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Role of cortisol in social and memory impairments in individuals with velocardiofacial syndrome (VCFS)

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Role of Cortisol in Social and Memory Impairments in Individuals with Velocardiofacial Syndrome (VCFS)

by

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Abstract

Velocardiofacial Syndrome (VCFS) is a genetic disorder characterized by numerous physiological and psychological symptoms. Little is known regarding the neuropsychological and hormonal substrates and the social functioning in individuals with VCFS. There is some evidence to suggest that the stress hormone cortisol contributes to social, cognitive, and communication deficits in related populations (Corbett, Schupp, Levine, & Mendoza, 2009). This study investigated the role that cortisol has on the social and cognitive impairments observed in children with VCFS. To this end, 11 children with confirmed VCFS were assessed for baseline cortisol levels and received neuropsychological testing that assessed attention, memory, language, and social functioning. These results were compared with the results from 11 controls that were matched according to age and sex. It was hypothesized that children with VCFS would have significantly higher baseline cortisol levels relative to control children. Additionally, these cortisol levels would be negatively correlated with measures of social functioning as measured by CBCL, ABAS-II, and RCMAS-2. Furthermore, it was hypothesized that cortisol levels would be negatively correlated with performance on cognitive tests. Specifically, it was hypothesized that cortisol levels in children with VCFS would be negatively correlated with tests of attention and memory as measured by the WRAML-2. Children with VCFS had significantly higher cortisol levels than control counterparts; $F(1, 20) = 5.436$, $p < .05$. Cortisol levels in VCFS were not related to measures of social functioning or measures of cognitive functioning. That said, a significant negative correlation was observed between the General Memory and Attention/Concentration indices of the WRAML-2 and cortisol concentrations in the control population: $r(11) = -.78$, $p < .05$; $r(11) = -.62$, $p < .05$. Additionally, the level of cortisol in control individuals was negatively correlated to the social

competency scale of the CBCL; $r(11) = -.64$, $p < .05$. These results support the role of neurohormonal substrates such as cortisol in social impairment and cognitive functioning in neurotypical children. More generally, these data provide evidence of a possible causal mechanism that underlies social impairments in other stress disorders known to involve cortisol dysregulation. Furthermore, these data add to the understanding of the interaction between stress, cortisol, and cognition are indicative of possible treatment targets for cognitive and social interventions.

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Introduction

Velocardiofacial syndrome (VCFS) is a genetic disorder that has a number of physiological and psychological symptoms. VCFS is one of the most common genetic disorders and one of the most common causes of learning disability and mild mental retardation (Eliez et al., 2001; Gothelf & Lombroso, 2001). Though the physiological symptoms associated with VCFS have been thoroughly studied, there is much to be understood with regard to the psychological features of the disorder. In particular, the neurochemical substrates underlying many of the social impairments expressed in these individuals have yet to be explored. Further examination of the neural substrates of social functioning in this population may provide insight into the brain-behavior relationships of social cognition. A review of the specific impairments associated with VCFS with an emphasis on their neuroanatomical and neurochemical substrates will be presented along with the role that cortisol plays in social impairment and psychopathology in other disorders. Specifically, this introduction will review the history of VCFS, the prevalence of the disorder, and the diagnostic tests used to identify the disorder. Furthermore, the physical, psychiatric, cognitive, and social deficits will be described along with the neurological, neurochemical, and neurohormonal anomalies associated with these symptoms. Finally, an experiment will be described in order to address a significant void in the literature regarding the etiology of social and cognitive impairments in this population.

History of VCFS Discovery

Velocardiofacial Syndrome was first described by Kirkpatrick and DiGeorge in 1968 as a constellation of immunologic deficiencies. It was renamed Shprintzen Syndrome following a re-categorization of the common presenting symptoms (Shprintzen et al., 1978). Shprintzen (1978)

described palate anomalies ("velo"), congenital cardiovascular defects ("cardio"), and mild facial dysmorphism ("facial") as characteristic of individuals with this particular disorder. Despite the consistent reemergence of the aforementioned symptoms, there is a great deal of symptom heterogeneity expressed in these individuals, which made it difficult to conclude that these individuals actually had the same disorder (Shprintzen et al., 1978). In order to definitively categorize these seemingly independent symptoms as being part of a single disorder, it was necessary to link the immunologic deficiencies noted by Kirkpatrick and the symptoms noted by Shprintzen with a common etiology. It was discovered that all of the individuals expressing some combination of the symptoms described by Kirkpatrick and Shprintzen had a microdeletion in the long arm of chromosome 22 at band 22q11.2 (Kelly et al., 1993). This finding allowed researchers to definitively conclude that the multitude of symptoms were part of the same syndrome. As research has progressed, the number of diagnostic symptoms has increased. At present, there are over 180 phenotypic characteristics of VCFS including congenital abnormalities, learning disabilities, and psychiatric disturbances (Gothelf, 2007). This has prompted researchers to begin using the title 22q11.2 deletion syndrome as opposed to VCFS, though the terms are still used interchangeably.

Prevalence and Diagnostic Tests

Despite consistent agreement among practitioners regarding the constellation of symptoms associated with VCFS, the exact prevalence of VCFS is difficult to ascertain. However, the best estimate to date is 1 in 5900 (Botto et al., 2003). The actual prevalence is in all likelihood much higher than this figure (Gothelf, 2007). Gothelf identifies several reasons for the inability to get an accurate prevalence rate. First, only "at-risk" infants are screened for the disorder. Second, the phenotypic and cognitive symptoms can be quite mild, resulting in a lack

of diagnosis or delayed diagnosis. Third, the heterogeneous symptom presentation often leads clinicians to make erroneous diagnoses.

Despite these barriers to gauging prevalence accurately, recent advances in genetic screening have enabled clinicians to determine with great accuracy whether a cluster of symptoms found in an individual is due to VCFS. Using Florescence In Situ Hybridization (FISH), clinicians can determine whether a micro-deletion exists on chromosome 22 (Driscoll et al., 1993). As a result, FISH has become the "gold standard" diagnostic test for VCFS. Though the diagnosis of VCFS can be made accurately and reliably, the cost of testing (approximately $1000 per test) is a major barrier to expansive infant screening. Future variants of the FISH test are likely to be more cost-effective, which would make widespread infant screening for the disorder feasible. Researchers will then be able to determine with greater accuracy the prevalence of the disorder. Despite difficulties accurately assessing prevalence, the physical symptoms associated with VCFS have been extensively examined. The following section will describe the physical anomalies associated with VCFS.

Common Physical Symptoms Associated with VCFS

There are a wide range of physical symptoms associated with VCFS. These symptoms can be grouped into 5 categories. The first symptom category is congenital cardiac anomalies. These anomalies occur in nearly 75% of all individuals with VCFS and include tetralogy of fallot, ventricular septal defects, and truncus arteriosus (Digilio et al., 2005). The second symptom category is abnormal faces (Gothelf, 2007). These facial anomalies are common in individuals with VCFS and include hypoplastic alae nasi, a prominent nasal root, elongation of the face with cheek flattening, narrow-set eyes, a small mouth, and a retruded chin. Many times these are the most prominent diagnostic features of VCFS. The third symptom category is

palatal abnormalities, which occur in approximately 75% of individuals with VCFS (Kirscher, 2005). Specifically, these anomalies include a defective palate and hypernasal speech. The fourth symptom category includes T-cell immunodeficiency (Gothelf, 2007). According to Kirkpatrick and DiGeorge (1968), this deficiency results from a hypoplastic parathyroid and thymus. The fifth symptom category consists of a heterogeneous set of other physical symptoms that covary with the disorder such as growth retardation, juvenile rheumatoid arthritis, and urinary tract abnormalities (Gothelf, 2005).

Etiological analysis of these symptoms has occurred on multiple levels. At the embryonic level, most of the symptoms observed appear to result from impaired migration of the neural crest cells (Prescott et al., 2005). These cells are the prelude to the formation of the mesenchyme of the third and fourth pharyngeal arches. From these arches emerge the face, cleft, thymus, parathyroid gland, and cardiovascular system. Thus, interference with the migration of the crest cells can result in a deleterious cascade in which numerous biological systems are impacted. Furthermore, this embryonic explanation accounts for the symptom heterogeneity observed in individuals with VCFS. That is, the level of impairment in the neural crest cell migration dictates the range of biological systems affected and the severity with which the pathology presents.

Another level of etiological analysis is genetic (Gothelf, 2007). One theory regarding the genetic etiology of VCFS is haploinsufficiency. Haploinsufficiency occurs when only a single functional copy of an essential gene is produced, resulting in insufficient gene product. Funke, Pandita, and Morrow (2001) noted that haploinsufficiency of one or more of a combination of genes may account for a multitude of the physical and psychiatric symptoms associated with VCFS (Funke, Pandita, & Morrow, 2001). In support of this, McDermid and Morrow (2002)

performed a knockout mutation for the TBX1 gene (McDermid & Morrow, 2002). TBX1 codes for a protein that is expressed in great quantity throughout numerous brain regions (Paylor et al., 2006). Compared to control mice, TBX1 "knockout" mice experienced numerous developmental abnormalities. Many of these abnormalities were correlates of the pathologies associated with VCFS. The deficits included cardiac abnormalities, abnormal facial structures, deformed vertebrae, and cleft palate. Thus, it is conceivable that VCFS might be related to genetic abnormalities of the TBX1 gene. Furthermore, the variable expression of the TBX1 protein explains the wide range of symptom heterogeneity in VCFS.

To conclude, there are two prominent theories regarding the etiology of impairment in this population. The first is the embryonic theory, which incorporates the concept of neural crest migration. The second theory is genetic, which suggests that limited protein expression from TBX1 mediates the symptomatology of VCFS. Both of these etiological theories account for the numerous symptoms associated with VCFS and the heterogeneity of symptom expression. However, more research is needed to determine how these two causal factors interact.

Psychiatric Symptoms of VCFS throughout the Lifespan

In addition to many physical disorders that are observed in individuals with VCFS, there are numerous comorbid psychiatric disorders (Arnold, Siegel-Bartelt, Cytrynbaum, Teshima, & Schachar, 2001; Feinstein, Eliez, Blasey, & Reiss, 2002; Fine et al., 2005; Gothelf et al., 2003; Gothelf et al., 2004; Lachman et al., 1996; Shprintzen, 2000). These psychiatric disturbances emerge at different times throughout the lifespan. For example, during childhood, one psychiatric disturbance in individuals with VCFS is ADHD (Arnold et al., 2001; Feinstein et al., 2002; Lajiness-O'Neill et al., 2006). This is one of the most common psychiatric correlates, with a prevalence rate of about 35% to 46% in individuals with VCFS. In addition to ADHD, a

significant proportion of children with VCFS present with non-verbal learning disorders (Lajiness-O'Neill et al., 2006). Anxiety disorders are also commonly diagnosed in children with VCFS (Gothelf et al., 2003; Prinzie et al., 2002). Specifically, VCFS children present with generalized anxiety disorder, separation anxiety disorder, obsessive compulsive disorder, and specific phobias. Affective disorders are also often comorbid with VCFS (Arnold et al., 2001; Feinstein et al., 2002). Arnold et al. (2001) noted high rates of depression among childhood samples of VCFS individuals. Furthermore, bipolar disorder was found in 66% of children with VCFS who demonstrated affective symptomatology (Papolos, Faedda, Veit, et al., 1996). Last, children with VCFS have a high prevalence rate of pervasive developmental disorders. For example, approximately 14%-45% of children who have VCFS also meet criteria for Autism Spectrum Disorder (ASD; Antshel et al., 2007; Fine et al., 2005; Niklasson, Rasmussen, Oskarsdottir, & Gillberg, 2001; Vorstman et al., 2006).

During late adolescence and throughout adulthood, a different pattern of psychiatric symptomatology emerges (Gothelf, 2007). For example, VCFS adults have much more severe, persistent anxiety than that observed in children with VCFS (Gothelf, Feinstein, et al., 2007). In addition, 32% of individuals with VCFS will develop schizophrenia or other psychotic disorders during late adolescence or early adulthood. Having 22q11.2 deletion syndrome is the single greatest genetic predictor of the development of schizophrenia (Murphy et al., 1999). These individuals are 25 times more likely to develop schizophrenia than the general population (Turner, 1989). Murphy (2002) noted that the prevalence rate of schizophrenia in individuals with VCFS is higher than in children with a schizophrenic parent (Murphy, 2002). This increased risk of schizophrenia and other psychiatric illness has led researchers to conceptualize VCFS as a significant genetic diathesis for the development of these disorders.

Cognitive Deficits and their Neurological Correlates in Patients with VCFS

In addition to the numerous psychiatric illnesses associated with VCFS, there are multiple cognitive impairments of note. For example, individuals with VCFS frequently have a Borderline IQ (Swillen et al., 1997). According to Swillen et al., approximately 25% to 40% have an IQ below 70 and meet criteria for Mild Mental Retardation.

Furthermore, deficits in attention, cognitive flexibility, and working memory are common (Gothelf, Furfaro, Penniman, Glover, & Reiss, 2005). Many of these deficits have been associated with impaired executive network efficiency (Sobin et al., 2004). The specific attention deficits can be ascribed to malformations of the head of the caudate nucleus (Sugama et al., 2000). Sugama (2000) noted that patients with VCFS had a larger right head of the caudate relative to the left head, whereas controls demonstrated the reverse trend. Interestingly, this same trend has been consistently identified in individuals with ADHD (Castellanos et al., 1994; Castellanos et al., 1996; Hynd et al., 1993). Taken together, these data suggest that this particular neuroanatomical anomaly mediates attention and other cognitive deficits in individuals with VCFS.

In addition to malformations of the caudate, it has been hypothesized that because people with VCFS have such large volumetric and activation differences in brain structures implicated in working memory compared to typically developing people, people with VCFS recruit a novel network of brain structures during performance of working memory tasks. Additionally, studies have demonstrated poor performance of tasks of verbal and visuo-spatial working memory by individuals with VCFS (Kates et al., 2006; Lajiness-O'Neill et al., 2005). Kates et al. (2007) sought to investigate this aberrant memory performance. They scanned the brains of 17 children and youth with VCFS, 10 of their siblings without the disorder and 10 healthy controls from the

wider community using fMRI. Their experimental design was similar to the commonly used Go/NoGo design. These researchers found no significant differences in reaction time or d-prime, a statistic based on a combination of the hit rate and false alarm rate. However, they found that controls activated the right middle and inferior frontal gyri, left and right inferior and superior parietal lobules, and left and right occipital lobes to a significantly greater degree than VCFS, whereas participants with VCFS activated the left orbitofrontal cortex, right cingulate, and the right cuneus and occipital cortex more. This lends credence to the hypothesis that people with VCFS activate a different neural network during working memory tasks than people without the disorder.

Another interesting study examining working memory function in people with VCFS used a directed forgetting paradigm (Debbané et al., 2008). In this method, a series of items are presented (e.g., words, pictures), some labeled "to be remembered" and some labeled "to be forgotten." This selective encoding strategy usually encourages greater encoding efficiency for the "to be remembered" items and leads to a directed forgetting effect (DF effect) for the "to be forgotten"' items. The researchers employed this paradigm and hypothesized that people with VCFS would show decreased DF effects, similar to previously obtained results of people with schizophrenia. The experimental groups were 33 individuals with VCFS aged 10-36 years old, and a control group of typically developing individuals in the same age range, matched on sex to the VCFS group. Repeated-measures analysis of variance (ANOVA) demonstrated a main effect for directed forgetting across groups, as would be expected. In other words, more "to be remembered" items were correctly identified than "to be forgotten items." However, there was no between-group effect, indicating the participants with VCFS were not significantly less likely to correctly identify the "to be remembered" items. This contradicts their hypothesis and

supports a view that people with VCFS are able to selectively process stimuli during encoding at a level similar to typically developing individuals, implying that working memory systems are not necessarily involved in memory impairments observed in VCFS.

In conjunction with deficits in attention and working memory, individuals with VCFS also display impairments in response inhibition. This response inhibition has been associated with abnormal activation of the parietal cortex (Eliez et al., 2001). Gothelf et al. (2007) noted that individuals with VCFS performed comparably to controls on the Go/No Go response inhibition task (Gothelf, Hoeft, et al., 2007). However, VCFS individuals showed additional activation in superior and inferior parietal regions in conjunction with frontal-striatal processes, suggesting that more neurocognitive resources were necessary in order to inhibit particular responding. These results, taken together with the observed attentional impairments, may account for the high rate of ADHD in individuals with VCFS.

In addition to response inhibition difficulties, individuals with VCFS also show impairment in visuospatial and numerical abilities. These impairments have been associated with structural deficits of the parietal lobe (Eliez et al., 2001; Kates et al., 2004). Eliez et al. (2001) observed increased activation of the left supramarginal gyrus (LSMG) as a function of computational difficulty in individuals with VCFS. No such trend was observed in age-matched controls. The authors concluded that the increased LSMG activation occurred as a result of parietal deficits and may contribute to limitations in numerical, mathematical, and spatial reasoning abilities in this population and account for the commonly observed NLD profile identified by Lajiness-O'Neill et al. (2005). Moreover, Lajiness-O'Neill et al. (2005) observed deficits in visual learning and facial memory in children with VCFS and their sibling controls and concluded that this specific cognitive profile may be the result of a disruption in the ventral-

temporal pathway that occurs independent of the microdeletion itself. Specific structures implicated by Lajiness-O'Neill (2005) that may be impacting memory performance were the fusiform gyrus and the parahippocampal/hippocamapal regions. Given that it is not the presence of VCFS alone mediating these specific cognitive deficits in this population, it is possible that other factors may contribute to this broader phenotype observed in the siblings of children with VCFS. Additionally, as will be discussed in the section on social processing, deficits in facial memory are associated with poor social competence and social skills deficits (Ennis & Whelton, 1994). Thus, the pervasive nonverbal memory deficits observed in this population may also contribute to the significant social impairment observed in this population.

In addition to the aforementioned nonverbal memory deficits observed in individuals with VCFS, this population also exhibits some verbal working memory deficits (Lajiness-O'Neill, 2005). On tests of verbal working memory, children with VCFS performed significantly worse than their sibling counterparts. These data suggest that the verbal working memory deficits observed in this population or more closely associated with the unique features of the microdeletion as opposed to a broader mechanism.

To summarize, individuals with VCFS have a number of cognitive deficits. These deficits include attention, working memory, memory, cognitive flexibility, response inhibition, visuospatial abilities, numerical processing, and language processing. Many of these deficits have neuroanatomical correlates that may account for poor performance on related tasks. Indeed, it is possible that many of these cognitive deficits and their neurological etiology contribute directly or indirectly to the many social deficits observed in individuals with VCFS. Specifically, it is likely that working memory deficits, language impairment, and verbal and

nonverbal learning deficits contribute significantly to social impairment. These social deficits will be the primary focus of the remainder of this introduction.

Social Impairments Observed in Individuals with VCFS

Though the physical symptomatology and genetic features of the disorder have been widely studied, there has been little advancement in the understanding of social phenotypes in VCFS and other genetic neurodevelopmental disorders (GNDD's; Gothelf, Feinstein, et al., 2007). One reason for this lack of research pertaining to the social deficits of these disorders is simply a failure to include assessments of social functioning when conducting research on these individuals. Frequently, the psychotic features, cognitive deficits, and genetic correlates take precedence when conducting research on GNDD's (Gothelf, Feinstein, et al., 2007). Furthermore, diagnostic overshadowing has been problematic in elucidating the specific social deficits in VCFS. Specifically, the cognitive impairments are viewed as the etiology of social features and therefore little concern is placed on determination of independent etiologies for these social deficits (Hodapp & Dykens, 2005; Jopp & Keys, 2001). Indeed, these cognitive impairments contribute to the observed deficits in social functioning; however, there is neuroanatomical evidence suggesting that there is an etiology for the social deficits in VCFS that is independent of their cognitive limitations. Moreover, there is evidence from other neurodevelopmental disorders that low IQ is unrelated to social functioning. For example, individuals with William's Syndrome exhibit highly social behavior despite having a lower IQ relative to normal controls (Steinlin, 2007). In other words, there is something about the specific cognitive profile of individuals with VCFS that contributes to the social impairment in this population.

Despite being overlooked throughout the history of VCFS research, recent research has identified a number of social deficits that commonly occur in individuals with VCFS. One such social deficit is impaired social communication. Interestingly, these individuals develop normal communication skills throughout the first two years of life, but then show significant delays acquiring the ability to use short phrases and sentences. Furthermore, their speech is typically unintelligible due to the emergence of compensatory articulation patterns (Golding-Kushner, Weller, & Shprintzen, 1985). The most common compensatory articulation in children with VCFS is the implementation of glottal stop substitutions (Shprintzen, 1997). As result of such communication difficulties, few utterances are understood and early speech attempts may not be reinforced or encouraged leading to further disintegration of communication abilities.

Another social deficit that may emerge as a result of these communication issues is extreme shyness and withdrawal (Shprintzen, 2000). Swillen et al. (2001) administered the Child Behavior Checklist (CBCL) to both individuals with VCFS and individuals with speech pathology and learning impairments. They found that individuals with VCFS exhibited significantly more withdrawal behavior, suggesting that the speech difficulties in VCFS interact with other features of the disorder to contribute to social withdrawal (Swillen, Devriendt, Ghesquiere, & Fryns, 2001).

One of the social deficits that may result from the shyness and social withdrawal is a failure to initiate interactions (Eliez et al., 2000). In their case study describing the language, speech, and psychological features of five individuals with VCFS, Eliez et al. (2001) noted a significant deficit in the number of attempts made to interact. The authors attributed this social deficit to communication difficulties and the tendency to engage in more withdrawn behaviors.

In addition to their initiation deficits, VCFS individuals also show deficits in facial recognition (Stiers et al., 2005). Stiers and colleagues (2005) noted that the substantial impairments in facial recognition correlated with decreased volume of the right inferior parietal and superior occipital lobe. In addition, there was a pronounced reduction of white matter behind the inferior frontal gyrus relative to controls. Thus, it is possible that the social deficits observed in these individuals relating to their inability to recognize faces may be mediated by abnormalities in these brain areas.

Further contributing to deficits in social functioning are impairments in processing facial expressions (van Amelsvoort et al., 2006). VCFS individuals perform significantly worse on facial emotional processing tasks compared to age- and IQ-matched controls. This deficit was associated with decreased activation in the right insula and frontal lobe. Alternatively, there was increased activation in occipital regions in VCFS patients relative to controls. The authors concluded that these genetically determined neuroanatomical anomalies might significantly contribute to social deficits due to their involvement in emotional processing.

In addition to their emotional processing difficulties, VCFS individuals have distinct personality characteristics that color many of their social interactions. VCFS individuals are less conscientious, less emotionally stable, more irritable, and more dependent than normal controls (Prinzie et al., 2002). Furthermore, VCFS individuals have been shown to have severe attachment and anxiety issues, which may contribute to or exacerbate their personality deficits. For example, Shprintzen (2000) noted age-inappropriate separation anxiety in VCFS individuals when compared with normal controls. Also, children with VCFS are more phobic and present with obsessive compulsive personality disorder more frequently than controls (Papolos et al.,

1996). Indeed, the personality characteristics and anxious symptoms of people with VCFS greatly impede social development.

Last, these individuals show affective abnormalities that interfere with social relations. People with VCFS have been shown to have a flatter affect than normal controls during engagement in social interactions. Furthermore, their facial response tends to be expressionless during social engagements that usually facilitate both affective and facial responses (Golding-Kushner et al., 1985). Indeed, this would make it difficult for individuals with VCSF to sustain conversations with others and provide reinforcing feedback to those with whom they are communicating. As a result, these individuals may have shorter interactions with individuals they are meeting for the first time.

In summary, individuals with VCFS have numerous features contributing to their social deficits. It is likely that many of the genetically prescribed neuroanatomical anomalies associated with VCFS contribute to these deficits. The following section will identify etiological theories for these social deficits.

Etiology of Social Impairments

One prominent theory regarding the etiology of social deficits in VCFS individuals suggests that the language deficits are the foundation for the other social impairments. Shprintzen (2000) argues that the language deficits observed in these individuals leads to ineffectual communication skills. These ineffectual communication skills then lead to social isolation due to lack of social reinforcement. Oftentimes these children are ridiculed for their poor communication skills, leading to withdrawal and the formation of social anxieties. In this way, language impairment can account for a number of other social peculiarities associated with the disorder.

Another physiological explanation for the etiology of social deficits is lesions. White matter hyperintensities and cysts have been consistently observed in individuals with VCFS (Mitnick, Bello, & Shprintzen, 1994). The individuals presenting with these abnormalities have the characteristic behavioral and social deficits commonly observed in this population. Though the lesions were not statistically related to the behavioral deficits, it is possible that they interact with other structural abnormalities to produce the social disturbances.

Additionally, there has been some work identifying specific brain structures and sub-nuclei that have been associated with the social deficits observed in this population. For example, one study found significant volumetric changes in amygdala, prefrontal cortex, and orbitofrontal cortex (Kates et al., 2006). These volumetric differences were positively correlated with impaired performance on social competency scales such as the CBCL and other parent report measures. Thus, as amygdala volume increased in size, symptom endorsement regarding social competency increased.

With regard to the amygdala, Kates et al. (2006) noted that after controlling for total brain size, the amygdala to prefrontal cortex ratio in VCFS patients was found to be significantly larger than in sibling controls. As the amygdala as been implicated in emotional processing (Davidson & Irwin, 1999; LeDoux, 2000; Phillips, Drevets, Rauch, & Lane, 2003), learning and memory (Maren, 1999), and facial processing (Adolphs, Tranel, Damasio, & Damasio, 1994; Hamann et al., 1996), this finding may account for many of the social features observed in individuals with VCFS. Though it is tempting to conclude that the genetic anomalies associated with this disorder mediate this neuroanatomical deviation, it has been proposed that this increase in amygdala volume could be the result of high anxiety levels in this population (Feinstein et al., 2002). Increased amygdala volumes have been observed in individuals with phobia and

generalized anxiety disorders (Bellis, 2000). Dong and Greenough (2004) found that this increased anxiety leads to experience-dependent neuronal remodeling in the amygdala, which may contribute to its increased volume (Dong & Greenough, 2004b). Thus, it is possible that the anxiety associated with these patients leads to the volumetric changes in the amygdala. These neuroanatomical changes then contribute to difficulties with emotional regulation, facial recognition, and affective style.

In addition to amygdala volume discrepancies, individuals with VCFS also have a prefrontal cortex (PFC) that is smaller than that of age-matched, non-clinical controls (Kates et al., 2006). The PFC has been implicated in the ability to modulate emotional stimuli and was found to be correlated with social impairments in individuals with VCFS (Kates et al., 2006). In particular, smaller volumes were observed in the orbitofrontal cortex relative to controls. The orbitofrontal cortex has been implicated in the modulation of anxiety (Breiter & Rauch, 1996; Rauch, Savage, Alpert, Fischman, & Jenike, 1997). Thus, it is possible that many of the attachment and anxiety issues in VCFS patients may be related to abnormalities in this particular structure.

Other sub-regions of the PFC have been implicated in social functioning. For example, the dorsolateral PFC (DLPFC) has been shown to be involved in the regulation of affect and has been implicated in the etiology of mood disorders such as depression (Gainotti, 1972; Robinson, Kubos, Starr, Rao, & Price, 1984; Sackeim, Decina, & Malitz, 1982). Thus, it is conceivable that aberrant functioning in this sub-region could mediate the mood symptoms that are frequently observed in individuals with VCFS. That said, there is some recent evidence to suggest that DLPFC plays only a secondary role in the etiology of mood disorders and is primarily involved in the regulation of attentional systems and complex cognitive processing (Kobel et al., 2010;

Seidman, Valera, & Bush, 2004; Seidman et al., 2006). Thus, though the DLPFC has historically been implicated in the regulation of affect, contemporary research using advanced imaging techniques suggests that the aberrant DLPFC functioning may underlie only the cognitive deficits associated with VCFS.

In addition to the dorsolateral PFC, the ventromedial PFC (VMPFC) also has a role in social functioning. The VMPFC has been implicated in the anticipation of future consequences (Bechara, Damasio, Damasio, & Anderson, 1994). Specifically, individuals with damage to this region showed an inability to accurately anticipate both positive and negative consequences. Interestingly, individuals with damage to this region showed reduced electromodal responses when they were presented with a risky choice relative to controls who demonstrated both conscious recognition of the risky choice and full electromodal responses (Bechara, Damasio, Tranel, & Damasio, 1997; Bechara, Tranel, Damasio, & Damasio, 1996). Indeed, it is possible that much of the impulsiveness and disinhibition observed in individuals with VCFS might be mediated by volumetric differences in this particular region. Despite this logical connection, there has been limited research with regard to this region of the frontal cortex in individuals with VCFS.

Taken together, these data suggest that anomalies in the prefrontal cortex mediate many of the social deficits observed in individuals with VCFS. Furthermore, specific sub-regions should be examined in individuals with VCFS in order to determine whether differences in these structures mediate the observed social deficits.

Additional neuroanatomical analysis in individuals with VCFS has found discrepancies in volume of the insular cortex (van Amelsvoort et al., 2001). In addition to this volumetric difference, van Amelsvoort and Schmitz (2006) also noted decreased activation in right insula

during tasks that involved facial processing (van Amelsvoort et al., 2006). The insula has been shown to be involved in the processing of general emotions and has been implicated as one of the etiological factors of anxiety (Rauch et al., 1995). Furthermore, it has been demonstrated that the insular cortex plays a critical role in regulating autonomic responses accompanying emotion. Also, the insula is a site that has been implicated in gustatory processing. It is especially active during exposure to disgusting foods and photos of faces with an expression of disgust, suggesting one of its functions is the recognition of distasteful stimuli (Rozin, 1997; Young, 1997). Last, given the known input and output pathways of the insular cortex, Davidson and Irwin (1993) concluded that the insula is likely associated with the physiological changes that occur following autonomic activation (Davidson & Irwin, 1999). In sum, given what is known regarding the functions of the insular cortex, the anatomical and activation abnormalities in this structure may underlie such social deficits as withdrawal, inhibition, poor attachment, and social anxiety. Though some speculation has been made regarding the insular cortex and social deficits in VCFS, no one to date has directly assessed the relationship between insula and social deficits.

In addition to the aforementioned structures, it is also possible that the STS mediates some of the social deficits observed in individuals with VCFS. The STS has been implicated in the development of theory of mind (ToM; Moriguchi, Ohnishi, Mori, Matsuda, & Komaki, 2007). Theory of mind is defined as the ability to attribute mental states to the self and others. As such, it has been implicated in the development of social deficits in a number of disorders including ASD and schizophrenia (Castelli, Frith, Happe, & Frith, 2002; Happe et al., 1996; Ohnishi et al., 2000). Given that individuals with VCFS experience a number of social deficits including impairments in controlling gaze, which has been hypothesized to be related to theory

of mind (Garrett, Menon, MacKenzie, & Reiss, 2004), it is plausible that these individuals may show volumetric differences in the STS relative to controls. Additionally, individuals with activation deficits in this structure have difficulty maintaining appropriate social posturing as is the case with individuals diagnosed with ASD and schizophrenia. Furthermore, the STS has been implicated in the processing of vocal sounds (Gervais et al., 2004). Autistic individuals showed decreased activation in this area during exposure to vocal sounds. Gervais et al. (2004) concluded that some of the social deficits observed in ASD might be attributed to deficits in vocal processing.

Yet another structure that has been implicated in social processing and may contribute to the social deficits observed is the anterior cingulate cortex (ACC). The ACC has been implicated in the ability to attend to emotional events (Posner, 1995). Additionally, Lang et al. (1997) showed increased activity in the ACC during tasks for which attention to emotional stimuli was required (Lane et al., 1997). When subjects were asked to attend to non-emotional stimuli, no such activation in the ACC was recruited. It was therefore concluded that one of the roles of the ACC was to facilitate attention to emotional stimuli. The ACC has also been implicated in the mediation of other affective and cognitive functions (Fujiwara et al., 2007). Specifically, the ACC helps modulate emotional responses and has been found to be an essential component in social cognition and mentalizing (Kopelman, Andreasen, & Nopoulos, 2005). Interestingly, volumetric differences in the ACC have been observed in schizophrenic patients relative to controls (Kopelman et al., 2005). The smaller ACC volume has also been correlated with specific deficits in social cognition (Fujiwara et al., 2007).

In addition to a structural level analysis of the etiology of psychopathology in individuals with VCFS, there has been some work done at the neurochemical level. For example, the

pathophysiological and neurochemical mechanisms that underlie depression in VCFS are believed to be the same as for major depression. Specifically, deficits in both serotonin (5HT) production and transmission may have been implicated in etiology of depression in this population. Such deficits in 5HT have been observed via microdialysis procedures in mouse models of VCFS (Tuathaigh et al., 2002). Furthermore, VCFS individuals suffering from depression, OCD, and generalized anxiety respond well to treatment with selective-serotonin reuptake inhibitors (SSRI's), suggesting a deficiency in this neurotransmitter.

Similarly, neurotransmitter abnormalities have been observed in norepinephrine (NE) in individuals with VCFS that present with mania (Papolos, Veit, Faedda, Saito, & Lachman, 1998). In addition to NE deficits, gamma-aminobutyric acid (GABA) deficiencies have also been implicated as one of the pathophysiological mechanisms in anxiety disorders. Though there has not been a direct examination of this neurotransmitter in individuals with VCFS who have anxiety symptoms, anxiolytics such as benzodiazepine, a GABA agonist, are effective in regulating anxiety levels in this population (Krishnan, 2005)

Additionally, cortisol and the subsequent production of glucocorticoids has been implicated in anxiety (Hoehn-Saric, McLeod, Lee, & Zimmerli, 1991), depression (Vythilingam et al., 2004), and some features of social impairment (Corbett et al., 2009) in related populations. Anomalous cortisol levels have not yet been demonstrated in this population, but have been shown in children with ASD (Curin et al., 2003; Naber et al., 2007; Richdale & Prior, 1992) and schizophrenia (Carroll, 1976; Walder, Walker, & Lewine, 2000), two disorders commonly associated with VCFS. The remainder of this literature review will emphasize the role of cortisol in the development of various psychopathology and social impairment.

Physiological Description of the Human Stress Response

It is a widely disseminated finding that there are two distinct types of stress. There is short-term, brief stress, which is generally thought to be adaptive, and necessary to sustaining and energizing the fight or flight response. This is the type of stress associated with brief sympathetic activation and is one of the autonomic physiological mechanisms that is said to have facilitated our survival throughout evolution. The stress response is quite complex, requiring the integration between numerous structures, hormones, and systems throughout the body (Sapolsky, 1996). First, a stressor occurs. This, in the short-term, usually involves some immediate danger that requires the body to engage in action. Following recognition of the stressor, a cascade of events occurs. Initially, there is a brief release of catecholamines. Specifically, epinephrine and norepinephrine are secreted by nerve cells approximately 2 seconds following exposure to the stressor (Sapolsky, Romero, & Munck, 2000). Examination using microdialysis has found that stressful situations are associated with higher concentrations of norepinephrine in the hypothalamus, frontal cortex, and lateral basal forebrain (Cenci, Kalen, Mandel, & Bjoerklund, 1992; Yokoo et al., 1990). Approximately 10 seconds later, the hypothalamus receives signals to release corticotropin releasing hormone (CRH) into general circulation (Horowitz, 1986). CRH is also released into the brain where it acts as a neuromodulator and neurotransmitter. This means that CRH can have direct impact on the regulation of simple and complex functions of the nervous system. For example, CRH has been shown to impact periaqueductal gray matter, the locus coeruleus, and the central nucleus of the amygdala (Moriceau, Roth, Okotoghaide, & Sullivan, 2004). The infusion of CRH into the central nervous system has behavioral consequences. Interestingly, these behavioral consequences mirror those associated with exposure to aversive stimuli. For example, injections of CRH into the central nervous system

lead to an increase in neophobia in rats (Britton, Koob, Rivier, & Vale, 1982). Additionally, CRH infusions to the central nervous system have been shown to facilitate acquisition of a classically conditioned fear response (Cole & Koob, 1988). Alternatively, introduction of CRH antagonists via intracerebroventricular injection decreases the experience of anxiety that is caused by an array of stressful situations (Kalin, Sherman, & Takahashi, 1988). Thus, the stress response does indeed have an impact on structures and circuits of the CNS.

Next, CRH bind to receptors on the pituitary gland stimulating the release of adreno-corticotropic hormone (ACTH; Kvetnansky et al., 1995). Following these changes, the hypothalamus decreases production and release of gonadotropin releasing hormone (GnRH) with subsequent decreases in pituitary gonadotropins (Sapolsky et al., 2000). This initial physiological response to immediate stressors occurs quickly, with only a small delay between endocrine up-regulation and activation of target tissues via a second messenger cascade. It is this initial physiological response to environmental threats that is responsible for changes associated with sympathetic activation, which includes pupil dilation, salivation inhibition, sweating stimulation, vasoconstriction, inhibition of digestion, and so on (Carlson, 2004). This initial response to a stressor is brief, usually terminating within an hour (Sapolsky et al., 2000).

The next step in the physiological stress response involves the release of glucocorticoids, in particular cortisol. Cortisol is released when ACTH, released by the pituitary gland, enters the general circulation and binds to the adrenal cortex stimulating glucocorticoid release. Under normal circumstances of low-grade stress, glucocorticoids facilitate the degradation of proteins and the subsequent conversion to glucose preparing nutrients for the organism to utilize. Additionally, the hormone helps increase blood flow, thereby increasing blood pressure. Also, glucocorticoids stimulate behavioral responsiveness in numerous biological systems (Carlson,

2004). Overall, glucocorticoids have numerous effects throughout the brain due to the ubiquity of glucocorticoid receptors throughout body tissues and brain.

These effects in the short term have evolved to ensure organismal survival. In fact, individuals with damage to their adrenal cortex are prone to death when encountering stressful stimuli and need to be administered exogenous glucocorticoids (Tyrell & Baxter, 1981). That said, the stress response evolved to manage short-term, immediate, and identifiable stressors. In contemporary society, stressors exist that are long-term and ambiguous. For example, financial concerns, work-related stress, family stressors, and stress associated with physical and emotional trauma are just a few types of stressors that activate the stress response frequently over long periods of time. Long-term exposure to stressful situations can significantly impact overall physical health and functioning. For example, holocaust survivors, particularly those who lived in concentration camps, have significantly poorer health in later stages of life than individuals not exposed to such extremely stressful conditions (Cohen, 1953). Further evidence of the deleterious impact of long-term stress on overall physical health is seen in subway drivers and air-traffic controllers. According to Theorell et al. (1992), subway drivers who were involved in accidents where individuals were maimed or killed developed more illnesses in several months following the incident than individuals not exposed to significant stressors (Theorell et al., 1992). Air-traffic controllers are also prone to stress-related health conditions and demonstrate a higher incidence of high blood pressure, ulcers, and diabetes (Cobb & Rose, 1973).

Physical Deficits Associated With Long-Term Stress Exposure

There is little debate as to the etiology of health deficits following prolonged exposure to stress. In a seminal work, Hans Selye (1976), implicated glucocorticoids as the primary cause of the deleterious health consequences of prolonged stress exposure (Selye, 1976). Subsequent

analyses of the effect of glucocorticoids on stress-related health decline have demonstrated that glucocorticoids can impact a variety of systems. In particular, prolonged glucocorticoid exposure has been implicated in increased blood pressure, damage to muscle tissue, diabetes, infertility, inhibition of growth, inhibition of the inflammatory response, and suppression of the immune system (Carlson, 2004).

Secondary effects of high blood pressure include increased risk of heart attack and stroke. According to DeVries et al. (2001), social stressors increase the risk of stroke and subsequent apoptosis-related damage by suppressing bcl-2 expression (DeVries et al., 2001). The bcl-2 gene is involved in the prevention of cellular necrosis and apoptosis. Social stressors, particularly those involving chronic intimidation, have a pronounced effect on bcl-2 protein production, increasing susceptibility to stroke and more severe damage and tissue loss during stroke. Thus, stress both increases the likelihood of a stroke by increasing blood pressure and then makes the impact of the stroke more damaging by decreasing expression of neural protective proteins.

As mentioned previously, stress can impact physical growth and development. In children, exposure to long-term stress can lead to decreased attainment in height and failure to thrive. The mechanism for decreased stature and body weight is complicated and influenced by numerous factors. However, on the simplest level, long-term exposure to glucocorticoids leads to suppression of the release of growth hormones leading to background growth failure without organic etiology (Skuse, Albanese, Stanhope, Gilmour, & Voss, 1996). Interestingly, once the children were removed from familial stressors, spontaneous "catch-up" occurred and growth hormonal levels normalized. Thus, childhood stress has a significant impact on growth and development.

Glucocorticoids have also been shown to inhibit the positive inflammatory response, resulting in increased healing time and permanent tissue damage and infection following injury. Some of the deleterious effects of stress were demonstrated by examining the healing rate of wounds on caregivers of patients suffering from Alzheimer's disease relative to controls (Kiecolt-Glaser, Marucha, Malarkey, Mercado, & Glaser, 1995). Measures of wound size/diameter and application of hydrogen peroxide indicated that care-giving related stress delays wound healing. As mentioned previously, mechanistically, this occurs via suppression of the inflammatory response.

There are other physical problems that occur in response to long-term exposure to stress; however, these are beyond the scope of this discussion. It is sufficient to conclude that glucocorticoids can impact nearly every system of the body as a result of diffuse receptor distribution.

Impact of Stress on Cognition

In addition to the deleterious effects of long-term glucocorticoid exposure on physical health, there are negative cognitive consequences to long term stress exposure. There are multiple cognitive consequences of stress including memory impairment, compromised learning ability, executive dysfunction, and attentional impairments.

Memory changes are one of the most prominent changes observed in response to long-term stress exposure. On a specific assessment of declarative memory performance in the elderly, researchers observed that exposure to a stressful task (public speaking) has a more negative impact on memory than exposure to a benign task (Lupien et al., 1997). Additionally, there seems to be some specificity with regard to the type of memory impaired during prolonged cortisol exposure. According to Newcomer et al. (1999), verbal declarative memory was

differentially impacted when compared with performance on nonverbal memory measures following exposure to long-term stress (Newcomer et al., 1999). Thus, though memory impairment is a common finding in examinations of the effects of long-term stress exposure, it appears there is some preservation of nonverbal memory abilities.

Not only does long-term psychological stress have an impact on encoding and retrieval, it also has an impact on memory strategies (Johnsen & Asbjornsen, 2009). According to researchers, individuals with Post-Traumatic Stress Disorder (PTSD), a disorder associated with high-resting cortisol levels, have difficulty employing effective list-learning strategies as evidenced by poor performance on the California Verbal Learning Test-II (CVLT-II). Specifically, individuals did not use semantic clustering strategies, but rather employed only a recency strategy during recall trials. According to the authors, this reflected an underlying deficit in the formation of effective encoding strategies. Thus, not only is there evidence of mechanistic deficit associated with encoding and retrieval, there are also deficits in the way the individual organizes information before encoding.

There are numerous hypotheses regarding the mechanism of memory impairment observed in individuals exposed to long-term stress and glucocorticoid exposure. One generally accepted mechanism is hippocampal atrophy. The hippocampus is an essential structure in learning and memory (Carlson, 2004). According to Sapolsky and McEwan (1995), long-term exposure to glucocorticoids damages neurons located in field CA1 of the hippocampus. Cellular atrophy occurs because corticosteroids prevent the entry of glucose into the cell as well as the reuptake of glutamate, two essential features for survival of the cell (McEwen & Sapolsky, 1995). Furthermore, due to the decreased reuptake of glutamate, there are excessive amounts of glutamate outside of the cell body. This extracellular glutamate opens an inordinate amount of

calcium channels leading to excitotoxicity and cell death. The cell death in this structure is so pronounced that volumetric changes can be observed using imaging technology (Gurvits et al., 1996). In combat exposed veterans with symptoms of PTSD, a 20 percent reduction in hippocampal volume was observed. Interestingly, the amount of tissue loss was positively correlated with the amount of combat exposure. A similar finding was observed in children who were the victims of child abuse. In this population, hippocampal atrophy has been implicated in the formation of false memories and inability to recall specific details of the abuse event (Bremner, Krystal, Southwick, & Charney, 1995).

The hippocampus plays another important function in the stress response by operating as a stop gap for stress hormone release (Sapolsky et al., 2000). That is, the hippocampus is part of a negative feedback loop that disengages the stress response. This mechanism works perfectly under conditions of brief exposure to stress. However, as exposure to stress hormones becomes prolonged, the hippocampus becomes more atrophied. As the atrophy progresses, the structure loses its ability to help regulate the stress response via biofeedback, and the stress hormones are able to circulate unimpeded throughout the various systems of the body. Thus, prolonged stress exposure leads to the inability to further regulate the stress response, which leads to more hippocampal damage, more memory impairment, and even less ability to regulate the stress hormone. In other words, the system gets converted from a system regulated by negative feedback to one that resembles a feed forward process.

The impact of stress on memory and learning is not entirely mediated by the impact of glucocorticoids and the hippocampus. The amygdala, a structure implicated in the coordination of stress behaviors and modulation of memory consolidation, also plays a role in the deleterious effects of the stress response (Kim, Lee, Han, & Packard, 2001). In an experiment examining

the role of the amygdala in stress-related memory and learning impairments, researchers observed that rats with lesions to the amygdala did not show learning deficits during performance of the Morris Water Maze following exposure to stress. Thus, elevated glucocorticoid levels alone are not sufficient to induce cognitive impairment; other structures within the limbic system facilitate the physiological and behavioral response to stress.

Psychological Problems Associated with Long-Term Exposure to Glucocorticoids

There have been numerous psychological disorders mediated by or exacerbated by long-term exposure to stress. Indeed, PTSD is one disorder characterized by stress-related atrophy to the hippocampus (Gurvits et al., 1996). Interestingly, individuals with PTSD do not show elevated cortisol secretion. In fact, they have a tendency to show decreased production and release of cortisol (Yehuda, 2001). Despite this decrease in cortisol production, individuals show an elevated stress response because of an increase in stress hormone receptor proliferation and sensitivity. That is, there are more receptors with increased sensitivity for the stress hormone. As such, there is less cortisol released by the adrenal cortex, but the negative effects are still observed due to increased binding efficiency.

In addition to PTSD, Generalized Anxiety Disorder (GAD) and Panic Disorder have also been associated with increased cortisol levels (Graeff, 2007; Hoehn-Saric et al., 1991). The amygdala is a neurological structure directly implicated in anxiety and depression. Corticosteroids have been shown to increase the CRH mRNA expression at the central nucleus of the amygdala (Merali, Anisman, James, Kent, & Schulkin, 2008). This expression increases activation in the amygdala, leading to a potentiation of the fear response and symptoms of anxiety. Thus, cortisol can affect the development of anxiolytic symptoms via promotion of CRH release in the central nucleus of the amygdala.

Mood disorders are also mediated by increased levels of corticosteroids. A more recent conceptualization of the neurobiological etiology of some forms of depression posits that a decrease in neurogenesis has a significant impact on mood stability. Neurogenesis is defined as the birth of new neurons (Carlson, 2004). To date, there are only a few sites in the brain that have been shown to be capable of producing new neurons. One of these structures is the hippocampus; the same region where atrophy takes place following prolonged exposure to glucocorticoids. Neurogenesis is facilitated by neurotrophic growth factors. Long-term cortisol exposure can inhibit production of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). This results in the inhibition of cell proliferation and integration into existing neurological circuits. Such decreases of cell proliferation have been associated with mood impairment in individuals with depression (Huang & Herbert, 2006). Thus, stress can lead to the decrease of neurogenesis, which contributes to the development of depression. Further evidence for the role of neurogenesis in the regulation of mood was demonstrated in an experiment assessing the mechanism of efficacy for fluoxetine, a selective serotonin reuptake inhibitor (SSRI) commonly used in the treatment of depression and anxiety. Findings suggest that fluoxetine facilitates the proliferation of progenitor cells, the precursors to hippocampal cells, in the dentate gyrus (Huang & Herbert, 2006). Following cellular proliferation, cognitive and mood symptoms associated with depression are alleviated, usually within 3 weeks, the same length of time it takes new cells to become integrated into neuronal circuits. In summary, cortisol inhibits neurogenesis, an integral process in the maintenance of cognitive functioning and mood stability. Moreover, it is this inhibition of neurogenesis and active degradation of neural tissues that may account for many of the aforementioned neuroanatomical anomalies found in individuals with VCFS.

To conclude, increased cortisol levels may contribute significantly to the psychopathology and cognitive deficits observed in individuals with VCFS. To date, there have been no studies examining the direct impact of cortisol on psychopathology in this particular population. However, given evidence from other populations, it is reasonable to conclude that some of the observed short-term memory deficits, mood impairment, anxiety, and psychotic symptoms may be mediated or exacerbated by the presence of high baseline cortisol levels in individuals with VCFS.

Specific Social Deficits Related to Abnormal Cortisol Levels

As previously mentioned, cortisol and the subsequent production of glucocorticoids has been implicated in anxiety (Hoehn-Saric et al., 1991), depression (Vythilingam et al., 2004), and social impairment (Corbett et al., 2009) in related populations. To date, anomalous cortisol levels have not yet been demonstrated in this population but have been shown in children with ASD (Curin et al., 2003; Naber et al., 2007; Richdale & Prior, 1992) and schizophrenia (Carroll, 1976; Walder et al., 2000), two disorders commonly associated with VCFS. Specifically, in children with ASD, higher ACTH levels were observed relative to control children, suggesting a significant disruption in the HPA axis (Curin et al., 2003). Additionally, higher cortisol levels in children with ASD were associated with greater sensory sensitivity and social stress (Corbett et al., 2009). Finally, cortisol levels in children with ASD were correlated with parental attachment. Aberrant cortisol levels in children with ASD were related to poorer attachment ratings as measured by the Strange Situation Procedure (SSP; Naber et al., 2007). Moreover, the severity of autistic symptoms was also correlated with baseline cortisol levels. Thus, given what is known about this hormone in children with ASD, it is conceivable that cortisol may have both a direct or indirect impact on overall social competency in VCFS, a population that exhibits

considerable social impairment. Indeed, additional research is necessary to determine cortisol's role in regulating mood, anxiety, and pro-social behavior in children with VCFS. From the scant research on the impact of cortisol in children with ASD, it is difficult to ascertain whether elevated cortisol levels are due to exposure to environmental stress or physiological predisposition to dysregulation of the HPA axis. However, in individuals with VCFS, previous research has demonstrated enlargement in structures involved in glucocorticoid regulation (Kates et al., 2006). That said, enlargement of the amygdala in this population does not rule out an environmental explanation of elevated stress hormones. Indeed, neuroplasticity occurs frequently in response to environmental contingencies (Dong & Greenough, 2004a). As mentioned previously, Dong and Greenough (2004) found that increased exposure to anxiety-provoking situations lead to experience-dependent neuronal remodeling in the amygdala, which may contribute to its increased volume (Dong & Greenough, 2004b). Thus, it is possible that the anxiety associated with these patients leads to the volumetric changes in the amygdala and the subsequent release of glucocorticoids. Thus, it is difficult to hypothesize whether environmental circumstances or physiological predispositions to HPA hyperactivation contribute to hypothesized cortisol elevations in individuals with VCFS and related disorders. Thus, it is important to note that one aim of the current study is not to provide evidence for the etiology of elevated cortisol levels in children with VCFS, but rather to examine the relationship between cortisol and measures of memory, social functioning, and adaptive functioning.

To conclude, there is considerable evidence from a variety of clinical groups that aberrant cortisol levels have an impact on neuroanatomical architecture, physical symptomatology, psychopathology, cognitive functioning, and social competency. Given that children with VCFS display behaviors and symptomatology similar to other groups known to have disruptions in

cortisol levels, it is reasonable to hypothesize that a similar cortisol disruption would be observed in this population as well. Moreover, the poor regulation of cortisol would be related to specific cognitive and social deficits in children with VCFS.

An understanding of the relationship between cortisol levels, cognitive impairment, and social competency is important for several reasons.

First, understanding causal mechanisms of social and cognitive impairment can help facilitate more accurate theories of the etiology of symptoms associated with neurodevelopmental disorders. Moreover, understanding of the relationship between aberrant cortisol levels and social and cognitive impairment can contribute to our current understanding of normal brain-behavior relationships.

Second, understanding the relationship between cortisol and various symptomatologies can inform treatment. That is, cortisol levels and rhythms can be normalized pharmacologically and behaviorally. Evidence for pharmacological regulation of glucocorticoids is observed in Cushing's patients who receive treatment with glucocorticoid antagonists such as ketoconazole (Nizoral), mitotane (Lysodren), and metyrapone (Metopirone; Carlson, 2004). Following treatment, many of the emotional and cognitive symptoms associated with Cushing's are reduced (Sonino & Fava, 1998). Behaviorally, the deleterious impact of cortisol on biological systems has been successfully treated with behavioral activation procedures. This process works via the promotion of neurotrophic factors and the facilitation of neurogenesis, which leads to increased hippocampal volumes and increased regulation of glucocorticoid release. Though there are few studies to date that have directly examined the role of behavioral activation therapy on the cortisol response in children and adults with VCFS, it is likely that increased exposure to reinforcing elements of the environment leads to an increase in neurogenesis, which helps to

alleviate the cognitive and mood symptoms associated with depression. For example, one study found that "rewarding" behaviors such as running, eating, and sexual activity all increase neurogenesis and function as antidepressants in animal models of depression (Brene et al., 2007). Thus, to date there has been research implicating that behavioral activation serves to alleviate depressive symptoms through the increase of neurogenesis and normalization of HPA functioning. Therefore, further understanding of hormonal regulation in individuals with VCFS can inform treatment recommendations and protocols and lead to enhanced prognosis.

Third, understanding of the relationship between neurohormones and behavior can lead to the development of physiological biomarkers that can facilitate determinations of severity and prognosis. In addition to imaging and genetic biomarkers, neurohormonal assays are frequently being employed as biomarkers indicative of psychopathology (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003). Recently, cortisol has been implicated in the susceptibility of individuals to Post-Traumatic Stress Disorder (PTSD; Bachmann et al., 2005; Yehuda, 1999), developmental deficits in children with ASD (Curin et al., 2003), and the etiology of schizophrenia and affective disorders (Carroll, 1976; Gerra et al., 2008). That said, to date there have been no studies examining the relationship between baseline cortisol levels and the social and cognitive deficits observed in VCFS. Indeed, an examination of cortisol in this population may help predict the type of deficits expected and may facilitate early, more specific intervention. That is, currently, the presentation of symptomatology in VCFS is highly variable. Research using neurohormonal assessments may unveil relationships between specific symptomatology and cortisol levels, which may help practitioners more accurately predict the presence or absence of symptoms and therefore design more efficient interventions.

In summary, there are multiple reasons for examining the role of cortisol in the development of cognitive and social impairment in children with neurodevelopmental disorders. Moreover, children with VCFS due to their significant deficits in social competency and comorbid cognitive impairment are an ideal group to study neurohormonal contributions to pathology given their specific range of deficits.

Hypotheses

Specific Aim 1 was to determine if there are differences between baseline cortisol levels in individuals with VCFS relative to controls. Given the role that cortisol plays in emotional processing and the experience of anxiety and the fact that children with VCFS have increased anxiety likely due to their neuronal architecture, cognitive interpretation of stressful events, and as they are less emotionally stable, it was hypothesized that VCFS individuals will have a significantly higher baseline cortisol levels relative to controls.

Specific Aim 2 was to determine whether individuals with VCFS perform significantly poorer on tests of memory and learning relative to controls. Given existing literature regarding cognitive functioning in individuals with VCFS, it was hypothesized that individuals with VCFS would exhibit significantly lower performance on measures of memory and learning. Specifically, individuals with VCFS would exhibit specific deficits in general memory ability, working memory ability, and attention and concentration as measured by the Wide Range Assessment of Memory and Learning (WRAML-2).

Specific Aim 3 was to determine if there are any differences in the level of social functioning between individuals with VCFS and controls. Given the significant social impairment commonly reported in studies involving children with VCFS, it was hypothesized that children with VCFS would perform significantly worse on measures of social functioning.

Specifically, ratings of social competency would be lower while ratings of social problems, thought problems, and anxiety/depression as measured by the CBCL would be significantly higher in children with VCFS. Additionally, individuals with VCFS would be rated significantly lower on the social scale and communication scale of ABAS-II. Finally, Children with VCFS would exhibit significantly higher levels of social anxiety and total anxiety as measured by the RCMAS-2 relative to controls.

Specific Aim 4 was to assess the relationships between cortisol levels and memory and social functioning in individuals with VCFS. Given the impact that elevated cortisol levels have on neurological structures supporting working memory, long-term memory, attention/concentration, and nonverbal memory storage, it was predicted that cortisol level would be negatively correlated with measures of cognitive performance in these domains. Additionally, there is evidence in the PDD literature suggesting that an augmented physiological stress response underlies many of the social competency issues associated with ASD and other PDD (Curin et al., 2003; Naber et al., 2007). As a result of the significant anxiety observed in individuals with VCFS, it was hypothesized that the cortisol levels would be correlated with measures of social competency and social functioning. Specifically, it was hypothesized that cortisol levels would be negatively associated with social competency as measured by the CBCL. Additionally, it was predicted that a positive association between cortisol levels and the anxious, depressed, social problems, and thought problems subscales of the CBCL would be observed. Moreover, a negative association would be observed between cortisol levels and the socialization and communication subscales of the ABAS-II. Finally, a positive association would be observed between the social anxiety scale and total anxiety of the RCMAS-2.

Measures

Child Behavior Checklist (CBCL): The CBCL is a standardized, parent-report measure of children's (ages 4-18) behavior problems and competencies. The problem portion of the measure consists of 118 specific problem items that compose the 9 subscales (Withdrawn = 9 items, Somatic Complaints = 3 items, Anxious/Depressed = 14 items, Social Problems = 8 items, Thought Problems = 7 items, Attention Problems = 11 items, Delinquent Behavior = 13 items, Aggressive Behavior = 20 items, and Other Problems including Sex Problems = 33 items). The competency portion of the measure contains 20 items that compose the 3 subscales (Activities = 5 items, Social = 6, School = 4 items, and Other = 5 open items not scored in the profile).

CBCL Subscales and Competency: For the problem scale, items are scored on a 3-point Likert-type response scale with the following anchors: 0 = *Not True*, 1 = *Somewhat or Sometimes True*, 2 = *Very True or Often True*. Problem Scale scores can range from 0 to 236. Raw scores are converted into T scores for clinical analysis. A high total problem score (>T = 70) indicates that the child is experiencing a clinically significant level of disordered behavior. Furthermore, a child can have a low total problem score but have an elevated sub-scale (>T = 70) indicating significant behavior problems in the specific domain. For the competency scale, items are scored on a mix of three (0, 1, 2), and four (0, 1, 2, 3, 4) option Likert-type items as well as number of dichotomously scored items (0 = *no*, 1 = *yes*). Competency Scale scores can range from 0 to 23. The raw scores are then converted into T scores for clinical analysis. High total competency scores indicate a high level of competence. Low total competency scores (<T = 30) indicate a low level of overall competence. Furthermore, a child can have relatively high competency scores but score low in one particular domain, indicating a lack of competency in the corresponding subscale.

Problem scales for the CBCL were derived from an item-level principal components analysis. Thus, the composition of items for each subscale is based on internal consistency of a group of items. The total problems scale has good overall internal consistency (α = .96). The problem subscales also have acceptable internal consistencies (Withdrawn, α = .80; Somatic, α = .72; Anxious/Depressed, α = .86; Social Problems, α = .76; Thought Problems, α = .68; Attention Problems, α = .83; Delinquent Behavior, α = .83; Aggressive Behavior, α = .92). The competency scales were derived in a similar fashion using principal component analyses. The total competency scale has less overall internal consistency than the total problem score (α = .64). Similarly, each competence subscale has lower internal consistency than the problem subscales (Activities, α = .42; Social, α = .60; School, α = .61). This lower internal reliability is likely due to the small number of items in each competency subscale relative to the problem subscales.

In addition to adequate internal consistency, the CBCL has high inter-interviewer reliability. Scores from three interviewers of 723 children were compared. The overall intra-class correlation coefficient (ICC) for problem items was .96. The overall ICC for the competence items was .93. Thus, inter-interviewer reliability is high.

The CBCL also has a high inter-parent reliability according to Cohen's (1988) criteria. The overall mean *r* for the total problems scale was .76. Each problem subscale also had a significant mean *r* indicating high inter-parent agreement (Withdrawn, *r* = .66; Somatic Complaints, *r* = .52; Anxious/Depressed, *r* = .66; Social Problems, *r* = .77; Thought Problems, *r* = .48; Attention Problems, *r* = .79; Delinquent Behavior, *r* = .78; Aggressive Behavior, *r* = .77; Sex Problems, *r* = .52). Similarly, the overall total competence scale has high inter-parent

reliability indicated by a mean *r* of .79. Each competence subscale also had a significant mean *r* indicating high inter-parent agreement (Activities, *r* = .59; Social, *r* = .73; School, *r* = .87).

Furthermore, the CBCL has high test-retest reliability across a seven-day interval. Scores from 80 subjects were used in the temporal stability analysis. The overall total competence scale had a mean *r* (averaged across all competency subscale scores) of .89. Each problem subscale also had a significant test-retest reliability (Withdrawn, *r* = .82; Somatic Complaints, *r* = .95; Anxious/Depressed, *r* = .86; Social Problems, *r* = .87; Thought Problems, *r* = .82; Attention Problems, *r* = .90; Delinquent Behavior, *r* = .86; Aggressive Behavior, *r* = .91; Sex Problems, *r* = .83). Similarly, the competence scale had high overall test-retest reliability in the analysis with a mean *r* of .87. Each competence subscale also had a significant mean *r* indicating high test-retest reliability (Activities, *r* = .80; Social, *r* =.70; School, *r* =.92).

In sum, the CBCL has high internal consistency, scores are stable over a brief time frame (one week), and there is acceptable inter-rater agreement for both interviewers and parents. Collectively, these data support the use of the CBCL in this experiment.

CBCL Validity: The problem scale of the CBCL has high convergent validity evidenced by high correlations with other behavioral measures including the Connor's Parent Questionnaire (1973) and the Quay-Peterson Revised Behavior Checklist (1983). The correlation between the total problem scale on the CBCL and the Connors Parent Questionnaire is .82 ($p < .0001$). The problem subscales were also highly correlated with related subscales on the Connors Parent Questionnaire (Somatic Complaints-Psychosomatic *r* =.70; Anxious/Depressed-Anxiety, *r* = .67; Attention Problems-Impulsivity/Hyperactivity, *r* =.59; Delinquent Behavior-Antisocial, *r* = .77; Aggressive Behavior-Conduct Problem, *r* = .86). The correlation between the total problem scale on the CBCL and Quay-Peterson Revised Behavior Checklist was *r* = .81 ($p < .0001$). The

problem subscales were also highly correlated with related subscales on the Quay-Peterson Revised Behavior Checklist (Withdrawn-Anxiety, r = .66, Anxious/Depressed-Anxiety, r =.78; Thought Problems-Psychotic, r =.64; Attention Problems-Attention Problems, r = .77; Delinquent Behavior-Socialized Aggression, r = .59; Aggressive Behavior-Conduct Disorder, r = .88). Thus, the CBCL is highly correlated with measures and subscales purporting to measure similar constructs. Statistics for discriminate validity were unavailable for this particular measure.

The criterion-related validity of the CBCL is supported by the fact that the CBCL quantitative scale can discriminate between referred and non-referred children after removing the effects of demographics. The overall total problem score accounts for 32% of the variance in referral status. Most problem subscales also account for a significant percentage of the variance in referral status (Withdrawn= 16%; Anxious/Depressed= 21%; Social Problems= 24%; Though Problems= 16%; Attention Problems= 31%; Delinquent Behavior r = 21%; Aggressive Behavior r = 24%). Similarly, the overall total competence score accounts for a significant amount of variance in referral status (26%). Additionally, two of the three subscales account for a significant amount of the variance in referral status (Social= 18%; School= 37%). Thus, because the CBCL can discriminate between clinical samples and non-clinical samples, it is said to have a high measure of criterion-related validity. In further support of criterion-related validity, clinical cut points on the scale scores were shown to discriminate between referred and non-referred children (Achenbach, 1991).

In sum, the CBCL has content validity, convergent validity, and criterion-related validity, which support construct validity. Thus, it use in this particular experiment to assess the behavioral problems and social competency of individuals with VCFS is justified. Overall, this

measure was chosen for its internal and external validity as well as its ability to capture a wide array of pathological behaviors. Additionally, there is a subscale on this measure specifically devoted to social competency, a construct that is of particular interest in this study and not well established on other measures of social functioning.

ABAS-II: The ABAS-II (Parent Form; Ages 5-21) is a diagnostic assessment measuring adaptive behaviors associated with daily functioning in both domestic and external social environments. It consists of 232 items that compose 10 primary skill areas: Communication, Community Use, Functional Academics, Home living, Health and Safety, Leisure, Self-Care, Self-Direction, Social, and Work. Item responses are selected based on a 4-point Likert scale with the following anchors: *0= Is Not Able; 1= Never or Almost Never When Needed; 2= Sometimes When Needed; 3= Always or Almost Always When Needed.* Overall, higher standard scores on the ABAS-II denote greater competency in a specific domain. Standard scores are based on a normal curve distribution.

Items on the ABAS-II were developed using pilot and tryout studies involving 1045 parents. Item statistics, differential item functioning, item guessing rate, item bias, skill area reliability, validity, clarity of instructions, and clinical usefulness were determined based on pilot data. Standardization was done using a representative sample stratified according to data from the 2000 census. Eleven age groups spanning ages 5-21 were utilized for standardization. An equal number of males and females were used in each of the 11 age groups. Racial groupings were stratified into the 11 age groups according to the proportions indicated by the census data. Finally, geographic regions were included into the age groups proportionally as indicated by the census data. Some of the relevant data will be reported here. For the complete statistical profile and item analyses see Harrison & Oakland (2003).

ABAS-II Reliability: With regard to internal consistency, coefficient alpha was calculated to determine inter-item relationships. Average reliability coefficients for the adaptive domains across the six standardization samples are between .80 and .97, indicating that there is a high degree of internal consistency for the measure.

In addition to a high degree of internal consistency, the ABAS-II also has a low standard error of measurement (SEM). Average SEM ranges from .86-1.38 for the skill areas, suggesting low overall measurement error for each of the domains assessed in the scale.

Test-retest reliability was examined by having parents rate the same child on two separate occasions with approximately 12 days between each rating. Correlations ranged from .84-.93 for skill areas, suggesting that the measure is reliable at two individual time points and that the test-retest reliability is adequate for this experiment.

Inter-rater reliability and cross-form consistency were calculated with Pearson's product-moment correlation. Inter-rater reliability correlations ranged from .76-.93, suggesting adequate consistency across raters. Cross-form consistency ranged from .51-.81, which is relatively high given that the ratings were taken in two distinct forms on two separate individuals settings.

In summary, the ABAS-II has good reliability characteristics that should facilitate the acquisition of consistent data in this experiment.

ABAS-II Validity: Content validity of the ABAS-II is supported by inclusion of theoretical skill areas purported to measure adaptive behavior that have been shown to be important in the development of independent living and social skills in previous research studies (Harrison & Oakland, 2003). Specifically, the 10 skill areas included in the measure have been shown in previous literature to be highly correlated with global functioning and age-appropriate activities of daily living. Additionally, in order to support content validity, items were selected

according to four principles. First, items selected needed to be clinically relevant and applicable to practice. Second, total item number was to be high enough to support reliability but low enough to promote respondent completion without interference from fatigue. Third, items included needed to be directly observable. Fourth, psychometric properties (previously reported) should be adequate for an observer-report measure. These guiding principles combined with use of theory-based skill area inclusion support content validity in this measure.

In addition to theory-based item selection, confirmatory factor analysis using the Tucker-Lewis Index (TLI) was conducted in order to ensure the content validity of the measure. A one-factor model was found to fit the data best (*TLI*= .91), suggesting that the 10 skill areas underlie a global adaptive ability index. In other words, the skills areas do in fact measure a unified construct of global adaptive functioning and therefore items in each of these skill areas are sufficiently measuring the construct.

The ABAS-II also has a high degree of convergence with scales purporting to measure general adaptive ability. Specifically, individual and global scales of the ABAS-II are highly correlated with related scales of the Vineland Adaptive Behavior Scale (VABS). Specifically, correlations on related skill areas ranged from .69 to .84, suggesting a high degree of convergent validity. Moreover, the ABAS-II has a weak relationship with other measures of behavior that serve as behavior screens or indicators of severe impairment. For example, correlation of the ABAS-II global composite with the Scales of Independent Behavior- Revised (SIB-R) broad independence score, a brief screen of independent daily activities, was fairly low (r = .18). Taken together, these data suggest that the ABAS-II has adequate convergent and discriminant validity.

Finally, the ABAS-II has adequate clinical validity as well. That is, it can distinguish well between clinical groups and normal controls. A reporting of data from these clinical groups is beyond the scope of this paper. However, it is important to note that children with ASD and other social deficits were reported as being considerably worse in the social domain relative to normal controls. Specifically, they were rated 36.25 points lower on average in this particular skill domain relative to controls ($p < .01$). In summary, the scale is sensitive with regard to differentiation of clinical populations. Moreover, the items composing the social skills scale are clearly sufficient to detect social deficits and therefore, its use in this particular experiment is justified.

To conclude, the ABAS-II exhibits both adequate reliability as evidenced by the high degree of internal consistency, test-retest reliability, and inter-rater reliability. Additionally, construct validity and overall validity are supported with evidence of content validity, convergent validity, discriminant validity, and clinical validity. As such, the test is an adequate measure of key aspects of social functioning and other activities of daily living.

WRAML-2: The WRAML-2 is a comprehensive assessment of memory and learning designed to assess memory abilities following traumatic brain injury, neurological insult, trauma, and other psychological issues (Sheslow & Adams, 2003). The battery takes less than 1 hour to administer in most cases and therefore is ideal for capturing global memory abilities in children with shorter attention spans or other disability. Also, due to its relative brevity the measure is of good use as a memory screener. The WRAML-2 is normalized for individuals age 8 to adult and therefore is ideal for studies involving both children and young adults. Generally, the WRAML assesses immediate and delayed recall, verbal memory, visual memory, and working memory. Primary indices include General Memory, Verbal Memory, Visual Memory, and

Attention-Concentration. These indices are composed of 17 subtests assessing different domains of cognitive functioning: Story Memory, Verbal Learning, Design Memory, Picture Memory, Finger Windows, Number/Letter Sequencing, Sentence Memory, Sound Symbol Test, Verbal Working Memory, Symbolic Working Memory, Story Memory, Verbal Learning Delay Recall, Sound Symbol Delay Recall, Story Memory Recognition, Verbal Learning Recognition, Design Memory Recognition, and Picture Memory Recognition. Test items and domain construction was based on previous editions of the WRAML and other tests purporting to measure specific areas of cognitive functioning (Sheslow & Adams, 2003).

WRAML-2 reliability. Cronbach's Alpha was used to determine the internal reliability of the primary indices composing the General Memory Index (.90-.96): Verbal Memory Index (.89-.94); Visual Memory Index (.82-.93); Attention/Concentration Index (.83-.91); and Screening Memory Index (.90-.95). These values indicate adequate internal consistency and high intercorrelations between related items of a specific index.

Test-retest reliability was evaluated by administration of the measure two times separated by an average interval of 49 days and calculating a reliability coefficient: Verbal Memory Index (.85); Visual Memory Index (.67); Attention/ Concentration Index (.68); General Memory Index (.81); Memory Screening Index (.78). Coefficients indicate a relatively stable measure across multiple administrations.

Inter-rater reliability is also relatively high for the WRAML-2. Inter-rater reliability was calculated using Cohen's Kappa. Kappa scores ranged from .977-.981, suggesting a high degree of inter-rater reliability.

Given the degree of internal consistency of the subscales, the test-retest reliability, and the inter-rater reliability, the measure has adequate reliability for the purposes of this experiment.

WRAML-2 Validity: Internal validity was examined by evaluation of the inter-correlations of the primary indices and subtests of the WRAML-2. All correlations were found to be significant at the $p = .01$ level. Thus, the items composing the subtests and indices of the WRAML-2 are likely measuring a relatively uniform construct.

Internal validity was also examined using exploratory factor analyses in order to determine the factor structure of the core subtests. A three-factor solution was found to fit the data best from the six core subtests. A follow-up confirmatory factor analysis also supported the three-factor model (AGFI=.973). According to the data from the analysis, over 70% of the variance was explained by the three-factor model. In other words, construction of the test using three primary indices composing the General Memory Index is supported by the factor analysis.

In addition to measures of internal validity, external validity of the WRAML-2 has also been established. Specifically, convergence has been established between the WRAML-2 and other related measures of memory and learning. For example, the WRAML-2 was correlated with the Children's Memory Scale (CMS), Test of Memory and Learning (TOMAL), and the California Verbal Learning Test-II (CVLT-2). Correlations between the General Memory Index and indices from other tests of memory and learning ranged from .44-.64, suggesting a moderate relationship between the WRAML-2 and other measures of memory and learning. Lower, non-significant correlations were observed between indices of the WRAML-2 and unrelated indices of memory and learning measures. The Visual Memory Index of the WRAML-2 was found to be unrelated to tests of verbal memory on other measures (.03). In summary, the WRAML-2 exhibits both convergent and discriminant validity, which supports the construct and overall external validity of this measure.

Clinical validity has also been demonstrated with this measure. Specifically, the WRAML-2 effectively distinguishes Alzheimer's patients, traumatic brain injury, Parkinson's, and chronic alcohol abusers from normal healthy controls.

In summary, the WRAML-2 has adequate internal and external validity justifying the use of this measure as an indicator of learning and memory abilities in this study.

RCMAS (Revised Children's Manifest Anxiety Scale): The Revised Children's Manifest Anxiety Scale (RCMAS) is a widely used self-report measure designed to assess the degree and nature of anxiety in children and adolescents aged 6 to 19 years old. This measure is administrated through a series of 37 items to which the child answers "yes" or "no." The "yes" responses are considered descriptive of the child's feelings or actions and are counted toward a Total Anxiety score comprised of 4 subscales (Physiological Anxiety = 10 items, Worry/Oversensitivity = 11 items, Social Concerns/Concentration = 7 items, and Lie = 9 items). Because each "yes" answer adds to the Total Anxiety score, a high score is indicative of a high level of anxiety in the child.

RCMAS Subscales and General Indexes: Three of the four subscales on the RCMAS are factor-based. The first of these, Physiological Anxiety, is an index of the child's physiological response when experiencing anxiety. A high score on this subscale indicates that the child has specific physiological responses that are normally experienced during moments of anxiety. The second of these subscales is Worry/Oversensitivity. The items from this subscale contain the word "worry" or insinuate in some way that the child is afraid, nervous, or generally oversensitive to environmental pressures. A high score on this index suggests that the child may internalize much of the anxiety experienced and become overwhelmed with trying to subside the anxiety. There may be a strong need here for the child to learn coping mechanisms in order to

verbalize feelings of anxiety more openly. The third factor-based subscale, Social Concerns/Concentration, assesses the child's concerns regarding face-to-face interactions with another person and evaluates the degree of difficulty the child has in concentrating. A high score on this subscale indicates anxiety that the child experiences because the child feels he or she cannot meet the expectations laid out for them by the adults in their lives. The final subscale, which is not factor-based, is the Lie subscale score, which is used to determine whether the child has a negative intent to deliver false information to the examiner of the RCMAS. This index includes items that say, "I like everyone I know," and "I never lie." Since these statements could seldom be true of any person, much less a child, this could potentially suggest activity of inaccurate self-reporting.

RCMAS Reliability: The Kuder-Richardson formula 20 (KR_{20}) was used to determine inter-item reliability given that items are scored dichotomously. The range for the average reliability coefficients across the six study samples involving children from varying ethnicities, sex, and ages is between .78 and .85. Thus, there is a high level of consistency across ethnicity, sex, and age, with the exception of black females, in which case, the reliability coefficients are remarkably lower at ages 6, 8, 10, and 11 than their white female cohorts. Overall, for most ages and most ethnicity and sex groups, the alpha value for the Total Anxiety score equals or surpasses .80, the alpha value most recommended by Wilson and Reynolds (1996) for use in decision making. Internal consistency demonstrates coherence across the board for a number of normal as well as special or unique study samples.

The RCMAS has not only a high level of internal consistency, but also has a consistently low standard error of measurement (SEM) for the Total Anxiety Score. The coefficient alpha values reported for a standardization sample of 5,000 children produced 1 SEM at a 68%

confidence interval, 1.96 SEM at a 95% confidence interval, and a 2.58 SEM at a 99% confidence interval. For the kindergarten children included in this sample, the SEM t-scores were 4.58 for boys, 3.87 for girls, and 4.24 for the combined group of boys and girls. These figures mirror the reliability estimates from earlier studies.

Test-retest reliability research has been done only for the Total Anxiety Score and the Lie Subscale. In a sample of 534 elementary school-aged children who were tested approximately 9 months apart, a test-retest reliability coefficient of .68 was reported. Meanwhile, among the same group of students, the Lie Subscale correlation was reported at .58 when tested 9 months apart. The results from the Total Anxiety Score give justifiable evidence of constancy of general trait anxiety over an elongated period of time. However, the results from the Lie subscale are less favorable, but promising nonetheless. These results support the material stability of the RCMAS and its function in assessing chronic anxiety in children.

In sum, the RCMAS has strong reliability attributes that should provide for facilitated acquisition of consistent data in this study.

RCMAS Validity: Factor analysis currently reinforces the presence of a strong general anxiety factor (Ag), represented by the Total Anxiety Score, and the factor-influenced subscales as well as the Lie subscale. The RCMAS has strong construct validity when evidenced by the comparison with other measures, including the State-Trait Anxiety Inventory for Children (1973). Means and standard deviation was calculated for each scale: RCMAS, STAIC-Trait, and STAIC-State; Pearson product-moment coefficients of correlation were used between each pair of variables. The RCMAS correlated strongly with the STAIC-Trait scale ($r=.85$, $p<.001$); however, the RCMAS did not correlate significantly with the STAIC-State scale ($r=.35$, $p<.05$).

These results give ample support for the construct validity of the RCMAS as a measure of chronic manifest anxiety, regardless of state or situational anxiety being experienced. Despite the correlations between the self-reported measures and observed behaviors being smaller than expected, overall the correlations produced were as anticipated. The RCMAS Total Anxiety Score correlated with the STAIC-Trait scale at r = .65 for males and r = .67 for females. Correlations between the RCMAS subscales and the five dimensions of behavior evaluated by the Walker Problem Behavior Identification Checklist (1971) were nearly all positive correlations, maintaining a total problem behavior score of r = .32 for males and r = .29 for females. Correlations between child-reported anxiety symptoms and teacher-observed behavior problems establish supportive evidence for the validity of the RCMAS.

In summary, the RCMAS has convergent and divergent validity, which gives credence to the construct validity of the scale. Thus, this is a highly reliable measure with which to evaluate the degree and nature of anxiety in children with VCFS, who frequently present with a number of anxiety disorders.

Methods

Participants

Subjects included 22 children (11 males and 11 females) ages 6-16 years. Eleven of these children (6 male and 5 female, mean age = 11.6) were individuals diagnosed with VCFS. The other 11 children (6 male and 5 female, mean age = 12.5) served as controls. The neurotypical children were all of average intellectual ability based on brief neuropsychological testing using a two subtest intelligence scale. Previous experiments using cortisol analysis have demonstrated large effect sizes (*d* = .66-.85), justifying the use of 20 subjects (Alpers, Abelson, Wilhelm, & Roth, 2003; Kuhlmann & Wolf, 2006). Children with VCFS were recruited

primarily through the VCFS network of support groups. Leaders of VCFS support groups were contacted. The study was explained in detail to group heads and contact information and an informational flyer was given to them. Following this, group leaders contacted their members and asked if the families were interested in participating in the study. Interested families contacted the experimenter directly, and formal informed consent procedures (described below) commenced. Controls were recruited through peer nomination. Peer nomination and sibling controls are two methods of recruitment commonly used to collect data on neurotypicals in genetic research (Antshel, Conchelos, Lanzetta, Fremont, & Kates, 2005; Antshel et al., 2006; Kates, Antshel et al., 2007). A list of a group of local children of matching ages was generated. From this, a random sampling was taken.

Inclusion criterion for the current study included a VCFS diagnosis confirmed by FISH. Controls were matched with VCFS individuals on the variables of age and sex. Exclusion criteria for children with VCFS included the presence of pre- or perinatal pathology, head injury, or substance abuse. In controls, the exclusion criteria consisted of the presence of learning disabilities or other neurological insults. Informed consent was obtained from the parents of children with VCFS and controls upon the first consultation following a demographic interview. Specifically, potential candidates were contacted and the study was explained in detail. First, a demographic interview was completed to ensure that participants met inclusion criteria. Next, the procedure was explained. Participants were told that the experiment would involve a brief, painless cortisol swab, which occurred at 11am on a weekend day followed by an afternoon of cognitive testing. Participants were told that cognitive testing would take approximately 3-4 hours and involved an assessment of the child's memory, social functioning, and adaptive behavior. Parents were informed that neither they nor the child would receive results of testing

or cortisol analysis and that the testing protocols would be de-identified, locked in a filing cabinet, and housed in a secured laboratory. Additionally, parents were informed that immediately following the cortisol analysis, swabs would be destroyed and no other information would be obtained from them. After ensuring that the participants fully understood the procedure, any potential risks, the scientific benefits, and the time investment of the current study, they were scheduled for an appointment, and at that time written informed consent was obtained from the parents of the children. As the children are minors, informed assent was also obtained following a demonstration and thorough explanation of the procedures.

Design

The design of this experiment was a quasi-experimental study comparing two groups: A VCFS group and a control group on cortisol levels, neurocognitive functioning, and social competence. It was a between-groups design despite the fact that subjects were matched on certain characteristics. Individuals were assigned to groups based on the presence or absence of VCFS.

Procedures

Neuropsychological Procedure: Neuropsychological testing was completed by a trained psychometrician at various testing locations. Assent and consent were obtained from the child and his or her legal guardian, respectively, prior to the assessment. These assessments included an evaluation of intellectual functioning, a comprehensive evaluation of verbal and visual memory, attention, neurobehavioral functioning, emotional functioning, and adaptive functioning. The children underwent the neuropsychological assessment in the late morning. Cortisol swabs were taken before the assessment at 11 am to control for neurohormonal responses to testing conditions. Testing order of experimental measures was as follows: (1)

WRAML-2; (2) CBCL; (3) ABAS-II; (4) RCMAS-2. Total length of testing was approximately 2.5 hours, a time interval short enough to reduce the impact of fatigue on cognitive performance (Lezak, 2008).

Cortisol Procedure: Subjects were interviewed prior to sample collection to determine if the individual had had any recent injuries to the mouth or had not maintained adequate oral hygiene over the last year, as both blood and other impurities can contaminate the sample and confound results. Additionally, during the interview, subjects were asked if they had been to the dentist within the last 48 hours or had brushed their teeth within the last 45 minutes as these activities can increase the likelihood of oral irritation, which can release hormones that contaminate the sample. Prior to collection, on the informed consent information packet, subjects were instructed to avoid alcohol 24 hours prior to testing, eating a major meal 60 minutes prior to collection, dairy products 20 minutes before sample collection, and foods with large amounts of sugar or caffeine 24 hours prior to collection. Finally, subjects were required to rinse their mouth out with water 20 minutes before collection in order to remove any solid particles from the oral cavity.

Cortisol samples were taken from children at approximately 11:00 a.m. Eleven in the morning was selected due to the relative stability of cortisol flux at this time of day (Tout, de Haan, Campbell, & Gunnar, 1998). Times earlier than 11 a.m. are more impacted by individual differences in the child's sleep-wake cycle and therefore can confound results (Dettling, Gunnar, & Donzella, 1999). A similar problem has been reported in studies acquiring samples during the late afternoon. In summary, acquiring time-locked samples reduces error associated with time of day and provides a cost-effective way of collecting samples. Samples were acquired with a standard buccal swab using the passive, unstimulated collection method. Children were directed

to tilt their head forward allowing the saliva to pool near their bottom lip. A swab intended for use with children 6 years or older was used to collect approximately 1ml of saliva. The material that forms the oral swab is a non-toxic polymer material, which is inert and hypoallergenic. The swab was placed in a collection vile made of polypropylene intended to preserve the molecular structure of the steroid. The sample was then marked with both a numerical and bar code identification tag, placed in an insulated container, and kept below 7-10 degrees Celsius. Temperature was monitored throughout the process using a standard body thermometer.

Following the field collection process, the samples were shipped within 24-48 hours in their insulated containers to the Salimetrics lab for salivary cortisol distribution analysis. Upon arrival to the lab, the samples were thawed and centrifuged for approximately 15 minutes at 3000 rpms in order to separate cortisol from solute. Using the standard immunoassay saliva extraction procedure, the steroid, cortisol, was then chemically isolated and concentrations per volume calculated and reported (Gatti et al., 2009). The immunoassay procedure is useful because it reduces the number of sample preparation steps including additional cycles of washing and centrifuging, leading to reduced processing times and reduced cost (Rowe, Deo, Shofner, Ensor, & Daunert, 2007). The immunoassay procedure works by isolating the free cortisol in the saliva using cortisol antibodies to bind the steroid. Cortisol antibodies were developed via genetic modification of aequorin. The saliva sample was then mixed with the aequorin to form an aequorin-cortisol conjugate. Once the conjugate is formed, bioluminescence was used to calculate the concentration of bound cortisol (Rowe et al., 2007). The immunoassay methodology was selected due to its ability to detect very small differences in free cortisol concentrations, 1×10^{-10} M (Rowe et al., 2007).

Collection materials and vials were purchased from the Salimetrics Laboratory. Figure 1 shows the swab that was utilized and the collection tube and is a graphical representation of the overall collection procedure. Cortisol samples were destroyed immediately following analysis to comply with personal health information regulations. Data were de-identified and password protected to comply with privacy regulations.

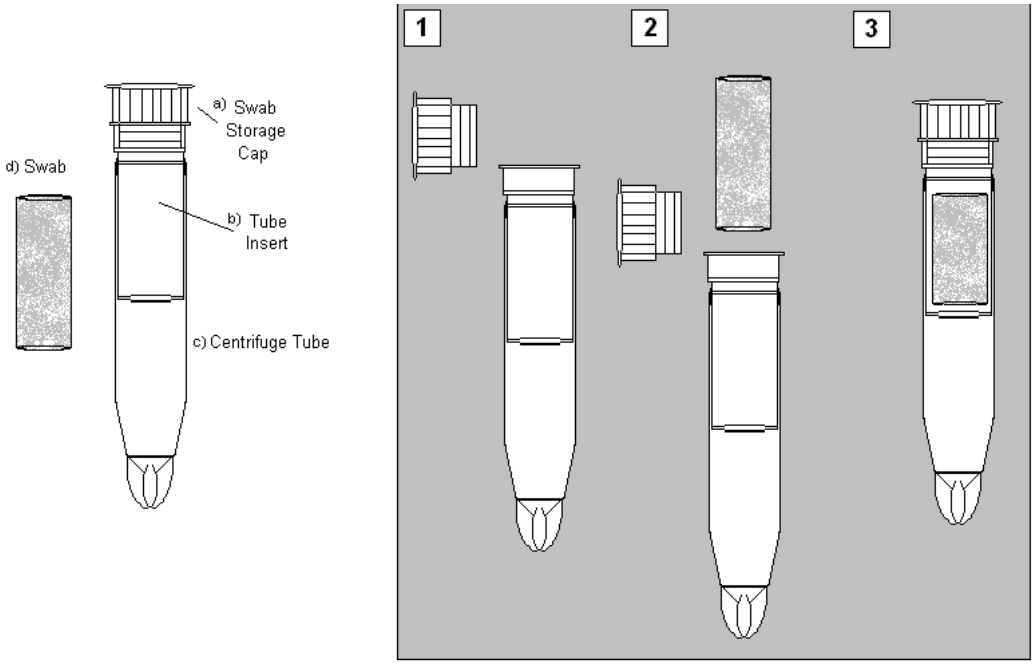

Figure 1. Salivary collection device and storage procedure. Following the swabbing procedure, the collection tube cap is opened (1). The swab is placed inside the tube directly above the centrifuge collection reservoir (2). The air-tight cap is then placed on the pre-labeled collection tube in preparation for freezing (3).

Analysis

Analysis methods for both cortisol concentrations and neuropsychological test data included standard descriptive techniques for continuous variables. Assumptions of normality were checked using standard techniques including tests for normality, skew, and kurtosis

(Tabachnick & Fidell, 1996). Effect sizes were also calculated and reported (H. Cohen et al., 2006; J. Cohen, 1988, 1992, 1994).

Initial analysis included a between-groups ANOVA to determine if there are any baseline cortisol differences (ug/dL), neurocognitive differences, or social/adaptive differences between individuals with VCFS and controls. ANOVA was used to reduce alpha inflation.

In order to examine the behavioral ramifications of cortisol anomalies in individuals with VCFS, *Pearson r* correlations assessing the relationship between cortisol levels and specific subscales of the CBCL, ABAS-II, and the WRAML-2 were conducted. Table 1 describes the specific analyses that were conducted in order to address the hypotheses in question and correlation results.

Table 1

Summary of Analyses and Correlation Results Between Cortisol Level and Subscales of the CBCL, ABAS-II, WRAML-2, and RCMAS-2.

Subscale	Correlation Between Cortisol and Psychological Measures in VCFS Patients
CBCL	*r*-value
Anxious/Depressed	.144
Social Problems	.169
Thought Problems	-.406
Social Competency	-.109
ABAS-II	
Socialization	.563
Communication	-.222
WRAML-2	
General Memory	-.342
Working Memory	.422
Attention/Concentration	-.516
RCMAS-2	
Social Anxiety	.563
Total Anxiety	.487

Note. CBCL = Child Behavior Checklist; ABAS-II= Adaptive Behaviors Assessment System 2nd Edition; RCMAS2= Revised Children's Manifest Anxiety Scale 2nd Edition; WRAML-2= Wide Range Assessment of Memory and Learning 2nd Edition.

Results

This project examined the concentration of a specific stress hormone, cortisol, in individuals with VCFS and its impact on cognitive and social functioning in individuals with VCFS. To do this, saliva samples were obtained using the aforementioned analysis procedures and the cortisol concentration calculated. Next, the performance on neuropsychological memory and attention measures along with measures of social functioning in individuals with VCFS was compared to control subjects. Finally, the relationship between the steroid hormone and scores on specific cognitive and behavioral measures were examined in individuals with VCFS in order to determine the impact of cortisol on these domains of functioning. Table 2 provides the mean cortisol concentration for each group and corresponding effect sizes for individuals with VCFS and Controls. Table 3 provides means and standard deviations for select subtests of the WRAML-2 in individuals with VCFS and Controls. Table 4 summarizes mean scores of behavioral measures of functioning including the CBCL, ABAS-II, and RCMAS-2 in individuals with VCFS and Controls.

Table 2

Mean Volume (cc) and SD of Cortisol in Individuals with VCFS and Controls

	VCFS		Controls		
Hormone	*M*	*SD*	M	*SD*	Effect Size *d*
Cortisol	.25354	.21342	.10408	.05443	1.01

Note. Cortisol concentrations are in micrograms per deciliter (ug/dL). Effect size is calculated as Cohen's *d.*

Table 3

Mean Index Scores, Standard Deviations, and Effect Sizes for WRAML-2 indices in individuals with VCFS and Controls

	VCFS		Controls		
WRAML-2 Index	*M*	*SD*	M	*SD*	Effect Size *d*
General Memory	74.03	12.76	109.45	14.22	-2.74
Working Memory	88.88	22.90	108.88	8.590	-1.70
Attn/Concentration	79.63	13.269	101.83	16.573	-1.55

Note. WRAML-2=Wide Range Assessment of Memory and Learning: 2nd edition. Effect size is calculated as Cohen's *d.*

Table 4

Means and Standard Deviations for Subscales of the CBCL, ABAS-II, and RCMAS in Children With VCFS.

	VCFS		Controls		
Subscale	*M*	*SD*	*M*	*SD*	Effect Size *d*
CBCL					
Anxious/Depressed	56.26	8.01	50.09	2.50	.95
Social Problems	57.13	8.57	50.54	.687	1.14
Thought Problems	58.82	8.91	52.00	3.06	1.07
Social Competency	46.00	11.55	51.00	8.30	-.52
ABAS-II					
Socialization	100.69	19.73	112.81	10.52	-.80
Communication	7.36	3.21	11.81	1.66	-1.83
RCMAS-2					
Social Anxiety	49.78	11.20	46.72	8.93	.33
Total Anxiety	50.43	11.47	46.81	9.61	.36

Note. CBCL = Child Behavior Checklist, M = 50, SD = 10 ; ABAS-II= Adaptive Behaviors Assessment System 2nd Edition, M = 100, SD = 15 for composite, M = 10, SD = 3 for individual scales; RCMAS2= Revised Children's Manifest Anxiety Scale 2nd Edition, M = 50, SD = 10.

Specific Aim 1

It was predicted that salivary cortisol concentrations would be higher in individuals with VCFS relative to controls. Figure 2 displays the mean cortisol concentration in individuals with

VCFS and controls. Results indicated that mean cortisol concentration was significantly larger in individuals with VCFS relative to controls, $F(1, 20) = 5.436$, $p < .05$.

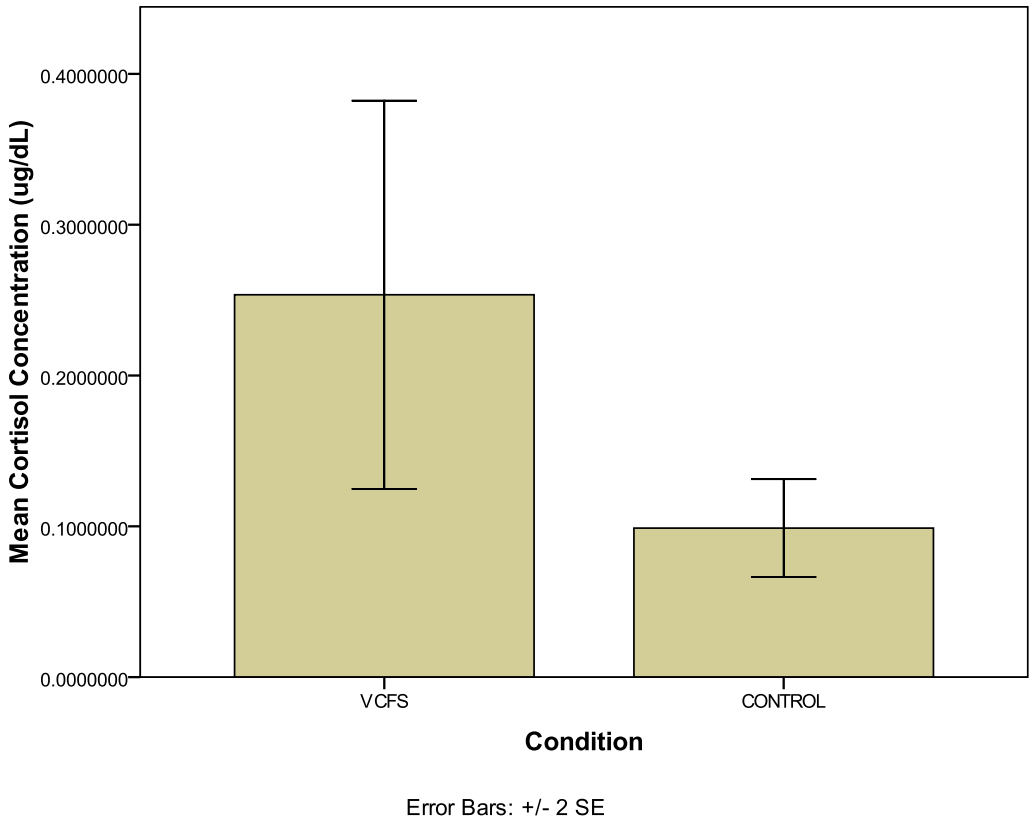

Figure 2. Mean cortisol concentrations (ug/dL) (+*SE*) for individuals with VCFS ($n = 11$) and control individuals ($n = 12$).

Specific Aim 2

It was predicted that individuals with VCFS would perform significantly worse on tests of memory learning as measured by the WRAML-2. In particular, general memory, attention and concentration, and working memory indices would be lower in individuals with VCFS. Results indicated significant difference in performance on these measures in individuals with VCFS when compared with controls: General Memory, $F(1, 20) = 37.875$, $p < .05$; Attention/Concentration, $F(1, 20) = 11.513$, $p < .05$; Working Memory, $F(1, 16) = 5.127$, $p <$

.05. Figure 3 displays the mean difference in standardized scores on these measures between individuals with VCFS and controls.

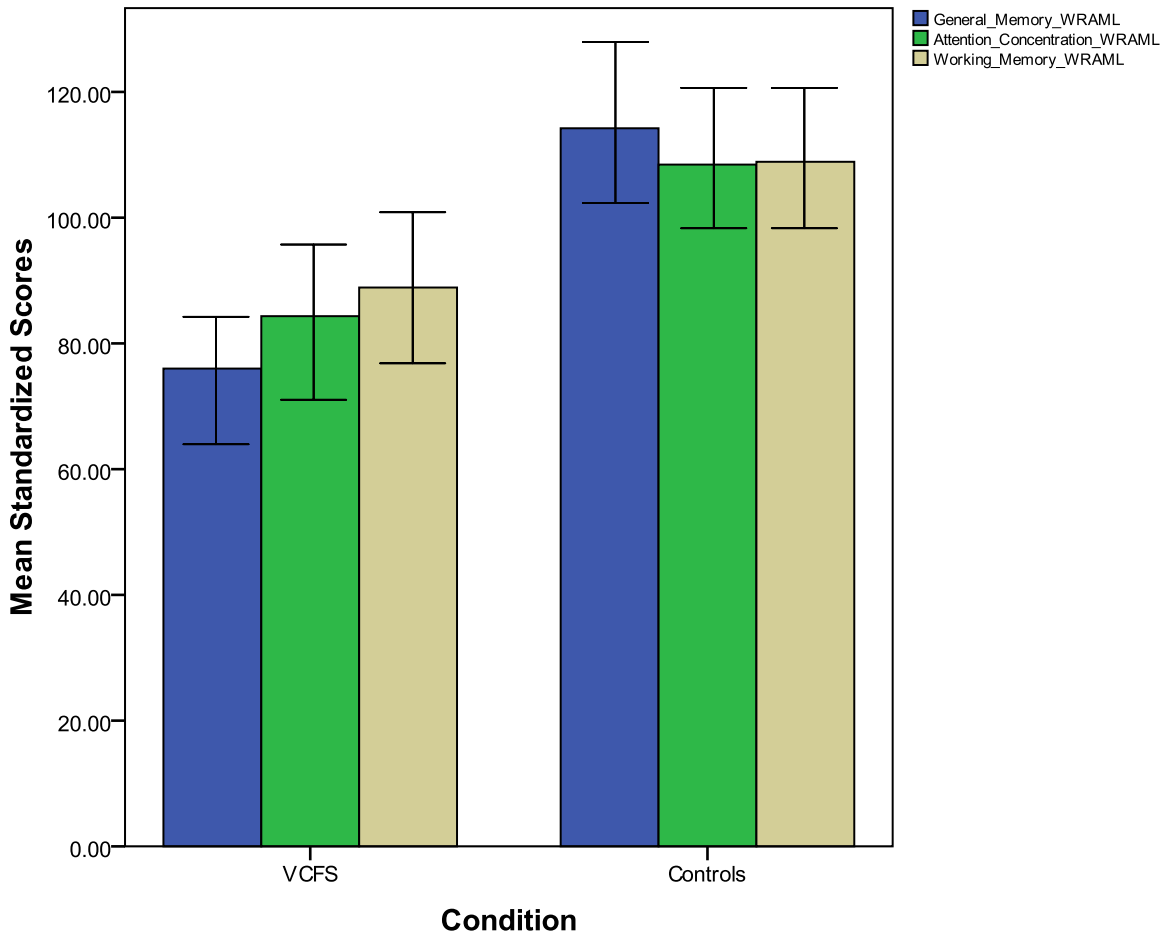

Figure 3. Mean index scores on neurocognitive measures for individuals with VCFS (n = 11) and control individuals (n = 11).

Specific Aim 3

It was hypothesized that children with VCFS would have lower ratings on measures of social skills**.** As anticipated, individuals with VCFS exhibited significantly more symptoms associated with lower social and adaptive functioning relative to controls: Anxious Depressed (CBCL), $F(1, 20) = 30.489$, $p < .05$; Social Problems (CBCL), $F(1, 20) = 5.375$, $p < .05$;

Thought Problems (CBCL), $F(1, 20) = 52.535$, $p < .05$; Social Competency (CBCL), $F(1, 20) = 5.826$, $p < .05$; Socialization (ABAS-II), $F(1, 20) = 16.327$, $p < .05$; Communication (ABAS-II), $F(1, 20) = 16.513$, $p < .05$. Figure 4 displays the mean difference in T-scores on subscales of the CBCL in individuals with VCFS and controls. Figure 5 displays the mean differences in standardized scores on the ABAS-II in individuals with VCFS and controls.

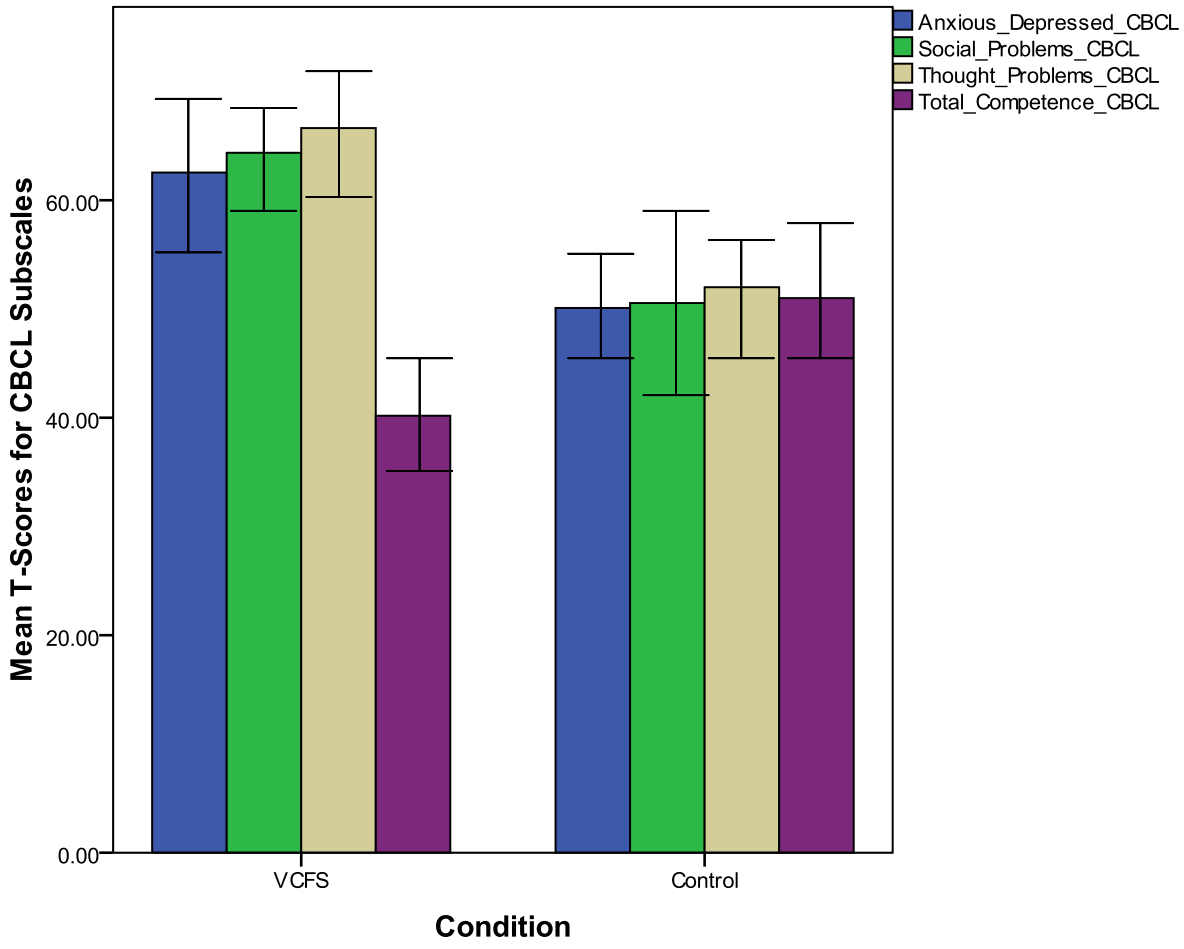

Figure 4. Mean t-scores on CBCL subscales for individuals with VCFS ($n = 11$) and control individuals ($n = 11$).

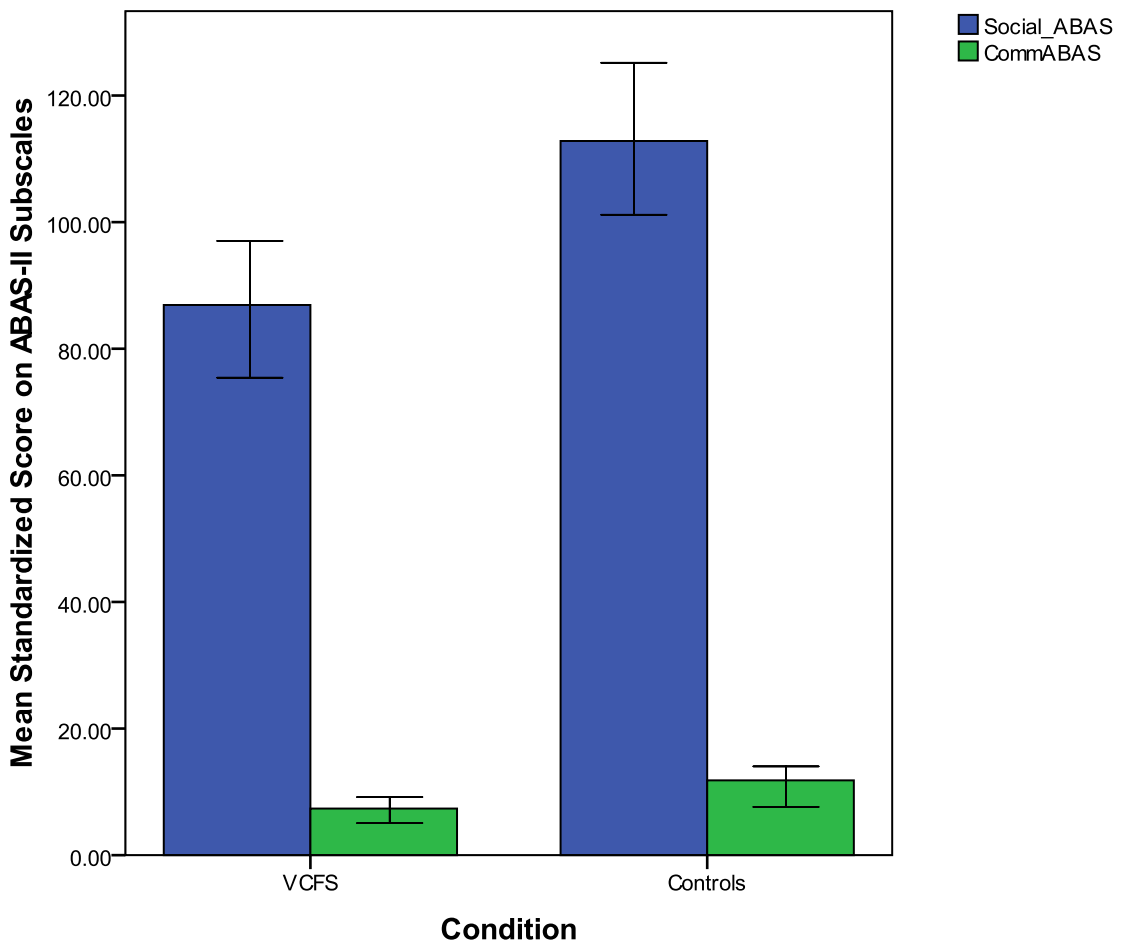

Figure 5. Mean standardized scores on ABAS-II subscales for individuals with VCFS (n = 11) and control individuals (n = 11).

Specific Aim 4

It was hypothesized that the cortisol level in individuals with VCFS would be significantly correlated with cognitive measures and measures of social and adaptive functioning. There were no significant correlations between cognitive measures and cortisol in individuals with VCFS. That said, a significant negative correlation was observed between the General Memory and Attention/Concentration indices of the WRAML-2 and cortisol concentrations in controls: $r(11) = -.778$, $p < .05$; $r(11) = -.618$, $p < .05$. Figures 6 and 7 graphically depict the

relationship between cortisol, the General Memory index score, and the Attention/Concentration index score.

There were no significant correlations observed in individuals with VCFS on select subscales of the ABAS-2, CBCL, and RCMAS-2. That said, the level of cortisol in control individuals was negatively correlated to the social competency scale of the CBCL, $r(11) = -.639$, $p < .05$. Figure 8 is a graphical representation of the correlation between cortisol concentration and the social competency subscale of the CBCL in controls. Taken together these data suggest that the individuals with VCFS have significantly larger concentrations of resting cortisol relative controls and significantly worse cognitive performance.

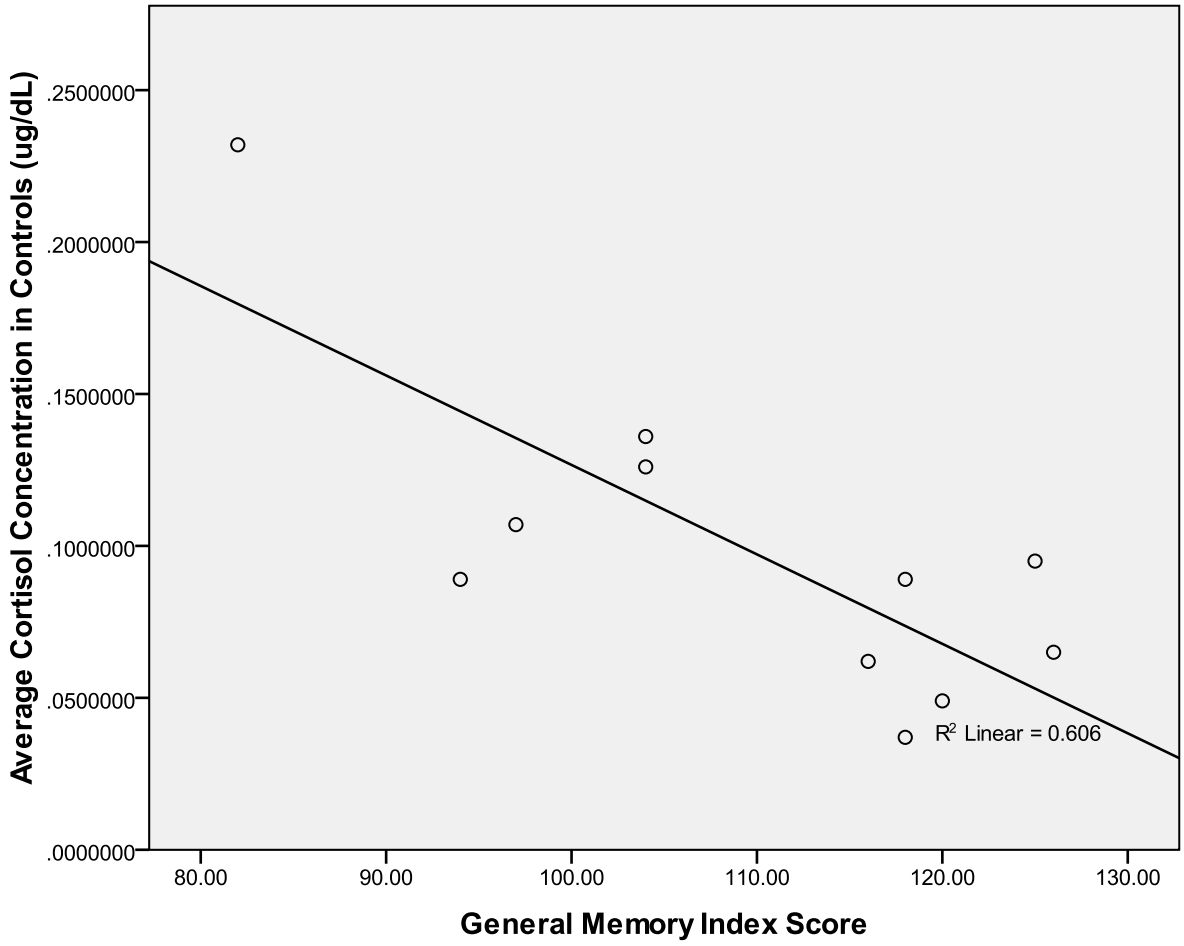

Figure 6. Relationship between average cortisol concentration (ug/dL) and the General Memory Index Score of the WRAML-2 in Controls.

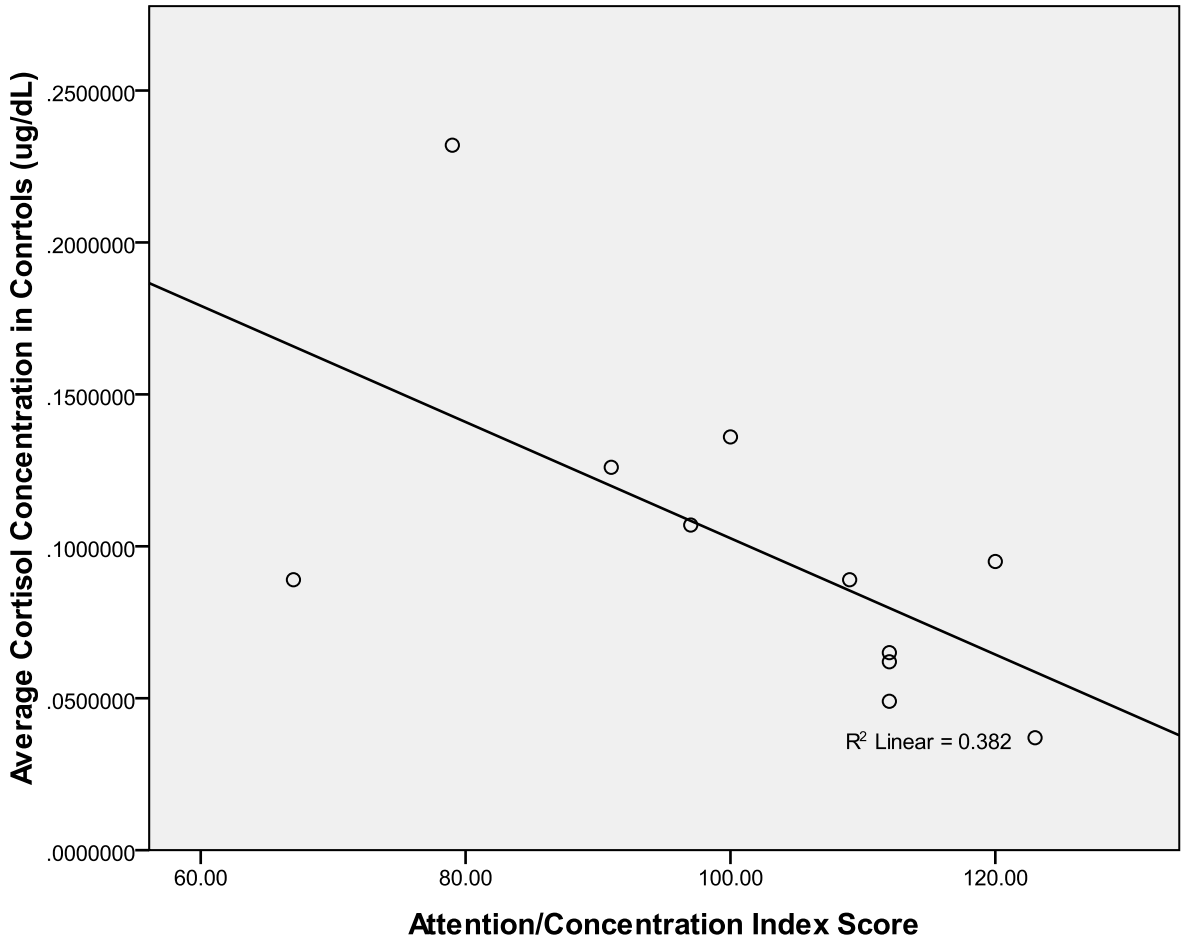

Figure 7. Relationship between average cortisol concentration (ug/dL) and the Attention/ Concentration Index Score of the WRAML-2 in Controls.

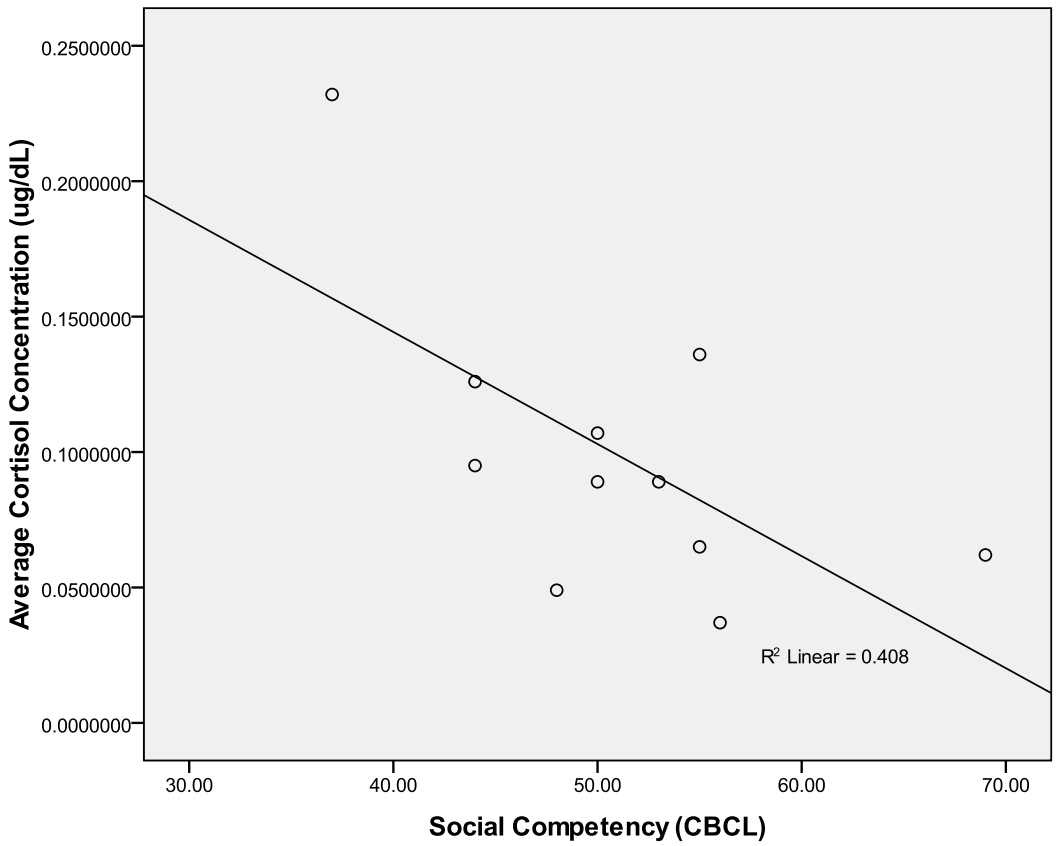

Figure 8. Relationship between average cortisol concentration (ug/dL) and the Total Competency subscale of the CBCL.

Discussion

This study sought to identify the relationship between cortisol and severity of cognitive and social impairments in individuals with VCFS. In particular, one goal of this research was to elucidate brain-behavior relationships within the context of social cognition. As social cognition has been implicated in numerous psychiatric disorders including pervasive developmental disorders, psychosis, and mood disorders, understanding the neural substrates involved is essential in determining the etiology of social deficits in these disorders, informing treatment, and enhancing outcome. Recent studies have implicated elevated cortisol levels in both cognitive and social impairments associated with the various psychological disorders comorbid

with VCFS (Tout et al., 1998; Vythilingam et al., 2004; Walder et al., 2000). In this study, salivary cortisol samples were taken from children with VCFS and neurotypicals in order to evaluate difference in resting cortisol levels between the groups. These cortisol levels were then correlated with measures of cognitive functioning, adaptive functioning, and social functioning in order to help determine the role cortisol plays in the development of cognitive and social deficits in this population.

Children with VCFS Show Elevated Cortisol Levels

The hypothesis that individuals with VCFS would have elevated resting cortisol levels relative to controls was supported. Though this has been the first study to demonstrate this elevation in a population of individuals with VCFS, it has been observed in related populations. For example, such elevated levels of cortisol have been observed in children with ASD who exhibit significant social and cognitive deficits (Richdale & Prior, 1992).

There are multiple theories regarding the etiology of cortisol elevation. The first theory involves physiological mechanisms. It may be possible that children with VCFS have an overactive hypothalamic-pituitary-adrenal axis that leads to the over-secretion of cortisol during both stressful and non-stressful events. Select groups such as individuals who are predisposed to the development of PTSD and children with neurodevelopmental disorders such as ASD exhibit an elevated cortisol response when presented with minor to moderate stressors relative to control counterparts (Carrion et al., 2002). Such a physiological profile may lead to increased startle response in individuals with PTSD and emotional regulation issues in children with ASD. Thus, hypersecretion of cortisol in response to minor stressors in daily living may account for the elevated levels of the steroid observed in children with VCFS.

Indeed, such hypersecretion of the HPA axis may be the result of traumatic events and the over-exposure to stressful stimuli; however, another recent study suggests that the inability to suppress cortisol is what mediates the deleterious neuroanatomical effects and psychopathology and cognitive deficits associated long-term glucocorticoid exposure (Watson, Thompson, Ritchie, Nicol Ferrier, & Young, 2006). Suppression of cortisol is one of the primary problems associated with Cushing's disease (Carlson, 2004), a disorder characterized by abnormally high levels of glucocorticoids, cognitive impairments, and permanent changes in social functioning (Heald et al., 2004). Cushing's disease and cortisol suppression can be assessed using a Dexamethasone Suppression Test. Dexamethasone is a steroid compound that is molecularly similar to cortisol and binds to glucocorticoid receptors. After low-dose administration of Dexamethosone, blood concentration of ACTH, an upstream precursor to glucocorticoids, is reduced via biofeedback mechanisms responsible for HPA regulation. In individuals with impairments in suppression of cortisol production, ACTH levels are not decreased, thereby signifying impairment in cortisol regulation. This mechanism of impaired suppression of cortisol may explain some of the difference between trauma-induced elevated cortisol levels and developmental or genetic factors impacting basal cortisol levels. That is, overproduction of cortisol, or hypersecretion, is often associated with stress exposure, while suppression impairment may be more developmental in nature.

There is neuroanatomical evidence that may implicate deficits in ACTH suppression as a mediating factor for elevated cortisol levels in children with VCFS. More specifically, the hippocampus of the temporal lobe has been found to be significantly smaller in this population (Jacobson, Rowe, & Lajiness-O'Neill, 2011; Kates et al., 2006). As the receptors get bound with ligands, the negative feedback loop that leads to the suppression of ACTH production gets

initiated via second messenger signals. As the hippocampus in children with VCFS is significantly smaller, it stands to reason that they have may have fewer receptors at this site meaning that regulation of ACTH production is going to be limited and temporally delayed. Thus, it is this well-documented anomaly of the hippocampus in the temporal lobe of children with VCFS that may contribute to deficits in suppression and the subsequent build-up of cortisol. That said, it still remains unclear as to whether children with VCFS were born with smaller hippocampi than controls or whether environmental factors such as exposure to significant amounts of stress, which have been known to cause hippocampal atrophy (Starkman et al., 1999; Suhr, Demireva, & Heffner, 2008), have contributed to the reduced hippocampal volumes. Due to the technological limitations, complex sedation protocols, and resultant ethical considerations involving imaging in infants, it is difficult to ascertain the directionality of the relationship between the temporal atrophy, stress, and cortisol. Future advances in technology may allow for early imaging in infants and may help determine the causal factors associated with HPA dysregulation in children with VCFS.

As aforementioned, in this particular study, it is difficult to determine whether elevated cortisol levels are developmentally mediated or caused by environmental stressors. That said, there were no differences observed between individuals with VCFS and controls on a self-report measure of total anxiety and social anxiety, suggesting that individuals with VCFS do not necessarily interpret engagement in social interaction as a stressful or anxiety-provoking event, nor are the children in this study endorsing significant levels of personal anxiety.

Though self-report measures did not indicate elevated anxiety or social anxiety, parent report measures did indicate significantly higher levels of anxiety and depression relative to controls. Thus, it may be possible that children with VCFS have difficulty labeling their own

emotions. Perhaps the children with VCFS who are experiencing physiological stress in response to certain situations though have become accustomed to the heightened arousal level and autonomic symptoms associated with the response and therefore do not recognize the situation as being particularly stressful. That is, the experience of stress may become commonplace for these individuals, and the baseline for stimuli interpreted as being stressful is raised, despite having the physiological responses associated with experiencing a stressor and the corresponding cortisol elevation. Such a process has been observed in rats that were exposed to a noxious audiologic stressor where, upon several exposures of the noxious stimuli, the rat stopped responding behaviorally to the stressor while still having physiological responses to the stimuli (Rabasa, Delgado-Morales, Munoz-Abellan, Nadal, & Armario, 2011). This is not unlike a learned helplessness model in which escape or avoidance behaviors give way to more docile acceptance (Seligman, 1972). In other words, the environment has not become any less stressful to the children, but their baseline for behavioral reaction has shifted up, which is why they are reporting less stress and anxiety than the corresponding observer report.

Another possible explanation for the cortisol elevation in children with VCFS involves an epigenetic model of HPA dysregulation in developing fetuses and newborns. That is, there might be an interaction between the maternal environment, the uterine environment, and the genetic coding of proteins that impacts the development and functioning of the HPA axis. There is some evidence from animal models to suggest that when a mother is exposed to significant stressors, permanent changes occur in the composition and functioning of the fetal HPA axis (Egliston, McMahon, & Austin, 2007; Wright, 2007). Specifically, regulation or suppression of the stress response is impacted in offspring of mothers who have experienced significant amounts of stress during pregnancy. Moreover, offspring exhibited higher resting cortisol levels

and delayed returns to baseline cortisol following exposure to mild stressors. Indeed, maternal stress may be one factor influencing the elevated cortisol levels in VCFS. More research is necessary in order to understand the impact of prenatal stress on the aberrant functioning of the HPA axis in individuals with VCFS.

In summary, it is unclear from these data whether children with VCFS are born with anomalous physiological mechanisms, which fail to suppress ACTH production or whether environmental stressors have a differential impact on the way these children secrete cortisol. Specific measures and records of stressful events and Dexamethosone Suppression Tests may be necessary in order to reach more definitive conclusions regarding the etiology of the elevated cortisol levels.

Children with VCFS Perform Significantly Poorer on Selected Tests of Memory and Learning

The hypothesis that children with VCFS would exhibit reduced cognitive abilities relative to control counterparts was supported. Specifically, children with VCFS exhibit poorer performance in general memory ability, attention and concentration ability, and working memory ability. This finding is not surprising given the high rate of ADHD and LD observed in this population. According to Antshel et al. (2007), children with VCFS exhibit an inattentive subtype of ADHD characterized by poor ability to focus and short attention span. One of the causal mechanisms for ADHD in this population is poor self-monitoring ability mediated by functional deficits in the frontal lobes and volumetric anomalies in the caudate nucleus (Castellanos et al., 1994) of children with VCFS. Specifically, children with VCFS show increased activation in the frontal lobe during a computerized continuous performance task. Such increased activation suggests that in order to perform well on the test, the individual needs

to work harder and recruit resources from other regions of the brain. Such recruitment is commonly observed in studies of functional neuroimaging in individuals with ADHD.

In addition to the frontal lobes, the caudate nucleus of the basal ganglia has been implicated in the regulation of attention and the ability to maintain vigilance (Garrett et al., 2008). Individuals with ADHD performing cancellation and continuous performance tasks show decreased activation in these areas during the test period (Schneider et al., 2010). Additionally, in control populations an asymmetry in this structure is observed (Hynd et al., 1993). Specifically, neurotypicals have a larger right caudate nucleus relative to the left. However, in children with ADHD, the asymmetry is absent and there is no significant difference between the right and the left caudate. Children with VCFS who exhibit symptoms associated with ADHD have a right and left caudate that are volumetrically indistinguishable (Sugama et al., 2000). Therefore, it is possible that volumetric anomalies in this structure are mediating some of the symptoms of inattention in this population.

Genetics may also play a role in the inattention observed in this population. Individuals with VCFS and comorbid ADHD have a low-activity COMT allele. In fact, individuals with VCFS have only a single copy of the COMT gene and therefore have the lowest COMT activity (Gothelf, Michaelovsky et al., 2007; Lachman et al., 1996). The COMT gene is responsible for coding for an enzyme associated with the breakdown of the neurotransmitter dopamine. As a result of the low activity variant in individuals with VCFS, too much dopamine accumulates in regions associated with the maintenance of attention such as the frontal lobes and select structures of the basal ganglia. Excessive dopamine in these regions have been associated with symptoms of inattention (Turic et al., 2005), rapid mood changes (Papolos et al., 1998), and psychosis (Murphy, Jones, & Owen, 1999). Given these findings, it is highly possible that the

poor performance on measures of inattention may be related to the COMT gene and subsequent dopamine modulation.

The inattention and inability to concentrate may be one of the factors impacting the poor social skills observed in children with VCFS. That is, the children do not attend to social cues such as facial expressions, eye gaze, and other social norms. As a result, the children do not learn to engage reciprocally in social interactions and have decreased overall social competence. Additionally, given the involvement of dopamine in social reciprocity and social responsiveness (Nagaraj, Singhi, & Malhi, 2006), it is likely that the low activity COMT variant expressed in children with VCFS may also be contributing to their social skills deficits. In other words, not only do the children have deficits attending to, learning, and using social cues, but they also may find social interaction less rewarding in general as a result of aberrant dopamine modulation. More research is necessary to determine the links between COMT, dopamine, cortisol, attention, and social functioning, in order to determine the magnitude and specific contributions of each of these variables.

In addition to inattention and concentration deficits, individuals with VCFS in this study exhibited general memory deficits. These data are consistent with previous findings examining the cognitive profile of children with VCFS (Debbane, Glaser, & Eliez, 2008; Kates, Krauss et al., 2007; Lajiness-O'Neill et al., 2006; Majerus, Van der Linden, Braissand, & Eliez, 2007; Swillen et al., 1999). Children with VCFS exhibit nonverbal learning deficits, recognition deficits for both verbal and nonverbal information, and difficulties suppressing irrelevant content, which all contribute to the general memory impairment commonly observed in this population. It has been reported that the general memory deficits observed in this population relate to neuroanatomical and functional inefficiencies in bilateral hippocampus of children with

VCFS. As previously stated, children with VCFS have decreased volume in their hippocampus (Jacobson et al., 2011; Kates et al., 2006). These volumetric anomalies have been correlated with decreased performance on tests of memory and learning relative to neurotypical children. In particular children with VCFS exhibit a NLD profile (Lajiness-O'Neill et al., 2006) though recently deficits have been observed in serial learning of verbal information and recognition of verbal information (Majerus et al., 2007). In summary, children with VCFS exhibit deficits in general ability characterized by nonverbal memory and learning deficits and circumscribed verbal learning deficits. It is likely that these deficits are caused by neuroanatomical and functional deficits in substructures of the temporal lobe.

Working memory abilities were also found to be reduced in children with VCFS relative to controls. These data have also been reported and therefore the data reported in this study is consistent with previous findings (Coman et al., 2010; De Smedt et al., 2008; Kates, Krauss et al., 2007; Lajiness-O'Neill, 2005; van Amelsvoort et al., 2004). Working memory requires a combination of neurological structures working in concert. Specifically, working memory is supported by sub-regions of the frontal lobe and the parietal lobe. For example, an examination of frontal lobe structures in individuals with decreased working memory ability found decreased activity in the frontal lobes. With regard to the parietal lobe, individuals with VCFS have exhibited volumetric and functional anomalies in this lobe of the brain (Eliez et al., 2000). Additionally, Diffusion Tensor Imaging (DTI) studies have examined the white matter pathways connecting the frontal and parietal lobes in this population and children with VCFS and found that the Fractional Anisotropy (FA) values in white matter tracts in the supramarginal gyrus and angular gyrus were positively correlated with performance on the arithmetic subscale of the WAIS/WISC, suggesting that aberrant connectivity between the frontal and parietal lobes may

contribute to spatial working memory deficits observed in this population (Barnea-Goraly, Eliez, Menon, Bammer, & Reiss, 2005). Moreover, studies have found decreased activation in the temporal lobe of patients with working memory deficits (White, Hongwanishkul, & Schmidt, 2011). Children with VCFS exhibit deficits and functional anomalies in all of these areas supporting working memory.

As aforementioned, decreased frontal volumes and aberrant activation of oribitofrontal and ventromedial prefrontal cortex has been observed in this population (Kates et al., 2010; Kates et al., 2006). The functional deficits associated with both the frontal lobe and temporal lobe have again been attributed to increased dopamine levels in this region, which impact both receptor sensitivity, overall efficiency of dopamine transmission, and production of factors essential to the maintenance of healthy cellular functioning and memory encoding (Gothelf, Schaer, & Eliez, 2008). It is possible that due to increased exposure to dopamine as a result of the inability to degrade dopamine, down regulation of D2 receptors occurs. D2 receptors have been shown to be essential for working memory (Goldman-Rakic, 1999; Wang, Vijayraghavan, & Goldman-Rakic, 2004). Moreover, the presence of excessive amounts of dopamine has been shown to lead to the down regulation of D2 receptors (Ginovart, Farde, Halldin, & Swahn, 1999; Zemlan, Hitzemann, Hirschowitz, & Garver, 1985). For example, in individuals with schizophrenia, release mediated down-regulation of central and cortical D2 receptors has been observed along with reduced receptor density (Ginovart et al., 1999; Zemlan et al., 1985). As working memory deficits have been observed in the schizophrenic population as well, it stands to reason that D2 receptor modulation may contribute to deficits observed in children with VCFS.

Additionally, evidence from cocaine abuse studies suggest that particular working memory deficits are mediated by the underproduction of Nuclear factor kappa B (NFkappaB),

which is a modulator of oxidative stress and a key component of short-term memory formation (Muriach et al., 2010). Following administration of high doses of exogenous cocaine, a synthetic dopamine agonist, NFkappaB was greatly reduced in frontal regions. Corresponding deficits in memory performance were also observed. Thus, it seems that an interaction between neuroanatomical deficits in the frontal cortex, parietal lobe, and temporal lobe along with overproduction of dopamine may mediate the significant difference in working memory performance in individuals with VCFS and controls. More research is necessary in order to understand the unique role that dopamine and NFkappaB plays in the development of working memory deficits in individuals with VCFS.

To summarize, it is unclear what the role of cortisol is in mediating cognitive deficits observed in this population. More research is necessary in order to determine whether elevated cortisol levels underlie some of the morphometric anomalies in children with VCFS. As will be shown, there may not be a direct relationship between elevated cortisol and other symptoms such as working memory attention, general memory. However, cortisol might share an indirect role or secondary role to dopamine in the impairment and symptomatology observed in this population.

The Relationship Between Cortisol and Cognitive and Behavioral Measures of Functioning

The hypothesis that cortisol levels would be correlated with select measures of cognitive functioning and social competence in individuals with VCFS was not supported. Specifically, no relationship between cortisol and general memory, attention and concentration, or working memory was observed. Moreover, a significant relationship was not observed between cortisol level and anxiety/depression, social problems, thought content problems, and social competency in individuals with VCFS. Additionally, cortisol levels were unrelated to measures of adaptive

functioning including social skills and communication. Finally, in children with VCFS cortisol was not related to measures of social anxiety or total anxiety. Though it is tempting to conclude that cortisol may be unrelated to these cognitive and behavioral measures, drawing such a conclusion is premature given the relationships observed in the control group. In the control group, a significant negative correlation was observed between cortisol and social competence as measured by the CBCL. Additionally, a negative correlation was observed between cortisol and the attention/concentration subscale of the WRAML-2. Thus, in controls, aspects of neurocognitive functioning are strongly correlated with resting cortisol levels. This may imply that in children with VCFS, another physiological or neuroanatomical mechanism may be influencing the strength and direction of the relationship between cortisol and neurocognitive and behavioral measures.

One possible explanation for the differences observed in the cortisol relationships between controls and individuals with VCFS is receptor sensitivity or down regulation. Down regulation of receptors is observed when there is a high presence of a certain endogenous or exogenous chemical messenger in the brain (Carlson, 2004). Essentially, receptor modulation is a physiological mechanism through which neurons attempt to maintain homeostasis. Put simply, receptors on the surface of cells are rendered inactive in response to high levels of transmitter. As a result, more transmitter is required in order to produce the same behavioral effect. Such receptor modulation governs the process of physiological and behavioral tolerance to certain drugs (Carlson, 2004). Glucocorticoid receptor down regulation has been observed in animal models. In response to long-term water exposure and restraint, the number of glucocorticoid receptors observed in the brains of rats were greatly reduced, leading to hyposuppression of the stress response (Huizenga et al., 2000; Mizoguchi et al., 2001). Given the long-term exposure to

elevated glucorticoids in individuals with VCFS, it may be possible that receptor down regulation may be occurring throughout the limbic system and prefrontal cortex. Such receptor down regulation may be the reason that the cortisol does not have a significant relationship with measures of cognitive and social functioning. It also explains the different findings between the two groups. Since individuals without VCFS are not exposed to the high levels of glucocorticoids for long periods of time, receptor down regulation in key areas of the brain associated with memory, learning, and social functioning may not have occurred, and performance on cognitive and behavioral measures is still affected. Thus, it may be that children with VCFS have developed somewhat of a tolerance to cortisol through receptor modulation and therefore do not exhibit the strong correlations between cortisol, cognition, and social functioning seen in controls. Further research examining the temporal relationship between receptor down regulation and cognitive and behavioral functioning in individuals with VCFS is necessary in order to determine if exposure to high levels of glucocorticoids throughout development impacts the effect that cortisol has on symptomatology.

Though glucocorticoid receptor modulation explains the differential cortisol findings in VCFS and controls, it does not explain the relativity poor cognitive performance and higher levels of symptomatology observed in the VCFS children in this study. Thus, an additional mechanism may also be acting along with cortisol and may significantly contribute to deficits observed in this population. One possible mechanism mediating the cognitive and social deficits associated with VCFS is dopamine. Due to the low-activity COMT variant expressed in children with VCFS, dopamine levels in this population are high (Gothelf et al., 2008; Papolos et al., 1998; van Amelsvoort et al., 2008; Yu, Zhang, Huang, Ding, & Li, 2007). Such high levels of dopamine have been associated with impaired memory performance (B. L. Murphy, Arnsten,

Goldman-Rakic, & Roth, 1996), attention/concentration deficits (Russell et al., 2006), and social deficits (Liddle, 2000). Thus, it is possible that both dopamine and cortisol act in concert to produce the deficits observed in VCFS. Moreover, given the COMT variant in children with VCFS ,it is likely that these children have significantly higher levels of dopamine than their control counterparts. These elevated dopamine levels may contribute to D1 and D2 receptor insensitivity in some regions of the brain associated with reward and attention while simultaneously contributing to symptoms associated with psychosis. The combination of dopamine and cortisol may have impacted the ability to observe a significant relationship between cortisol alone and the neuropsychological functioning of the children with VCFS. That is, the elevated dopamine levels may have exacerbated deficits and impacted test scores in such a way as to make the relationship with cortisol non-linear.

Treatment Implications

Given what is observed in neurotypical children regarding the relationship between cortisol level, cognitive performance, and social competence, this study has some relevant treatment implications. Reduction of cortisol level has been shown to improve mood impairment and cognitive functioning (Baker et al., 2010). When used to treat bipolar disorder, mifepristone, an antiglucocorticoid drug, was effective in reducing hypercortisolaemia. Following the reduction of glucocorticoids, verbal fluency, spatial recognition abilities, and working memory abilities improved significantly relative to a no-treatment control group (Young et al., 2004). In addition, mood symptoms were also reduced. To date there have not been any published studies examining the impact of mifepristone on cognitive functioning and social competency in individuals with VCFS. Given the high rate of bipolar disorder in this population and the relative effectiveness of the drug to treat both mood and cognitive symptoms, it may be a useful

treatment in this population, and further research is necessary in order to determine the efficacy of the drug in the alleviation of symptoms in this population.

In addition to drug therapy, behavioral interventions have also been shown to reduce cortisol levels and improve cognitive functioning (Baker et al., 2010). Following a regimen of daily moderate exercise, individuals with Alzheimer's disease showed an increase in their ability to regulate the HPA axis and a corresponding decrease in cortisol level. Performance on neurocogntive measures in the area of executive functioning and short-term memory were significantly improved relative to controls. These data further suggest that regulation of cortisol is effective in reducing cognitive symptoms.

In summary, regulation of cortisol may be an important treatment target for both individuals with VCFS and other populations exhibiting high levels of cortisol. Both behavioral and physiological treatments have been shown to reduce cortisol levels. More research is necessary in order to determine the optimal levels of cortisol and the specific impact that a reduction in cortisol levels would have on cognition and social functioning in patients with VCFS and other neurodevelopmental disorders.

Limitations

One of the primary limitations of this study was sample size. Indeed, the sample size was sufficient to be able to detect differences in resting cortisol levels. However, given the individual variability of neurocognitive performance, a larger sample size would have enhanced the power and decreased the likelihood of making a type-II error.

Sample composition is another limitation of this study. Due to the fact that subjects were recruited through the VCFS support group, there may be confounds associated with individuals who have access to the group. Additionally, these individuals are often apprised of recent

treatments, and it is possible that the children in the support group may have received more surgical, pharmacological, and psychological treatment than VCFS children not in the support group.

Another limitation is that only one physiological variable was examined in this study. It is likely that multiple factors contribute to the high levels of cortisol levels and cognitive and behavioral symptomatology observed in this population. It would be informative to incorporate multiple variables to determine the relative contribution of several possible etiological variables.

Finally, another limitation and confound in this study was the inability to control for various medications. Due to the wide range of medications taken in the VCFS sample and the inability to have patients not take their medication for any extended period of time, it was difficult to statistically control for any one type of medication (i.e. psychostimulants, antibiotics, antidepressants, anxiolytics, etc.). Future studies of cognitive and social functioning in this population should examine and control for the specific effects of the medication the individuals are taking. This would require a significantly larger sample size and a more consistent medication record between patients.

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