VARIABILITY OF NEURAL RESPONSES
IN THE LATERAL PREFRONTAL CORTEX

BY

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LIST OF ABBREVIATIONS

Analysis of Variance – ANOVA

Attention Deficit Hyperactivity Disorder – ADHD

Brodmann Area – BA

Delayed Discrimination Response – DDR

Dorsolateral Prefrontal Cortex – dl-PFC

Lateral Prefrontal Cortex – lPFC

Magnetic Resonance – MR

Oculomotor Delayed Response – ODR

Peri-stimulus time histogram – PSTH

Prefrontal Cortex – PFC

Variance of the Conditional Expectation – VarCE
ABSTRACT

The neurophysiological signature of visuospatial working memory is a conspicuous persistent activity throughout a delay period in which the stimulus is absent. The dorsolateral prefrontal cortex has long been implicated as the seat of visuospatial working memory, and undergoes dramatic increases in volume and functional connectivity during and around the time of puberty. However, much of the neurophysiological changes between the two developmental periods remains obscured, including that of how response variability changes. This is important, since recent studies have shown that mean firing rate alone may not be sufficient to fully explain neural responses as a function of task demands and stimulus intensity. Here, we address two primary questions: First, does response variability change significantly as a function of pubertal development? And second, does task difficulty modulate response variability in any meaningful way? We also looked at possible effects of the hemifield of the response on variability. What we found was a modest effect of development on Fano factor during the cue epoch and a generally higher Fano factor for ipsilateral responses in adult monkeys. Task difficulty had no definitive effects on response variability as measured by the Fano factor here.
CHAPTER I

INTRODUCTION

BY

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“Working memory” refers to the ability to maintain stimulus information in mind throughout a period of time in which that stimulus is not readily available to a given sense (Baddeley, 1986). Working memory could be thought of as a complex array of other cognitive attributes (selective attention, response inhibition) rather than a standalone property (Baddeley 2003), making working memory functioning a useful benchmark of overall cognitive ability and a diagnostic criterion for many cognitive and developmental disorders (Silver et al. 2003, Marquand et al. 2008). The dorsolateral prefrontal cortex (dl-PFC) and the parietal region most strongly connected to it, the posterior parietal cortex (Champod & Petrides 2007, Katsuki & Constantinidis 2012, Rawley & Constantinidis 2009, Cavada and Goldman-Rakic 1989, Constantinidis & Procyk 2004), have been implicated as key regions for visuospatial working memory representations via direct neurophysiology in animal models (Fuster & Alexander 1971, Funahashi et al. 1989, Levy & Goldman-Rakic 1999). However, the dl-PFC has been shown to be better able to hold salient stimulus representations in working memory while ignoring distractors than the posterior parietal cortex (Suzuki & Gottlieb 2013, Qi et al. 2010, Constantinidis & Steinmetz 1996) and has resulted in marked deficits in ability to maintain task information when lesioned (Buckley et al. 2009). Cross-sectional studies using fMRI and PET imaging have also confirmed the recruitment of areas throughout the PFC in working memory tasks performed by human adults, with the dorsolateral PFC being a notably important region for several cognitive functions (D’Esposito et al. 2000, D’Esposito 2007, Chugani et al. 1987, Sowell et al. 2001). Due to these properties, the dl-PFC is widely considered to be the seat of visuospatial working memory.
Variability as a Function of Task Difficulty

Visuospatial working memory representations are characterized by a conspicuous level of persistent activity in the dl-PFC throughout a delay period in which the stimulus is not available for viewing (Fuster & Alexander 1971, Funahashi et al. 1989). Previously, it was shown that firing rate during the delay period of a delayed discrimination response (DDR) task was not modulated by the difficulty of the task stimuli when the target was in the receptive field of a task-responsive neuron, while the firing rate was modulated by the luminance of the diametrically-located distractor of variable luminance when it was present in the receptive field (Constantinidis et al. 2001). Irrespective of task difficulty, neuronal responses during the delay period were maintained at a high level when the target was in the receptive field and were significantly higher in firing rate than responses to distractor stimuli, indicating that working memory representations of task responsive neurons are being driven by the remembered sensory trace of the target stimulus (which was always higher in luminance except for one condition in which it was the same luminance as the distractor). However, recent findings have indicated that mean firing rates may not fully explicate the effects of a stimulus or task demands on neural responses (Churchalnd et al. 2010).

The mean firing rate of a neuron or populations of neurons is one of the more common reporting methods for displaying responses to stimuli or task demands. However, mean firing rates vary widely in the cortex (Softky & Koch 1993, Vogel et al. 2005) such that, for a serially repeated stimulus, a cortical neuron will likely respond with a different number of spikes and different temporal distribution of spikes within a given window of time with each presentation (Shadlen & Newsome 1998). Some of the firing
rate variability evinced during the course of a given task will be the result of either relatively uninformative sensory noise caused by random fluctuations amplified early on in the transduction step in a sensory modality’s pathway (Faisal 2008); however, at least some of the variability evinced may have something to say about cortical state (Scholvinck et al. 2015, Harris & Thiele 2011) or even about stimulus information (Scaglione et al. 2011, Averbeck et al. 2006). In Churchland et al. (2010), it was illustrated that variability saw an immediate suppression as firing rate increased dramatically with stimulus presentation. This effect was seen across multiple cortical areas, at various states of alertness, at differing levels of stimulus salience, and in different test species, indicating suppression of variability with the presence of a stimulus possibly being a global attribute of the cortex or an indicator of a very widespread cortical network. Either possibility could fit in with current theories about neural network mechanisms of variability reduction (Deco & Hugues 2012).

To assess neuronal response variability, a commonly used metric is known as the Fano factor \( \left( \frac{\sigma^2_w}{\mu_w} \right) \), or the across-trial variance of the neuronal firing rate \( \sigma^2 \) divided by the mean of the neuronal firing rate \( \mu \) within a given window of time \( W \). The Fano factor easily expresses how well a response fits a Poisson process (Eden & Kramer 2010), the process most often used for spike modeling. Additionally, the Fano factor possesses the added flexibility of being about to quickly look at variability at different timescales by varying the window of collected spikes (Teich et al. 1997). Other methods to test response variability are common (Amarasingham et al. 2006, Churchland et al. 2011, Nawrot et al. 2008), but not as common as the Fano factor for the aforementioned reasons.
Recently, work by Ruff & Cohen (2014) showed a suppression of Fano factor variability with increasing task difficulty alongside lowered noise correlations in area V4 as Rhesus macaques (*Macaca mulatta*) performed discrimination task with variable levels of difficulty. While the effect was only seen to any degree of significance at the hardest difficulty levels, the authors interpreted this as the deployment of attention as a global cognitive variable modulating the variability of responses. This is in line with previous work showing that the allocation of attention to a task may have a role in reducing variability of single-neuron responses and covariability between neurons in the same brain area (Cohen & Maunsell 2009, Mitchell et al. 2007). This result motivated further analysis of the dataset by Constantinidis et al. (2001) to assess whether or not variability as measured by the Fano factor might be modulated as a function of difficulty. Other work had shown that increasing the number of choices in a task also increase the variability (Churchland et al. 2011), but few studies have looked at response variability using multiple levels of difficulty. The DDR task used originally in Constantinidis et al. (2001) is perfect for this, since luminance contrasts can be made to vary widely and give a broad range of difficulty conditions to analyze. In addition to this, the stable spiking across difficulty conditions during the delay period for when the target is presented inside the receptive field is an interesting detail into which analysis variability might lend further insight. It is hypothesized here that there will be a modulation of Fano factor variability as a function of task difficulty, with a soft assumption that variability would increase with increasing task difficulty. Very few studies have directly looked at prefrontal measures of response variability (Hussar & Pasternak 2010), so any findings here could help further characterize the region.
Variability as a Function of Development

During and around puberty, a noticeable increase in the ability to perform working memory tasks is evidenced (Luna et al. 2001). This coincides with a more robust recruitment of the fronto-parietal network (Crone et al. 2006, Tau & Peterson 2010) and a refinement of specific recruited regions compared to late childhood (Konrad et al. 2005, Geier et al. 2009) while engaged in a cognitive task. Assessing the changes between the adolescent and adult state for working memory has the potential to be particularly informative due to improvement in working memory tasks being highly correlated with other cognitive attributes’ enhancement as well (Luciana & Nelson 1998). The developmental changes that occur in the prefrontal cortex (PFC) during and around the period of puberty and into early adulthood are associated with increases in gray and white matter volumes in PFC and connecting regions of the brain such as the posterior parietal cortex (Olesen et al. 2003), increases in strength and number of connections from the PFC to other cortical and subcortical areas (Hwang et al. 2010), and changes in connectivity between neurons of the PFC (Zhou et al. 2014). It is also known that there is a refinement of areas recruited during complex cognitive tasks during puberty (Konrad et al. 2005, Geier et al. 2009) alongside strengthening of the connections of the fronto-parietal network (Crone et al. 2006) and changes in connectivity within the PFC (Zhou 2014). However, concomitant changes in neural representations of working memory stimuli and how those stimuli inform decision-making continue to be more poorly understood. The heavier recruitment of the fronto-parietal network as a result of puberty is most clearly seen in cases where there is a relatively difficult task present (Crone 2006,
Tau & Peterson 2010), and continues to be strengthened into adulthood for visuospatial working memory tasks.

The particular suite of changes that single PFC neurons and neuronal populations undergo between juvenile and adult life stages remains poorly understood, since characterizations of these properties in a longitudinal study using the same subjects has been rare. In particular, the variability of these representations and how they inform decisions could be fundamentally different between adolescent and adult states as a result of anatomical maturation, which would have direct implications on stimulus encoding (Averbeck et al. 2006). Variability may also be informative about distinctions between stimuli by populations of cells (Serences et al 2009).

In an attempt to understand how variability of neuronal responses might change with maturation of the prefrontal cortex, monkeys were trained to perform an oculomotor delayed response (ODR) task and neurophysiological recordings were collected during two different developmental periods for the same subjects. Since the transmission of information by single neurons would benefit the most by a lowering of the Fano factor to near-Poisson process spiking, it was postulated that neuronal response variability would decrease as a function of developmental stage, leaving adults with overall less variable neuronal responses.
CHAPTER II

VARIABILITY OF PREFRONTAL NEURON ACTIVITY AS A FUNCTION OF TASK DIFFICULTY AND STIMULUS LUMINANCE

BY

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ABSTRACT

Variability in neural firing rates has long been a target for analyses across several areas of the cortex, with high levels response variability being seen for repeated presentation of the same stimuli. Some of this firing rate variability is the result of relatively uninformative sensory noise. Recent work has supported both the idea that suppression of this noise with stimulus presentation is a general feature of cortical neurons and that changes in variability may have something to say about cognitive variables such as task difficulty. As there have been very few studies that have looked beyond sensory and association regions to variability evinced in frontal regions, here we present analyses of variability using the Fano factor for a dataset drawn from responses in the lateral prefrontal cortex (PFC) that exhibits little change in firing rate with different task difficulties. The present analysis found no significant between-condition differences of Fano factor variability; this lack of significance held true when the Fano factor was separated based on task epoch (fixation, cue, and cue delay periods) as well as when separated based on location of receptive field response. This ran counter to our initial expectations, since previous findings indicated that there could be a modest modulation of the Fano factor with increasing task difficulty and attention. The results presented here suggest that while cognitive factors may modulate variability independently of firing rate, they do not necessarily do so across all experimental conditions.
INTRODUCTION

Information about stimulus properties and cognitive states is most often conceived as conveyed by the mean firing rates of neurons. However, the variability of neuronal discharges may also be informative. For this reason, variability in neural firing rates has long been a target for analyses across several areas of the cortex, with particular emphasis on observing the change of firing rate variability under specific conditions or task demands. Cortical neurons tend to have high levels of response variability to similar stimuli (Softky & Koch 1993, Vogel et al 2005), making changes in variability somewhat difficult to interpret. Some of the firing rate variability evinced during the course of a given task will be the result of either relatively uninformative sensory noise caused by random fluctuations amplified early on in the transduction step in a sensory modality’s pathway (Faisal 2008) or the result of more complex synaptic noise possibly involved with other processes like long-term potentiation (Faure et al. 2000).

While much of the ambient variability seen seems throughout cortical regions appears to be roughly constant (Shadlen & Newsome 1998), subcortical regions of the brain appear to have lower response variability. This difference in variability between cortical and subcortical regions has been seen in the visual pathways of anesthetized monkeys and appears to be relative invariant with increasing response strength (Edwards et al. 1995; Croner et al. 1993). Though it has been reported that much of the variability evidenced in V1 neurons of alert monkeys is reduced when the effects of eye movements are accounted for (Gur et al. 1997), this phenomenon has often been characterized as a built-in threshold (Faisal 2008) that salient sensory signals must overcome to successfully inform decisions.
How the brain actually deals with noise that has little informative value and cannot be removed from the neural signal is as important as the noise itself. In general, the nervous system utilizes two methods to reduce the negative impacts of noise (Faisal 2008). The first of these, averaging, is seen at multiple levels but most starkly at earlier levels of sensory pathways (Kandel & Schwartz 2012). Averaging, as might be guessed, involves averaging signal from cells carrying the same information but different, ostensibly independent sources of noise. However, noise in the nervous system is generally correlated (Averbeck 2006), even in cases in which that correlation has an overall small effect on information (Averbeck & Lee 2004). Additionally, averaging works better under conditions where all signal sources possess the same quantity of noise (Faisal 2008), thus making averaging a poor tool on its own for use in reducing negative noise. Prior knowledge, the second of the nervous system’s methods to reduce negative noise, involves the use of known parameters or structure of a signal to produce a best estimate (Kording & Wolpert 2004). This sort of prior knowledge has been shown to be useful for producing probabilistic decisions in a bayesian manner (Keshvari et al. 2012, Kording & Wolpert 2004, Yang & Shadlen 2007, Beck et al. 2008) superior to decisions made with sensory information alone. In this case, prior knowledge within the brain about the structure of the inputs –that is, which inputs are noisier and which inputs have a higher signal-to-noise ratio – would allow the brain to appropriately weight the proper inputs when averaging. This might not necessarily mean that correlated inputs with noise would receive weaker weights, since neurons with correlated noise can actually sometimes carry more information (Averbeck 2006), but that knowledge of the structure can allow weighted averages that take into account only useful signal and useful noise. It
has also been illustrated that at least some of the noise present in neural systems could be beneficial by enhancing sensitivity to signals that are otherwise low in power (Jones 2005; Wiesenfeld & Moss 1995). Variability may also be informative about distinctions between stimuli, potentially reflecting more information being transmitted both by single cells (which would also increase redundancy) and populations of cells (Vogel et al 2009). Furthermore, sensory variability could be used by the brain in a probabilistic fashion combined with prior knowledge to produce optimal decisions (Jones 2005; Knill & Pouget 2004; Wolpert 2004; Loeb & Fishel 2014) as supported by human studies (Thurman & Lu 2014; Knill 1998; Saunders & Knill 2001; Kaya & Elhilali 2014).

Regardless of how this observed variability is utilized by the brain, it is at least clear that there may be some value in it beyond what is captured by mean firing rates.

Support has been lent to the idea that the onset of a stimulus during the course of a trial consistently drives variability down irrespective of the salience of the stimuli to a behavioral task, the state of alertness observed in the test animal (anesthetized versus awake), whether variability is being observed at the level of firing rates or the level of membrane potentials, or even the cortical area being recorded from. (Churchland et al 2010). Churchland et al. (2010) suggest that a decline in variability upon stimulus onset is either an indication of a widespread cortical network being present or that a stimulus-driven decline in variability is a natural general property of the cortex. A recent study in humans utilizing electrocorticography while subjects performed a visual stimulus detection task showed a similar reduction in post-stimulus variability (He & Zempel 2013). Additionally, the temporal component of the stimuli (whether it is constant versus dynamic/artificial versus natural) appears to have little effect on the variability evidenced,
indicating that variability is not reduced with ethological versus artificial stimuli
(Scaglione et al 2014; Warzecha & Egelhaaf 1999).

Aside from its ultimate role in the nervous system, how response variability might be modulated depending on context is still a largely unresolved question. Previous studies have often shown concomitant changes in variability with changes in mean firing rate (Churchland et al. 2010, Churchland et al 2011); however, other studies have observed changes in variability without significant changes in firing rate (Cohen et al. 2007), and recently it was found that increasing difficulty in a discrimination task decreased co-variability of pairs of neurons in macaque visual cortex alongside a slight lowering of Fano factor for individual neurons (Ruff & Cohen 2014b). Attentive processes have also been implicated in reducing Fano factor of neural responses and co-variability between neurons in area V4 (Cohen & Maunsell 2009, Mitchell et al. 2007) for salient stimuli in the receptive fields of specific neuron-pairs (Ruff & Cohen 2014a). This is important, since this variability could have direct effects on encoding (Averbeck et al. 2006) and perhaps underlie differences in behavioral outcomes in a task.

The prefrontal cortex (PFC) has long been implicated as essential in a suite of key cognitive abilities such as working memory (Miller & Cohen 2001). With this being the case, variability of neural firing rates in higher cortical areas such as the dorsolateral PFC might be valuable to discern how task stimuli of different difficulties bias behavioral outcomes even in cases in which mean firing rate changes very little. Very few studies thus far have looked at response variability in frontal regions of the brain (Cohen et al. 2007, Hussar & Pasternak 2010, Qi & Constantinidis 2012), with even much of the recent literature focusing on earlier regions in primary and associative sensory cortices.
(Steinmetz & Moore 2010, Abolafia et al. 2013, Ponce-Alvarez et al. 2013, Ruff & Cohen 2014a, Ruff & Cohen 2014b, Scaglione et al. 2014, Scholvinck et al. 2015, Mochol et al. 2015). In an attempt to better characterize possible changes in response variability due to a cognitive variable such as task difficulty, rhesus macaques (Macaca mulatta) were trained to perform a delayed discrimination response (DDR) task with variable difficulty conditions (as originally reported in Constantinidis et al. 2001). Neural data obtained from the lateral prefrontal cortex was then used to calculate a metric of across-trial variability, the Fano factor, to assess changes in response variability to the task’s changing difficulty. We hypothesized modulation of response variability at the highest task difficulties (Ruff & Cohen 2014) in conditions where the stimulus always appeared in the receptive field and which exhibited little difference in firing rate.
METHODS

Behavioral data was collected from monkeys trained to perform variants of working memory tasks and neurophysiological data were collected from their prefrontal cortex (figure 1). Additionally, secondary analysis was performed on a dataset collected previously, in a similar experiment (Constantinidis et al. 2001). Methodology for this work is briefly summarized here alongside analyses used for current work.

Behavioral task.

Monkeys were trained to perform an oculomotor delayed response (ODR) task (figure 2c) and a variant of that task, the delayed discrimination response (DDR) task (figure 2a). The basic ODR task required brief fixation of a central point for 500 ms, followed by the presentation of a cue (1° in size) in one of eight possible locations found equally spaced in the periphery for 500 ms, then a variable delay period of 1500-3000 ms, and finally a decision period in which a saccade must be made to the location of the presented cue stimulus within 500 ms after extinction of the fixation point. This common task had been used in previous research as a basic means of testing visuospatial working memory in behaving rhesus macaques (*Macaca mulatta*) (Funahashi, Bruce & Goldman-Rakic 1989; Funahashi, Bruce, & Goldman-Rakic 1991). The DDR task was built as a variant of the ODR task in which the time course of events (500 ms fixation, 500 ms cue presentation, 1500-3000 ms delay period, 500 ms maximum decision period) was retained with the addition of a secondary, distractor stimulus in the cue presentation period. The distractor could be one of eight possible contrast ratio values between 0 % (easiest) and 100% (hardest) as measured by the luminance difference of the distractor from the background.
(ΔL₄) divided by the luminance difference of the target from the background (ΔLᵣ). This distractor stimulus would appear concurrently with the target stimulus (randomly assigned to one of two locations each trial) in the diametrically opposed location (14° eccentricity) and would vary in luminance between background luminance and the target luminance (110 cd/m² or 150 cd/m²). The monkey would be required to ignore this stimulus and make a saccade to the remembered position of the brighter target stimulus during the decision period of the task to receive a juice reward. Performance seen in a monkey trained to perform this task for an upcoming experiment can be seen in Figure 3. Cases in which the stimulus and distractor were equiluminant were catch trials meant to demonstrate that there was no significant bias toward systematically choosing one location over the other. For these trials, the monkey would randomly receive a reward upon making a saccade to either stimulus. The DDR task was constructed for this experiment to illustrate two principles. First, mnemonic activity (measured here as delay period activity representative of working memory) is reflected in differences of firing rate dependent on the contrast strength of the target versus the distractor. Second, these reflected differences of firing rate have a strong relationship with psychophysical performance (Constantinidis et al. 2001).

Electrophysiological recordings and set-up.

All eye-position tracking was accomplished through the use of a non-invasive, infrared eye tracking system. In the earlier experiment (Constantinidis et al., 2001), an implanted scleral eye coil was used. To facilitate extracellular recordings, a craniotomy was
performed to expose a section of the prefrontal cortex around the posterior third of the principal sulcus. Additionally, a head-restraining device was implanted to keep the monkey’s head as well as the recording apparatus stable during behavioral training and electrophysiological recording. Once implanted, one to four varnish-coated tungsten electrodes (1-4 Mega-Ohms at 1 kHz) were advanced into the cortex and recording was performed at 25 µs sampling resolution (30 µs in the original study, Constantinidis et al., 2001). All animal training, surgeries and experimental procedures were done in accordance with NIH guidelines, and approved by the Wake Forest Animal Care and Use Committee.

Data analysis

Recorded spike data in the dataset of Constantinidis et al. (2001) that had been shown to be significantly above baseline during the course of at least one task epoch and had displayed distinct neural responses to a stimulus presented at each location of the DDR task were used in the primary analysis of variability seen here. Neurons with primarily cue-related or saccade-related were included as part of the analyzed sample alongside neurons with principally delay-period activity.

We performed variability analysis using the Fano factor ($\frac{\sigma^2_w}{\mu_w}$, where W represents the window of time at which the calculation is being performed), which was accomplished using adapted MATLAB (Mathworks) code from Churchland et al. (2010). Fano factor was computed using a 100 ms sliding window moving in 50 ms steps and plotted across task epochs and across condition both datasets. Fano factor values were
also computed for the entire fixation, cue, and delay epochs. A one-way ANOVA was then employed to test for significant differences of epoch-long Fano factor across conditions (varying luminance of distractor / location of stimulus) of the DDR task as well the ODR task (control analysis for difficulty-related variability). In addition, a Wilcoxon rank-sum test was employed to further investigate possible differences between contralateral (N=35) and ipsilateral (N=17) neuronal subpopulations included in the study.
RESULTS

Behavior

A contrast ratio was previously calculated by taking the difference between the luminance of the distractor and the background, $\Delta L_d$, divided by the difference between the luminance of the target and the background, $\Delta L_t (\Delta L_d / \Delta L_t)$ (Constantinidis et al. 2001). In order of increasing difficulty (left to right), the distractor stimuli used for the DDR task are shown in Figure 2b. Behavioral performance for one monkey performing this task with a range of luminances can be seen in Figure 3. Behavioral performance dropped rapidly after initial easy conditions, but did not reach the 50% correct behavior for the hardest condition in which stimulus and distractor are equiluminant. This is due to a feature of the current training task that requires the monkey to successfully complete a certain number of a given condition before the training session is over; this allows situations in which the monkey will serially be presented with the same condition until the correct choice is chosen, causing the increased performance for the equiluminant distractor-target condition. This is to be amended for recording sessions, with data recorded expected to match previous behavioral curves from Constantinidis et al. (2001) with behavior heading to 50% (random) for equiluminant conditions.

Neural Results

Analysis centered on 52 previously (Constantinidis et al. 2001) isolated neurons of the lateral prefrontal cortex (BA 8 & BA 46, Figure 1) of two rhesus macaques (*Macaca mulatta*). This region of the brain was selected for its responsiveness during working
memory tasks, particularly those for visuospatial working memory in the dorsal portion of this region (Funahashi, Bruce & Goldman-Rakic 1989). Since some neurons sampled appeared to be taken from regions inside of the inferior convexity, an area more ventral to the ventral lip of the principal sulcus than what is commonly considered dorsolateral prefrontal cortex, it is likely that at least some of the sampled neurons were additionally responsive to non-spatial stimuli as well as salient spatial stimuli (Wilson, Scalaidhe, & Goldman-Rakic 1993, Rao, Rainer, & Miller 1997).

**Variability across task difficulty**

While the monkeys were engaged in the DDR task (Figure 2a), 52 neurons’ responses were recorded; 35 of these responded best when the target was being presented at a contralateral location, while 17 responded best while the target was being presented in an ipsilateral location. Concurrent responses to distractor stimuli were also captured during this period for both locations for all neurons. The Fano factor ($\sigma^2_w/\mu_w$), a widely used metric of variability useful for its capacity to quickly indicate how closely a distribution obeys a Poisson process (Eden & Kramer 2010), was calculated for all 52 neurons using a 100 ms sliding window with 50 ms steps. This produced 100 measures (bins) of Fano factor over 5000 ms for 16 stimulus conditions for contralateral (N=35) and ipsilateral (N=17) neurons.

For contralateral neurons, stimulus conditions 1 through 8 represented contrasts of increasing difficulty (more difficulty discriminating target and distractor) for responses to the target, while 9 through 16 corresponded to the same distribution of contrast difficulty
for responses to the distractor. For ipsilateral neurons, stimulus conditions 1 through 8 represented contrasts of increasing difficulty for responses to the distractor, while conditions 9 through 16 represented corresponding responses to the target. Averages for bins corresponding to fixation (bins 1 through 20), cue (bins 21 through 30), and delay (bins 31 through 90) periods were then plotted by condition corresponding to levels of distractor luminance (Figure 2b) for responses recorded either while the target was eliciting responses inside the receptive field (Figure 4) or while the distractor was eliciting responses inside the receptive field (Figure 5). Consistent with previous findings (Churchland et al. 2010), there appeared to be a decrease in Fano factor for cue period relative to the preceding fixation period across most task difficulty conditions (Figures 4a-b & 5a-b). This decrease for cue period across stimulus difficulty was observed for all responses seen while the target was in the receptive field (Figure 4c), but most strongly for the contralateral subset of neurons (Figure 4a). Compared to Fano factor for responses to the target, Fano factor for responses to the distractor (Figure 5a-c) had delay period variability that appeared to be much higher than cue period variability across stimulus conditions.

It has been shown previously that task difficulty can influence neural response variability (Ruff & Cohen 2014). Furthermore, the very act of attending to something salient (Mitchell et al. 2007) or more cognitively difficult (Ruff & Cohen 2014) than being at rest may aid in reducing the Fano factor to a level closer to what is representative of Poisson firing (Fano factor = 1, variance equals mean spike rate). To test for significant differences of Fano factor variability between conditions of differing difficulty, a one-way ANOVA was employed using data from either responses to the
target or to the distractor. For mean Fano factor for responses to the target within the receptive field, there was no significant difference between conditions for fixation (P=0.75), cue (P=0.90), or delay (P=0.37). In cases in which the distractor was eliciting responses in the receptive field, a similar lack of between-condition difference in variability was observed for fixation (P=0.87), cue (P=0.93), and delay (P=0.97). This shows that task difficulty had no apparent effect on the Fano factor. This ran counter to our initial expectations, since previous findings indicated that response variability may be modulated incidental to increases in task difficulty (Mitchell et al. 2007, Ruff & Cohen 2014).

Variability of ipsilateral and contralateral neurons

In an additional step of analysis, the Fano factors for all responses either to the target within (Figure 6a) or the distractor within (Figure 6b) the receptive field were plotted over time alongside means for the ipsilateral and contralateral subpopulations. Each subpopulation had visibly different mean responses outside of the time around cue presentation, when each average was pushed close to a Fano factor value of 1 (Poisson spiking). This held true for responses both to the target and to the distractor in the receptive field. A series of t-tests were performed to test for differences between contralateral and ipsilateral subpopulations for when either the target or the distractor was in the receptive field for fixation, cue, and cue delay epochs. For fixation and cue epochs, there was no significant difference between ipsilateral and contralateral neurons for either target (fixation, P=0.50; cue, P=0.37) or distractor (fixation, P=0.36; cue, P=0.80) responses. The cue delay epoch approached significance for the target response (P=0.10),
but was still insignificant along with the distractor response (P=0.28). A Wilcoxon rank-sum test was also used and returned results that agreed that there were no significant differences between any of the distributions. All responses (both inside and outside the receptive field) were also plotted together depending on whether they were in the contralateral location (Figure 7a) or in the diametric ipsilateral location (Figure 7b). In each case, mean Fano factor for responses to the target and distractor inside of the receptive field were close in value to each other and the Fano factor profiles of all averaged conditions’ responses over time were similar. This is reflected in the p-values obtained from the means for each group when a t-test was applied. For the ipsilateral neurons, there was no significant difference between the responses to the target and the responses to the distractor (fixation, P=0.95; cue, P=0.74; cue delay, P=0.93). This was true for the contralateral neurons as well, with no significant differences between target and distractor for any epoch (fixation, P=0.66; cue, P=0.75; cue delay, P=0.54). The lack of significant difference was again confirmed using a Wilcoxon rank-sum test in each case. Notably, however, the contralateral and ipsilateral distributions of target and distractor responses were less significantly different from one another than when ipsilateral and contralateral neuronal responses were plotted together for target and distractor responses. This, alongside the visible difference in figure 6, seems to indicate a general, non-significant difference in variability based on hemifield.
DISCUSSION

The present analysis found no significant between-condition difference of Fano factor variability for data obtained from Constantinidis et al. (2001). This lack of significance held true when the Fano factor was separated based on task epoch (fixation, cue, and cue delay periods) as well as when separated based on location of receptive field response. This ran counter to initial expectations, since previous findings indicated that there could be a modest modulation of the Fano factor with increasing task difficulty (Ruff & Cohen 2014b) and attention (Mitchell et al. 2007, Ruff & Cohen 2014a). Furthermore, engagement in a cognitively demanding task has previously shown both an expected decrease of Fano factor with stimulus onset (Churchland et al. 2010) as well as increased information encoding preferential to task-relevant stimuli (Abolafia et al. 2013).

Why no change in Fano factor was evidenced between stimulus conditions is especially puzzling in light of a potential direct effect of neural response variability on the amount of information conveyed (Averbeck 2006). It has been previously illustrated in middle temporal (MT) neurons of rhesus macaques (*Macaca mulatta*) that response rate variability varies alongside noise correlation with different stimulus directions in a motion direction experiment (Ponce-Alvarez et al. 2013). Beyond this, it was found that stimulus-evoked covariance contributed to information transmission and encoding, which agrees well with previous studies (Averbeck and Lee 2006, Averbeck et al. 2006), but with a reported effect that was somewhat larger (Averbeck and Lee 2006, Averbeck and Lee 2004) to the point of making the population code as accurate as a population of independent neurons. Ponce-Alvarez et al. (2013) also explored the effects of varying luminance contrasts on the Fano factor, both in the preferred and anti-preferred directions.
of their task. Critically, they found a roughly monotonic decrease in population Fano factor with increasing Michelson luminance contrast (defined as the luminance of the grating minus the background divided by the luminance of the grating plus the background) for the preferred direction, indicating a decrease in Fano factor with increased ability to discriminate stimuli. As the DDR task utilized in our analysis relied on the monkey’s ability to reliably discriminate stimuli of varying luminance contrasts, a similar effect for population Fano factor across conditions might have been expected in at least one of the task epochs. Instead, what is seen is a general, non-significantly lowered Fano factor across all luminance conditions for the cue epoch in all neurons when the target elicits a response in the receptive field, as seen in figure 4C. At face value, this seems to indicate that the behavioral results plotted in figure 3 (taken from current round of experiments, comparable to psychometric curves reported in Constantinidis et al. 2001) may be explicable by firing rate alone.

When taking into account ipsilateral representations for both target and distractor for each condition (figures 4b & 5b), it becomes apparent that there is both a generally higher Fano factor reported for all epochs and that the cue delay Fano factors for each condition are much higher than their contralateral counterparts. This is underscored by the differences between the two subpopulations seen in Figure 6, in which the mean rates for all conditions are plotted over time, and by the p-value closest to significance being seen for the difference between the two subpopulations for target responses (P=0.10). However, the overall size of each subpopulations (35 neurons for contralateral, 17 for ipsilateral), likely keeps any of these differences from achieving significance and contributes to the much larger error bars seen for ipsilateral response conditions.
Another possibility to be considered is that the Fano factor has been driven low enough by stimulus presentation so as to be effectively at or near floor levels of variability and render it less sensitive to difficulty-dependent changes in variability during the cue and delay period epochs. This would partially explain the relatively flat profile of figures 4 & 5, especially for the contralateral subpopulation’s Fano factors for each condition (4a & 5a). This alternative, however, seems less likely when taking into account the finding from Ruff & Cohen (2014b) that variability should be driven down as the difficulty of the task increases irrespective of firing rate, at least for the highest difficult levels. An earlier finding (Churchland et al. 2011) shows an opposite trend for variability as measured by the variation of conditional expectation (VarCE) for two different versions of a task (one easy, one hard), but there was a modulation of response variability based on difficulty, nonetheless. In the aforementioned study, Churchland et al. (2011), the use of VarCE might have allowed further insight into trial-to-trial variability not given by use of the Fano factor. This is owing to the latter metric’s heavy dependence on the mean firing rate (Cohen & Kohn 2011). It is at least possible that its use in tandem (Brostek et al. 2013) with the Fano factor may have given a more complete estimate of trial-to-trial response variability. However, both metrics are most useful for interpreting responses within the context of a standard Poisson model wherein the spike count variance would be equal in value to mean spike count in a window of time. It has been suggested recently that a modulated Poisson spiking model in which both sensory-driven variability (which would be expected to be captured well by the Fano factor) as well as a stimulus-independent variability based on a fluctuating gain variable are taken into account would better explain overall variability seen (Goris et al. 2014).
Regardless, this lack of sensitivity due to being at floor state is possible when taking into account previous work in visual cortex indicating that response variance often remains markedly above mean firing and does not generally get driven down to Poisson (ratio of variance to mean equals one) behavior (Tolhurst et al. 1981). This is further supported by the idea that much of the variability seen is inherited from earlier parts of the sensory pathway (Sadagopan & Ferster 2012), meaning that frontal regions should inherit a floor state from earlier sensory representations if gain across cortical regions is invariant. However, whether or not this is the case is somewhat in doubt, since Goris et al. (2014) has shown that a modeled gain variable that increases variability over cortical regions as the visual hierarchy is ascended agrees well with experimental data. Additionally, other recent studies have revealed that cortical states (Scholvinck et al. 2015) and cognitive variables like attention (Ruff & Cohen 2014b) modulate response variability and covariability of neuron pairs. It is possible that sensory input from previous areas has less of an effect on variability than cognitive factors such as attention and task difficulty, cortical states defined by synchronization and frequency of neuronal responses, and a modulatory gain function that increases irrespective to stimulation along a sensory pathway. Given the continued lack of consensus regarding the role variability in the nervous system and how it should best be represented, further investigation with a larger dataset and other avenues of analysis will likely be necessary to draw further conclusions about prefrontal neuronal variability.
Figure 1: Macaque Brain Schematic

Schematic of the cortical area of the macaque brain being studied, emphasis on the lateral prefrontal cortex (LPFC). Recordings were limited to BA 8 and BA 46 on the anterior lip of the arcuate sulcus (AS) and on both banks of the posterior portion of the principal sulcus (PS).
Figure 2: Behavioral Task

a) Sequence of events for the Delayed Discrimination Response (DDR) task. A variant of the ODR task, the monkey was required to fixate on a central fixation point, observe the presentation of two stimuli (one of variable luminance, a distractor, the other of constant luminance, the target) that follow, maintain stimulus information through a delay period in which the stimuli disappear, and finally make a saccade toward the location where the brighter of the two stimuli resided when the central fixation point disappears.

b) Stimulus set for the DDR task. The task made use of distractors of varying contrast ratios (calculated as the difference between the luminance of the distractor and the background, $\Delta L_d$, divided by the difference between the luminance of the target and the background, $\Delta L_t$). From upper left to bottom right the average contrast ratios used were: 0%, 0.009%, 0.13%, 0.96%, 3.76%, 12.9%, 37%, and 100%.

c) Sequence of events for the Oculomotor Delayed Response (ODR) task. This task required the monkey to fixate on a central target, remember the spatial location of the following cue, maintain the memory trace of that cue through a delay period in which the cue is absent, and finally make a saccade toward the remembered location of the cue when the central fixation point disappears.

d) The spatial cue locations used for the ODR task. Each cue location was positioned at an eccentricity of 14° from the central fixation point, with eight possible locations total. All cues for this task were 1° in size.
Figure 3: Behavior Plotted across levels of Contrast Ratio

Percentage of correct trials for current DDR task, plotted across contrast ratio ($\Delta L_d / \Delta L_o$).

Increasingly difficult contrast ratios are shown from left to right.
Figure 4: Between-condition Fano Factor for the DDR task, target in the receptive field

a) The average Fano Factor for all contralateral neurons with responses to the target in their receptive fields, separated based on epoch (fixation, 1-1000ms, in red; cue, 1001-1500ms, in blue; delay, 1501-4500ms, in black).

b) The average Fano Factor for all ipsilateral neurons with responses to the target in their receptive fields, separated based on epoch (fixation, 1-1000ms, in red; cue, 1001-1500ms, in blue; delay, 1501-4500ms, in black).

c) The average Fano Factor for all neurons with responses to the target in their receptive fields, separated based on epoch (fixation, 1-1000ms, in red; cue, 1001-1500ms, in blue; delay, 1501-4500ms, in black).
Figure 5: Between-condition Fano Factor for the DDR task, distractor in the receptive field

a) The average Fano Factor for all contralateral neurons with responses to the distractor in their receptive fields, separated based on epoch (fixation, 1-1000ms, in red; cue, 1001-1500ms, in blue; delay, 1501-4500ms, in black).

b) The average Fano Factor for all ipsilateral neurons with responses to the distractor inside their receptive fields, separated based on epoch (fixation, 1-1000ms, in red; cue, 1001-1500ms, in blue; delay, 1501-4500ms, in black).

c) The average Fano Factor for all neurons with responses to the distractor inside of their receptive fields, separated based on epoch (fixation, 1-1000ms, in red; cue, 1001-1500ms, in blue; delay, 1501-4500ms, in black).
a) Fano Factor when Distractor is in Contralateral Receptive Field

b) Fano Factor when Distractor is in Ipsilateral Receptive Field

c) Fano Factor when Distractor is in the Receptive Field
Figure 6: Fano Factor over time for the DDR task while Target or Distractor is in the Receptive Field

a) Fano Factor for all neurons with responses to the target in the receptive field shown over the full time course of the task. Ipsilateral neurons’ individual Fano factors are shown in cyan with mean Fano factor shown in navy blue. Contralateral neurons’ individual Fano factor are shown in red with mean Fano factor shown in black.

b) Fano Factor for all neurons with responses to the distractor inside the receptive field shown over the full time course of the task. Ipsilateral neurons’ individual Fano factors are shown in cyan with mean Fano factor shown in navy blue. Contralateral neurons’ individual Fano factor are shown in red with mean Fano factor shown in black.
6a Fano Factor - Target in the Receptive Field

6b Fano Factor - Distractor in the Receptive Field
Figure 7: Fano Factor over time for Contralateral and Ipsilateral Receptive Fields

a) Fano Factor for all contralateral neurons shown over the full time course of the task. Neurons with responses to the target in the receptive field individual Fano factors are shown in cyan with mean Fano factor shown in navy blue. Neurons with responses to the target outside of the receptive field individual Fano factors are shown in red with mean Fano factor shown in black.

b) Fano Factor for all ipsilateral neurons shown over the full time course of the task. Neurons with responses to the target in the receptive field individual Fano factors are shown in cyan with mean Fano factor shown in navy blue. Neurons with responses to the target outside of the receptive field individual Fano factors are shown in red with mean Fano factor shown in black.
CHAPTER III

CHANGES IN FIRING RATE AND VARIABILITY OF PREFRONTAL RESPONSES IN ADOLESCENCE IN MACAQUES

BY

SAMSON G. KING
ABSTRACT

Changes in connectivity between neurons within the prefrontal cortex (PFC) as a result of development after the time of puberty have been previously illustrated. These changes coincide with improvements in working memory and other cognitive functions as well as a refinement in the pattern of brain regions activated by cognitive tasks after the time of puberty over the same critical developmental period. These findings could, in principle, be accounted for by changes in the nature of variability in the prefrontal cortex between adult and juvenile periods. How this variability might differ between juvenile and adult states continues to be an open question, and has several possibilities ranging from less shared noise thanks to noisier inputs being pruned to no actual direct change in response variability but a greater resistance to stochastic error. To assess this in part, monkeys were trained on an oculomotor delayed response (ODR) task and then were recorded from as they behaved. Mean firing rate, percent correct, and Fano factor variability all increased in adulthood. The increase in Fano factor was surprising, since it was thought likely that variability as measured by the Fano factor might decrease with age for better single-neuron signal encoding/transmission. Additional analysis found no effect for the hemifield of response. These results suggest that improvements in performance of cognitive tasks after puberty are likely not due to decreases in neuronal variability.
INTRODUCTION

There is a longstanding consensus that developmental changes in the prefrontal cortex occur for a prolonged period after puberty and into early adulthood. In particular, there is a tremendous increase in gray and white matter volumes in prefrontal cortex and connecting regions of the brain such as the posterior parietal cortex that comprise the primary components of the fronto-parietal network (Olesen et al. 2003, Crone et al. 2006). Additionally, changes in connectivity between neurons within the PFC as a result of development have been previously illustrated (Zhou et al. 2014). These changes parallel improvements in working memory and other cognitive functions (Luciana & Nelson 1998, Gathercole et al. 2004, Fry & Hale 2000) as well as a refinement of the pattern of brain regions activated during cognitive functions (Konrad et al. 2005, Geier et al. 2009) over the same critical developmental period. Cross-sectional studies using fMRI and PET imaging have also confirmed the recruitment of areas throughout the PFC in working memory tasks performed by human adults, with the lateral PFC being a notably important region for several cognitive functions (D’Esposito et al. 2000, Chugani et al. 1987, Sowell et al. 2001).

The physiological properties of prefrontal neurons that subserve many cognitive functions as well as how these properties are altered with maturation continue to remain less well characterized. It has been recently illustrated that there is an overall increase in functional connectivity between neuron pairs in the prefrontal cortex of Rhesus macaques (Macaca mulatta) with maturation to adulthood, attributed to a net decrease in inhibitory
connections in the prefrontal cortex (Zhou et al. 2014). This is critical, as increasing functional connectivity—and thereby correlation of responses—could have immense effects on information encoding and transmission in the prefrontal cortex (Averbeck et al. 2006). A previous finding in Zhou et al. (2013) raised the possibility that peripubertal monkeys could experience higher rates of failure despite otherwise robust activity of prefrontal neurons while engaged in a working memory task due to a greater vulnerability to variability. Taken together, these findings indicate a possible change in the nature of variability in the prefrontal cortex between adult and juvenile periods.

The variability of individual neuronal responses to repeatedly presented identical stimuli and repeated motor acts is a well-known characteristic of cortical neurons (Shadlen and Newsome 1998), along with a generally much higher levels of floor variability compared to subcortical regions (Kara et al. 2000, Goris et al. 2014, Scholvinck et al. 2015). There have been several proposals as to how the brain might make use of this noise, with some suggesting that it is might be used in a probabilistic fashion to make optimal choices (Knill & Pouget 2004) or as a means to prevent entrainment of neuronal responses to a stimulus (Stein et al. 2005). Regardless of its ultimate function (or non-function), how this variability might differ between juvenile and adult states continues to be an open question, and has several possibilities ranging from less shared noise thanks to noisier inputs being pruned to no actual direct change in response variability but a greater resistance to stochastic error.

To better assess the nature of variability changes in the dorsolateral prefrontal cortex due to adolescence, monkeys were trained to perform an oculomotor delayed response (ODR) task during two different developmental states (young and adult).
Neurophysiological recordings and behavioral performance were then collected during each period of development while the monkeys performed the task and analyzed for changes in response variability. The expectation was that variability as measured by the Fano factor should decrease significantly between puberty and adulthood to better enhance the fidelity of salient signal among non-salient noise. Additionally, since there was a general, non-significant difference in Fano factor variability seen for hemifield in chapter II, the possibility that response variability could differ for this dataset was also addressed. The assumption for the latter topic for analysis was that ipsilateral response variability would be higher than contralateral response variability (as was shown generally for the previous chapter’s dataset).
METHODS

Subjects

Non-human primates provide an attractive model for investigating the developmental changes evident in the PFC during and around puberty. For rhesus macaques (*Macaca mulatta*), a pubertal period (3.5-5 years of age) has already been established (Plant et al. 2005) that, alongside median lifespan, can be used to make comparative neurophysiological assumptions about human pubertal development (11-16 years of age) based on sexual maturity (Roth et al. 2004, Mattison et al. 2011). It is also known that rhesus macaques continue to undergo maturation of the brain throughout adolescence and early adulthood, which is mirrored over a much longer timescale in humans (Fuster 2002). Additionally, there is precedent for use of rhesus macaques in longitudinal studies addressing peri-adolescent development in other neural systems (Soto et al. 2012).

Four male monkeys were used throughout the duration of this experiment. To ascertain peripubertal developmental stage using a more precise metric than age, morphometric measures of development (body weight, crown-to-rump length, chest circumference, ulnar and femur length, testicular volume, presence or absence of erupted canines, bone maturation, and serum concentration of circulating hormones) were taken for each monkey approximately every three months. Measurements lasted from prior to neurophysiological recordings to until after recordings had ceased.
Behavioral Task.

Four monkeys were trained to perform a basic Oculomotor Delayed Response (ODR) Task similar to that described in Chapter II (Figure 1). For successful completion of a trial, the monkeys were first required to fixate on a central point for 1.0s for the rest of the trial to commence, followed by a presentation of a 1° white square stimulus cue that could appear at one of eight locations arranged on a circle of 10° eccentricity around the fixation point, a delay period that lasts for 1.5 s in which the monkey must maintain the memory of the cued location, and finally make a saccade to the remembered location of the cue within 600 ms of the fixation point being extinguished at the end of the delay period. To be considered a correct trial, the monkey must end its eye movement within a 5–6° radius window centered on where the stimulus was previously displayed, and the monkey was required to hold fixation within this window for 100 ms. Animals were rewarded with fruit juice for successful completion of a trial. Eye position was monitored with an infrared eye tracking system (ISCAN, RK-716; ISCAN, Burlington, MA). Breaking fixation at any point before the offset of the fixation point aborted the trial and resulted in no reward. The stimulus presentation and online behavioral control was achieved by in-house software (Meyer & Constantinidis 2005).

Surgery and neurophysiology.

The monkeys were initially naïve to behavioral training or task execution of any kind. They were first trained in the ODR task during the young stage. Once the animals had reached asymptotic performance, a 20-mm diameter recording cylinder was implanted
over the prefrontal cortex of each animal, localized via magnetic resonance imaging over areas 8a and 46 of the dorsolateral prefrontal cortex (Figure 3). Epoxylite-coated Tungsten electrodes with a diameter of 250 μm and an impedance of 4 MΩ at 1 KHz (FHC Bowdoin, ME) were used for all penetrations performed for neurophysiological recording. Electrical signals recorded from the brain were amplified, band-pass filtered between 500 and 8 kHz, and stored through a modular data acquisition system at 25 μs resolution (APM system, FHC, Bowdoin, ME).

At the conclusion of neurophysiological recordings for the juvenile period of development, the animals were put on break and did not train on any task for 18 months. At the end of this period, all monkeys were considered to be in the adult stage of development based on morphometric indices of development periodically collected. With this being the case, all subjects again underwent training in working memory tasks including the ODR task; all four were able to quickly rebuild proficiency in the tasks, allowing a new phase of recordings to commence using identical methods. All surgical and animal use procedures were reviewed and approved by the Wake Forest University Institutional Animal Care and Use Committee, in accordance with the U.S. Public Health Service Policy on humane care and use of laboratory animals and the National Research Council’s Guide for the care and use of laboratory animals.

Behavioral Data Analysis.

We analyzed performance in the ODR task as the percentage of trials with correct responses, defined as trials that had an eye movement to the area of the remembered
stimulus after the fixation point is extinguished. This proportion of correct responses is shown for each monkey in Figure 2. Some trials resulted in breaks in fixation, blinks, or premature saccades, prior to offset of the fixation point; these were counted as errors and are not reported or used in further analysis here.

Neural Data Analysis.

Recorded spike waveforms were sorted into separate units using an automated cluster analysis method based on the KlustaKwik algorithm (Harris et al. 2000). Firing rate of units was then determined by averaging spikes in each task epoch. In the ODR task, we identified neurons with significant elevation of firing rate within 500 ms of cue presentation. Firing rate in this period was compared to the 1 s baseline fixation period prior to the presentation of the cue, and neurons with significant difference in firing rate were identified (paired t-test, p<0.05). Neurons with significant responses during the stimulus presentation were used in further analysis presented here. Population peri-stimulus time histograms (PSTH) were constructed averaging responses of multiple neurons. Additionally, Fano factor ($\sigma^2_w/\mu_w$) analysis of variability was accomplished using MATLAB (Mathworks) code adapted from Churchland et al. 2010. Fano factor was computed using a 100 ms sliding window moving in 20 ms steps and plotted across task epochs of the ODR dataset. We identified neurons with maximal responses to the target that were either purely contralateral (conditions 1, 2 & 8) or purely ipsilateral (conditions 4, 5, & 6). Neurons with a maximal response at a location along the midline vertical meridian were not included in this analysis. Ipsilateral and contralateral neurons
were then separated out to investigate potential differences in Fano factor between the two groups for young and adult phases. A two-tailed t-test was then employed to test for significant differences of the Fano factor within specific periods of the task (fixation, cue, delay). In addition, an unbalanced 2-way ANOVA was performed for fixation, cue, and delay periods to further investigate possible differences between purely contralateral and purely ipsilateral neurons sampled at young (N=191 contralateral, 23 ipsilateral) and adult (N=216 contralateral, 22 ipsilateral) stages.
RESULTS

Neural & Behavioral Result

A total of 607 neurons from areas 8a and 46 of the dorsolateral prefrontal cortex (Figure 1) in the young stage (33, 133, 158 and 283 neurons from the four monkeys, respectively) were initially recorded from. Subsequently recordings were performed from 830 neurons in the adult stage from the same monkeys (133, 41, 238 and 418, respectively) occurring after an 18 month interim period. Analysis was focused on 309 neurons in the young stage and 324 neurons in the adult stage with significantly elevated responses to at least one stimulus location (paired t-test, p<0.05). For behavior, there was an improvement in working memory task performance that was observed in adulthood (as shown in Figure 2). In addition, neural data indicated that working memory activity during the delay period of the ODR task was significantly higher after maturation (Figure 3A & B).

Fano Factor Analysis

In an additional step of analysis, the Fano factor ($\sigma^2_w/\mu_w$) was computed to assess the variability of neural responses seen throughout the course of the ODR task. As a metric, the Fano factor was so chosen for its capacity to quickly say something about how closely a distribution obeys a Poisson process (Eden & Kramer 2010). A 100 ms sliding window moving in 20 ms steps was used in the calculation of the Fano factor, and the result for both young and adult periods was plotted across time (Figure 4). As the analysis of Chapter II indicated that responses to contralateral and ipsilateral stimuli might be generally different in their variability (at least in the small sample of neurons tested for
that analysis), two sets of data were isolated of either purely contralateral (conditions 1, 2 & 8) or purely ipsilateral (conditions 4-6) responses for further analysis of Fano factor variability. These responses were further subdivided into responses taken from the young period of development or the adult period of development and plotted across time (Figure 5a-d).

A two-tailed t-test was then employed to test for significant differences of the Fano factor within specific periods of the task (fixation, cue, delay) and across combinations of hemifield of response (contralateral and ipsilateral) and developmental stage (adult and young). For young contralateral (N=191) and adult contralateral (N=216) data sets, there was a significant difference seen in variability for the cue period (t-test, P=0.045) but not for the fixation (P=0.26) and delay (P=0.23) periods, indicating a possible difference in Fano factor in the contralateral hemifield as a result of age. For young ipsilateral (N=23) and adult ipsilateral (N=22) data sets, there were no significant differences seen in the fixation (P=0.24), cue (P=0.11), or delay (P=0.38) periods, indicating no observed difference due to developmental stage. This lack of significant difference held true when young contralateral and young ipsilateral sets were matched (fixation, P=0.68; cue, P=0.54; delay, P=0.94), indicating no within-developmental-stage difference in either hemifield’s Fano factor. When adult contralateral and adult ipsilateral sets were compared, there were again no observed significant differences between hemifield Fano factors with the adult stage (fixation, P=0.38; cue, P=0.50; delay, P=0.39). This finding points to a possible effect of age on variability in responses to contralateral stimuli, but no other significant effects were observed. To further investigate this, an unbalanced two-way ANOVA was performed for fixation, cue, and delay periods using
hemifield (contralateral/ipsilateral) and developmental period (young/adult) as groups. For the fixation period, there was a trend towards a difference for developmental period (P=0.10). We also found no significant effect of hemifield (P=0.70) and no significant interaction (P=0.35). The cue saw significant differences for developmental period (P=0.028), but no significance for hemifield (P=0.92) or interaction (P=0.37). The delay period saw no significance for developmental period (P=0.20), hemifield of stimulus location (P=0.56), or interaction effects (P=0.51). This seems to indicate that whatever differences in Fano factor variability seen between the contralateral young and contralateral adult sets for cue were dependent more on the effects of developmental stage rather than the effect of stimulus location. This result may be related to current findings regarding the importance of the peripubertal period for cognitive tasks and previous findings regarding preferential but not exclusive encoding of contralateral spatial information versus ipsilateral information in the PFC (Rainer & Ranganath 2002).
DISCUSSION

Findings for the current analyses indicate a modest overall increase in Fano factor variability after puberty (Figure 5). This ran counter to initial expectations, since it was assumed that much of the variability would be reduced to better overcome the noise threshold (Faisal 2008) and thereby allow better single-neuron stimulus representations to propagate. However, since many studies cite the importance of correlations between neurons of the prefrontal cortex (Zhou et al. 2014) and others call into question the idea that noise correlations necessarily always decrease information transmission (Averbeck et al. 2006), it should hardly come as a surprise that what might be best for single-neuron information transmission is not what is actually seen in real data. Indeed, previous work has shown that correlated fluctuations between and across multiple populations of neurons tuned to different stimuli can increase the fidelity of the signal (Romo et al. 2003). When applied to the persistent activity seen during the course of a working memory task, similar findings have been presented; when correlated variability was applied globally over populations of distinct mutually inhibitory neurons, persistent activity was more stable (Polk et al. 2012).

Proximally, this might also underscore the importance of cortical state in shaping global variability (Scholvinck et al. 2015, Mochol et al. 2015), which may in turn also be shaped by desynchronization caused by attention (Harris & Thiele 2011, Mochol et al. 2015). Whether or not this is the case may actually be critical for certain developmental
disorders like ADHD, since a previous study indicated attenuated alpha-beta desynchronization for afflicted individuals versus normally developing controls while performing a working memory task (Lenartowicz et al. 2014). It is postulated that the increased functional connectivity through fewer inhibitory inputs reported in Zhou et al. (2014) might increase correlated ‘global’ variability between distinct prefrontal populations and stabilize persistent activity (and perhaps even make desynchronization by attention easier). This would help account for the overall higher firing rate seen in figure 4b for the adult state not being affected by the concomitantly higher variability as measured by the Fano factor seen in figure 5, and allowing for a higher proportion correct on the ODR task for each monkey during the adult state (figure 2). Further analysis is warranted to better assess this.

Alongside this, the second stage of analysis revealed very little difference in variability between hemifields. However, though there were no indications of significant within-age differences in Fano factor between ipsilateral and contralateral hemifields, there was a significant difference between Fano factors for contralateral young data and contralateral adult data during the cue period that was not observed for ipsilateral young and adult data. This difference was further analyzed with an unbalanced two-way analysis of variance, which pointed to no significant effects for hemifield of response on Fano factor variability during the cue, but a significant effect for developmental period (P=0.03). This indicates that while responses seen in the contralateral hemifield were the responses that changed the most in variability between young and adult stages, the change had less to do with the location of the responses and more to do with the developmental stage. Critically, our predictions about ipsilateral Fano factor values being
higher than contralateral Fano factor values proved to be generally true, albeit only for the adult stage. This matches well with chapter II’s findings of a generally higher level of variability in ipsilateral representations in male macaques, but it also might suffer from the same affliction: relatively low sample size for two distributions (both ipsilateral). Additional analysis using a higher number of collected ipsilateral neurons is desirable to see if more statistical power would lead a near-significant result to becoming significant.
Figure 1: Oculomotor Delayed Response (ODR) Task

Sequence of events for the Oculomotor Delayed Response (ODR) task. This task required the monkey to fixate on a central target, remember the spatial location of the following cue, maintain the memory trace of that cue through a delay period in which the cue is absent, and finally make a saccade toward the remembered location of the cue when the central fixation point disappears.
Figure 2: Behavior during the ODR task in each developmental stage

Proportion of correct responses as defined as those that end in a saccade to the correctly remembered location when the fixation point is extinguished at the end of the delay period. The data for each monkey is divided by developmental stage with the young stage being shown in blue and the adult shown in red.
Figure 3: Magnetic resonance image of a Macaque brain.

Pictured here, a BrainSight-processed MR image of Monkey 1708’s brain. In red, the boundary of the 20-mm diameter recording cylinder is shown. The exposed area of cortex includes BA areas 8a and 46.
Figure 4: Working memory firing rate before and after maturation

A. Juvenile developmental stage peri-stimulus time histogram of firing rate during the course of the ODR task. Correct responses made into the receptive field are seen in the solid trace; responses to stimuli outside of the receptive field are seen in the dotted trace. Stimulus presentation is represented by the gray bar, insets depict stimulus location and saccade direction.

B. Same conventions as A, but for adult stage.
Figure 5: Total population Fano factor for each developmental stage throughout the course of the ODR task. Young developmental stage is shown in blue, adult stage is shown in red. Gray bar coincides with the cue presentation period.
Figure 6: Fano factors of combined contralateral and ipsilateral data sets for both juvenile and adult developmental plotted over the course of the ODR task.

A. Young (in blue) and Adult (in red) Fano factors for responses in the ipsilateral hemifield.

B. Young (in blue) and Adult (in red) Fano factor for responses in the contralateral hemifield.

C. Contralateral (in blue) and ipsilateral (in red) Fano factor for responses during the young developmental period.

D. Contralateral (in blue) and ipsilateral (in red) Fano factor for responses during the adult developmental period.
CHAPTER IV

DISCUSSION

BY

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Far from seeing increasing variability across trials as a function of difficulty (Churchland et al 2011) as hypothesized previously, the present round of analyses using neural data from Constantinidis et al. (2001) found no significant between-condition differences in Fano factor variability that would indicate any sort of systematic modulation of variability when monkeys are subjected to a range of difficulty levels in a delayed discrimination task. This lack of significance held true when the Fano factor was divided based on fixation, cue, and delay periods. This ran counter to initial expectations, since previous findings indicated that there could be a modest modulation of variability as measured by the Fano factor and variation of conditional expectation with increasing task difficulty (Ruff & Cohen 2014b, Churchland et al. 2011) and attention (Mitchell et al 2007, Ruff & Cohen 2014a). This is especially true of the task from Ruff & Cohen (2014b), since that study made use of a delayed contrast discrimination task with stimuli of varying contrasts not unlike the delayed discrimination response (DDR) task used in Constantinidis et al. (2001). The primary differences between the two tasks were that the more recent study used a variable delay throughout recording and training sessions and that it blocked trials of a particular difficulty together where the earlier study randomized difficulty from trial to trial. With this in mind, there was no immediate reason to suspect that a task with a broad range of stimulus difficulties like the DDR task would display very little variability between its conditions.

The second set of analyses started with two hypotheses. First, Fano factor would decrease as a function of age, reflecting more efficient single-neuron transmission of information. Second, ipsilateral hemifield responses would display higher rates of variability compared to contralateral responses. Hemifield analyses were originally
motivated by the generally higher, albeit insignificantly so, Fano factor variability for ipsilateral responses when compared to contralateral responses from the previous analysis investigating the effects of task difficulty on neuronal response variability. For this developmental dataset recorded while monkeys performed an oculomotor delayed response (ODR) task, responses were again split into hemifield after looking at total population variability to see if the general effect held true and whether the effect seen in this task might be more significant than the previous finding. For the data analyzed here, there were no significant within-age differences in variability during fixation, cue, or cue delay epochs between ipsilateral and contralateral neuronal responses. However, there was a generally higher ipsilateral Fano factor for the adult stage when compared to contralateral responses that was absent in the juvenile. The higher general ipsilateral variability for the adult stage matches well with our second hypothesis (and reproduces the previous finding), though the effect was non-significant. The fact that ipsilateral and contralateral response variability was much closer together for the young stage than for the adult was somewhat surprising, but intriguing for what it might indicate concerning changes in variability with development. Counter to the initial expectation that response variability would decrease with development, Fano factor actually showed an increase across all epochs of the ODR task. Curiously, only contralateral responses showed any significant differences between the two periods, and then only for the cue epoch (P=0.045). To further analyze the effect of age and hemifield on Fano factor variability, an unbalanced two-way analysis of variance was used with ‘hemifield’ and ‘age’ as the groups. This analysis pointed to no significant effects for hemifield, a significant effect for developmental period during the cue, and no significant interactions. Overall, this
indicates that while responses seen in the contralateral hemifield were the responses that changed the most in variability between young and adult stages, the change in variability probably had little to do with the location of the responses and more to do with the developmental stage; development drove observed modest changes in variability.

Current analyses found a modest effect of developmental period for changes in response variability as measured by the Fano factor that were absent or non-significant for task difficulty and the visual hemifield of the response. It is likely, however, that further analysis using larger datasets is needed to address cases in which there are near-significant or general differences. Additionally, it is possible that there actually is a significant amount of response variability present, but it that it is not seen as robustly for single-neuron responses that the Fano factor addresses, but more strongly as covariability between pairs of neurons (Cohen & Maunsell 2009). Indeed, most recent studies look at noise correlations of neuron pairs either exclusively or alongside individual response variability (Ruff & Cohen 2014a, Ruff & Cohen 2014b, Scholvinck et al. 2015, Goris et al. 2014), and it has long been known that these correlations could affect information encoding and transmission either positively or negatively (Averbeck et al. 2006). Beyond this, while the Fano factor is a good metric of how well neural data might fit to a spiking model that uses a Poisson process (Eden & Kramer 2010) and is highly flexible concerning which timescale it is calculated in (Teich et al. 1997), there is mounting evidence that much of the cortex displays rates of variability higher than that expected by a Poisson process (Tolhurst et al. 1981), and that a modulated Poisson process that takes into account across-cortex fluctuations in variability may be more realistic (Goris et al. 2014). Within the context of development, noise correlations between neurons may be
much more relevant than changes in single-neuron variability, since previous work has indicated that there is a functional increase in connectivity as a result of development in and around puberty as a result of lower inhibitory input (Zhou et al. 2014).

Previous findings have shown that mean firing rate, though a commonly reported metric, may not fully explicate the effects of a stimulus or task demands on neural responses and that the variability of the response may informative as well (Churchland et al. 2010, White et al. 2012, Scaglione et al. 2014). Here, we attempted to untangle how variability measured by the Fano factor might differ within the context of varying task difficulty, between different visual hemifields, and as a result of development. Current results indicate a modest difference in response variability between juvenile and adult states for neural responses in the contralateral hemifield during the cue presentation period and a generally higher response variability for neural responses in the ipsilateral hemifield when compared to responses in the contralateral hemifield for the adult state. Proximally, it is concluded that development may have a role in changing response variability in the dorsolateral prefrontal cortex and that response variability may differ more greatly across different spatial hemifields for visuospatial working memory tasks in adults than in juveniles; however, additional analysis is required to better support this.
REFERENCES


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EDUCATION

M.S. Wake Forest School of Medicine, Winston-Salem, NC, May 2015. Major Field: Biomedical Sciences; Concentration: Neuroscience.

B.S. with Honors. Southern Arkansas University, Magnolia, AR, May 2013. Major Field: Biological Sciences.

RESEARCH EXPERIENCE

Graduate Research, Wake Forest School of Medicine. December 2014-ongoing.

Advisor: Christos Constantinidis. Stimulation of the Nucleus Basalis of Meynert. Pre-implantation of stimulating electrode stage.

Graduate Research, Wake Forest School of Medicine. August 2013-ongoing. Advisor: Christos Constantinidis. PFC Development Project. Research in working memory changes between two developmental stages in rhesus macaques (*Macaca mulatta*).

Undergraduate Research, Wake Forest Primate Center, Wake Forest School of Medicine. December 2012-January 2013. Worked as an intern in Dr. Carol Shively laboratory. Primary roles included checking of databased data and assisting with the behavioral monitoring and enrichment of Cynomolgous monkeys (*Macaca fascicularis*).

Undergraduate Research, Rancho Santa Ana Botanical Gardens. August 2012. Work included selection of specimens, DNA extraction, wax-embedding and sectioning of plant material, phylogenetic analysis of specimen, mounting and labeling of specimen, and presentation of summarized conclusion about the specimen.

Undergraduate Research, Natural Resources Research Center, Southern Arkansas University. November 2011-February 2012, September-November 2012. Primary goals were to analyze nectar composition of *Passiflora* specimens utilizing high-performance liquid chromatography (HPLC).

Undergraduate Research, Southern Arkansas University. August 2011-May 2013. Worked as a laboratory assistant in the lab of Dr. Shawn Krosnick, with duties including running PCRs to amplify the CRC gene of *Passiflora* species in the subgenus Decaloba, electrophoresis and imaging of gels, quantification & amplification of PCR products, optimization of primers, and organization/databasing of data. Additional work included
using fluorescence microscopy to analyze the resistance to self-pollination among *Passiflora* species and general laboratory upkeep.

**Research Grants**

Southern Arkansas University Undergraduate Research Grant, Fall 2012. Winter break research in behavior at Wake Forest Primate Center/Center for Comparative Medicine. $1250 (S. King).

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Preceptor, Vertebrate Zoology Laboratory. SAU, January-May 2013.

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Laboratory Instruction of basic molecular techniques, January –March 2012.

Supplemental Instructor for Genetics 3033, August –December 2011.

**MEMBERSHIP IN PROFESSIONAL SOCIETIES**

Western North Carolina Chapter of the Society for Neuroscience

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Member of the Wake Forest University Brain Awareness Council, August 2014 — present

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Member of Beta-Beta-Beta, spring 2010 – spring 2013

Honors College Association Academic Chair, spring 2010.

SKILLS & OTHER EXPERIENCE

MATLAB programming

Experience handling and working with non-human primates including Macaca mulatta (August 2013-ongoing) and Macaca fascicularis (December 2012-January 2013).

REFERENCES

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