaviour based on how the reward is given. This is supported by the fact that some human patients with prefrontal lesions have difficulty assessing how their actions relate to long-term goals. This could result from a loss of the neurons coding reward dependent responses that would impair their ability to predict the value of their actions on future events and outcomes. Watanabe also demonstrated that there is differential delay activity in macaque PFC neurons depending on whether or not a reward is expected and that these cells also code the consequence of the response (reinforcement or error). Furthermore in three other publications Watanabee and colleagues concluded that 1) the lateral PFC integrates motivational reward information from the OFC while coding spatial locations ("where") and reward expectancy ("what") therefore acting as a integrating center of cognitive and motivational aspects of the expectancy of reward outcome (Hikosaka and Watanabe, 2000). 2) The

lateral PFC integrates reward and cognitive information leading to enhancement of receiving reward since neurons which code spatial selectivity were also far more likely to code reward expectancy than reward omission during an instructed outcome expectancy task (Watanabe et al., 2005). And 3) cells of the PFC respond differently to the expectancy of positive and negative reinforces. Of the recorded cells 24.3% respond to positive reward, 5.6% respond to aversive reward 10.7% respond regardless of the expected reward. Thus the data suggests that dlPFC is more sensitive to rewarding versus aversive outcomes (Kobayashi et al., 2006). These findings have laid much of the foundation for the research into the role of the PFC in reward processing and demonstrate further the multifaceted role of the PFC in the integration of cognitive control and reward value signals.

Leon and Shadlen (1999) supported the findings of Watanabe and colleagues in another study that identified reward expectancy related signals in the FEF and area 46 of awake and behaving macaques during a memory guided saccade task. The authors also indicated that behaviour was modulated by reward value. The trials that were associated with low reward had double the number of early fixation breaks than highly rewarded trials. Recording in the dlPFC the authors demonstrated that cells in this region increase their spike rate relative to the onset of reward related information irrespective of when this information is given. In conditions where reward information is given before or after spatial cues, these cells show an increase in firing when informed of reward value. Even if the cell is selective for spatial location already prior to receiving information about the target value some cells were shown to have an increase in

activity following the addition of reward information, some only transiently and others having a sustained response (Leon and Shadlen, 1999). This again highlights the role of the PFC in signaling of reward expectancy information in a value dependent manner that in turn modulates the behavioural proficiency of the subject during the task.

In any discussion of reward the negative counterparts cost and risk must also be considered. These factors can be exemplified from one perspective by the time between the promise of reward and the decision to be made. As the delay between reward and choice increases the risk of not receiving the reward also increases, as such the influence of the potential reward is diminished (Rushworth and Behrens, 2008). This process of risk evaluation has been observed in fMRI studies where human subjects were faced with decisions based on a delay period with respect to reward. These studies show significant activity in the lPFC and OFC (Tanaka et al., 2004; McClure et al., 2004). Importantly, lPFC neurons, due to their ability to also code environmental states and features, can process the steps required to proceed from the current state to the desired goal and potentially determine whether or not those steps are worth the additional effort (Mushiake et al., 2006). This prioritization of conditional reward stimulus association is of utmost importance in order to maximize reward opportunities over the long term and direct attention to optimal targets. Furthermore, in both humans (Yoshida and Ishii, 2006) and macaques (Averbeck et al., 2006) engaged in navigating a maze, the lPFC has been shown to represent levels of uncertainty about current environmental conditions. Although this does not show direct reward representation, these signals are of vital importance to processing

potential reward outcomes in a manner that optimizes immediate reward versus the potential of greater future rewards.

It is this rich functionality of value and reward-expectancy modulated activity as well as the coding of cost versus reward associations in combination with the other cognitive roles that place the PFC as one of the chief areas of interest for this study. As mentioned earlier our hypothesis indicates that we will expect to find clear reward-value modulation during cued reward expectancy periods in the various areas of the PFC (9, 8, 46). These findings would support those demonstrated by previous studies in macaques. Furthermore, by mapping these functional correlates of reward across the fronto-cingulate axis we hope to provide localized functions to the anatomical circuitry of reward processing in the frontal cortex indicated by Haber and Knuston (2010).

1.9 Topographical Overlap and Functional Hubs of Integration

For optimal allocation of spatial attention to occur there must be integration of a variety of information within the brain during periods of top-down attentional shifting. The signals of reward and spatial attention as outlined above are two of the key executive signals that must be combined for behaviour to be most favourable. Furthermore, for these functional signals to integrate in the brain there must be regional overlap of function within brain areas (Kable and Glimcher, 2009). The ACC and PFC, due to their unique ability to code a variety of functions and the vast amount of connectivity to and from these areas, are uniquely positioned to act as topographical and functional hubs of integration for

reward and attention (Haber and Knuston, 2010; Averbeck and Seo, 2008). However, the nature of this functional integration overlap still remains elusive.

As outlined, both of these regions have been implicated in the reward and attention circuitry. However their precise roles from a networks perspective still remain unknown. Our hypothesis puts forth that cellular correlates of reward and attention will be found in both the ACC and PFC and that these signals will show regions of cortical overlap indicative of functional integration. Furthermore, we hypothesize that these cellular signals are vital to the behavioural outcome of the subject, where lack of these signals will result in errors. Averbeck and Seo (2008) suggested that these fronto-cingulate regions host the signals that support decision-making. The combination of both sensory and limbic information as well as the influence of motor connections in these regions allows for the expression of these decisions in their ultimate goal, action (Averbeck and Seo, 2008).

The desire for high reward or the knowledge of the location of a potentially salient target is not enough to result in positive behavioural outcomes. One must be able to locate the target as well as evaluate the value of this target to dictate behavioural measures. For example soccer players cannot simply want to win a soccer match or know the location of the ball; they must have an understanding of the rules of the game, the muscle memory of how to control the ball in a variety of scenarios and the know-how to interact variably with the other individuals on the field be it the referee, team-mates or opponents. This real world example illustrates the importance of merging various information types in order to optimize performance in a context dependent way.

For this integration to occur there must be anatomical and functional overlap between the reward based cortico-basal ganglia loops and other cognitive and motor systems of the brain (Kable and Glimcher, 2009). Haber and Knutson (2010) discuss in depth the required integration of various basal ganglia loops in order to optimize behavioural outcomes and various authors suggest the roles of the ACC in this process (Wallis and Kenerley, 2010; Kennerley et al., 2009; Hayden et al., 2011; Hayden et al., 2009; Amiez et al., 2005; Matsumoto et al., 2007; Rushworth and Behrens, 2008). Similarly, the fact that PFC neurons are involved in retaining spatial information (Everling et al., 2002; Lebedev et al., 2004; Rossi et al., 2007; Petrides, 2005) and reward expectancy (Watanabe, 1996; Watanabe et al., 2005; Leon and Shadlen, 2004; Hikosaka and Watanabe, 2000) indicates that the PFC is processing different kinds of information in parallel. These findings provide strong support that implicates these two regions as potential locations for anatomical overlap and functional integration (Rushworth and Behrens, 2008). Motivational drive from desire of highly-valued stimuli and localization of stimuli from the environment based on sensory information or previous rule based learning or other associative processes are two of the chief components of optimizing behaviour in a case-dependent fashion. This is demonstrated anatomically by the variety of cortical circuits involved in reward processing and spatial attention. The purpose of this study is to help resolve some long-standing debates about the function of fronto-cortical areas involved in these processes and will provide a novel technique of mapping the correlates of cognitive function in cells of the frontal cortex.

1.1.0 Summary

The above introduction has reviewed some of the major insights and controversies with respect to reward value and spatial attention processing in the ACC and PFC. These discussions have laid the foundation of this study that is aimed at demonstrating the neural correlates of reward value-expectancy and spatial attention in the frontal cortex during periods of attentional orienting. Our hypothesis aims to discern whether or not there are spatial attention signals in the ACC of macaques, where in the ACC these signals are located, and how they differ anatomically from reward related activity. Furthermore, our experiment will show how these signals of reward and spatial attention evolve in the frontal cortex during optimal and suboptimal behaviour. It is our hope that this experiment will provide new insights into the roles of cortical sub-areas and to demonstrate isolated areas of overlap where both attention and reward coexist in the frontal cortex. These findings will allow us to raise new questions about the functional correlation between these areas paving the way for further studies that could reveal the inner workings of these neural attention and reward networks.

2.1 Methods:

To test our hypothesis we recorded single neuron activity in two male Rhesus monkeys (*Macaca mulatta*) ages 5 (monkey M) and 9 years (monkey R), weighing 5.5 and 12.0 kg respectively at the time of recording. All procedures were conducted according to the guidelines set forth by the Canadian Council of Animal Care on the use of laboratory animals and the University of Western Ontario's Council on Animal Care.

2.2 Surgical Techniques

Monkeys were initially anaesthetized with an intramuscular injection of 5mg/kg ketamine, 0.125mg/kg medetomidine then transported to the operation room. They were then intubated and given an intravenous bolus of propofol. Anaesthesia was maintained by a combination of intravenous propofol and with inhaled isofluorane (1.5-2%) and the animals were placed in stereotaxic frame via referencing of their ear canals. Atropine (0.05 mg/kg) was administered subcutaneously to reduce secretions and block tachycardia and a loading dose of metacam was given subcutaneously as an anti-inflamitory. Both monkeys were implanted with standard head posts using dental acrylic centrally on top of their skull during one surgical session and then with their respective recording chambers at a later date (following training) using the same procedure. Post surgery, the monkeys were given a one week recovery period which included oral doses of metacam on the first two days post operation and intramuscular injections of buprenorphine for pain on a decreasing regimen of 0.03, 0.02 0.01 mg/kg for 3 days post operation. Finally ceazolin (25 mg/kg) was given 3 times

daily for 5 days to prevent any infections. All surgical procedures underwent approval by The Animal Use Subcommittee at The University of Western Ontario.

2.3 Training and Pre-recoding Procedures

The monkey's fluid intake was regulated to maintain motivation on the task and they were trained to sit in custom-made primate chairs. All experimental procedures were conducted inside custom-made isolation chambers. These chambers were constructed to minimize external auditory stimuli and regulate luminance internally from the rest of the room maintaining a dark workspace to enhance contrast of the view screen for the monkeys. The primate chair was slid into the box and was clamped into position to ensure that the monkey was returned to the same spot in the box everyday. The subjects were then head-restrained (see surgical procedures below) and trained to foveate on a central fixation point on a view monitor, their eye positions tracked by an eye-tracker (details below). Correct responses were rewarded with fluid rewards. Once the monkeys were able to fixate, the training on the task began. The task (described below) was broken down into smaller components and the monkeys were trained in a stepwise manner to perform the task. It was ensured that the monkeys were able to successfully perform the task at a high level of proficiency that was reliable over several days before data acquisition began.

Before recordings began, anatomical 7T MRIs were obtained from both monkeys with ear channels made visible with vitamin E capsules for later horizontal alignment, and with visualization of possible electrode trajectories in the recording grid using iodine (see figure 3). The slice image interval was 1mm

and each chamber was clearly visualized by the scan. This procedure allowed precise reconstruction of electrode recording locations (see below). We insured minimal artifacts during imaging by anaesthetizing the monkeys before scanning and using a minimal amount of titanium screws during surgical implantation of the head post and chamber. Ceramic screws were used as an alternative.

2.4 Extracellular Recordings

Extracellular recordings were conducted through standard recording chambers 19 mm inner diameter implanted over the right hemisphere in both monkeys. For monkey R, we initially recorded in the right hemisphere from 30 sites through an additional chamber implanted on the left hemisphere with an oblique angle over the midline. This chamber allowed a perpendicular penetration of the PS, but at the risk of penetrating the dura at an extreme angle and damaging major blood vessels. These constraints prevented further usage of that chamber. For monkey M, we re-positioned the recording chamber moving it 5 mm anterior after recording from several sites in its original position (n=55). This allowed access to more anterior regions of the prefrontal cortex and cingulate sulcus, and aligned recordings to the same anterior-to-posterior axis of the frontal cortex as covered with recordings obtained in monkey R.

During recording sessions between one and six individual tungsten electrodes (impedance 1.2-2.2 MΩ, diameter 125-250 μm, *FHC, Bowdoinham, ME*) were lowered through stainless steel guide tubes with software controlled precision micro-drives (*NAN Instruments Ltd., Israel*). The location of the penetration was documented by placing the guide tubes within a recording grid

with 1 mm inter-hole spacing placed over the recording chamber. The angle of the recoding drive on the chamber with respect to the anterior-posterior and medial-lateral axis of the monkey's skull was noted. The chamber and electrode grid position were also documented on a daily basis along with the depth of recordings and pattern of neural activity for each channel in order to facilitate later reconstruction of the recording sites (more below).

2.5 Data Acquisition

The monkey chambers and implants were cleaned on a daily basis pre and post recording according to the standard operating procedures set forth by The University of Western Ontario, Animal Use Subcommittee. This was to ensure that the dura was kept clean and free of any infection prior to the recording session and to maintain the health of the subjects.

The monkey's eye position was tracked continuously with an infrared system (*ISCAN*, Woburn, US) running on a DOS platform, with eye fixation controlled within a 1.4-2.0 degree radius window at a sampling rate of 60Hz. Cellular data amplification, filtering, and acquisition were done with a multi - channel processor (Map System, Plexon, Inc.), using headstages with unit gain. Spiking activity was obtained following a 100-8000 Hz passband filter, further amplification and digitization was done at a 40 kHz sampling rate. The electrodes were lowered slowly (0.02-0.003 μm/s) into the cortex to target locations. The first indication of neural activity was noted as well as the pattern of activity to the region of interest. Quiet zones and all major single and multi-unit activity depths were documented. To ensure minimal noise entering the system the drive and

recording apparatus was all shielded using a Faraday cage and each guide tube was individually grounded to a main ground cable. Prior to recording, the threshold for each neural signal was adjusted manually to always have a low proportion of multiunit activity visible against which we could separate single neuron action potentials in a 0.85 to 1.1 ms time window. Once favourable single units were isolated a period of 10-30 minutes was allowed to ensure the stability of the unit before recording began. We identified a favourable single unit as one that is visually separable in amplitude from the background activity online while recording. The monkey's eye position was then calibrated using preprogrammed Monkeylogic software (http://www.monkeylogic.net/) and the task was initiated. During the task any changes in cellular activity or waveforms were documented. Typical recording sessions lasted for approximately 45 min -2 h. After preliminary online sorting of unit waveforms, we resorted and isolated single unit activity offline with the Plexon Offline Sorter (*Plexon Inc., Dallas, TX*), based on principal component analysis of the spike waveforms, taking great care to limit unit isolation to periods with clear temporal stability and separation of unique waveforms from other isolated neurons and multiunit activity.

2.6 Visual Stimulation and Experimental Paradigm

Stimuli were presented on a 19 inch CRT monitor placed 57 cm from the monkeys eyes and running at 1024x768 pixel resolution and 85 Hz refresh rate. Behavioral control and visual stimulation was accomplished with Pentium III PCs running the open-source software Monkeylogic (http://www.monkeylogic.net/). We used two circular grating stimuli (identical to those gratings shown in figure

2), moving within the circular aperture at 1.0 degree per sec, a spatial frequency of 1.4 degrees and radius of 1.5-2.2 degrees. Gratings were presented at 4.2 degrees eccentricity to the left and right of fixation. The grating on the left side always moved within the aperture upwards at -45 degrees and those on the right moved upwards at +45 degrees relative to vertical. Monkeys had to detect a transient smooth clockwise/counterclockwise rotation of the grating movement (see below). The rotation was adjusted to ensure ≥ 85% of overall correct responses to the grating and ranged between ±13 and ± 19 degrees. Note that we obtained complete psychometric curves prior to recording neuronal activity to determine the degree of rotation, which ensured enough correct trials, while maintaining constant task difficulty. The rotation proceeded smoothly from standard direction of motion towards maximum tilt within 60 ms, staying at maximum tilt for 235 ms, and rotating back to the standard direction within 60 ms, and continued moving at the standard ±45 degrees thereafter.

The monkeys performed a selective attention task requiring a forced-choice discrimination of two targets for the cued stimulus (figure 2).

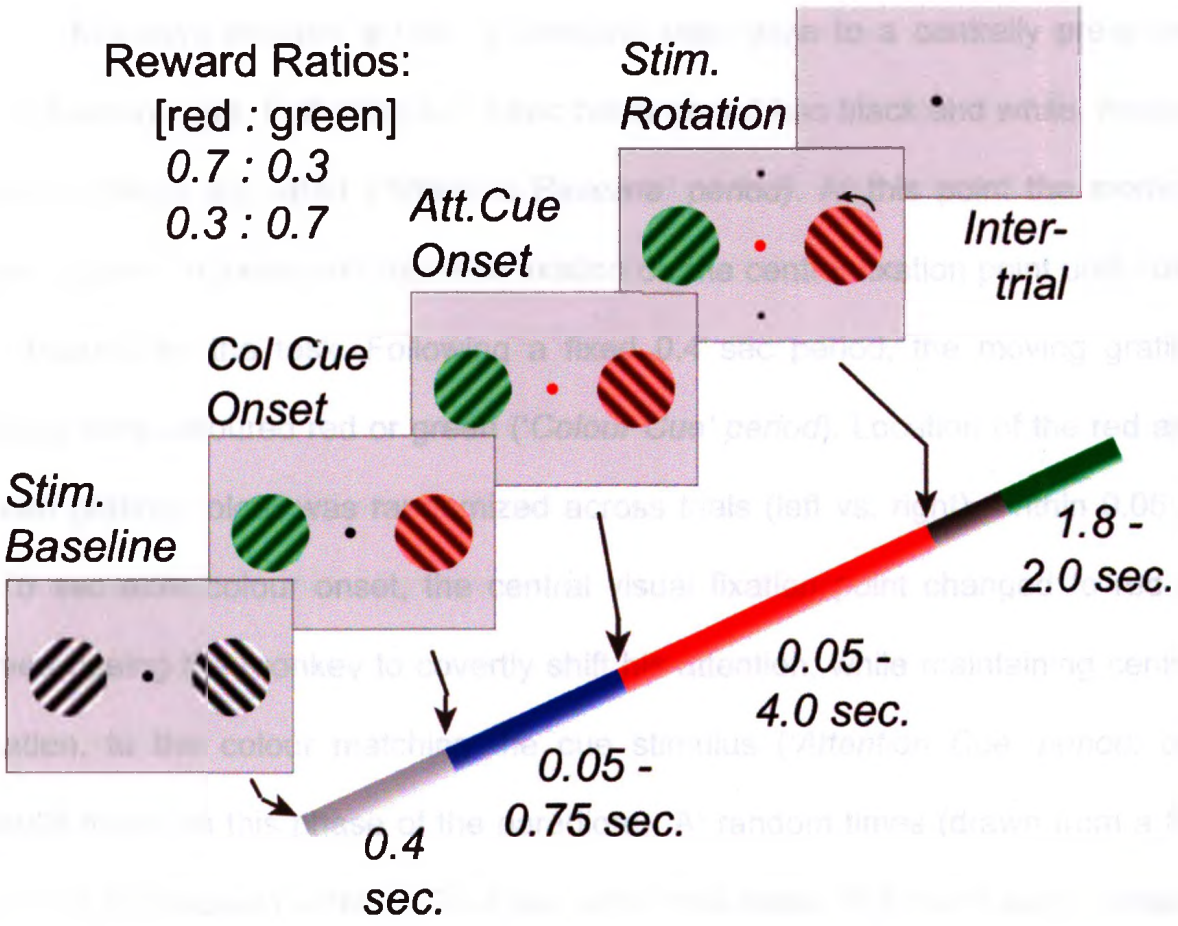

Figure 2. Paradigm: Monkeys initiated a trial by directing and keeping their gaze on a centrally presented fixation point. Following 0.3 sec two moving grating stimuli appeared (*stimulus baseline*), which were coloured red/green after 0.4 sec (*colour cue onset*). Within 0.05 to 0.75 sec after colour onset the central fixation point changed to red or green cueing the monkeys to covertly shift attention towards the location with the colour-matching stimulus (*attention cue onset*). At random times within 0.05-4.0 sec the cued target grating smoothly rotated clockwise or counterclockwise. In half of the trials the uncued distractor changed before the target. Monkeys discriminated the rotation of the target stimuli by saccading up- or downwards to the cued response target. Note that stimulus colour was associated with high/low liquid reward with reward ratio (0.7 : 0.3) changing every 30 correct trials to keep reward expectancy salient.

Monkeys initiated a trial by directing their gaze to a centrally presented grey fixation point. Following a 0.3 sec blank period two black and white, moving grating stimuli appeared (*'Stimulus Baseline' period)*. At this point the monkey was required to fixate and maintain fixation on the central fixation point until cued to respond by the task. Following a fixed 0.4 sec period, the moving grating stimuli were coloured red or green (*'Colour Cue' period*). Location of the red and green grating colour was randomized across trials (left vs. right). Within 0.05 to 0.75 sec after colour onset, the central visual fixation point changed to red or green cueing the monkey to covertly shift his attention, while maintaining central fixation, to the colour matching the cue stimulus (*'Attention Cue' period;* our results focus on this phase of the paradigm). At random times (drawn from a flat random distribution) within 0.05-4 sec after cue onset, the cued target grating transiently rotated clockwise or counterclockwise as described above. In half of the trials the un-cued distractor grating transiently rotated before the cued target. Monkeys had to discriminate the rotation of the target stimulus by making a saccadic eye movement up or down to one of two response locations within 70-550 ms following rotation of cued target (monkey R: clockwise/counterclockwise required up/down saccade, while the response mapping was reverse for monkey M). Monkeys had to keep fixation on the response target after the saccade for 50 ms. After a further delay of 0.4 sec after a correct saccadic response the monkeys received fluid reward through a sipper tube. The opening of a mechanical valve of a distantly placed custom-made air-compression controlled reward system, dispensed the reward and minimized auditory sounds associated with reward. Responses to the incorrect target were counted as errors. Likewise,

breaks of fixation outside the 70-500 ms response time window resulted in abortion of the trial, as did the failure to respond in the allotted time frame. For the analysis of errors, only error trials were considered where fixation broke *after* a stimulus change, that is, either after the onset of the distractor change when it changed before the target, or after the onset of the target change.

Furthermore the stimulus colour was associated with the magnitude of liquid reward that the monkey received upon correct completion of a trial. The reward ratio for the red : green stimuli was set to 0.7 : 0.3, reversing in blocks of 30 correctly performed trials, so that reward ratios for red : green became 0.3 : 0.7. The number of correctly performed trials on red/green and high/low rewarded trials was controlled to be equal with a custom coded condition-selection function, which drew trials to equalize the count of correct trials with a variable trial lag of 2-5, which successfully prevented re-occurrences of sequences of target combinations of location (right/left), reward (high/low), and rotation- (clock-/ counterclockwise). Between sets of trials with constant reward ratio, we introduced five 'neutral' trials, in which the monkeys were only required to maintain fixation to a yellow fixation point to obtain a small liquid reward (which resulted in 60-75% of the lowest reward given during the attentional trials). These 'neutral' trials were of particular help during initial training to keep monkeys performing the attention-demanding task as both monkeys proved during training to be sensitive to the requirement of attending to a stimuli that gave lower liquid reward (the lower rewarded stimulus colour) and would work for fewer trials. We kept these neutral trials also after prolonged training, because they made the reward differences between targets more salient.

2.7 Reconstruction of Recording Sites

The anatomical site of each recorded neuron was reconstructed and projected onto the flat map representation of a standardized, macaque brain (Van Essen et al., 2001) by following a sequence of steps as highlighted for two examples sites in (figure 3).

The example sites were reconstructed to lie within area 32 (figure 3A) and area 46 (figure 3B) according to the fronto-cingulate subdivisions scheme outlined by Barbas and Zikopoulus (2007). A similar area assignment likewise follows when considering anatomical subdivision schemes proposed by two other major anatomical labs by Saleem et al. (2008) and Petrides and Pandya (2007), are illustrated in figure 4.

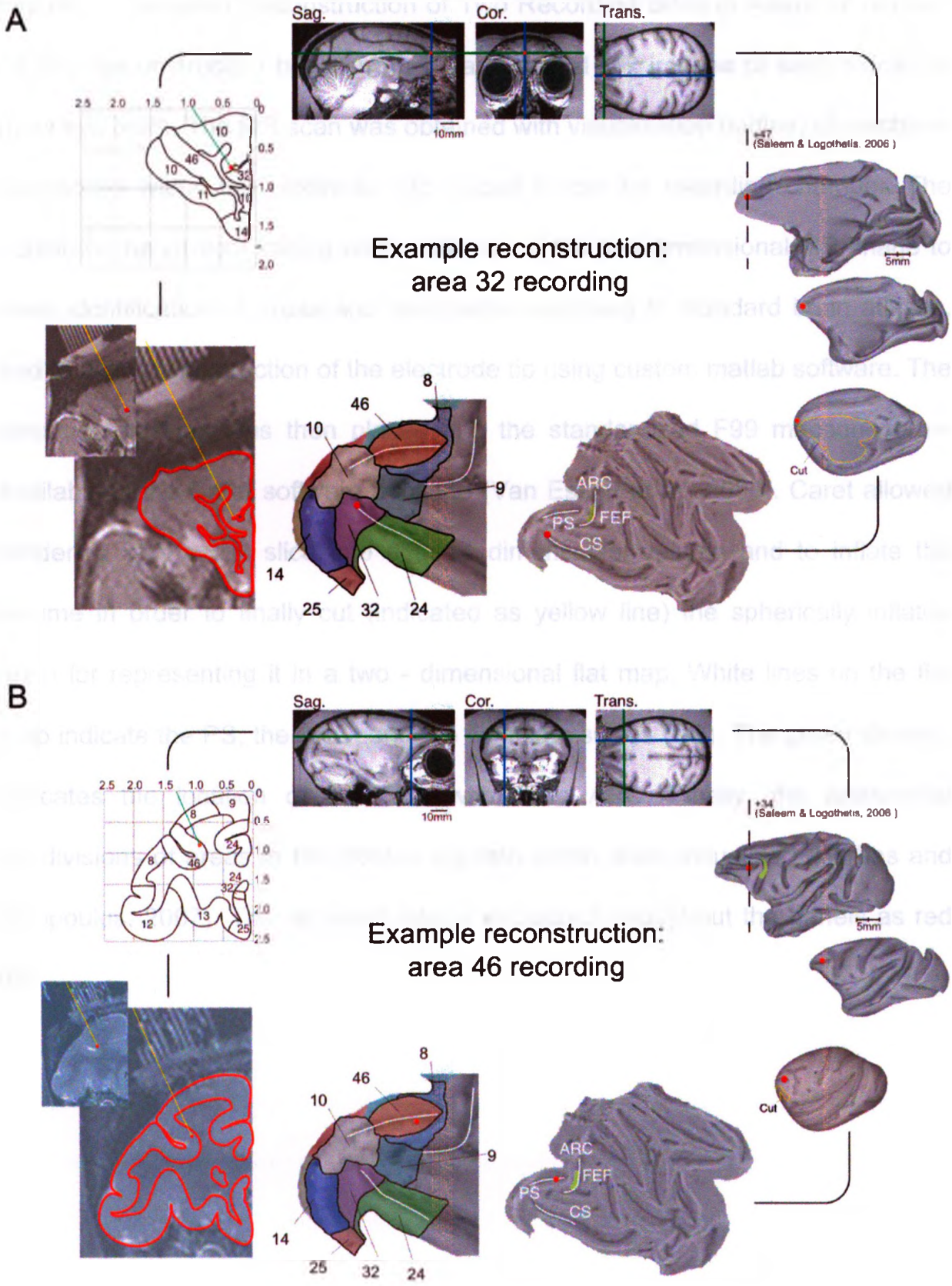
A
Sag.
Cor.
Trans.
10mm
(Saleem & Logothetis, 2006)
5mm
Example reconstruction:
area 32 recording
8
46
10
9
14
25
32
24
ARC
FEF
PS
CS
Cut
B
Sag.
Cor.
Trans.
10mm
(Saleem & Logothetis, 2006)
5mm
Example reconstruction:
area 46 recording
8
46
10
9
14
25
32
24
ARC
FEF
PS
CS
Cut

Figure 3. Stepwise Reconstruction of Two Recording Sites in Areas 32 (A) and 46 (B). Reconstruction began from 7T anatomical MR images of each individual monkeys brain. The MR scan was obtained with visualization (iodine) of electrode trajectories within the electrode grid placed inside the recording chamber. The outline of the cortical folding was sketched on the two-dimensional MR image to ease identification of areas and landmarks according to standard brain atlases, and to align reconstruction of the electrode tip using custom matlab software. The electrode position was then placed into the standardized F99 macaque brain available in the Caret software package (Van Essen et al., 2001). Caret allowed rendering of the MR slice into a three dimensional volume and to inflate the volume in order to finally cut (indicated as yellow line) the spherically inflated brain for representing it in a two - dimensional flat map. White lines on the flat map indicate the PS, the ARC, and the cingulate sulcus (CS). The green shading indicates the location of the FEF within the ARC. Finally, the anatomical subdivisions of areas in the fronto-cingulate cortex were visualized (Barbas and Zikopoulus, 2007). The recorded site is visualized throughout the panels as red dot.

Reconstruction began by projecting each electrodes trajectory onto the two dimensional brain slice obtained from 7T anatomical MR images, using the open-source OsiriX Imaging software (Rosset, et al., 2004) and custom-written Matlab programs (*Mathworks Inc.*), utilizing the iodine visualized electrode trajectory within the electrode grid placed in the recording chamber during the anatomical MRI scan. We drew the coronal outline of the cortical folding from the MRI grey scale image to ease the comparison of the individual monkey brain slices to standard anatomical atlases. This also assisted us in using major landmarks to guide projection of the electrode tip position into the standardized F99 brain available in Caret (Van Essen et al., 2001). Note that we initially reproduced the individual monkey brains within the Caret software to validate similarity and derive the scaling factors to match the lower resolution monkey MRIs to the higher resolution standard F99 brain. We then projected manually and under visual guidance the electrode position to the matched location in the standard F99 brain in Caret (Van Essen, 2002). We estimate that the complete procedure from documenting precisely the recording depth, identification of the recording location in the monkeys MRI slice, referencing the recording position with the documented cellular activity profile from online recording sessions, up to the placement of the electrode position in the standard F99 brain introduces a potential maximal error of 3mm. However, we felt that despite this potential distortion, which we cannot rule out despite our confidence that the typical (unsystematic) error is more in the 1mm range, the assignment of recording locations to standard brains is highly beneficial. Anatomical reconstruction was conducted entirely independent of the analysis of neuronal data and the

projecting functional results onto the anatomical 2D map, thus the results did not influence the anatomical reconstruction.

After identifying all recording sites within the standard F99 brain, we used the Caret software package to render the standard brain into a three dimensional volume, which was then spherically inflated and cut in order to unfold the brain into two dimensional space (figure 3). In an independent procedure we visualized major anatomical subdivision schemes of the fronto-cingulate cortex, using the scheme from Barbas and Zikopoulus, (2007) as a major reference throughout the manuscript on to the F99 brain in Caret. Two alternative schemes that are shown in figure 4, illustrate a high level of overall correspondence between area assignments to anatomical locations. The area subdivision from Barbas and Zikopoulus, (2007) showed the most general mapping scheme with a large area 46 spanning the whole length of the PS, a large area 8 which extents posterior to the PS and up to the ARC and a large area 9. The most relevant difference for the current study of the Barbas map to the anatomical map from Petrides and Pandya, (2007) concerns finer areal subdivisions around the lateral PFC: Petrides and Pandya (2007) propose a smaller area 9, an intermediate area 9/46 distinction around the posterior extent of the PS, and subdivide area 8 into dorsolateral and dorsal areas 8a and 8b. The most apparent difference of relevance for the current manuscript of the map by Saleem and colleagues (2008) to the Barbas (2007) subdivisions concerns a more anterior extending area 9, and an explicit (and generally agreed upon) subdivision of area 24 according to the more dysgranular nature of the cortex as one moves ventrally along the cingulate sulcus.

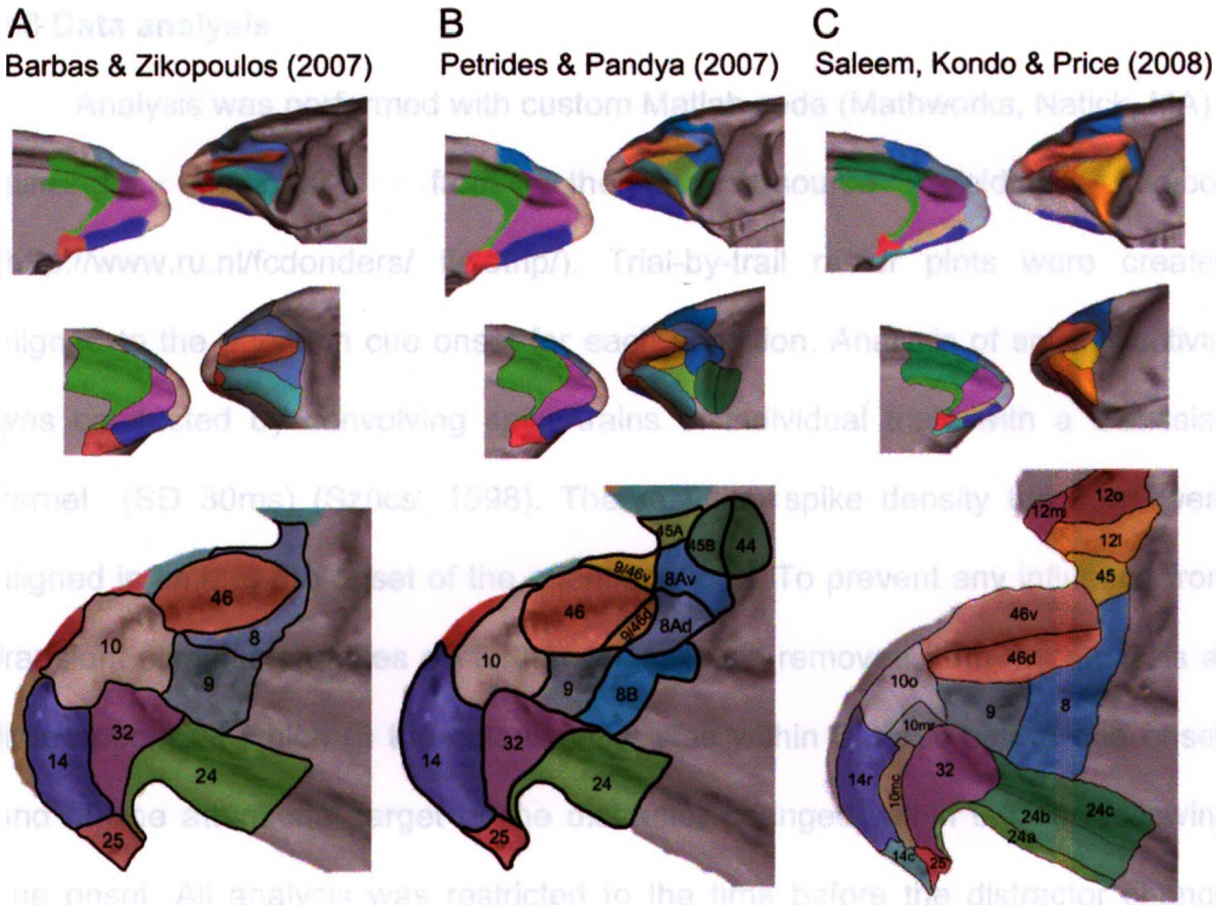

Figure 4. Three major anatomical schemes subdividing the fronto-cingulate cortex into areas according to difference in cytoarchitecture and identified afferent and efferent connectivity. (A) Subdivisions proposed by Barbas and Zikopoulus (2007), entered as colour ed shadings into the standard F99 macaque brain available in Caret rendered in 3D (*top panel*), semi-inflated 3D volume (*middle panel*), and flattened into a 2D map representation using the Caret software package (Van Essen et al., 2001). B and C same format as in A but with area subdivisions proposed by Petrides and Pandya (2007) (B) and by Saleem, Kondo and Price (2008) (C). Note the overall agreement across subdivision schemes from different labs.

2.8 Data analysis

Analysis was performed with custom Matlab code (Mathworks, Natick, MA), utilizing functionality from the open-source fieldtrip toolbox (http://www.ru.nl/fcdonders/ fieldtrip/). Trial-by-trail raster plots were created aligned to the attention cue onset for each condition. Analysis of spiking activity was conducted by convolving spike-trains of individual trials with a Gaussian kernel (SD 30ms) (Szücs, 1998). The resulting spike density functions were aligned in time to the onset of the attentional cue. To prevent any influence from transient stimulus changes on further analysis we removed from the analysis all time epochs at which (i) the colour onset was within 0.3 sec before cue onset, and (ii) the attentional target or the distractor changed within 0.3 sec following cue onset. All analysis was restricted to the time before the distractor change (which happened in half of the trials before the target), because after a distractor change attentional demands would be released from being necessarily spatially selective.

2.9 Single Neuron Statistics

To analyze whether the activity of single neurons was statistically different between conditions, we performed two major analyses, standard *ANOVA*s, and permutation statistics on *ROC* (receiver operating characteristics) values, both based on average firing rates obtained in 0.3 sec time windows within 0.15 to 0.65 sec following cue onset. For initial characterization of each cell to be modulated by spatial attention or attentional target value, one-way ANOVAs were applied with the factors target location (left / right), and target value (high/low),

considering the F-test for a main effect to be significant at $p \leq 0.05$. For the ROC analysis we first computed the ROC for the experimentally observed spike-trains in the respective conditions (attend left vs. right; and attend high rewarded vs. low rewarded target).

To test for significance of the ROC values, we performed a bootstrap analysis by obtaining a random distribution of n=1000 ROC values by randomly assigning spike-trains from trials to either condition (without replacement). The mean and standard deviation of this random distribution reflect the values obtained when there is no systematic trial-by-trial difference between the mean spike rates between conditions, e.g. with the ROC mean being close to 0.5. We then considered the observed ROC value as statistically significant when it was larger, or smaller, than the 95th percentile confidence limits of the random distribution. We used ROC analysis, because a significant ROC value allows us to infer that an ideal observer of the spike rates is able to predict with statistical certainty on a trial-by-trial basis which condition gave rise to that spike rate fluctuation. The ROC results we report were very similar when we compared it to 'explained variance' (results not shown). We decided to use ROC values, however, because they are a more conservative evaluation which provide a more direct measure of the trial-by-trial based predictability of spike rate fluctuation, while not requiring particular formulas to adjust for differences in sample size (number of trials), not being systematically biased by the total number of samples, and because ROC computations are computationally more efficient when conducting permutation statistics. They also allowed us to account for an uneven distribution of recording locations across the frontal cortex and do not

assume a normal distribution in the spike trains of the cells.

2.1.0 Spatial Clustering of ROC Significant Neurons

To analyze whether the proportion of neurons with significant ROC values predicting attentional target location, or target value, were spatially clustered within fronto-cingulate cortex, we binned the anatomical space in a grid with an inter-pixel distance of 2mm. We then counted within each pixel (grid intersection) neurons recorded within a circular radius of 4mm (figure 5 C).

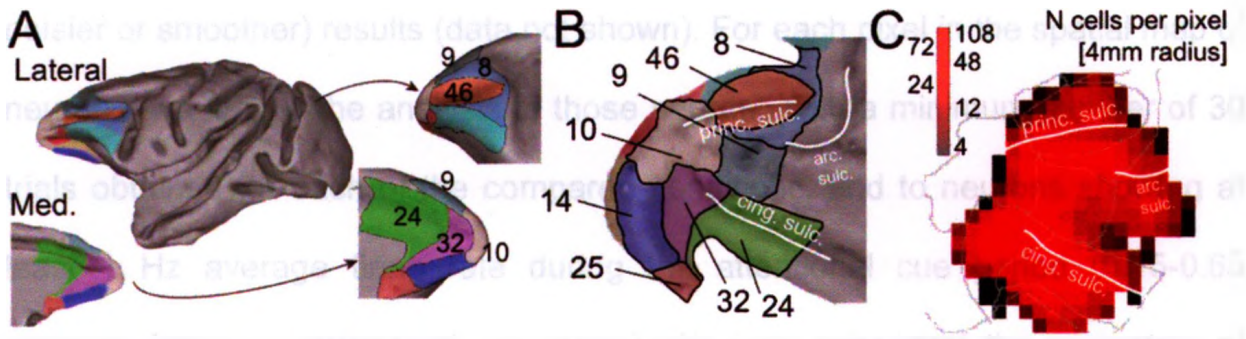

Figure 5. Fronto-cingulate anatomy and Recording Coverage. (A) Standard 3D to a partially inflated macaque brain, lateral and medial view with anatomical subdivisions (coloured) according to Barbas and Zikopoulus (2007). (B) Flat map representation of the fronto-cingulate cortex shown in A, covering ACC (areas 24 and 32) and lPFC (areas 10, 9, 46, and 8). (C) Number of cells recorded across areas overlaid on the contour of areal subdivisions (in grey) from the flat map in B. For each pixel in the map we counted the recorded cells within 4 mm radius (in steps of 2mm). White lines indicate sulci.

For all results reported in this study, we likewise tested finer (3mm) and coarser (5mm) resolutions and observed qualitatively similar (although either noisier or smoother) results (data not shown). For each pixel in the spatial map of neurons, we limited the analysis to those neurons with a minimum number of 30 trials obtained for each of the compared conditions, and to neurons showing at least 1 Hz average firing rate during the attentional cue period (0.15-0.65 seconds following attentional cue onset). We then calculated the proportion of ROC significant neurons for each pixel in the map. For further statistical analysis pixels containing $n \leq 5$ neurons (i.e. at the rims of the map) were disregarded.

To test whether any map pixel contained a higher proportion of ROC significant neurons than expected by chance, we applied a permutation test, which explicitly controlled for uneven sampling across the map. We tested the null hypothesis that the proportion of ROC significant neurons at any map pixel did not differ across the entire map by obtaining a random distribution from randomly assigning (n=1000) each neuron to a map location while keeping the number of neurons per pixel identical to the observed number of neurons at that pixel. This test ensures that only those regions in the map with reliably highest proportion of single neurons survive the statistical criterion ($p \leq 0.05$). We applied this test on single site significance of the ROC values, which allows on a trial-by-trial basis to infer that neuronal spike rates predict the attentional target location or value at that pixel on the map.

3.1 Results

3.2 Behavioral Performance

In our task, monkeys were successfully applying the correct cue-target rule (cue red: attend red vs. cue green: attend green) to spatially select the target stimulus with an average 78.6 % accuracy (figure 6 top panel). The effect of stimulus value was illustrated in behaviour such that when the monkeys were cued to select a target with high stimulus value, performance was significantly better compared to targets with lower stimulus value. However, this effect was restricted to the attentional shift period immediately after the cue (0.15 - 0.4 sec) (figure 6 top panel). Analysis of reaction times for the choice of the attentional target did not vary for high vs. low value targets. The reaction times did however reach an asymptotic low level only for those choices made 0.8 sec, or later, after cue onset (figure 6 bottom panel).

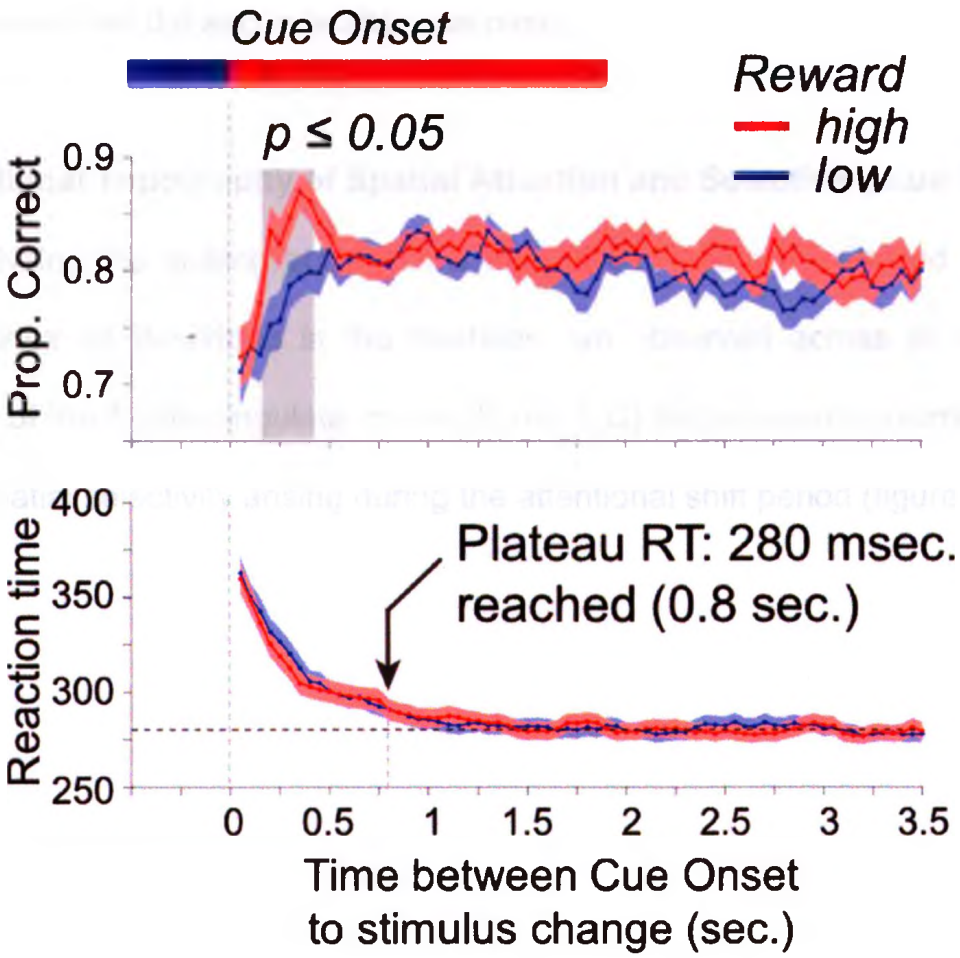

Figure 6. Behavioural results from cue onset to the cue change signaling response. colour shading shows SEM. Zero is the cue onset. Target stimulus value high/low (red/blue). *Top panel:* The graph shows the proportion of correct trials for discriminating the target rotation as a function of the time at which the target started to rotate relative to the cue onset. Reward value shows a behavioural effect in the first 0.15-0.4s following cue onset and plateaus after. High rewarded cues show more percent correct responses than low in this early time window. Grey bar indicates where differences are significant (paired t-test: $p \leq 0.05$). *Bottom panel*: Saccadic reaction time for discriminating the target rotation as a function of the time at which the target rotated relative to the cue onset. Reaction time does not vary with reward value and an asymptotic low of

280 ms is reached 0.8 seconds after cue onset.

3.3 Functional Topography of Spatial Attention and Selective Value Signals

Analyzing the spiking activity of a total of 811 neurons sampled from the frontal cortex as described in the methods, we observed across all recorded subareas of the fronto-cingulate cortex (figure 5 C) single neuron examples with reliable spatial selectivity arising during the attentional shift period (figure 7).

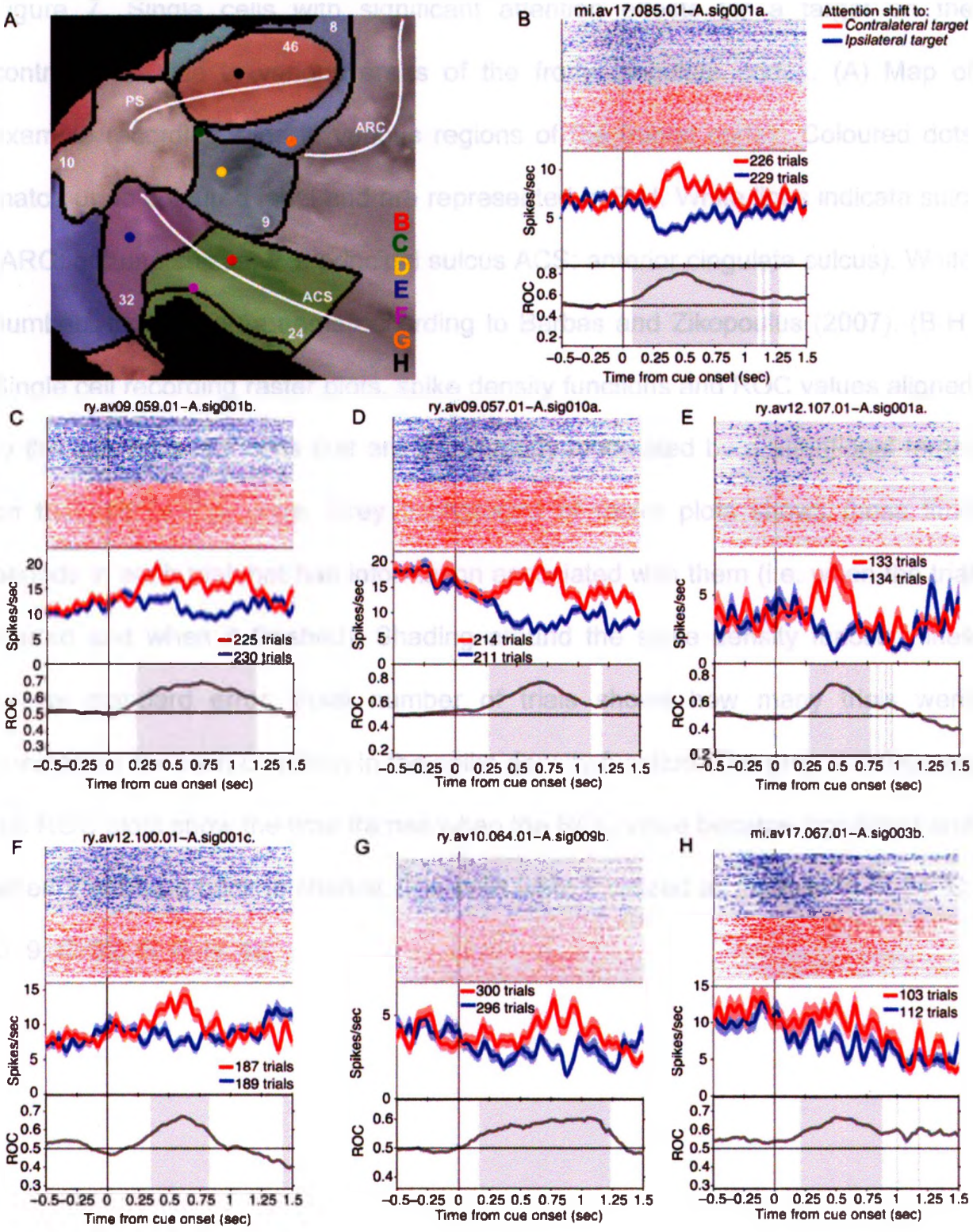
A
8
46
PS
ARC
10
9
32
ACS
24
B
C
D
E
F
G
H
B
mi.av17.085.01–A.sig001a.
Attention shift to:
Contralateral target
Ipsilateral target
226 trials
229 trials
Spikes/sec
ROC
Time from cue onset (sec)
C
ry.av09.059.01–A.sig001b.
225 trials
230 trials
D
ry.av09.057.01–A.sig010a.
214 trials
211 trials
E
ry.av12.107.01–A.sig001a.
133 trials
134 trials
F
ry.av12.100.01–A.sig001c.
187 trials
189 trials
G
ry.av10.064.01–A.sig009b.
300 trials
296 trials
H
mi.av17.067.01–A.sig003b.
103 trials
112 trials

Figure 7. Single cells with significant attention effects for a target on the contralateral side in various areas of the fronto-cingulate cortex. (A) Map of example recording sites in various regions of the frontal cortex. Coloured dots match up to coloured label and are represented in B-H. White lines indicate sulci (ARC: arcuate sulcus, PS: principal sulcus ACS: anterior cingulate sulcus). White numbers indicate area name according to Barbas and Zikopoulus (2007). (B-H) Single cell recording raster plots, spike density functions and ROC values aligned to the cue onset for cells that are significantly modulated by a attentional target on the contralateral side. Grey background of raster plots shows those time periods in each trial that had information associated with them (i.e. when the trial started and when it finished). Shading around the spike density function lines shows standard error. Inset number of trials shows how many trials were considered for each condition in the spike density function. The grey shadings on the ROC plots show the time frames when the ROC value became significant and when it returned back to chance. The cells were localized as follows: B, F: 24; C, D: 9; E: 32; G: 8; H: 46.

The proportion of these attentional shift signals however varied systematically across anatomical space. Figure 8 illustrates the spatial topography of the proportion of neurons with a significant main effect (*ANOVA*, $p \leq 0.05$) for spatial target location (contralateral. vs. ipsilateral. target) and for target value (high vs. low predicted reward) in a 0.3 sec window following 0.15 sec after cue onset. Spatial attention signals were significant at the single neuron level in more than 50% of all cells and clustered within three large areas: area 24 (ACC), an anterior band spanning areas 46/9/8, and in area 8 (about 5mm anterior to the frontal eye fields) (figure 8 A). A different spatial clustering was found for neurons significantly modulated by target value. These cells were localized largely to area 32 and extending laterally into area 24 and dorsally into areas 10 and 9 (figure 8 B).

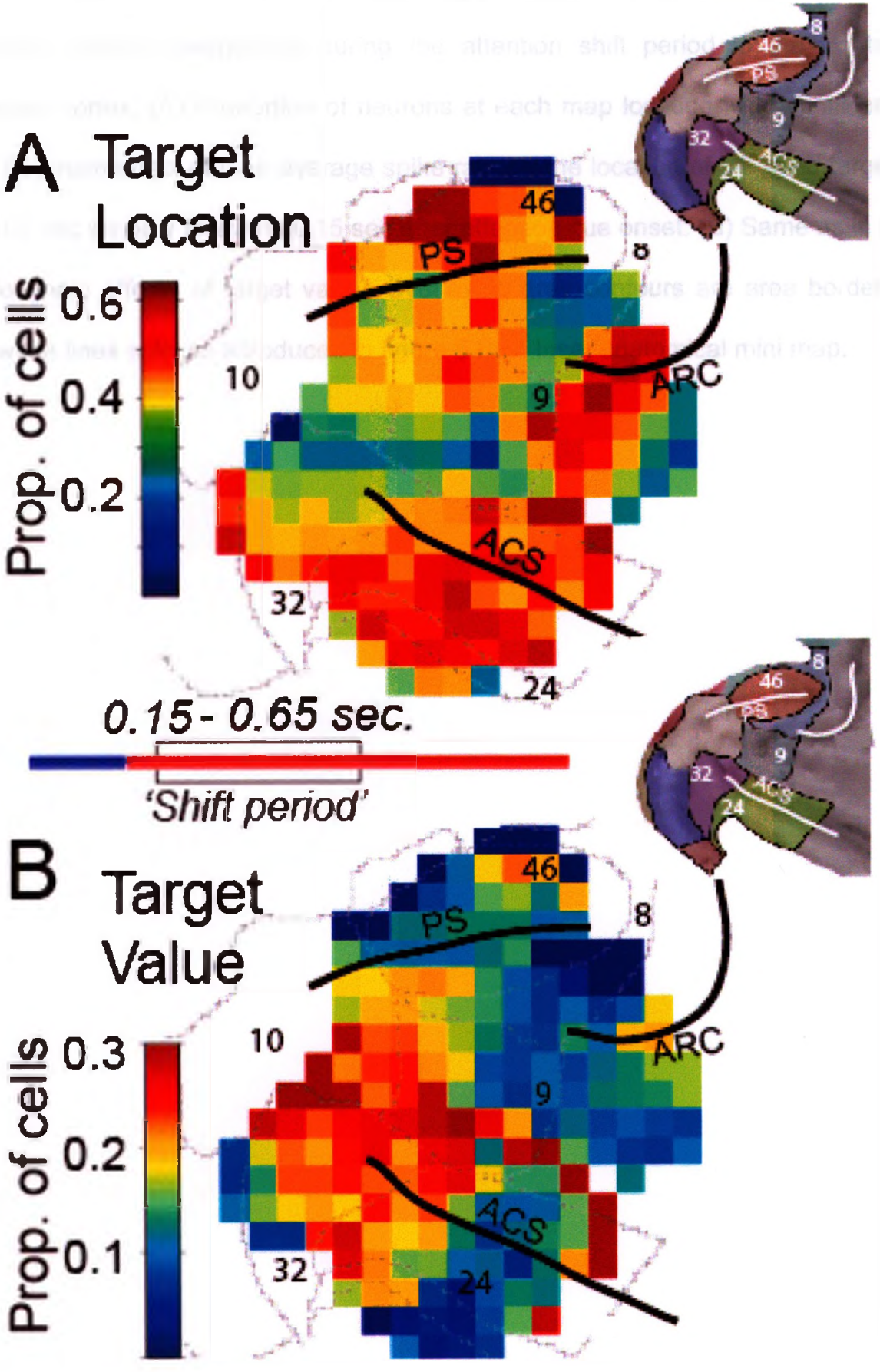
A Target Location
Prop. of cells
0.6
0.4
0.2
46
PS
8
10
9
ARC
ACS
32
24
0.15 - 0.65 sec.
'Shift period'
B Target Value
Prop. of cells
0.3
0.2
0.1
46
PS
8
10
9
ARC
ACS
32
24

Figure 8. Topographical representation of significant (ANOVA) spatial- and value-selective neuronal responses during the attention shift period in the fronto-cingulate cortex. (A) Proportion of neurons at each map location with significant ($p \leq 0.05$) main effect of their average spike rate for the location of the cued target in a 0.3 sec window following 0.15 sec after attention cue onset. (B) Same as in A but for main effects of target value. Overlaying grey contours are area borders and white lines sulci as introduced in figure 5 B C. Inset anatomical mini map.

Furthermore we analyzed those cells that have an interaction effect between attention and reward; that is those cells that fire significantly only for contra-high, contra-low, ipsi-high, or ipisi-low. We identified confined regions where attention and reward interact in single cells. These localized regions contained cells that code both attention and value at the rim of dorsolateral area 9, posterior parts of area 46, and at the intersecting region of 32 and 24 (figure 9) indicating anatomical hubs of signal convergence with potential functional relevance.

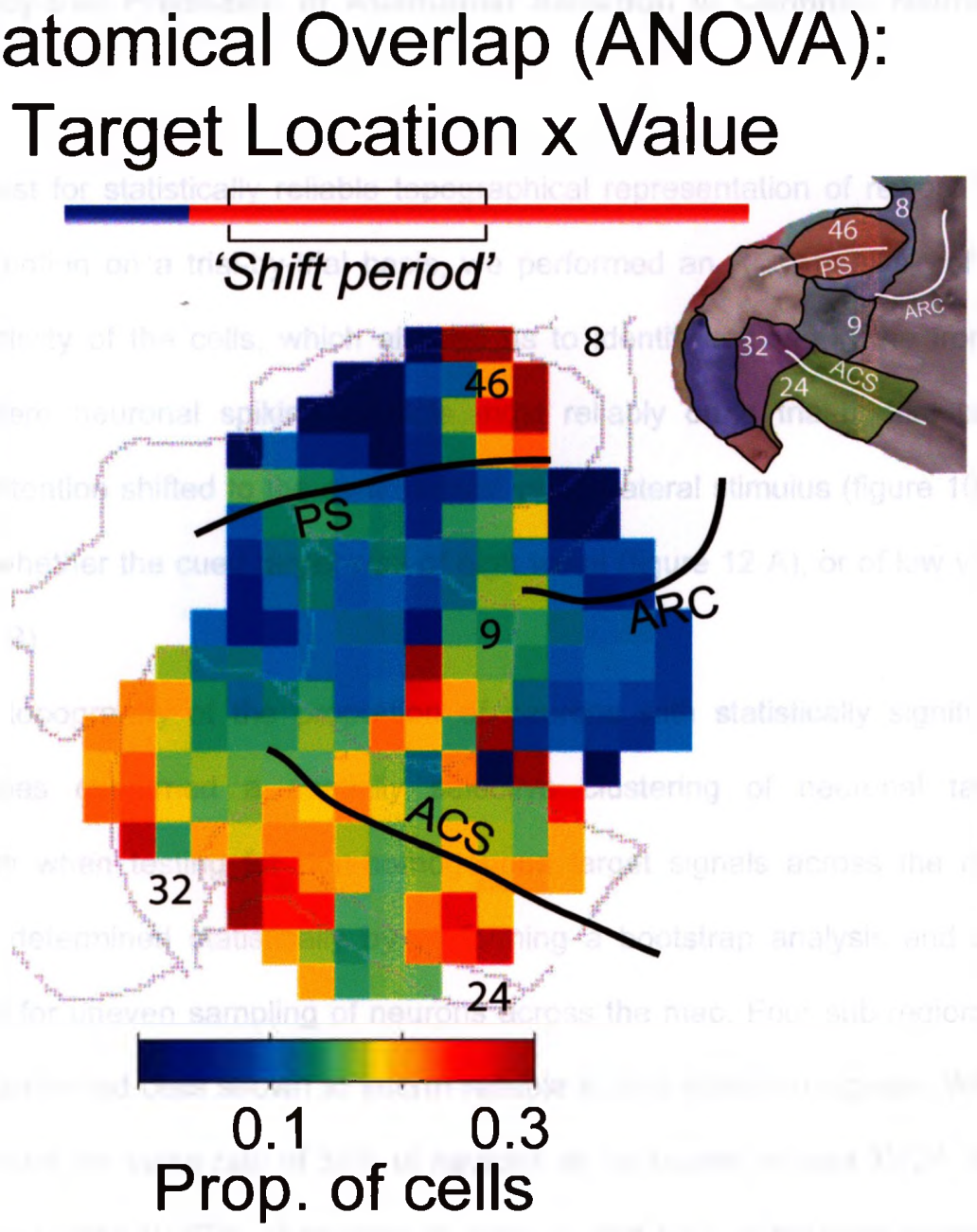

Figure 9. ANOVA demarcation of cells with overlapping functional responses for spatial attention and reward. Hubs of functional integration housing cells with overlapping function are seen in the fronto-cingulate cortex in confined areas. At the margin of areas 24 -32 as well as the margin of areas 9-24, and within the posterior section of area 46 these functional hubs are present. Inset anatomical mini map, black lines are sulci.

3.4 Trial-by-trial Prediction of Attentional Selection in Confined Neuronal Clusters

To test for statistically reliable topographical representation of reward and spatial attention on a trial-by-trial basis, we performed an ROC analysis of the spiking activity of the cells, which allowed us to identify regions in the frontal-cortex where neuronal spiking predicts most reliably on a trial-by-trial basis whether attention shifted to the contralateral vs. ipsilateral stimulus (figure 10 A), and also whether the cued target was of high value (figure 12 A), or of low value (figure 12 B).

The topography of the proportion of neurons with statistically significant ROC values confirmed a spatially selective clustering of neuronal target information when testing for non-homogenous target signals across the map. This was determined statistically by performing a bootstrap analysis and also accounted for uneven sampling of neurons across the map. Four sub-regions of the map contained cells shown to inform reliable spatial selection signals. Within these clusters the spike rate of 38% of neurons at the border of area 32/24, 42% of neurons in area 9, 57% of neurons in area 46 and 54% of neurons in area 8 predicted that attention is shifted to the contralateral target stimulus. That is, this proportion of cells, in these defined clusters, increased their firing to a statistically significant degree when the subject attended to a target on the contralateral side of recorded hemisphere versus the ipsilateral side. In these clusters neurons transiently enhanced their firing rate during the attention shift period (0.15-0.65 seconds after cue onset) to the contralateral target (figure 10 B-E).

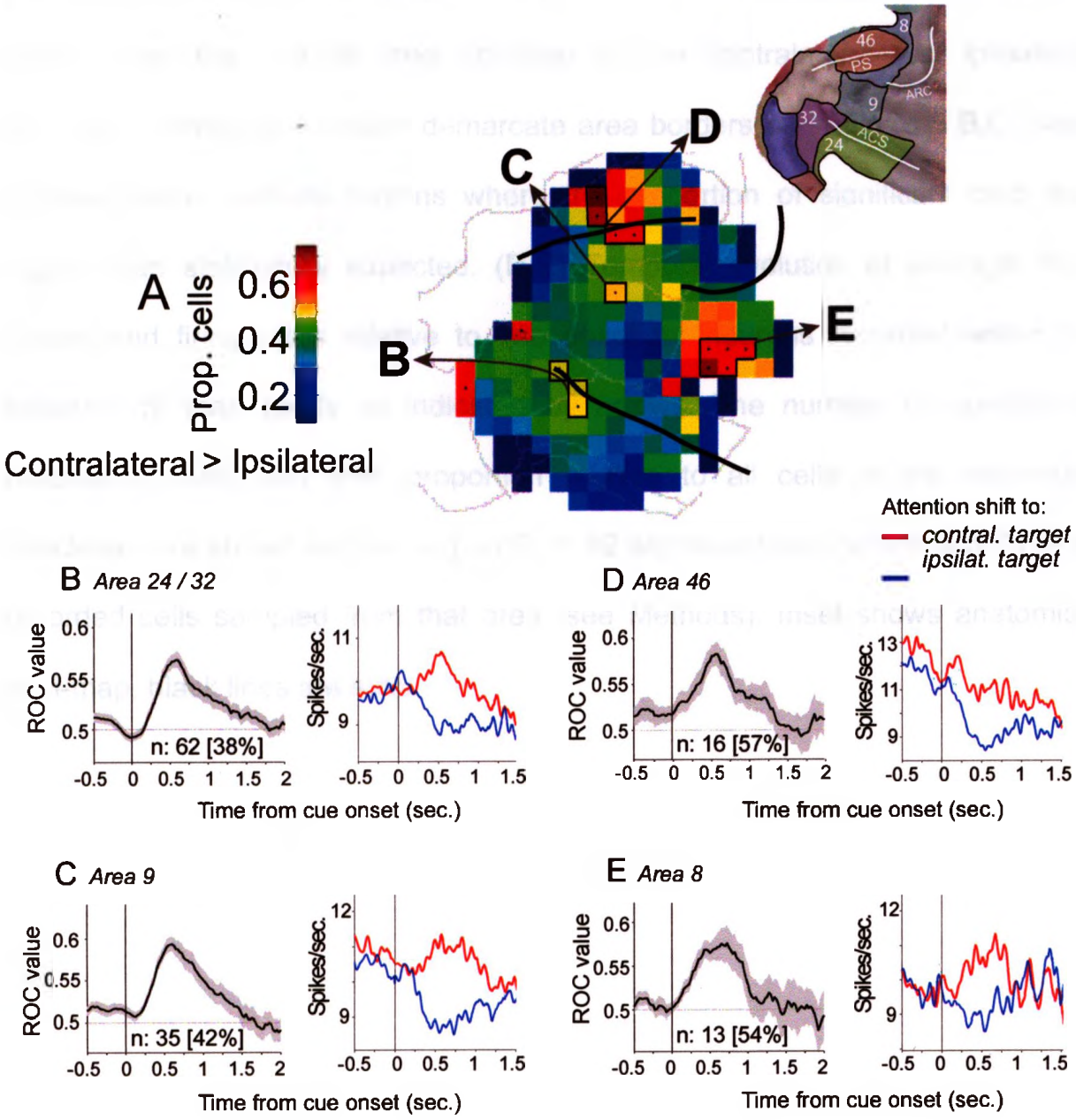
A
Prop. cells
0.6
0.4
0.2
Contralateral > Ipsilateral
B
C
D
E
8
46
PS
ARC
9
32
ACS
24
Attention shift to:
contral. target
ipsilat. target
B Area 24 / 32
ROC value
n: 62 [38%]
Spikes/sec.
Time from cue onset (sec.)
D Area 46
n: 16 [57%]
Time from cue onset (sec.)
C Area 9
n: 35 [42%]
Time from cue onset (sec.)
E Area 8
n: 13 [54%]
Time from cue onset (sec.)

Figure 10. Predicting Attentional Shifts to Stimuli in Space. (A) Proportion of cells with statistically a significant higher ROC value within 0.15-0.65 sec following cue onset when the cue directed attention to the contralateral (vs. ipsilateral) stimulus. Overlaying contours demarcate area borders as in figure 5 B,C. Black squared areas indicate regions where the proportion of significant cells was higher than statistically expected. (B-E) Temporal evolution of average ROC values and firing rates relative to cue onset for neurons recorded within the squared off map pixels as indicated by arrows. The number of significantly modulated cells, and their proportion relative to all cells in the respective subcluster are shown as text, e.g. in B, n: 62 significant cells which is 38% of all recorded cells sampled from that area (see Methods). Inset shows anatomical mini-map, black lines are sulci.

Conversely, in two spatially separate, adjacent sub-clusters (posterior sub-regions of area 24 and 9) neurons selectively reduced their activity during shifts to the contralateral target (figure 11, A). In the area 9 posterior subcluster, 45% of neurons (Figure 11 B) were shown to significantly decrease their firing for the contralateral stimulus and in area 24 two sub-regions with 37/39% of their cells having this response (Figure 11 C, D). This decrease in activity was determined to be statistically significant by the same permutation tests as above.

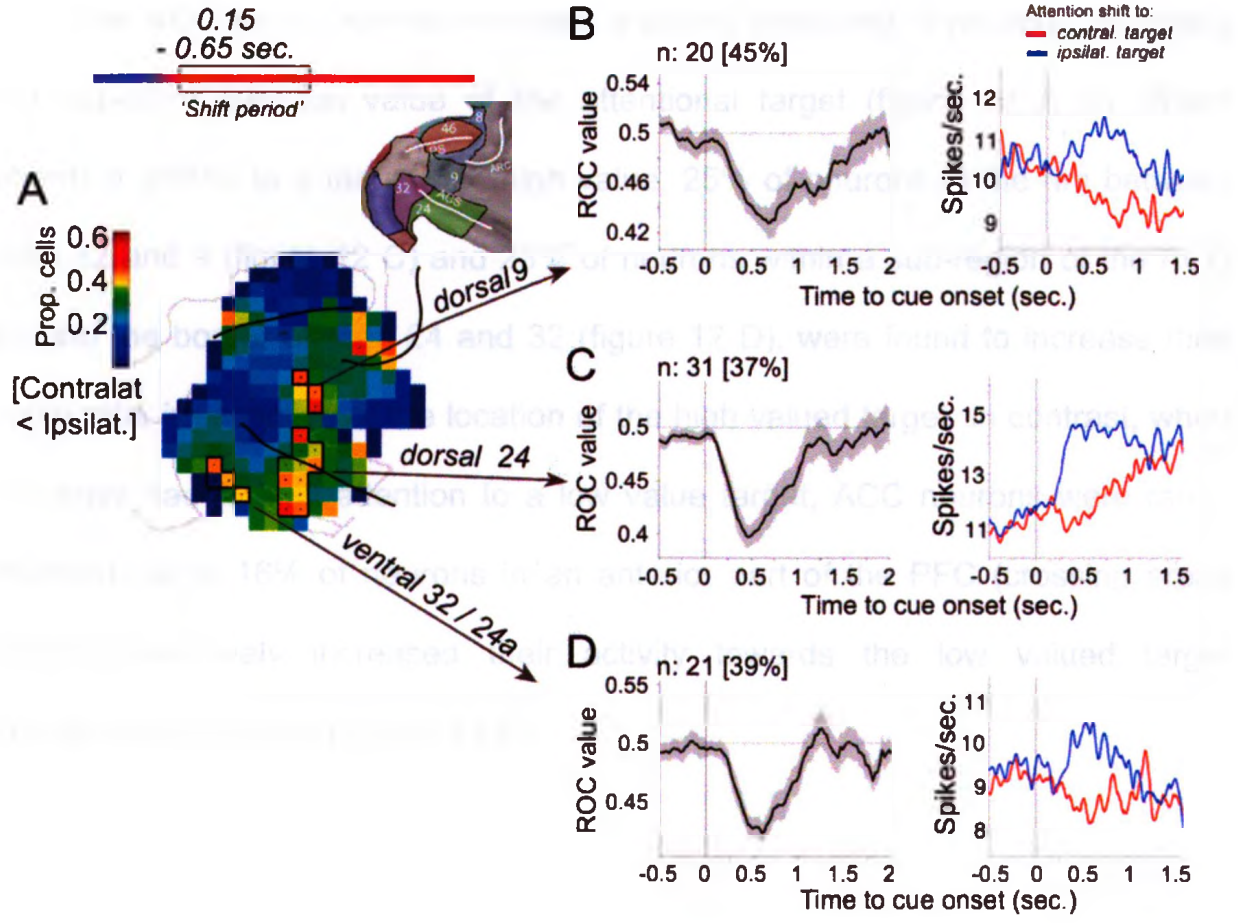

Figure 11. Proportion of neurons with significantly reduced activity when the cue directs attention to the contralateral stimulus. (A) Same format as figure 10, showing the proportion of cells with statistically significant lower ROC value within 0.15-0.65 sec. following cue onset when the cue directed attention to the contralateral (vs. ipsilateral) stimulus. (B-D) Temporal evolution of average ROC values and firing rates relative to cue onset for neurons with significant ROC values recorded within the map pixels with black squares indicated by arrows. Inset shows corresponding anatomical mini-map, black lines show corresponding sluci.

The ROC maps likewise revealed a spatial clustering of neurons predicting the expected stimulus value of the attentional target (figure 12 A B). When attention shifted to a target with high value, 25% of neurons at the rim between area 32 and 9 (figure 12 C) and 23% of neurons within a sub-region of the ACC around the border of area 24 and 32 (figure 12 D), were found to increase their spike rate, irrespective of the location of the high valued target. In contrast, when monkeys had to shift attention to a low value target, ACC neurons were rarely involved, while 16% of neurons in an anterior part of the PFC (crossing areas 46/9/8) selectively increased their activity towards the low valued target irrespective of location (figure 12 E).

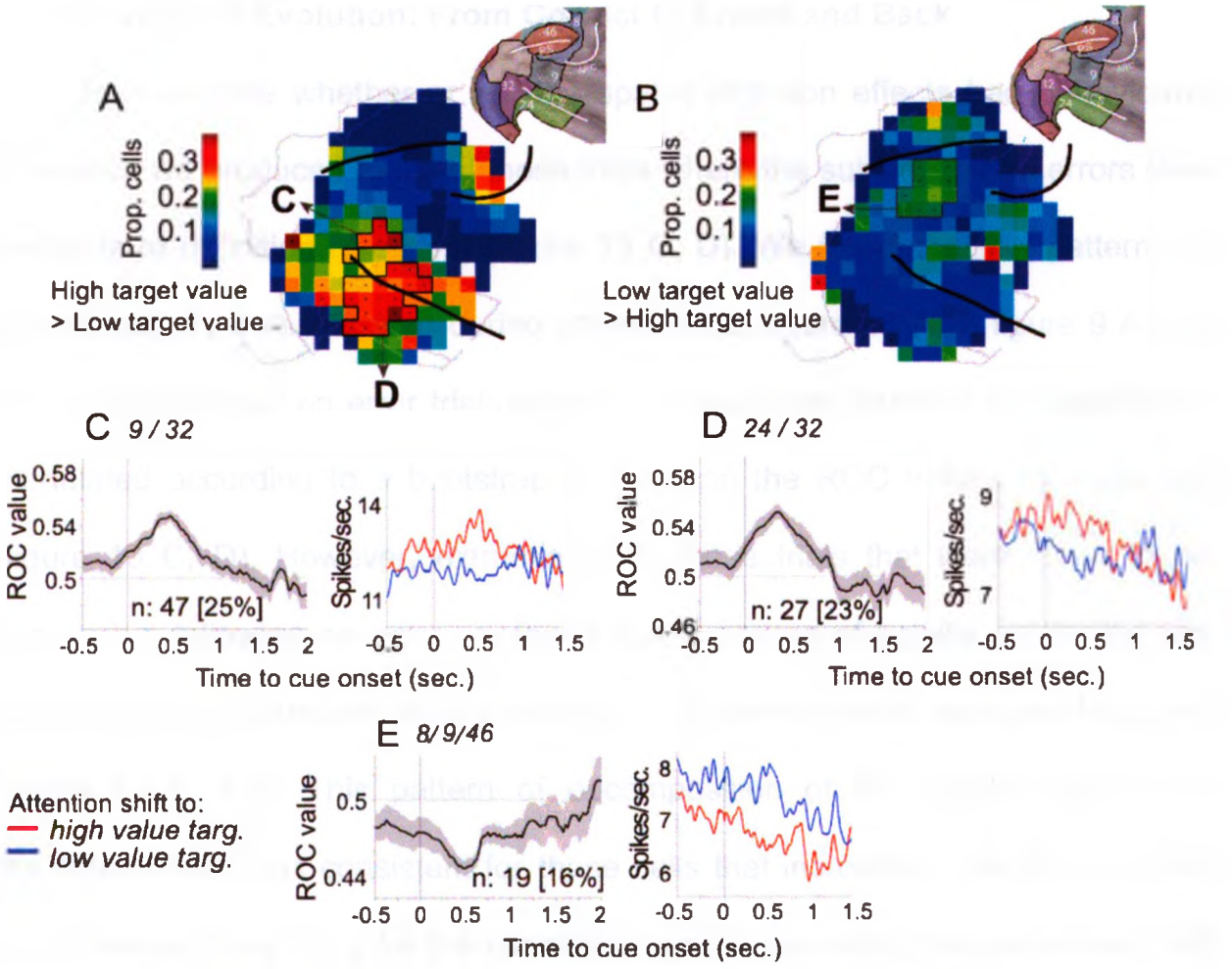

Figure 12. Predicting Attentional Shifts to Targets with High/Low Stimulus Value. (A, B) Spatial topography of the proportion of ROC significant neurons predicting that the attentional target has a high (A) or low (B) value. (C-D) Same format as figure 9 B-E, showing the average ROC value and firing rate for those map regions showing a statistically significant concentration of neurons conveying attentional target value information. Inset shows anatomical mini-map. Black lines are sulci.

3.5 Behavioural Evolution: From Correct to Errors and Back

To determine whether or not the spatial attention effects had behavioural relevance we produced maps of those trials where the subjects made errors (see methods to definition of errors) (figure 13 C, D). We found that the patterns of cellular activity demonstrated during correct trials (figure 13 A, B figure 9 A and 11 A) decomposed on error trials where no areas were found to be significantly modulated according to a bootstrap analysis on the ROC values for each cell (figure 13 C, D). However, extracting only those trials that were found to be correct but following an error we found that a pattern of activity similar but not identical (see discussion) to the activity of all correct trials, was reestablished (figure 13 E, F). This pattern of decomposition of the cellular signal and reestablishment was consistent for those cells that increased their firing as well as decreased their firing for the contralateral stimulus during the attentional shift period. This activity pattern is a good indication that these cells are playing an active role in attentional selection, as their firing rate seems to dictate the behavioural outcome of the task on a trial-by-trial basis.

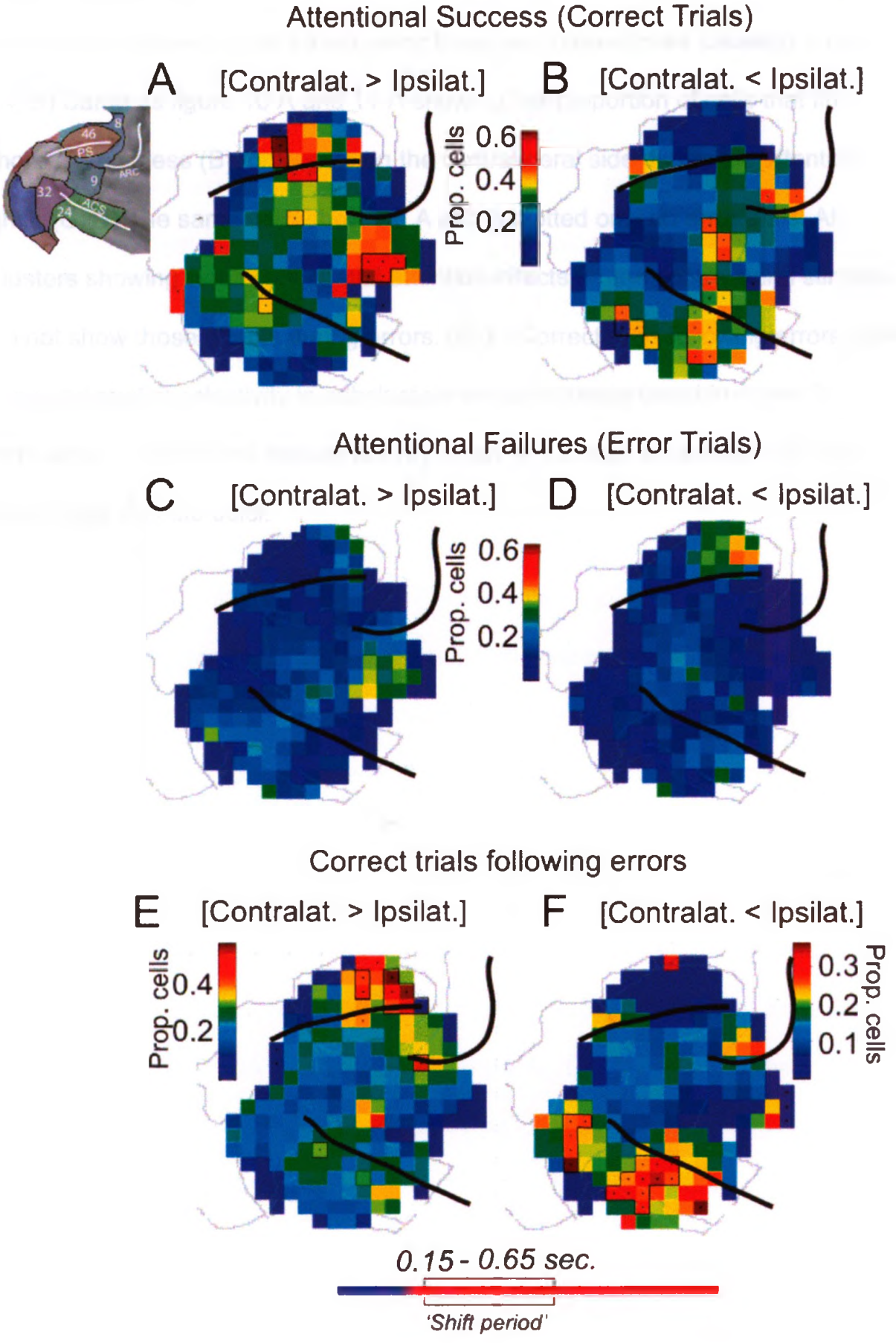
Attentional Success (Correct Trials)
A [Contralat. > Ipsilat.]
B [Contralat. < Ipsilat.]
46
8
PS
ARC
9
32
ACS
24
Prop. cells
0.6
0.4
0.2
Attentional Failures (Error Trials)
C [Contralat. > Ipsilat.]
D [Contralat. < Ipsilat.]
Prop. cells
0.6
0.4
0.2
Correct trials following errors
E [Contralat. > Ipsilat.]
F [Contralat. < Ipsilat.]
Prop. cells
0.4
0.2
0.3
0.2
0.1
Prop. cells
0.15 - 0.65 sec.
'Shift period'

Figure 13. Statistical map of the evolution of spiking activity, related to spatial selectivity, between correct trials, error trials, and correct trials following errors. (A, B) Same as figure 10 A and 11 A showing the proportion of cells that fire more (A) and less (B) for targets on the contralateral side during the attention shift. (C, D) The same cells shown in A and B plotted only on error trials. All clusters showing significant spatial attention effects for the contralateral stimulus do not show those effects during errors. (E, F) Correct trials following errors show resumed spatial selectivity in subclusters similar to those found in A and B indicating reestablished cellular activity. Inset in A shows anatomical mini-map, black lines indicate sulci.

3.6 Time Course of Attentional Selection Within Topographical Hubs of Functional Activity

To determine whether or not there were regions of topographical integration between the clusters housing selective value signals and those conveying spatial attention information, we performed an ANOVA that selected for those pixels that contained cells that fired for both spatial location and value (figure, 9). Two spatially distinct regions were revealed where both attentional dimensions (space and value) converge (figure 14 A). In the ACC at the intersection of areas 24 and 32, a proportion of neurons greater than expected by chance, enhance their firing rate during shifts to a contralateral target when the expected value is high. In clear spatial separation, neurons in PFC (localized to area 9) conveyed spatial attention information together with selective increases of activity when the target stimulus was expected to be of low value. These clusters with interaction effects between reward and attention, as well as those clusters that were non-interacting are visualized on figure 14A.

We were also interested in the time course that these effects take on a cluster population level. We found that the proportion of neurons conveying statistically significant (ROC) target information (space and value) rises sharply after cue onset in both conditions (figure 14 B, C, D). This suggests that the cellular activity is a functionally relevant signature underlying successful attentional shifts. A closer inspection of the latencies of significant attentional shift signals in these zones of functional convergence showed that spatial selectivity emerged only after the onset of the spatially informative cue (figure 14 A) where

value information associated with the attention shift period was already present prior to the attentional shift but continued to ramp up following the attentional cue.

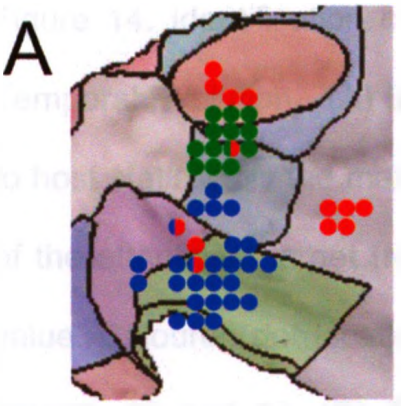
A
— Prop. of cells with significant ROC values
— Prop. of cells which became significantly active at that time bin
— Median latency of significantly active cells
(Latency= first time with sign. ROC)
Regions with significant ROC effect for:
Target Location
High Target Value
Low Target Value
B Spatially selective clusters
area 46
280ms
[SE 50]
area 9
380 ms
[SE 20]
area 8
380 ms
[SE 40]
area 24/32
380 ms
[SE 20]
Time to cue onset (sec.)
C High value selective clusters
area 24/32
350 ms
[SE 30]
area 9
400 ms
[SE 20]
Time to cue onset (sec.)
D Low value selective clusters
area 8/9/46
350 ms
[SE 40]
Time to cue onset (sec.)

Figure 14. Identification of Value and Attention Information Clusters and Their Temporal Evolution. (A) Summary sketch of those regions in the map identified to host statistically the maximum concentration of neurons predicting the location of the attentional target (red), or a high (blue), and low (green) expected target value. Coloured dot locations correspond to the boxed subclusters illustrated in figures, 10 and 12. (B) The proportion of significant ROC values (black line) predicting target location in all four panels each sampled from a independent hub indicated on mini map as a function of time relative to cue onset. Each panel considers only those neurons from those map pixels sketched as full- colour circles to the left of each panel. Red vertical line illustrates the median latency and is reported in text at the top right of each panel. The violet bar histograms indicate the earliest time epochs at which single neurons in each cluster became significantly modulated. (C) Same format as in B, but grouping together neurons from clusters conveying high target value. (D) Same format as B-C, but showing neuronal clusters predominantly conveyed low target value.

4.1 Discussion

The results of this study identify three key findings demonstrating the existence of spatially concentrated clusters of spatial attention control and reward value expectancy. First, we demonstrated by means of the ROC analysis that there are concentrated clusters of spatial attention and reward value processing in the fronto-cingulate cortex. The ROC analysis allows an ideal observer the ability to reliably predict the information coding of each cell (and thus region on the flat map) on a trial-by-trial basis. To our knowledge this is the first time that single cell recordings in rhesus macaques have been reconstructed in this manner using proven computer-generated statistical representations of the standard macaque brain (Van Essen et al., 2001; Van Essen et al., 2005). Second, we demonstrated that a spatially-confined cluster of cells have an interaction effect between reward and attention; that is, they significantly increased or decreased firing (ANOVA $p \leq 0.05$) for attention contralateral-high reward, contra-low, ipsi-high or ipsi-low conditions only. Two such clusters of cells were isolated, one to the border areas of 24-32, identified as an 'attention contralateral-high reward' cluster and a second to the border of 24-9 and 'attention contralateral-low reward' cluster. Finally, we report that the attention orienting signals present during the attention shift period of the paradigm are necessary for optimal behaviour. When these neuronal patterns of activity fail to be recruited, the monkey's behaviour resulted in errors. Their performance was reestablished only when those regions coding for spatial location were reactivated.

To date few studies in macaques have independently modulated the effects of reward and spatial attention during an attention shift and reward-expectancy period (Wallis and Kennerley, 2010). Our study provides compelling evidence that neural correlates of spatial attention and reward processing exist in the fronto-cingulate cortex. Furthermore these results suggest that the highlighted areas could potentially form an integrated network relevant to successful processing of attentional monitoring, a network centered around the area 32-24/24-9 hubs of attention-reward integration. As mentioned earlier, the anterior regions of the ACC as well as lateral prefrontal regions have been suggested as possible hubs of signal integration by various authors (Mansouri et al., 2009; Kable and Glimcher, 2009; Averbeck and Seo, 2008; Haber and Knutson , 2010; Watanabe, 1996; Rushworth and Behrens, 2008).

Our paradigm elicited strong attentional effects in the frontal cortex as well as identified localized spatial attention signals in the ACC, a finding that is novel to our knowledge in the macaque and is contrary to the findings of Kennerley et al., (2009). This difference in results could be accounted for by differences in recording location and experimental design. As we illustrated, the spatial-attention effects in the frontal cortex seem to be isolated to confined clusters in the cingulate and prefrontal cortices including those areas explored by Kennerley et al. (2009). In their experiment, Kennerley and colleagues (2009) recorded from cells in the dorsal bank of the ACC (24c, Saleem and Price, 2008), a region considered by anatomists as PFC area 9 (Petrides and Pandaya, 1997; Vogt et al., 2005). Unlike in our study, Kennerley et al. did not record from ventral ACC regions 32, (Barbas and Zikopolous, 2007) and 24a/b (Saleem et al., 2008) thus

minimizing their sampling of the frontal regions in the ACC in which we report spatial attention signals. Additionally, in their reconstruction of the recording locations Kennerley et al. (2009) used 1.5T (versus our 7T) anatomical MRI images and visual estimation to reconstruct their recording locations. This could potentially result in a large amount of variability in their reconstruction and possibly lead to assigning functions of some ACC cells to the OFC. Most importantly however, it is their experimental design that is geared more towards eliciting spatial working memory than spatial attention effects. In their task, macaque subjects were informed of a spatial location that they had to maintain in working memory until cued to respond. The duration between cue and response varied from 1 second to 2.5 seconds depending on if they were given reward information prior to spatial information. In this task, the subjects were not required to covertly distinguish between potential attentional targets as required in our paradigm. Furthermore, our task required up to 4 seconds of selective covert attention at times towards a particular target while ignoring distractor stimuli. Importantly, variants of our paradigm in the past have been shown to elicit robust spatial attention effects in the FEF as well as dorsal and ventral areas of the visual cortex (Womelsdorf et al., 2006; Gregoriou et al., 2009). As we hypothesized earlier, the fact that the ACC is so tightly-connected to the attention circuitry of the brain (Corbetta et al. 2008; Posner et al., 1988; Haber and Knutson, 2010) we excpected that spatial attention signals would be present in the ACC. Kennerley and colleagues (2009) report of a lack of spatially-related activity in the dorsal bank of the ACC is likely due to the fact that their paradigm elicits spatial working memory more so than covert top down spatial attention.

In addition to demonstrating the presence of neuronal signatures for spatial attention in confined spatial areas of the ACC we illustrated that the variation of reward value caused behavioural enhancement. This occurred exclusively during time frames when the subject had to respond early. This result is of particular interest as it illustrates that the macaques can discern which target is of higher value at a ratio of 0.3-0.7. However, their proficiency in the task shows that they have the ability to forgo the desire for higher reward for the guarantee of a smaller reward. A similar pattern of results has been reported by Watanabe et al. (2001). Interestingly this behavioural effect can be accounted for by a natural priming that the subject receives for the higher rewarded stimulus during the colour-cue period of the task prior to the attentional-cue. Leon and Shadlen (1999) reported a similar result when macaques were found to make more errors on low rewarded trials in a variant of a memory-guided saccade task. In our task when the colour cue comes on, the subject is informed of the location of the high and low rewarded stimuli and likely has a motivational bias for the highly rewarded target (Watanabe et al., 2001). As such in those trials when the cued target fulfils this motivational bias, the subject shows enhanced behaviour. Conversely when the cue directs attention to the lower rewarded stimulus, our subjects show the ability to forgo the desire for high reward and work for the assurance of a lower reward.

However, the fact that this behavioural effect is not determined by a significant variation in reaction time between high and low valued rewards illustrates that the reward value is not causing any kind of reflexive bias towards either stimulus and that the subjects are capable of processing the variables

relevant to the task (attend contra or ipsi / high or low). Leon and Shadlen (1999), also reported minimal variation in reaction time in their task when reward value was altered. Additionally, our results show that during those early phases when the target change cues the response, the reaction times are actually slower than the peak reaction time that plateaus at 280ms some 800ms into the trial. This plateau effect occurs after the behavioural effect has worn off. This further illustrates that during these early attentional shift cue periods, neural processing is occurring that allows the subject to determine the rules of the current trial and overcome motivational bias prior to making their decision to receive the reward. On the contrary however, Watanabe et al. (2001), showed that for preferred vs. non-preferred stimuli there is a behavioural difference in reaction time. They reported that trials that cued reward expectancy for preferred rewards resulted in shorter reaction times than those signaling non-preferred reward. These differences could be accounted for by the fact that Watanabe et al. 2001, used a paradigm that required arm movements versus ours that cued a visual response. They also used different types of reward (both different foods and liquids) compared to our task that offered a subtle yet evidently noticeable difference in the same liquid reward. Accounting for these differences and the fact that other studies that cued saccadic responses (Leon and Shadlen, 1999) support our findings of equal reaction times leads us to conclude that the attentional cueing of different valued targets in our paradigm causes behavioural enhancement without changes in reaction time.

In addition to the behavioural significance demonstrated by the variance of reward value, we were interested in mapping the neuronal activity correlated with

spatial attention and reward value in the ACC and PFC. We demonstrated in this study for the first time in this manner (to our knowledge), separate functional areas in the frontal cortex that flexibly convey information about location and value, sub-serving shifts in selective attention. The two patterns of activity for directing spatial attention illustrated by a higher (mostly in PFC) or lower (mostly in ACC) firing rate towards the contralateral target, suggests a differential role with respect to spatial information processing for each of these regions. Spatially separate nodes in the frontal cortex increasing their activity to targets on the contralateral and ipsilateral sides of space suggest that different hemisphere regions have different roles in selection of spatial attention targets. These patterns of activity are supported by various findings regarding neuronal activity in each of these regions. The PFC known for selective cognitive control and decision-making has been shown to house positive correlates of spatial attention (Lebedev et al., 2004; Petrides, 2005; Asaad et al., 1998). Conversely, single unit recordings and error related negativities from EEG studies have shown that the ACC hosts clear negative feedback signals from the detection of errors as well as search periods (Quilodran et al., 2007; Mansouri et al., 2009). These findings are similar to the localization of ipsilateral spatial attention information found mostly in the ACC as demonstrated by our study. Importantly, these signals could have easily been missed at a coarser population level, such as in fMRI, without voxel wise comparisons (Haynes and Rees, 2006; Kahnt et al., 2010) since we observed these cells in close proximity, enhancing their activity for different conditions (contralateral vs. ipsilateral). Additionally the effects we illustrated are confined to a time scale of 0.50s, as such, population data from fMRI would be

required to sample at a high rate in conjunction with EEG in order to illustrate the variability in the system at this temporal scale (Ogawa et al., 2000). The response to spatially distinct attentional targets from different regions of the frontal cortex is a pattern we find during periods of shifting attention. This highlights the various spatial attention effects in clustered regions of the frontal cortex. These findings reveal that separable sub-regions within the fronto-cingulate architecture regulate various different attentional effects.

In addition to the clustering of spatial-attention effects in the frontal cortex a similar pattern of activity is demonstrated for different loci conveying the expectancy of reward-value. For both high and low reward value, activation of different areas during the attentional shift period suggests that these regions differentially contribute to reward information processing. The involvement of cingulate (32/24) areas in high reward processing is supported by the dense connections to the limbic structures (amygdala, VS) and its role as a key player in the reward cortico-basal-ganglia loop (Haber and Knutson , 2010; Averbeck and Seo, 2008; Ferry et al., 2000; Ghashghaei et al., 2007). Conversely the involvement of PFC regions coding for low target value (8/9/46) is supported by the PFC's role in response inhibition and cognitive control processes (Everling et al., 2002; Kuwajima and Sawaguchi, 2007). In a model of decision making Gold and Shadlen (2007), argue that the critical variables for guiding attentional selection should incorporate a comparison of predicted values available for the stimuli. Our results point to a neural correlate of this comparison of reward prediction as soon as attention is directed towards a target stimulus. Typically, our behaviour is directed toward stimuli with highest expected value, which is not

always the stimulus with maximal positive incentive. Therefore, when attention is shifted towards low-valued targets, motivational biases towards more salient stimuli need to be over-ruled by an overarching attentional rule e.g. target left – attend left, irrespective of value. This attentional rule must win the competition against the motivational bias in order for the selection of a sub-optimal target to occur. The results we report are consistent with a recent human fMRI study that reports strong activation in the lPFC when subjects select healthier but less tasteful food options over unhealthy but tasteful options (Hare et al., 2009). In this study those trials where subjects demonstrated self control in their choice of food was associated with higher lPFC activity regardless of their predisposition to healthy or non-healthy selections. The authors suggest that the lPFC could play an important role in modulating valuation signal that come from regions of the ACC (25, 32) (Hare et al., 2009). The PFC has also been discussed to contain the necessary signals to make risk-reward calculations, which allow the subject to determine whether the outcome of a given trial is worth the effort required to receive the reward (Rushworth and Behrens, 2008). Our findings of reward related activity in the lPFC of macaques is thus supported by these functional associations of reward processing in the lPFC that relate to the ability to display self control and make calculated decisions.

In addition to the functional PFC region (8/9/46) signaling low reward value, a similar yet spatially distinct cluster in the ACC signals high reward value. This area is located at the intersection of two ACC subareas 32 and 24, which may pertain to the affective and cognitive subdivisions of the ACC, respectively, as identified with human fMRI (Bush et al., 2000). Additionally, this region of the

ACC has been highlighted as a reward centre which is involved in complex aspects of emotion such as functioning during social interaction, due to its connections with temporal and subcortical areas (Rudebeck et al., 2008). The ACC in combination with the OFC broadly defines a ventromedial prefrontal cortex network that has been shown to assign and compute subjective and economic values in order to optimize behavioural choices (Kable and Glimcher, 2009; Kennerley et al., 2009; Schultz, 2006; Kahnt et al., 2010; Averbeck et al., 2006).

A constant re-evaluation of response-outcome possibilities can serve to enhance decision-making in a context dependent manner (Gold and Shadlen, 2007). As such communication between these reward areas dictating high or low reward value is invaluable to enhance behavioural outcomes. This evaluation of reward information must be incorporated with spatial attention information in order to make correct responses (Kable and Glimcher, 2009). Our results demonstrate integration zones for spatial and reward information in anatomically confined hubs, which merge attention and high reward separately from attention and low reward. The zones of convergence between reward and attention suggest functional integration hubs of a frontal attention-reward network. Since each area involved in the network requires the information from other variables in order for the system as a whole to be able to conduct efficient information coding, these zones of functional integration are of critical importance to behaviour, and as we report they are inactive on error trials. Additionally, we demonstrate that each of these and the other functional clusters show differential latencies in the recruitment of significantly active cells. Timing differences in the recruitment of

the various clusters further supports the notion that these areas are involved in a functional attention-reward network. Since the timing of recruitment between spatial attention and reward-associated events differ, this suggests that each area is brought into play at different times to incorporate the various signals into a behavioural outcome. The results show that spatial attention signals are recruited after the onset of the attentional cue; a result we would expect as target location is not known prior to the attention cue onset. Conversely, reward related activity already shows between ten to twenty percent significant recruitment prior to the onset of the attentional cue. This as well could be an expected result as reward information is provided during the colour-cue period where the subject is informed of the location of both the high and low rewarded targets. Furthermore in both conditions, attention and reward, the number of significantly modulated cells is show to increase to a maximum then decrease some 0.5 to 0.7 seconds following cue onset. This further suggests the role of these neurons during the attention shift period of our paradigm. Further investigation of these time courses and their relevance to the activation of the signal for each region should be conducted in order to determine the time course of signal evolution between these functional hubs.

The behavioural and neuronal results gleaned thus far lead us to question the functions of the cells in the frontal cortex during attentional shift periods on error trials and compare those results to the clusters identified on correct trials. Our results illustrated that a failure to recruit the necessary spatial attention signals would result in erroneous behaviour, suggesting that these patterns of cortical activity are necessary for informing the correct response to the attentional

target. An analysis of those correct trials succeeding errors illustrated the re-establishment of activity in those functional loci conveying spatial attention information. Although these results do not perfectly mimic those found on the average of all correct trials (potentially due to a smaller sample size since the animals were quite proficient at the task and made few errors (see Methods for definition of "errors")) the results are spatially similar. Therefore we can suggest that these areas of the brain are playing some critical role in determining the allocation of spatial-attention that therefore makes some contribution towards behavioural outcomes between correct and error trials. This pattern of cellular activation and deactivation on error trials has been shown by many studies at a single cell and population level but has never been mapped out across a broad cortical region in this manner (Asaad et al 1998; Mansouri et al., 2006). Furthermore, these results are supported in part by human fMRI experiments where activity breaks down building up to an error. In one study authors demonstrated that informative cues in the ACC, directing anticipatory control leading to correct performance, show greater activity when compared to non-informative cues that lead to errors. That is from one perspective a measure of uncertainty within a network that dictates that the outcome will likely be an error (Aarts et al., 2008). Furthermore another human imaging study has demonstrated that decreases in the deactivation of the default mode network during a task, up to 30 seconds before an error occurs, is a pattern of activity in those correct trials leading up to an error. This pattern suggests a build up of activity on a population level that modulates behavioural outcomes on a trial-by-trial basis (Eichele et al., 2007).

Furthermore, inspection of figure 13 D which illustrates the activity of those cells decreasing their firing for a contralateral stimulus shows an increase in activity (although not significant potentially due to small sample size) in area 46. This pattern is similar to that found in figure 13 A which shows a functional region that increases firing for spatial attention to the contralateral side. This increased firing on error trial could be accounted for if the animal were attending (and responding) to the wrong stimulus; that is, the ipsilateral stimulus, which could potentially recruit that region as shown in figure 13 A. Additional investigation is required to determine the inner workings of these functional networks in order to better correlate their patterns to behaviour.

5.1 Summary and Conclusions

The findings highlighted in this study pave the way for further investigation as to the role of each of theses functional zones within an active attention-reward network in the frontal cortex. The clustering of spatial and value information is prevalent for attentional-control in larger network models of flexible goal-directed behaviour (Kable and Glimcher, 2009). These networks rely on contextual rules that form the general scaffold for guiding interpretation of incoming sensory information (Gold and Shadlen, 2007). Our results suggest that value information is flexibly recruited from functional hubs in the frontal cortex to sub-serve relevant shifts in spatial attention. The relative weight of activity from each of these nodes could be the determining factor that dictates how much attention a particular target is given and should be determined by continued investigation. Additionally the spatial separation of selective value signals for high and low reward suggests

that attentional top-down control may originate from a comparison of available value information encoded not within a single homogenous area but by the interaction of two separable neuronal populations (Corbetta et al., 2008). Further research is required to determine how each of these regions act within the confines of an attention-reward network and how their interactions lead to successful attentional orienting and thereby influence optimal behaviour.

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Appendix 1 – Ethics and protocol approval

Western

Dec. 30, 2008

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2012

Dear Dr. Everling

Your Animal Use Protocol form entitled:
Role of Frontal Cortex in cognitive control
Funding Agency: CIHR - Grant #R3104A12, NSERC DISCOVERY - Grant - PYFRR

has been approved by the University Council on Animal Care. This approval is valid from **Dec. 30, 2008** to **Dec. 31, 2009**. The protocol number for this project is **#2008-125 and replaces #2004-099-12.**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 4 Years

Species	Strain	Other Detail	Pain Level	Animal # Total for 4 Years
Other, add to detail	NHP - Macaca mullata or Macaca fascicularis	3-16 kg	D	20

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

c.c. Approved Protocol - S. Everling, T. Admans
Approval Letter - S. Everling, T. Admans

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, ● London, Ontario ● CANADA N6A 5C1
PH: 519-661-2111 ext. 86770 ● FL: 519-661-2028 ● www.uwo.ca / animal

Western

01.01.11
*This is the 2nd Renewal of this protocol
*A Full Protocol submission will be required in 2013

Dear Dr. Everling

Your Animal Use Protocol form entitled:

Role of Frontal Cortex in Cognitive Control

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from 01.01.11 to 01.01.12

The protocol number for this project remains as 2008-125

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.
The holder of this *Animal Use Protocol* is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c. B. Soper

The University *of* Western Ontario
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