A Neuroanatomical Approach to Examining Changes in Cognition: The long-term effects of Chronic Cocaine Use

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Cognitive deficits exhibited by human cocaine users have been shown to predict outcome of therapeutic treatments, so understanding the structural basis of these deficits is clinically important. Longitudinal cognitive assessments were made, using touch-screen based tasks, in two groups of rhesus monkeys: a chronic cocaine self-administration group, and a control water self-administration group. Structural changes in regions of the prefrontal cortex over time were determined by measuring changes in the volumes of gray matter from magnetic resonance images taken pre- and post-treatment. Linear regressions were run to correlate changes in cognition with structural changes over time. A positive correlation was observed between changes in visual working memory performance and changes in structure in the dorsolateral prefrontal cortex, area 8, area 10, and area 12 in the cocaine group, but not in the control group. In human studies, it is impossible to know if structural changes are a direct result of cocaine use or result from differences prior to drug use or other factors. The structural differences identified in this study strongly support a link between cocaine use and cognitive deficits, and will guide post-mortem studies into possible neurobiological mechanisms underlying the observed anatomical changes.

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1.0 INTRODUCTION

Many psychiatric illnesses are exacerbated by chronic drug use, and the incidence of relapse is high in human cocaine users. These aspects of drug use may be rooted in neurobiological changes in cortical brain structures, which in nonhuman primates are similar to humans, making them an effective model for study. Cognitive deficits exhibited by human cocaine users have been shown to predict the outcome of therapeutic treatments, so understanding the structural basis of these deficits is clinically important (Aharonovich et al., 2006; Patkar et al., 2004; Teichner, Horner, Roitzsch, Herron, & Thevos, 2002). The aim of this study was to elucidate the link between cocaine use and cognitive deficits by determining if there are structural differences in our nonhuman primate models that correlate with previously defined cognitive changes.

The literature indicates that there are structural differences between the brains of human cocaine users and those who do not use drugs, and that these differences occur in regions associated with cognitive function that is impaired in human cocaine users, such as behavioral inhibition and decision making (Franklin et al., 2002). Structural differences have been identified using longitudinal magnetic resonance images (MRI) in clinical studies of aging, where gray matter was found to decrease with age in humans (Fjell et al., 2010; R. S. Liu et al., 2003) and monkeys (Knickmeyer et al., 2010). In clinical studies of cocaine use, MRI has been used to compare cortical volumes of users with normal controls (Bartzokis et al., 2002; Franklin et al., 2002; Lim et al., 2008; Sim et al., 2007). The use of MR imaging allows for structural changes to be determined by tissue type, leading to more focused interpretation of changes

observed. Atlas-based segmentation is one method used to determine tissue type in MR images, and has been used in human studies of brain morphometry (Carmichael et al., 2005; Wu, Carmichael, Lopez-Garcia, Carter, & Aizenstein, 2006). The longitudinal MR imaging using a nonhuman primate model in this study allow for translation of results to a clinical population.

In human studies, it is difficult to know if structural changes are a direct result of cocaine use or can be attributed to differences prior to drug use or other factors. Polydrug use is a challenge in clinical studies, as many cocaine users use alcohol, tobacco or other drugs simultaneously. Alcohol (O'Neill, Cardenas, & Meyerhoff, 2001), heroin (Connolly, Bell, Foxe, & Garavan, 2013) and other drugs have also been show to affect gray matter and white matter volumes in certain brain regions, posing the challenge of determining which drugs may be causing which structural changes. Alcohol is particularly detrimental to attention and executive function (Goldstein et al., 2004). Deficits which have been attributed to cocaine use could, in fact, be a result of alcohol consumption by the users studied. Identifying the source of structural changes requires a group of subjects that have abused only one of these drugs. The limitations on control in clinical studies necessitate an animal model of cocaine use.

It is also difficult to account for pre-existing conditions in clinical studies, as data on brain structure and function before the start of drug use is not available for human users. Deficits in inhibitory control and executive function have been shown to be predictive of substance abuse, and may be pre-existing traits rather than a result of drug abuse (Ersche et al., 2012). Ersche et. al. observed that siblings of drug users did not have the same PFC gray matter deficits seen in users, indicating changes in the PFC are likely a result of drug abuse and not a pre-existing condition (Ersche et al., 2012). To fully account for pre-existing conditions as a

potential factor in structural and cognitive changes in drug use, animal models with extensive baseline data are necessary.

Understanding the root of behavioral deficits could help guide therapies that would help patients improve function in multiple aspects of living. Users that perform worse on tasks that test cognitive skills such as attention, memory, spatial ability, and global function were found to drop out of treatment before completion. (Aharonovich et al., 2006). Sometimes the extent of cognitive deficits is correlated with years of use (Aharonovich et al., 2006) but not always (Bolla, Rothman, & Cadet, 1999), which makes it difficult to determine the significance of cocaine use in causing these deficits. In addition, drug users often have comorbid disorders such as depression or ADHD which are not always statistically accounted for, and may affect results of clinical studies (Aharonovich et al., 2006; Ersche, Clark, London, Robbins, & Sahakian, 2006; Franklin et al., 2002; Goldstein et al., 2004). These aspects of clinical studies further frame the need for animal models in which cocaine use is the only varying factor between groups.

The cognitive domains focused on in this study were associative learning, reversal learning and visual working memory. The literature has shown that cocaine users exhibit impairments in reversal learning (Clarke, Robbins, & Roberts, 2008; Izquierdo, Suda, & Murray, 2004; Schoenbaum, Saddoris, Ramus, Shaham, & Setlow, 2004) but not associative learning (Clarke et al., 2008; Izquierdo et al., 2004). Working memory impairments have also been observed in cocaine users (Bechara & Martin, 2004; Goldstein et al., 2004), although Ersche et. al. found that only male subjects were impaired in visual memory (Ersche et al., 2006) while a cross-sectional study using a nonhuman primate model with a small sample size found that working memory was not impaired across delays up to ten seconds (S. Liu, Heitz, Sampson,

Zhang, & Bradberry, 2008). Subjects' performance on tasks in our study indicate that cocaine users are impaired at both reversal learning and visual working memory.

The significance of this research to clinical populations is revealed in the relationship between cognitive abilities as observed on task performance, and cognitive abilities as observed in social settings and day-to-day functioning. Reversal learning performance has been shown to correlate with successful function in day to day life (Fellows & Farah, 2003). Also described as cognitive control and flexibility, reversal performance is a skill used to change behavior after reward or punishment, which is essential in social settings, economic decision-making and daily function (Fellows & Farah, 2003; Rolls, Hornak, Wade, & McGrath, 1994). Deficits in reversal learning performance in a laboratory test reflect disinhibited or inappropriate behavior socially (Rolls et al., 1994).

Associations between cognitive domains and specific cortical regions have been shown to exist using clinical studies of brain damage (Fellows & Farah, 2003; Hornak et al., 2004), clinical activation studies (Cools, Clark, Owen, & Robbins, 2002; Elliott & Dolan, 1999), nonhuman primate lesion studies (Clarke et al., 2008) and single unit studies (Bechara & Martin, 2004; Schoenbaum et al., 2004; Wilson, Scalaidhe, & Goldman-Rakic, 1993). What is not well-evaluated is whether cognitive deficits observed in users result from altered structure in these regions and whether changes seen in these regions can be attributed to chronic cocaine use. Studies on structural changes in cocaine users have focused on reward circuitry and the prefrontal cortex (PFC). Reductions in gray matter occur in areas that are activated by cocaine-related stimuli such as the orbitofrontal cortex (OFC) (Childress et al., 1999; Franklin et al., 2002; Grant et al., 1996; Maas et al., 1998; Matochik, London, Eldreth, Cadet, & Bolla, 2003; Sim et al., 2007). Cocaine abuse may enhance the gray matter reductions and decrease the

normal gain in white matter that naturally occurs with aging in the PFC (Bartzokis et al., 2000; Lim et al., 2008). Changes in volume in terms of tissue type are critical to interpreting the mechanism of cortical change and how that change might be reflected in behavior.

Visual working memory function has been associated with the ventrolateral prefrontal cortex (vlPFC), sometimes referred to as the inferior dorsolateral prefrontal cortex (dlPFC) (Bechara & Martin, 2004; Elliott & Dolan, 1999; Goldstein et al., 2004; Wilson et al., 1993). It is not clear whether this association represents vlPFC involvement in the executive process of working memory or in short-term storage of information (Bechara & Martin, 2004; Petrides, 2000). Projections from the vlPFC to the hippocampus and surrounding temporal cortex indicate the association could also involve vlPFC influence on the memory functions of these areas (Bechara & Martin, 2004).

Impairments in reversal learning have been associated with damage to another part of the prefrontal cortex, the OFC (Dias, Robbins, & Roberts, 1996; Fellows & Farah, 2003; Hornak et al., 2004; Schoenbaum et al., 2004). OFC activation has been correlated with the amount of reward received, independent of reward type (Hornak et al., 2004; Padoa-Schioppa & Assad, 2006; Tremblay & Schultz, 1999; Wallis & Miller, 2003). Thus the OFC seems to link visual stimuli with reward, encoding reward value, but there is more to understand about the role this plays in reversal learning (Murray & Izquierdo, 2007).

The purpose of relating cognitive changes to specific anatomical regions is that post-mortem studies can be performed to determine the neurobiological basis of changes in these areas. The role of cocaine abuse in activation of the nervous system's innate immune response has been presented as a possible mechanism of neuroinflammation that may be underlying the structural changes observed (Clark, Wiley, & Bradberry, 2013). The current project will serve to

guide potential post-mortem studies in the cocaine exposed primates that will evaluate region specific indications of neuroinflammation.

This study compares two groups of rhesus monkeys: a cocaine group, in which monkeys self-administered cocaine for approximately one year, and a control group, in which monkeys self-administered a water reward. High-quality structural MR images, pre and post cocaine self-administration, were used to compare changes in brain structure over time in individual animals. Previous analysis of the data collected from performance on cognitive tasks indicated that the cocaine group had significant deficits in reversal learning and visual working memory after beginning self-administration (Porter et al., 2011). A significant difference in associative learning was not indicated (Porter et al., 2011). This study examines changes in both cognitive performance and brain structure over time to test the hypothesis that changes in these regions may be contributing to cognitive deficits such as those impairments seen clinically in human cocaine users. The results of this study are critical to our understanding of the effects of chronic cocaine use and our understanding of the role of prefrontal cortex in cognitive behavior.

2.0 METHODS

2.1 SUBJECTS

Fourteen male rhesus macaque monkeys were used in this study: six in the control group and eight in the experimental group. None of the subjects had previous behavioral training or drug exposure. Subjects worked for a water reward when performing cognitive tests and were water regulated five days per week at a weekly average of 25 ml/kg per day, with free access to water over the weekend. Subjects performed a self-administration task Tuesday through Friday each week, in which cocaine subjects worked for cocaine and control subjects worked for water. Cognitive tasks were performed on Mondays after 24 hours of resumed water regulation and 72 hours drug-free.

Each subject had a subcutaneous vascular access port surgically implanted midscapula with a catheter into the internal jugular vein, through which cocaine was self-administered by the cocaine subjects. The port decreased the risk of infection since nothing lies outside the skin. Subjects worked on a 15-inch touch-screen computer in a sound-attenuated chamber. E-prime software was used to create and run each task and record responses.

2.2 MRI ACQUISITION

Magnetic resonance images were acquired using a Siemens 3 Tesla Allegra scanner using a custom-designed dual stereotaxic holder and secondary coil designed by Dr. Seong Gi Kim and colleagues (University of Pittsburgh). The first sets of images, pre self-administration, were acquired using a 128 mm field of view: TR = 1790 ms; TE = 3.04 ms; TI = 800 ms; flip angle = 8 degrees; slice thickness = 0.5 mm; voxel size = 0.5x0.5x0.5 mm; number of slices = 208. A second set of images were acquired for each individual, post self-administration. Subjects 11706 and 12206 received their second images under the same scan parameters as the first set of images. Due to wrap around artifacts, all other subjects received their second set of images using a 153 mm field of view: TR = 1680 ms; TE = 3.04 ms; TI = 800 ms; flip angle = 8 degrees; slice thickness = 0.6 mm; voxel size = 0.6x0.6x0.6 mm; number of slices = 192. Data from the first set of images were multiplied by a correction factor before analysis took place, to account for the difference in voxel size between image sets.

2.3 IMAGE PROCESSING

Each individual subject's MR scan at each time-point was processed, using semi-automated atlas-based segmentation, to estimate regional gray matter volumes for select prefrontal regions of interest (ROIs). The atlas-based segmentation followed methods previously described for human brain morphometry (Carmichael et al., 2005; Wu et al., 2006). The method relies on a template (i.e. atlas), which was warped using a highly-deformable method to each rhesus brain MRI. The specific template used in this study was generated by aligning all of the

brain MRIs from this study to a common rhesus-brain space. The individual ROIs were manually drawn on the common rhesus template using the program MRIcron. ROIs on the template were then warped to the individual subject MRIs. Images were de-noised with an ITK N4 inhomogeneity correction using the Slicer program to minimize the variable contrast across the image. The warped ROIs were then masked with gray matter tissue segmentation. This tissue segmentation was done using the FAST program from the FSL software.

A difference value was calculated for each subject by subtracting the gray matter voxel count of ROIs of the pre self-administration images from the post self-administration images. This allowed a determination of each individual's change in volume over time, so that gray matter changes could be compared to changes in that individual's cognitive performance. These correlations were determined using a Pearson's linear regression in Sigma Stat within each group, for various ROI/task combinations. Mann Whitney rank sum tests were conducted to examine change in volume between groups, in certain regions of interest.

2.4 SELF-ADMINISTRATION TASK

The self-administration task required subjects to touch a visual cue in the form of a shape that appeared on the screen a certain number of times in order to receive the reward. After receiving the reward there was a timeout phase. A distinct border was visible around the edge of the screen, and a tone (ascending or descending) was heard throughout each session, except during reward delivery. When the reward was administered, a green border appeared around the screen and the sound stopped. These contextual visual and audio cues of drug administration were used for later studies on environmental stimuli and their potential effects on cognition.

Subjects' baseline performance was measured on each cognitive task and subjects were divided into groups based on this performance and their age. Cocaine subjects began the self-administration task by administering, via the vascular access port, up to six infusions of 0.5 mg/kg of cocaine followed by a 10-minute timeout. This was run on an FR-30 schedule, or fixed-response schedule in which the subject must touch the stimulus 30 times to receive the reward. Typically, all six infusions were received for a cumulative intake of 3.0 mg/kg in each session.

Control subjects received their water reward via a sipper tube that was placed in front of their mouths in the chamber. Subjects began by receiving six rewards of 0.3 mL/kg for an average of 1.8 mL/kg on a variable ratio schedule. Subjects were then increased to eighteen rewards of 0.66 mL/kg, followed by a 3.3 minute timeout on a random 5-15 touch schedule. The difference in reinforcement schedule between groups minimized the effects of boredom in control subjects during the timeout phase, as water does not necessitate time to recover from its effects. These schedules encouraged subjects in both groups to attend to the task and also have similar average total times spent working in the chamber.

2.5 STIMULUS DISCRIMINATION/REVERSAL TASK

Associative learning and cognitive control were tested using a stimulus discrimination with reversal task. In the first phase of this task, three unique visual stimuli were paired with a low, medium or high reward, in the form of water. Subjects were presented with two of the three stimuli in each trial, with each trial providing a different and random combination of the stimuli. A response was considered correct if the subject chose the stimulus worth a higher reward, which

indicated that the subject associated the stimulus with the reward value. When a subject achieved 27 correct responses out of 30 consecutive trials, the task continued into the second phase. In the second phase of the task the stimuli paired with the high and low rewards were switched, with the stimulus paired with the medium reward remaining the same. In this phase, subjects had to reverse the associations made in the previous phase, thus correct responses were indicative of successful cognitive control, also referred to as reversal learning.

Initially, low, medium and high rewards corresponded to 0.02 mL/kg, 0.05 mL/kg and 0.1 mL/kg of water respectively. Ten baseline sessions and 9 post self-administration sessions were collected at these low-contrast reward values. During the post self-administration period, all of the control subjects met the criteria but only five of the eight cocaine subjects did. To increase incentive, reward values were changed such that the difference between high and low rewards was greater. For the subsequent nine sessions, high-contrast reward values were 0.001 mL/kg, 0.03 mL/kg and 0.12 mL.kg of water. All subjects in each group met the task criteria at these high-contrast values.

Associative learning from the stimulus discrimination portion of this task was analyzed by taking the accuracy of the first 15 trials, or choices between two stimuli, from each session. These trials were binned by three to reduce the noise seen in binary data. Low reward-contrast data were analyzed with a 3-way repeated measures ANOVA between groups with bin number and period (baseline versus post self-administration) as the repeating factors. High-contrast sessions were analyzed using a 2-way repeated measures ANOVA with bin number as the repeating factor. These data were not compared to baseline data due to the difference in reward values. Cognitive control and flexibility was analyzed using the first 15 trials from the reversal

phase of the task from the high-contrast sessions. A 2-way repeated measures ANOVA was run with the number of bins as the repeated factor.

2.6 DELAYED MATCH TO SAMPLE TASK

A delayed match-to-sample task (DMS) was used to test visual working memory. In this task, the subject was presented with a visual stimulus which was followed by a delay, or period containing no stimuli, of 0, 10, 20 or 40 seconds. After the delay, the previously presented stimulus and a new unique stimulus appeared on the screen. A response was considered correct when the subject selected the previously presented stimulus by touching the image, which is indicative of visual working memory. A water reward of 0.075 mL/kg was administered after a correct response. After a correct response there was a two second inter-trial interval. After an incorrect response there was a seven second inter-trial interval.

Trials for this task were only included when the subject completed greater than half of the 160 total trials per session and were within a side-bias range where there were more than 25% and less than 75% of all responses per side. Of the 152 total cocaine sessions, 33 were excluded due to these criteria, while 16 of 114 control sessions were excluded. For each subject, 10 baseline sessions were collected and 19 self-administration sessions were collected. A 3-way repeated measures ANOVA was run on delay interval and exposure between groups. A 2-way repeated measures ANOVA was run between groups with period and delay as repeating factors.

2.7 TASK ANALYSIS

In order to correlate the behavioral data with the structural data, the behavioral data was analyzed to derive one number that would represent a change in behavior over time. Slope values were calculated to represent performance on each task, as described below. For each type of behavior, a difference between slope values was calculated by subtracting slope values from the pre self-administration period from slope values from the post self-administration period.

To evaluate visual working memory performance, delay-dependent accuracy was determined across 19 sessions. The slope of the accuracy across delays was calculated for each individual subject for both the pre and post self-administration periods, as seen in Figures 10 and 11. A steeper slope indicates a greater deficit in visual working memory performance as accuracy decreases to a greater extent with a longer delay. A difference value was calculated by subtracting the post self-administration slope from the pre self-administration slope, to represent a change in visual working memory performance over time.

For reversal learning performance, a trial is the term for each time two stimuli appear on the screen and the subject must make a choice, and a session represents each time the subject performs the stimulus discrimination reversal task, which is a set of 200 trials. Post self-administration reversal accuracy was averaged across nine sessions, then plotted versus trial bin number, as seen in Figures 12 and 13. The slope across trials was calculated. If reversal performance improved as more trials were performed, this resulted in a steeper slope value as accuracy increases across fewer trials. A difference value was calculated by subtracting the post self-administration slope from the pre self-administration slope, to represent a change in reversal performance over time. Slope values were calculated for reversal performance for each subject during both the pre and post self-administration periods.

3.0 RESULTS

Regions of interest (ROIs) were manually drawn onto a population-specific rhesus macaque template using MRIcron. Figures 1-5 show the ROIs used in this study on the template in three dimensions.

Previous analysis of task performance, as described in Porter et. al. 2011, indicated that there was no difference between groups on associative learning accuracy, as seen in Figure 6. Cocaine subjects were, however, impaired at reversal learning during the post self-administration period compared to control subjects, as seen in Figure 7. Cocaine subjects were also impaired at visual working memory compared to their baseline performance, as seen in Figure 9. Control subjects were not impaired at visual working memory compared to their baseline performance, as seen in Figure 8. Pre and post self-administration slopes for the delayed match to sample task and the reversal portion of the stimulus discrimination/reversal task are shown in Figures 10-13 for representative subjects, one cocaine and one control subject for each task.

The atlas-based segmentation technique allowed volumetric analyses to be conducted based on tissue type. Significant differences in gray matter change between groups were not observed in the regions of interest, as seen in Figure 14.

Though no between group differences in gray matter changes over time were observed, we performed linear regressions within each groupto see if there was a relationship between changes in task performance and changes in gray matter volume across individuals in the regions

of interest (see Table 1). Strong relationships were revealed in the cocaine group only. Change in visual working memory performance was shown to correlate positively with change in gray matter in dorsolateral prefrontal cortex (dlPFC; p= 0.001; Figure 15), area 8 (p=0.021; Figure 16), and area 12 (p=0.037; Figure 18). The cocaine subjects with larger gray matter losses in these areas performed worse on the delayed match to sample task. Change in visual working memory performance was shown to correlate positively with change in gray matter volume in area 10 with marginal significance (p=0.063; Figure 17). Change in visual working memory performance was not shown to correlate with change in gray matter volume in area 11,13,14 (orbitofrontal cortex, Fig 19). The control group showed no significant relationship between altered cognition and gray matter volume in any region examined.

4.0 DISCUSSION

This study reveals a unique impact of cocaine use on the relationship between changes in brain structure and function. Our nonhuman primate model of chronic cocaine abuse revealed impairments in reversal learning and visual working memory performance. The visual working memory deficits were shown to correlate with decreases in gray matter volume in specific regions of the prefrontal cortex in the cocaine subjects. Changes in visual working memory were shown to significantly correlate with changes in volume in the dorsolateral prefrontal cortex (dlPFC), area 8, area 10 (marginally), and area 12 of cocaine monkeys. These observed relationships are important in understanding the effects of cocaine but also in further understanding the role of the prefrontal cortex in cognition.

The hypothesis of this study, that previously identified cognitive deficits are rooted in gray matter changes in the prefrontal cortex, was based on observations from previous studies. Clinical studies of cocaine use have indicated that drug users exhibit structural differences in prefrontal cortex (PFC) compared to normal controls (Bartzokis et al., 2002; Franklin et al., 2002; Lim et al., 2008) and also experience deficits in cognition, specifically in visual working memory and reversal learning. In addition, animal models and various clinical studies, have indicated that a relationship exists between specific prefrontal cortical regions and cognitive abilities (Clarke et al., 2008; Cools et al., 2002; Elliott & Dolan, 1999; Fellows & Farah, 2003; Hornak et al., 2004; Wilson et al., 1993). Previous studies were limited in concluding that

cocaine causes the observed deficits. Clinical studies of drug use typically do not have baseline data, and results could therefore be attributed to pre-existing differences between groups, or some other factor such as simultaneous use of alcohol or other drugs by participants (Aharonovich et al., 2006; Bartzokis et al., 2000; Goldstein et al., 2004; Lim et al., 2008). All subjects in this study were exposed to the same environmental factors so that any observed changes were attributable to cocaine use. Extensive baseline data were collected on cortical size and cognitive abilities for both groups so longitudinal comparisons could be calculated. Comparing each individual subject to themselves allowed relationships to be revealed even given the small number of subjects in the study.

Visual working memory has been associated with the lateral prefrontal cortex (Bechara & Martin, 2004; Goldstein et al., 2004; Wilson et al., 1993). Spatial working memory has been associated with the dorsolateral prefrontal cortex (dlPFC), while object-oriented working memory, as is examined in this study, has been associated with the vlPFC (Wilson et al., 1993). Neurons in each area are preferentially activated during the type of working memory tasks they support (Wilson et al., 1993). Thus the relationship observed in this study between visual working memory performance and area 12 reinforces previous observations on visual working memory and the vlPFC.

Deficits on the delayed match to sample task can be interpreted in several ways. One alternate explanation for deficits on the task, rather than impaired visual working memory, is impaired response inhibition. Nonhuman primates and human infants (unlike human adults) have a natural tendency to respond to novel stimuli (Rodriguez & Paule, 2009). In the delayed match to sample task, the subject is expected to respond to the stimulus that was previously viewed, ignoring the novel stimulus. If cocaine use impairs response inhibition, as has been

indicated in previous literature, this could cause subjects to revert to impulsively responding to the novel stimuli, reducing accuracy (Bechara & Martin, 2004).

Bechara and Martin (2004) also hypothesized that deficits on the delayed match to sample task observed in drug users could not be attributed solely to working memory. They did not observe an increase in impairment as length of delay increased and concluded that drug users were experiencing deficits in attention or executive function (Bechara & Martin, 2004). Our study indicated an increase in impairment across delays, with the greatest impairment seen at the 40-second delay interval. If the impairment was solely in attention or executive function, the deficit would tend to be consistent across delays, rather than increasing as the delay increases. The increasing deficit based on delay-length indicates an inability for working memory to hold the stimuli 'online' for increasing lengths of time. Impaired performance on the delayed match to sample task seen in this study, then, cannot be explained by only considering deficits in attention, as visual working memory appears to play a role.

The second major cognitive impairment observed in cocaine subjects in this study was reversal learning. Reversal learning performance is clinically relevant as it can be predictive of behavior and success in therapy (Fellows & Farah, 2003; Rolls et al., 1994). One study found that the number of times a patient touched a previously-rewarded stimulus was related to socially inappropriate behavior; both of these represent a lack of inhibition (Rolls et al., 1994). This lack of inhibition represents an inability to alter behavior in response to feedback, such as an inability to stop behaving inappropriately after negative responses from a peer (Rolls et al., 1994). Similar dysfunction may also be observed in socioeconomic decision making, as reversal learning has been shown to be impaired in gambling tasks (Fellows & Farah, 2003).

Understanding the specific cognitive deficits that are related to inappropriate behavior allows for the development of therapies that target those deficits.

Reversal learning ability has been associated with the orbitofrontal cortex (OFC), as observed in nonhuman primate lesion studies, studies of lesions in clinical populations, and studies of single neuron firing rates (Clarke et al., 2008; Dias et al., 1996; Fellows & Farah, 2003; Hornak et al., 2004). The OFC is thought to hold the reward value of stimuli and thus respond to the changes in reward value that occur during reversal learning tasks (Hornak et al., 2004). This study, however, did not reveal a significant relationship between the OFC (area 11,13,14) and reversal performance in the cocaine group.

Other brain regions, in addition to the OFC, have been implicated in reversal learning impairments as well. One hypothesis states that the basolateral amygdala registers the change in value that occurs at the start of a reversal phase; the OFC then holds the new reward values after the change has been registered by the amygdala (Murray & Izquierdo, 2007). Deficits in reversal learning have also been observed in association with damage to striatal areas (Goldman & Rosvold, 1972; Reading, Dunnett, & Robbins, 1991). The OFC has been specifically implicated in responding to both positive and negative feedback, while damage to striatal areas only accounts for impaired responding to negative feedback (Clarke et al., 2008). In this study, changes in reversal performance did not correlate with changes in amygdala or striatal size in either group, indicating the influence of these areas cannot account for the deficits observed in reversal learning. In addition, a rat model of cocaine use revealed deficits on a task that tested OFC function exclusively (Schoenbaum et al., 2004). These observations indicate the OFC has a role in reversal performance that is implicated in cocaine use despite the fact that a relationship between changes in OFC volume and changes in reversal performance are not observed in this

study. The differences in the relationship between cognitive changes and accepted underlying neural substrates of reversal performance and visual working memory is striking, but unexplained at present. The fact that a relationship was not observed in this study is unlikely due to a small sample size, given the strong relationships found with visual working memory.

Plasticity in the prefrontal cortex has been shown to reflect the demands of the task that has been learned (Freedman, Riesenhuber, Poggio, & Miller, 2001). The encoding of abstract rules, disinhibition, and working memory have all been shown to map across both the lateral PFC and the OFC (Duncan, 2001; Rao, Rainer, & Miller, 1997; Wallis, Anderson, & Miller, 2001). Duncan et. al. (2001) created a map of the PFC to outline which areas have been shown to correspond to different types of function. This map indicates that there are certain critical regions of the PFC, such as dorsolateral, ventrolateral and orbital surfaces of the PFC, that encode cognitive abilities, but that the specific cognitive abilities engaged are spread throughout those critical regions (Duncan, 2001). In essence, function is represented broadly, but primarily across several key areas, and attempts to localize cognitive abilities to one region alone may limit the interpretation of these results (Goldman-Rakic, 1988). One region that is particularly attuned to the demands of the relevant task is the lateral PFC (Freedman et al., 2001; Wallis et al., 2001).

The results from this study may be interpreted using this theory of broadly distributed function among critical cortical areas. In this study, like most nonhuman primate studies, the subjects were extensively trained on the cognitive tasks, then performed the tasks consistently once every two weeks for a year during self-administration. This amount of exposure to the tasks is much greater than would be seen in a typical clinical study. Training prior to self-administration, may have contributed to a broad distribution of visual working memory across

cortical areas; PFC neuron activation would have altered to encode the skills required by the delayed match to sample task.

Although the structural changes observed in this study were similar between groups, the cocaine group exhibited a deficit in cognitive abilities that correlates with gray matter volume loss, while the control group does not. Something is causing a deficit in performance in the cocaine group that is linked to gray matter volume, even though changes in volume observed in these regions do not differ between groups.

The relationships observed between structure and function in the cocaine group may result from a disruption of compensatory changes during aging. In normal aging, the continuous practice on cognitive tasks would allow the neurons necessary for these abilities to continue to communicate effectively while pruning synapses that are not being used (Hebb 1949). Any structural changes observed in the control group would minimally affect performance on cognitive tasks, because neurons being used for tasks are not lost, or because adaptive increases in efficiency, perhaps based in distributed interactions, are able to compensate for natural aging. In the cocaine group, the changes in structure while using cocaine may have occurred in a way that interferes with ongoing adaptive aging. Measures of functional connectivity could be informative in evaluating whether there are differences in distributed neural interactions between groups.

Substantial support for the presence of structural changes resulting from cocaine use is present in the literature. Gray matter loss was accelerated in cocaine users compared to what is observed in normal aging (Lim et al., 2008), while normal white matter expansion in the PFC was lost in cocaine users (Bartzokis et al., 2002). Alterations in the release of glutamate and dopamine in the prefrontal cortex have been observed in cocaine-treated rats (Bell, Duffy, &

Kalivas, 2000), and a decrease in blood flow to the PFC was observed in human users (Volkow et al., 1998). Neuroinflammation, as part of the innate immune response to cocaine use, may also be a mechanism for damage (Clark et al., 2013). Together the studies cited above suggest that cocaine causes structural changes in the prefrontal cortex. While the lack of change observed in this study may be a consequence of limited statistical power, the possibility also remains that structural differences observed in cross-sectional studies were pre-existing.

Identifying underlying changes in structure and/or distributed interactions that normally support adaptive aging could help the ultimate goal of improving therapies for addiction. Studies have shown that cognitive deficits in cocaine users persist for a month or longer into abstinence (Bolla et al., 1999), while alcohol studies showed that abstinent alcoholics had larger gray matter volumes than heavy drinkers. This suggests there may be recovery of structure during abstinence (O'Neill et al., 2001), or alternatively, indicates that those with greater gray matter volume were more likely to make the decision to quit. The present study has revealed a relationship between cocaine-induced cognitive changes and structural changes to areas of the prefrontal cortex, which will inform post-mortem studies looking for markers of neuroinflammation such as those reviewed in Clark et. al., 2013 (Clark et al., 2013).

This study examined changes in both cognitive performance and brain structure over time to test the hypothesis that changes in these regions may be contributing to cognitive deficits such as those impairments seen clinically in human cocaine users. Baseline and post self-administration performance on cognitive tasks, and longitudinal MRIs allowed for comparisons to be made across time within individual subjects; such comparisons cannot be made in clinical populations which do not have baseline data. One limitation of this study was the small sample size, which may have masked changes in structure that resulted from cocaine use. A relationship

between structural changes in orbitofrontal cortex and impairments in reversal performance was not observed, despite the literature that indicates that a relationship exists between reversal performance and the orbitofrontal cortex. In contrast, the relationships revealed between changes in structure in dorso- and ventrolateral prefrontal cortex and changes in function are consistent with the literature indicating these areas in the prefrontal cortex normally support visual working memory. These findings will guide post-mortem studies for region dependent changes and functional connectivity studies of distributed neural interactions associated with cocaine use.

5.0 TABLES AND FIGURES

5.1 MRICRON IMAGES

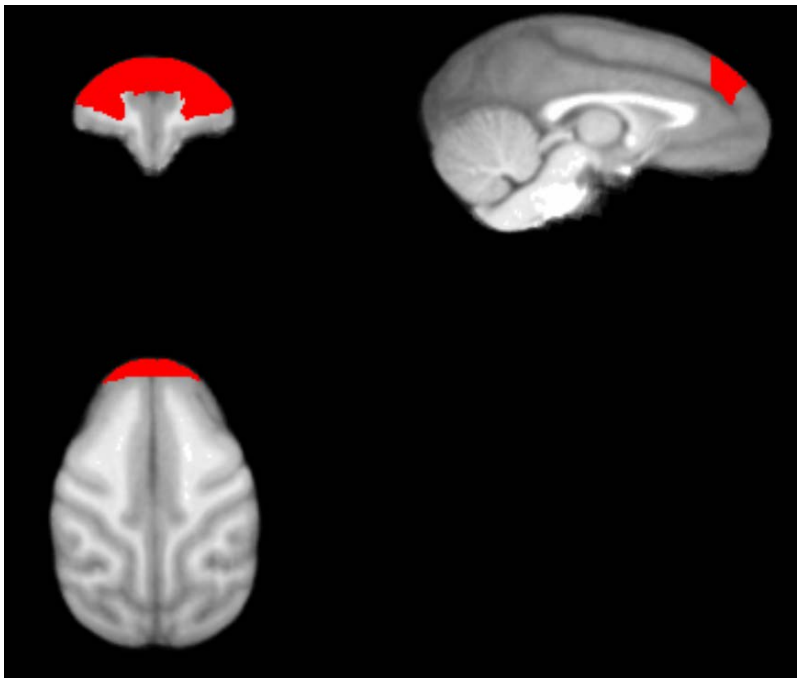

Figure 1: The dorsolateral prefrontal cortex (dlPFC)

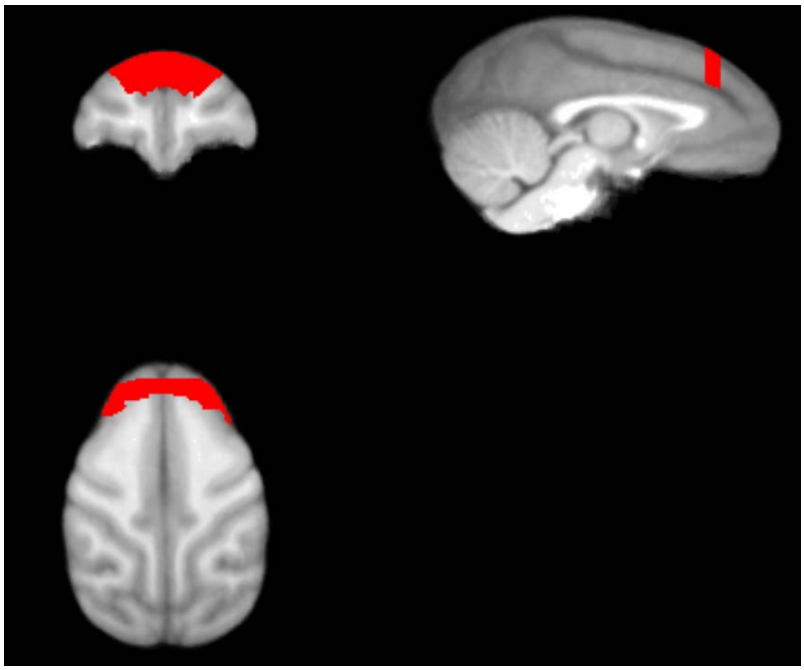

Figure 2: Area 8

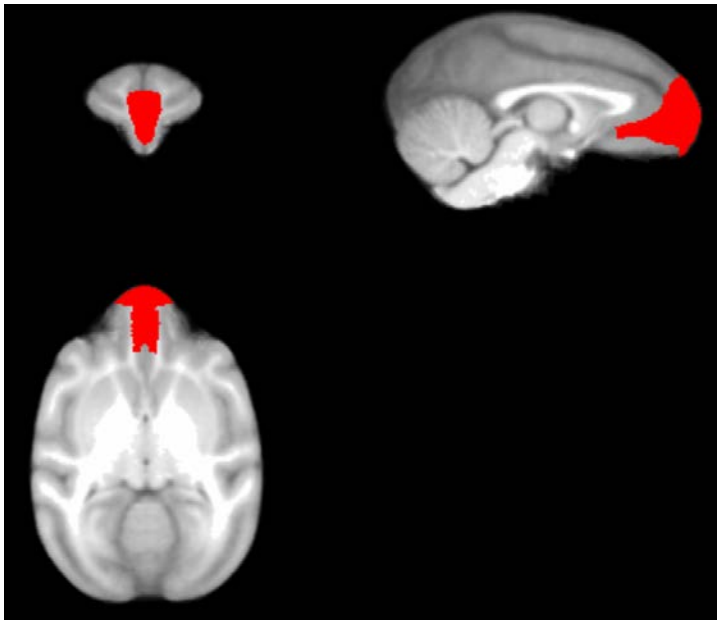

Figure 3: Area 10

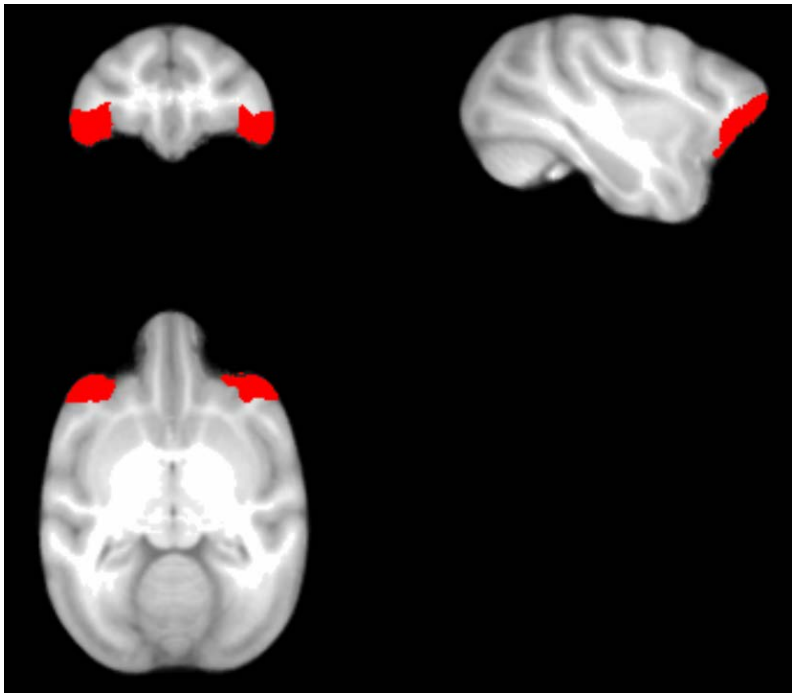

Figure 4: Area 12

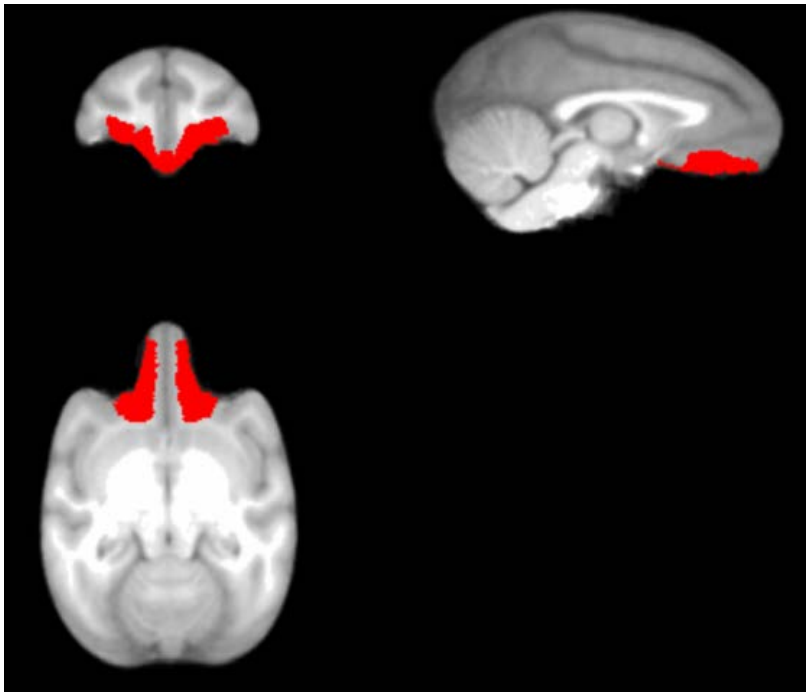

Figure 5: Area 11,13,14 represents the orbitofrontal cortex (OFC)

5.2 FIGURES FROM PORTER ET. AL. 2011

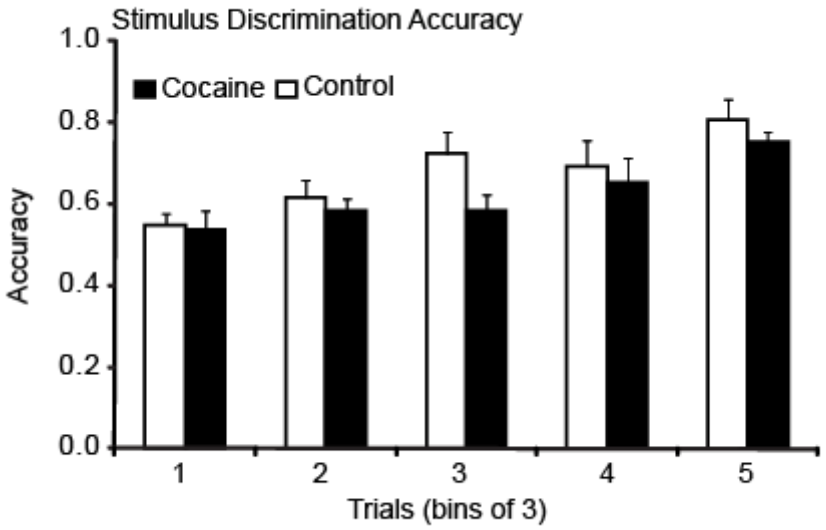

Figure 6: There was no significant difference between groups on stimulus discrimination performance post self-administration.

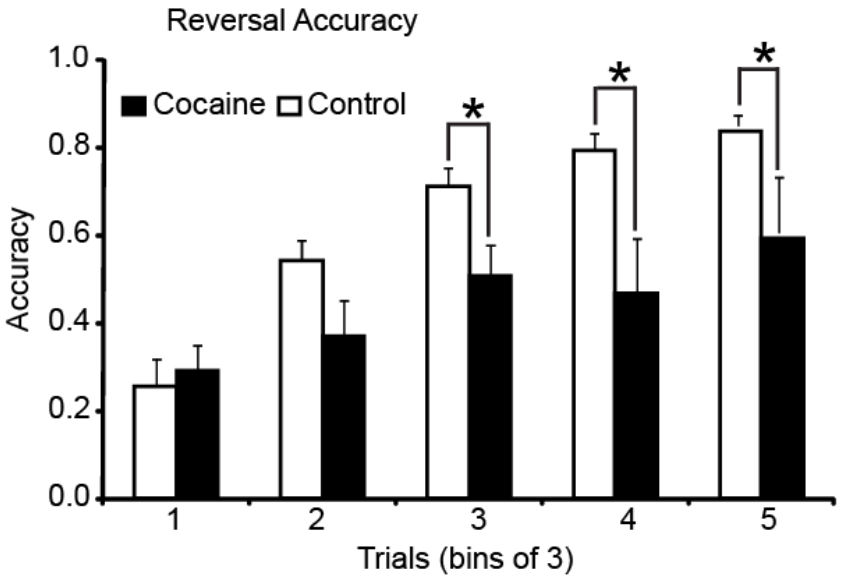

Figure 7: The cocaine group exhibited impaired reversal learning during the post self-administration period compared to the control group.

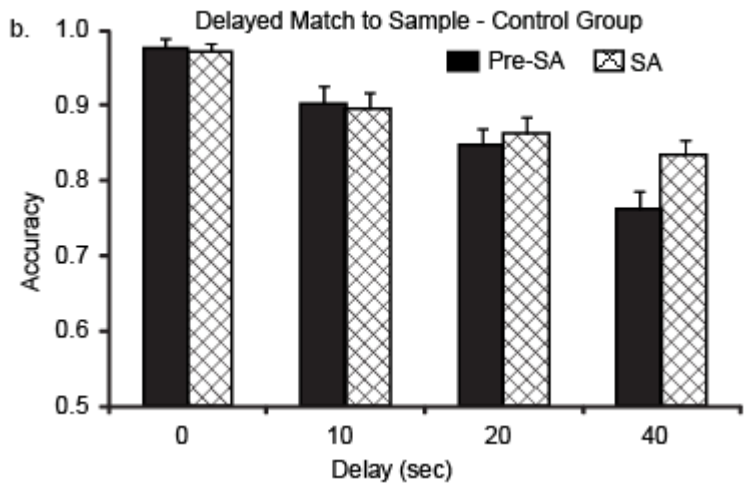

Figure 8: The control group was not impaired at visual working memory post self-administration.

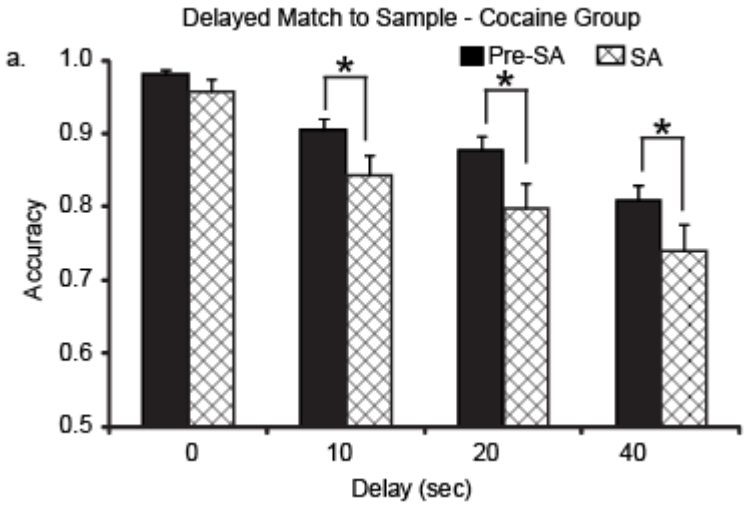

Figure 9: The cocaine group was impaired at visual working memory during the post self-administration period compared to pre self-administration performance.

5.3 REPRESENTATIVE SUBJECTS OF TASK PERFORMANCE

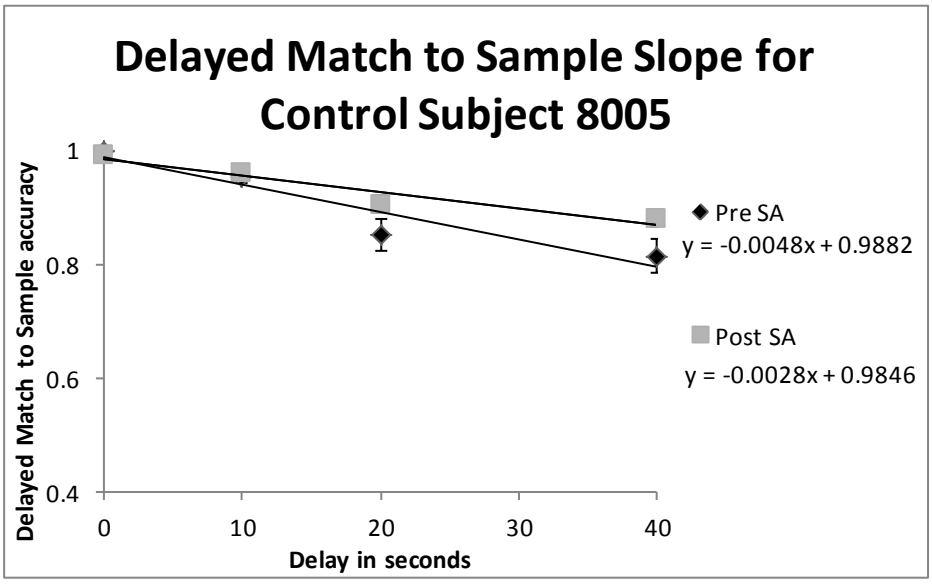

Figure 10: Control subject 8005 has a slope that is less steep during the post self-administration period, indicating greater visual working memory ability during this time.

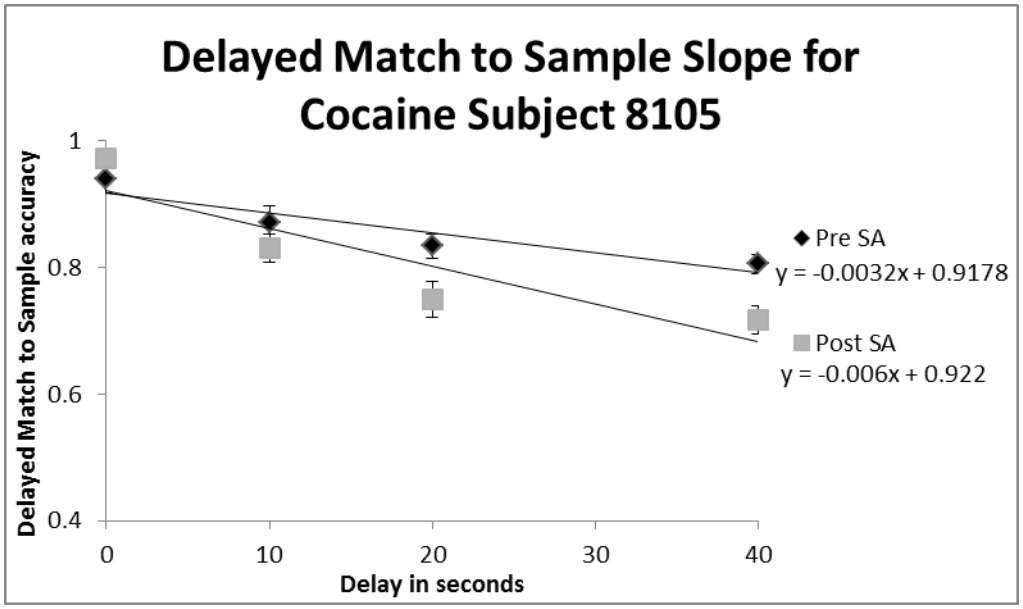

Figure 11: Cocaine subject 8105 exhibited deficits in visual working memory performance during the post self-administration period compared to the pre self-administration period, as indicated by a steeper slope across delay times.

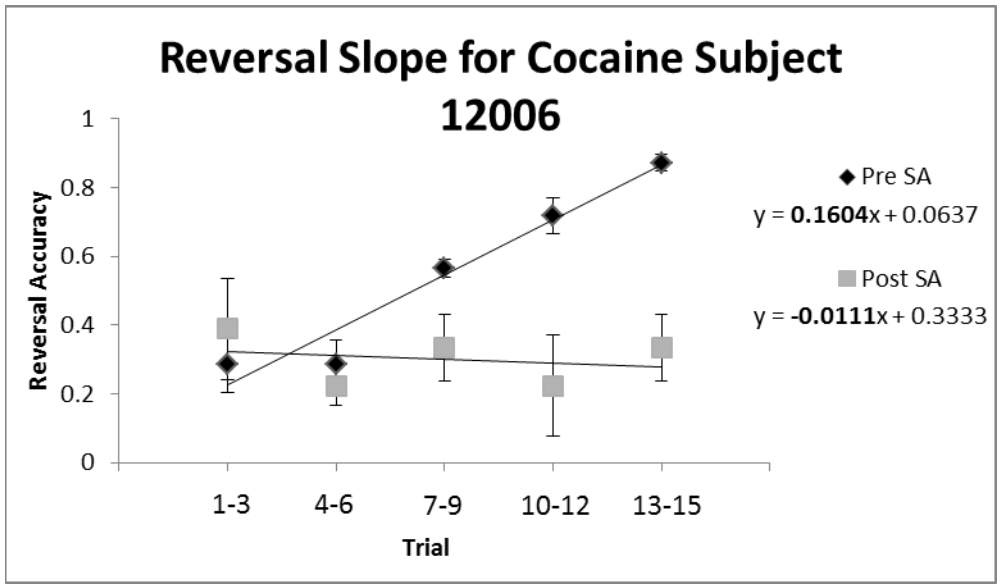

Figure 12: Cocaine subjects 12006 exhibited deficits in reversal performance when compared to the pre self-administration period, as indicated by a decrease in slope across trials.

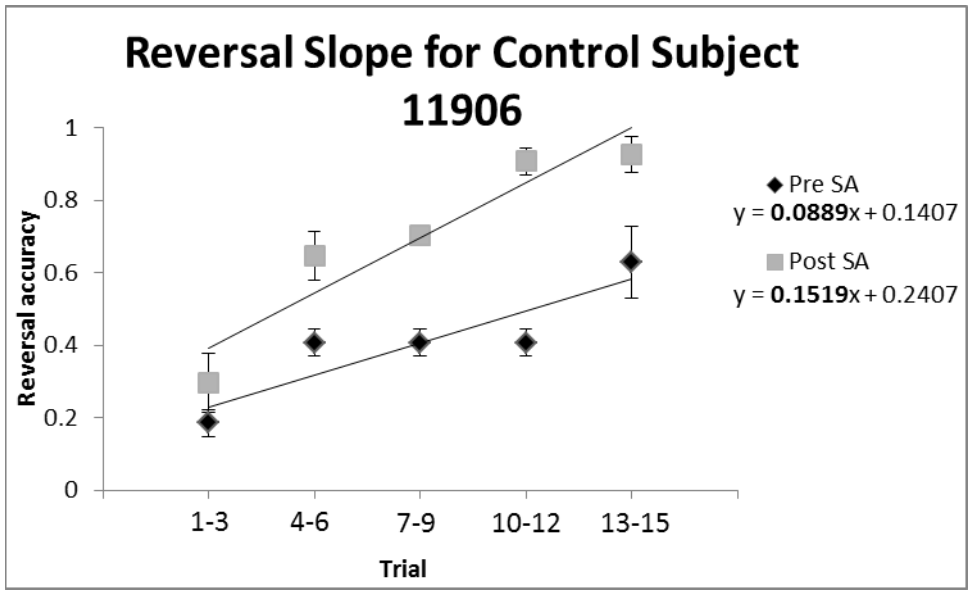

Figure 13: Control subject 11906 exhibited improved reversal learning performance during the post self-administration period as indicated by a greater increase in slope across trials.

5.4 STRUCTURAL CHANGES

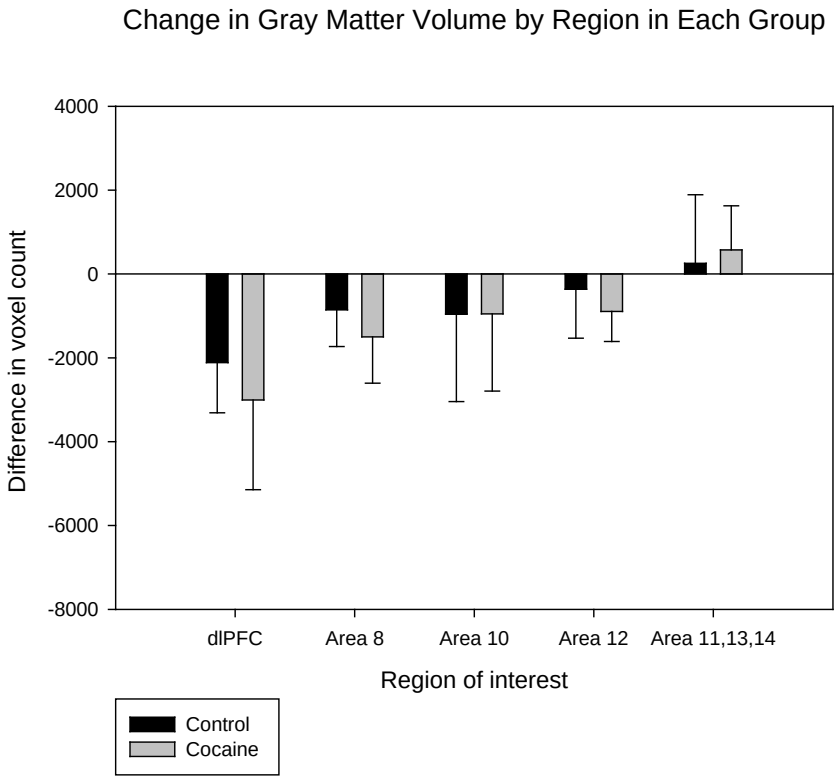

Figure 14: A significant difference between groups in changes in gray matter over time was not observed.

Table 1: Linear regressions were run to correlate changes in task performance over time with changes in gray matter volume by subtracting the pre self-administration data from the post self-administration data.

Column1	Cocaine	Control
dlPFC bilateral and visual working memory	0.001	0.94
Area 8 and visual working memory	0.021	0.817
Area 10 and visual working memory	0.063	0.766
Area 12 and visual working memory	0.037	0.927
Area 11,13,14 and visual working memory	0.67	0.935

5.5 LINEAR REGRESSIONS BETWEEN CHANGES IN STRUCTURE AND CHANGES IN FUNCTION

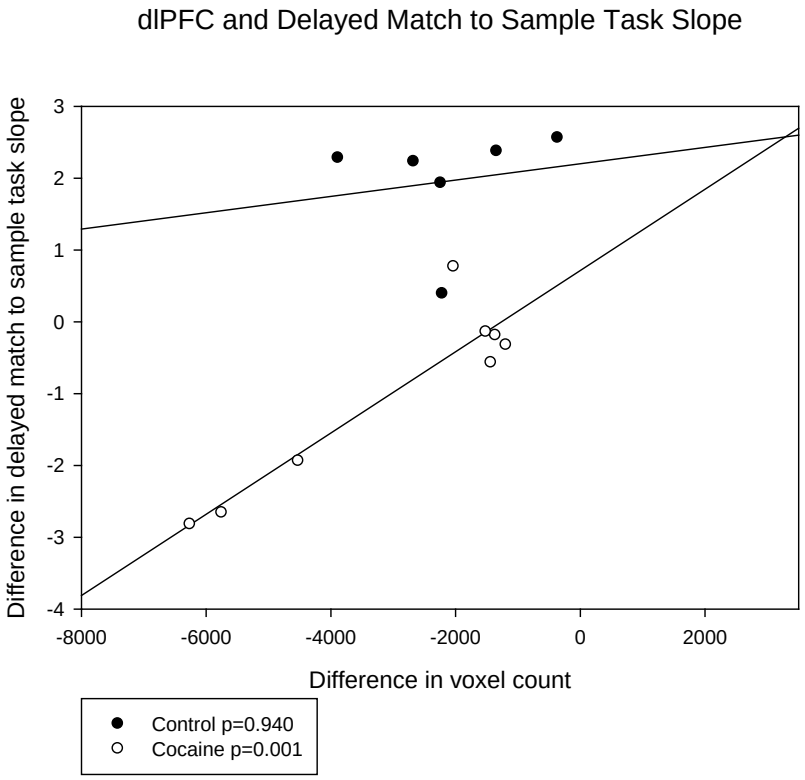

Figure 15: Regressions between dorsolateral prefrontal cortex (dlPFC) and performance on the delayed match to sample task for each group

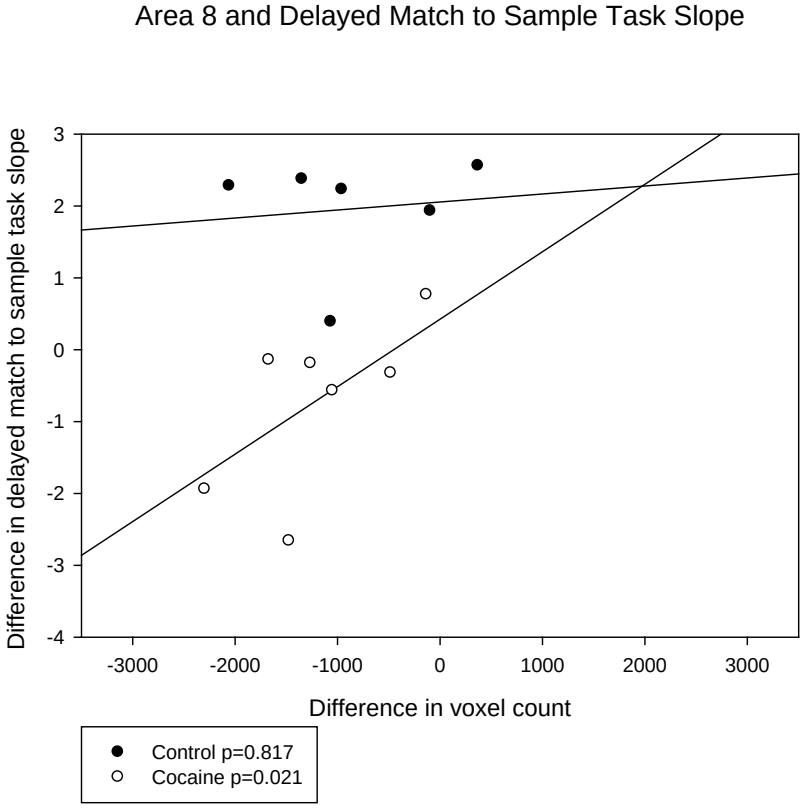

Figure 16: Regressions between area 8 and performance on the delayed match to sample task for each group

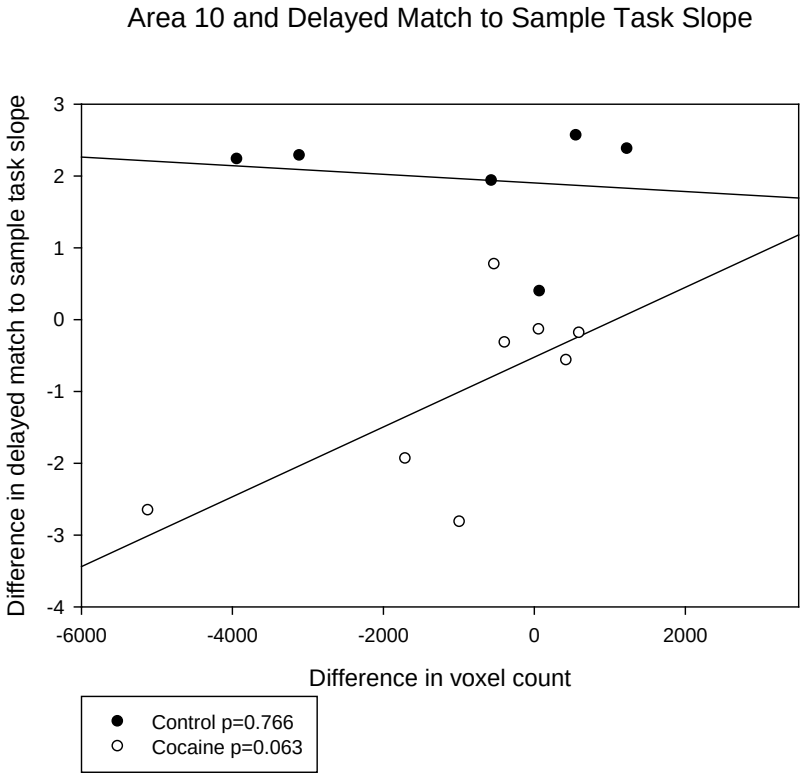

Figure 17: Regressions between area 10 and performance on the delayed match to sample task for each group

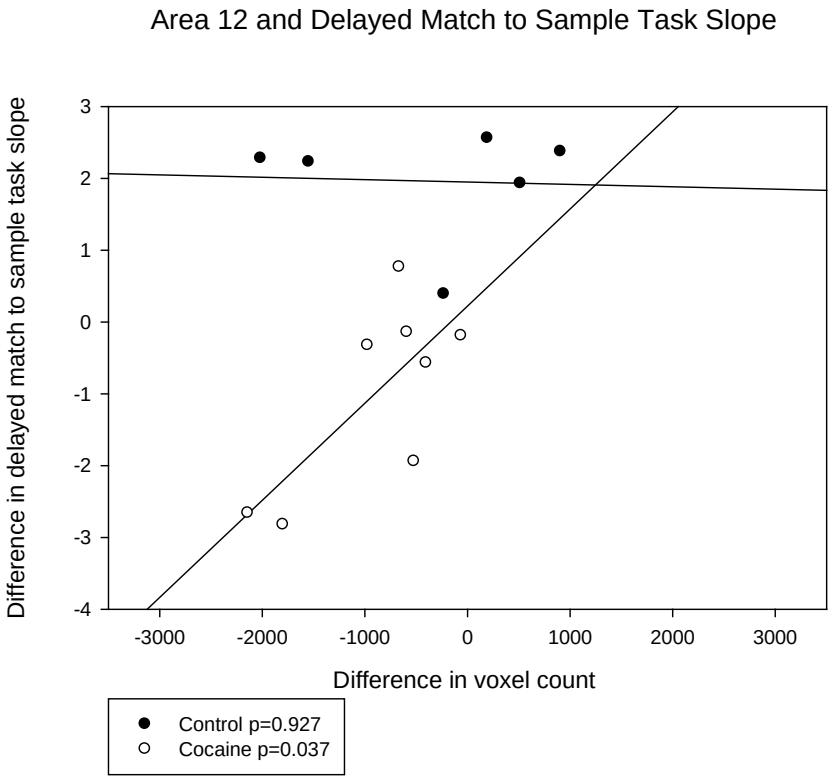

Figure 18: Regressions between area 12 and performance on the delayed match to sample task for each group

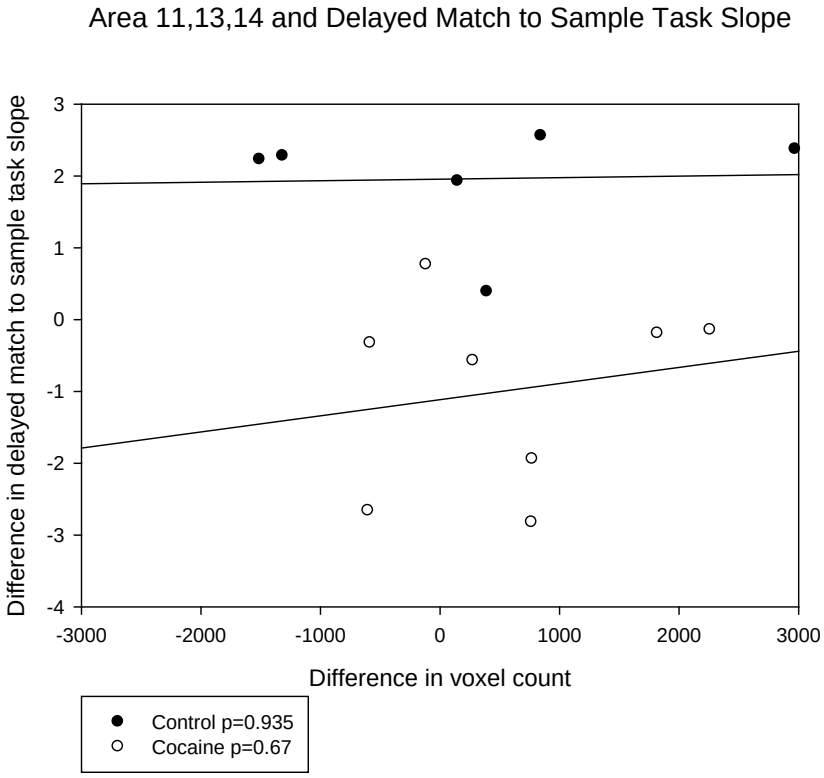

Figure 19: Regressions between area 11,13,14 (orbitofrontal cortex, OFC) and performance on the delayed match to sample task for each group

BIBLIOGRAPHY

Aharonovich, E., Hasin, D. S., Brooks, A. C., Liu, X., Bisaga, A., & Nunes, E. V. (2006). Cognitive deficits predict low treatment retention in cocaine dependent patients. *Drug Alcohol Depend, 81*(3), 313-322.

Bartzokis, G., Beckson, M., Lu, P. H., Edwards, N., Bridge, P., & Mintz, J. (2002). Brain maturation may be arrested in chronic cocaine addicts. *Biol Psychiatry, 51*(8), 605-611.

Bartzokis, G., Lu, P. H., Beckson, M., Rapoport, R., Grant, S., Wiseman, E. J., & London, E. D. (2000). Abstinence from cocaine reduces high-risk responses on a gambling task. *Neuropsychopharmacology, 22*(1), 102-103.

Bechara, A., & Martin, E. M. (2004). Impaired decision making related to working memory deficits in individuals with substance addictions. *Neuropsychology, 18*(1), 152-162.

Bell, K., Duffy, P., & Kalivas, P. W. (2000). Context-specific enhancement of glutamate transmission by cocaine. *Neuropsychopharmacology, 23*(3), 335-344. doi: 10.1016/S0893-133X(00)00100-7

Bolla, K. I., Rothman, R., & Cadet, J. L. (1999). Dose-related neurobehavioral effects of chronic cocaine use. *Journal of Neuropsychiatry & Clinical Neurosciences., 11*(3), 361-369.

Carmichael, O. T., Aizenstein, H. A., Davis, S. W., Becker, J. T., Thompson, P. M., Meltzer, C. C., & Liu, Y. (2005). Atlas-based hippocampus segmentation in Alzheimer's disease and mild cognitive impairment. *Neuroimage, 27*(4), 979-990. doi: 10.1016/j.neuroimage.2005.05.005

Childress, A. R., Mozley, P. D., McElgin, W., Fitzgerald, J., Reivich, M., & O'Brien, C. P. (1999). Limbic activation during cue-induced cocaine craving. *Am J Psychiatry, 156*(1), 11-18.

Clark, K. H., Wiley, C. A., & Bradberry, C. W. (2013). Psychostimulant abuse and neuroinflammation: emerging evidence of their interconnection. *Neurotox Res, 23*(2), 174-188. doi: 10.1007/s12640-012-9334-7

Clarke, H. F., Robbins, T. W., & Roberts, A. C. (2008). Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. *J Neurosci, 28*(43), 10972-10982.

Connolly, C. G., Bell, R. P., Foxe, J. J., & Garavan, H. (2013). Dissociated grey matter changes with prolonged addiction and extended abstinence in cocaine users. *PLoS One, 8*(3), e59645. doi: 10.1371/journal.pone.0059645

Cools, R., Clark, L., Owen, A. M., & Robbins, T. W. (2002). Defining the neural mechanisms of probabilistic reversal learning using event-related functional magnetic resonance imaging. *J Neurosci, 22*(11), 4563-4567.

Dias, R., Robbins, T. W., & Roberts, A. C. (1996). Dissociation in prefrontal cortex of affective and attentional shifts. *Nature, 380*(6569), 69-72.

Duncan, J. (2001). An adaptive coding model of neural function in prefrontal cortex. *Nat Rev Neurosci, 2*(11), 820-829.

Elliott, R., & Dolan, R. J. (1999). Differential neural responses during performance of matching and nonmatching to sample tasks at two delay intervals. *J Neurosci, 19*(12), 5066-5073.

Ersche, K. D., Clark, L., London, M., Robbins, T. W., & Sahakian, B. J. (2006). Profile of executive and memory function associated with amphetamine and opiate dependence. *Neuropsychopharmacology, 31*(5), 1036-1047.

Ersche, K. D., Turton, A. J., Chamberlain, S. R., Muller, U., Bullmore, E. T., & Robbins, T. W. (2012). Cognitive dysfunction and anxious-impulsive personality traits are endophenotypes for drug dependence. *Am J Psychiatry, 169*(9), 926-936. doi: 10.1176/appi.ajp.2012.11091421

Fellows, L. K., & Farah, M. J. (2003). Ventromedial frontal cortex mediates affective shifting in humans: evidence from a reversal learning paradigm. *Brain., 126*(Pt 8), 1830-1837.

Fjell, A. M., Westlye, L. T., Espeseth, T., Reinvang, I., Dale, A. M., Holland, D., & Walhovd, K. B. (2010). Cortical gray matter atrophy in healthy aging cannot be explained by undetected incipient cognitive disorders: a comment on Burgmans et al. (2009). *Neuropsychology, 24*(2), 258-263; discussion 264-266. doi: 10.1037/a0018827

Franklin, Teresa R., Acton, Paul D., Maldjian, Joseph A., Gray, Jason D., Croft, Jason R., Dackis, Charles A., . . . Childress, Anna Rose. (2002). Decreased gray matter concentration in the insular, orbitofrontal, cingulate, and temporal cortices of cocaine patients. *Biological Psychiatry, 51*(2), 134-142.

Freedman, D. J., Riesenhuber, M., Poggio, T., & Miller, E. K. (2001). Categorical representation of visual stimuli in the primate prefrontal cortex. *Science, 291*(5502), 312-316. doi: 10.1126/science.291.5502.312

Goldman-Rakic, P. S. (1988). Topography of cognition: parallel distributed networks in primate association cortex. [Review]. *Annual Review of Neuroscience, 11*, 137-156.

Goldman, P. S., & Rosvold, H. E. (1972). The effects of selective caudate lesions in infant and juvenile Rhesus monkeys. *Brain Res, 43*(1), 53-66.

Goldstein, R. Z., Leskovjan, A. C., Hoff, A. L., Hitzemann, R., Bashan, F., Khalsa, S. S., . . . Volkow, N. D. (2004). Severity of neuropsychological impairment in cocaine and alcohol addiction: association with metabolism in the prefrontal cortex. *Neuropsychologia, 42*(11), 1447-1458.

Grant, S., London, E. D., Newlin, D. B., Villemagne, V. L., Liu, X., Contoreggi, C., . . . Margolin, A. (1996). Activation of memory circuits during cue-elicited cocaine craving. *Proc Natl Acad Sci U S A, 93*(21), 12040-12045.

Hornak, J., O'Doherty, J., Bramham, J., Rolls, E. T., Morris, R. G., Bullock, P. R., & Polkey, C. E. (2004). Reward-related reversal learning after surgical excisions in orbito-frontal or dorsolateral prefrontal cortex in humans. *J Cogn Neurosci, 16*(3), 463-478.

Izquierdo, A., Suda, R. K., & Murray, E. A. (2004). Bilateral orbital prefrontal cortex lesions in rhesus monkeys disrupt choices guided by both reward value and reward contingency. *J Neurosci, 24*(34), 7540-7548.

Knickmeyer, R. C., Styner, M., Short, S. J., Lubach, G. R., Kang, C., Hamer, R., . . . Gilmore, J. H. (2010). Maturational trajectories of cortical brain development through the pubertal transition: unique species and sex differences in the monkey revealed through structural magnetic resonance imaging. *Cereb Cortex, 20*(5), 1053-1063. doi: 10.1093/cercor/bhp166

Lim, K. O., Wozniak, J. R., Mueller, B. A., Franc, D. T., Specker, S. M., Rodriguez, C. P., . . . Rotrosen, J. P. (2008). Brain macrostructural and microstructural abnormalities in cocaine dependence. *Drug Alcohol Depend, 92*(1-3), 164-172.

Liu, R. S., Lemieux, L., Bell, G. S., Sisodiya, S. M., Shorvon, S. D., Sander, J. W., & Duncan, J. S. (2003). A longitudinal study of brain morphometrics using quantitative magnetic resonance imaging and difference image analysis. *Neuroimage, 20*(1), 22-33.

Liu, S., Heitz, R. P., Sampson, A. R., Zhang, W., & Bradberry, C. W. (2008). Evidence of temporal cortical dysfunction in rhesus monkeys following chronic cocaine self-administration. *Cereb Cortex, 18*(9), 2109-2116.

Maas, L. C., Lukas, S. E., Kaufman, M. J., Weiss, R. D., Daniels, S. L., Rogers, V. W., . . . Renshaw, P. F. (1998). Functional magnetic resonance imaging of human brain activation during cue-induced cocaine craving. *Am J Psychiatry, 155*(1), 124-126.

Matochik, J. A., London, E. D., Eldreth, D. A., Cadet, J. L., & Bolla, K. I. (2003). Frontal cortical tissue composition in abstinent cocaine abusers: a magnetic resonance imaging study. *Neuroimage, 19*(3), 1095-1102.

Murray, Elisabeth A., & Izquierdo, Alicia. (2007). Orbitofrontal cortex and amygdala contributions to affect and action in primates. *Annals of the New York Academy of Sciences, 1121*, 273-296.

O'Neill, J., Cardenas, V. A., & Meyerhoff, D. J. (2001). Effects of abstinence on the brain: quantitative magnetic resonance imaging and magnetic resonance spectroscopic imaging in chronic alcohol abuse. *Alcohol Clin Exp Res, 25*(11), 1673-1682.

Padoa-Schioppa, C., & Assad, J. A. (2006). Neurons in the orbitofrontal cortex encode economic value. *Nature, 441*(7090), 223-226.

Patkar, A. A., Murray, H. W., Mannelli, P., Gottheil, E., Weinstein, S. P., & Vergare, M. J. (2004). Pre-treatment measures of impulsivity, aggression and sensation seeking are associated with treatment outcome for African-American cocaine-dependent patients. *J Addict Dis, 23*(2), 109-122.

Petrides, M. (2000). Dissociable roles of mid-dorsolateral prefrontal and anterior inferotemporal cortex in visual working memory. *J Neurosci, 20*(19), 7496-7503.

Porter, J. N., Olsen, A. S., Gurnsey, K., Dugan, B. P., Jedema, H. P., & Bradberry, C. W. (2011). Chronic cocaine self-administration in rhesus monkeys: impact on associative learning, cognitive control, and working memory. *J Neurosci, 31*(13), 4926-4934.

Rao, S. C., Rainer, G., & Miller, E. K. (1997). Integration of what and where in the primate prefrontal cortex. *Science, 276*(5313), 821-824.

Reading, P. J., Dunnett, S. B., & Robbins, T. W. (1991). Dissociable roles of the ventral, medial and lateral striatum on the acquisition and performance of a complex visual stimulus-response habit. *Behav Brain Res, 45*(2), 147-161.

Rodriguez, J. S., & Paule, M. G. (2009). Working Memory Delayed Response Tasks in Monkeys. In J. J. Buccafusco (Ed.), *Methods of Behavior Analysis in Neuroscience* (2nd ed.). Boca Raton (FL).

Rolls, E. T., Hornak, J., Wade, D., & McGrath, J. (1994). Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage. *J Neurol Neurosurg Psychiatry, 57*(12), 1518-1524.

Schoenbaum, G., Saddoris, M. P., Ramus, S. J., Shaham, Y., & Setlow, B. (2004). Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. *Eur J Neurosci, 19*(7), 1997-2002.

Sim, M. E., Lyoo, I. K., Streeter, C. C., Covell, J., Sarid-Segal, O., Ciraulo, D. A., . . . Renshaw, P. F. (2007). Cerebellar gray matter volume correlates with duration of cocaine use in cocaine-dependent subjects. *Neuropsychopharmacology, 32*(10), 2229-2237.

Teichner, G., Horner, M. D., Roitzsch, J. C., Herron, J., & Thevos, A. (2002). Substance abuse treatment outcomes for cognitively impaired and intact outpatients. *Addict Behav, 27*(5), 751-763.

Tremblay, L., & Schultz, W. (1999). Relative reward preference in primate orbitofrontal cortex [see comments]. *Nature, 398*(6729), 704-708.

Volkow, N. D., Wang, G. J., Fowler, J. S., Hitzemann, R., Gatley, J., Ding, Y. S., . . . Pappas, N. (1998). Differences in regional brain metabolic responses between single and repeated doses of methylphenidate. *Psychiatry Res, 83*(1), 29-36.

Wallis, J. D., Anderson, K. C., & Miller, E. K. (2001). Single neurons in prefrontal cortex encode abstract rules. *Nature, 411*(6840), 953-956. doi: 10.1038/35082081

Wallis, J. D., & Miller, E. K. (2003). Neuronal activity in primate dorsolateral and orbital prefrontal cortex during performance of a reward preference task. *European Journal of Neuroscience., 18*(7), 2069-2081.

Wilson, F. A., Scalaidhe, S. P., & Goldman-Rakic, P. S. (1993). Dissociation of object and spatial processing domains in primate prefrontal cortex [see comments]. *Science, 260*(5116), 1955-1958.

Wu, M., Carmichael, O., Lopez-Garcia, P., Carter, C. S., & Aizenstein, H. J. (2006). Quantitative comparison of AIR, SPM, and the fully deformable model for atlas-based segmentation of functional and structural MR images. *Hum Brain Mapp, 27*(9), 747-754.