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The Relation of Cannabis and Alcohol Use with Cortisol and Symptom Severity in Youth At-Risk for Psychosis

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An abstract of

A thesis submitted to the Faculty of Emory College of Arts and Sciences

of Emory University in partial fulfillment

of the requirements of the degree of

Bachelor of Arts with Honors

Department of Psychology

2010

Abstract

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By Sara M. Feldman

Approximately half of the patients suffering from schizophrenia have a lifetime comorbid substance use disorder (SUD) diagnosis. Substance use among psychotic patients is twice the rate of use among healthy controls in the general population. SUDs in schizophrenic patients are associated with poorer clinical outcomes. Research shows that 25 to 45% of individuals who meet criteria for the prodrome to schizophrenia convert to a full-blown psychotic disorder within 2 to 5 years. Further, individuals who meet prodromal criteria are increasingly likely to convert to a psychotic disorder if they use cannabis. Experimental studies where Delta-9-THC was administered show acute increases in cortisol levels. The present study examined the relation of alcohol (AU) and cannabis use (CU) with cortisol levels and symptoms in 33 healthy controls, 56 prodromal patients, and 40 psychiatric controls. The current study hypothesized that prodromal subjects who report CU will show elevated cortisol levels, as well as more severe positive symptoms. The present study indicated no relation of AU with cortisol levels, symptoms, or conversion to psychosis. Further, the findings indicated that CU was not associated with symptom severity or progression. However, CU was linked with reduced cortisol levels. The results are interpreted in light of past findings indicating that youth with lower baseline cortisol levels are more likely to become cannabis users in the future, and that longer term CU is linked with reduced cortisol levels. Thus, although CU results in an acute increase in cortisol and positive symptoms, initial CU and prolonged CU appears to be associated with lower cortisol levels.

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Table of Contents

The Relation of Cannabis and Alcohol Use with Cortisol and Symptom Severity in Youth At-Risk for Psychosis

Approximately half of the patients suffering from schizophrenia have a lifetime comorbid substance use disorder (SUD) diagnosis (Barnett et al., 2007). Substance use (SU) among psychotic patients is twice the rate of use among the general population. For patients with schizophrenia, the rate of alcohol use disorder (AUD) is three times higher than in the general population. Similarly, the odds of having another SUD other than an AUD were six times higher (Gregg, Barrowclough, & Haddock, 2007). In one study, the prevalence rate for current cannabis use (CU) was found to be approximately 23% in schizophrenic patients. Lifetime prevalence rates for CU by schizophrenia patients were even higher—an astounding 42 % (Green, Young, & Kavanagh, 2005; Gregg et al., 2007). This is in contrast to the 31% lifetime CU in the general population. Substance using schizophrenic patients are more likely to be male and younger when compared to schizophrenia patients who are nonusers (Barnett et al., 2007; Swofford, Scheller-Gilkey, Miller, Woolwine, & Mance, 2000).

Our understanding of the determinants and consequences of SU in schizophrenia is somewhat limited. Further, obtaining accurate self-report information about substance use is challenging, and published rates of use are likely to be underestimates (Bloye, Ramzan, Leach, Davies, & Hilton, 2003). Moreover, because the data are correlational in nature, causal relations are not easily discerned. Some have suggested, for example, that the elevated rate of substance use in schizophrenia is due to attempts at "self-medication." Other data indicate that substance use worsens symptoms and prognosis, and recent research indicates that it might contribute to vulnerability. These interpretive frameworks are revisited following an overview of the research literature.

As described below, there is evidence that substance use, especially cannabis, may increase risk for schizophrenia and other psychotic disorders. In light of this, it is important to examine the relation of substance use with the progression of symptoms in individuals who are at clinical risk for these disorders. Further, some recent experimental findings indicate that cannabis may have the potential to exacerbate symptom progression because it augments the biological response to stress. More specifically, exposure to the active ingredient in cannabis (THC) produces an increase in the secretion of cortisol, an important component of the neurohormonal response to stress. The present study examines the relation of CU and alcohol use (AU) with symptom severity and symptom progression in youth at clinical risk for psychotic disorders. In addition, levels of cortisol secretion at baseline and follow-up are measured in order to determine whether substance use, especially cannabis, is contributing to stress sensitivity in at-risk youth.

Correlates of Substance Use by Psychotic Patients

Much of the research on the relation of substance use with psychotic disorders does not differentiate among subtypes of substances. Nonetheless, the research findings have shed light on the general correlates of SU in patients with psychosis. The literature suggests that the correlates of SUD in people with schizophrenia may be much more complex than the factors associated with substance abuse in the general population.

A study by Barnett et al., (2007) recorded current and lifetime substance use for people who were referred to a specialist early in their treatment of their first psychotic episode. Many drug use problems were found. Specifically, cannabis abuse was found in 51% of patients, and alcohol abuse was reported by 43% of patients. The age of first use of cannabis, cocaine, ecstasy, and amphetamines were significantly correlated with the age at first psychotic symptom.

These results indicate that patients who begin using drugs at a younger age are likely to be younger when they experience their first psychotic break. Early drug users also wait longer than patients who took drugs at a later age to seek treatment for their first psychotic symptom.

A similar study conducted by Fowler et al. (1998) found that the 6-month and lifetime prevalence of substance abuse or dependence was 26.8% and 59.8% respectively, among schizophrenia patients. Alcohol (77.3%), cannabis, and amphetamines were the most commonly abused substances by this population. Patients with current or lifetime diagnoses of substance abuse or dependence were younger, and were more likely to be single males, with higher rates of criminal charges. Patients with SUDs were also younger at first treatment and showed more symptoms than participants with no past or current substance use.

A meta-analysis of nine studies reporting Positive and Negative Syndrome Scale ratings in schizophrenia patients with and without SUDs found that those with a SUD had higher positive symptoms, but not negative symptoms, than those without a SUD (Talamo, Centorrino, Tondo, Dimitri, Henne, & Baldessarini, 2006). As described below, this pattern also holds for CU specifically.

It is known that SUDs in schizophrenic patients are associated with poorer clinical outcomes (Barnett et al., 2007; Compton, 2005; Fowler et al., 1998; Swofford et al., 2000; Volkow, 2009). In a study by Swofford et al. (2000), for example, symptoms, hospitalizations, compliance, and demographic variables were investigated in schizophrenia patients who were users and nonusers of any recreational drug or alcohol. In patients who were drug users, she found an increased likelihood for relapse, hospitalizations, and worsening of symptoms. Substance users also had a higher rate of missed appointments, which was positively correlated with increased hospitalizations. Drug users were more likely than AUrs to have more negative

symptoms, except when the AUrs needed treatment for alcoholism. Present drug users had higher tardive dyskinesia scores, more cognitive impairments, less education, and higher average neuroleptic dose than in nonusers and AUrs. These findings suggest a harmful impact of drug use on schizophrenia patients. The findings propose that if drug use can be reduced or halted altogether, clinical outcomes may improve for this population.

Research by Compton (2005) found that after adjusting for sociodemographic variables and for a schizophrenia-spectrum disorder (SSD) subtypes, SUDs were associated with occupational problems, housing problems, economic problems, problems with access to healthcare services, and problems related to interaction with the legal system. These issues may make it even more difficult for a person with both a SSD and a SUD to seek treatment and improve his or her life.

When compared to schizophrenia patients without substance use, dual diagnosis schizophrenia (DDS) patients have worse clinical outcomes in terms of engagement and retention in treatment, neuroleptic compliance and responsiveness, psychosocial aspects and complications, and in terms of overall management difficulty and long-term prognosis (Goswami Mattoo, Basu, & Singh, 2004). While there is evidence from cross-sectional studies that drug-using schizophrenia patients have less severe negative symptoms than nonusers, longitudinal studies show that drug use does worsen the course of the psychotic illness (Akerele & Levin, 2002; Barnett et al., 2007; Compton, 2005; Fowler et al., 1998; Swofford et al., 2000; Volkow, 2009). This pattern of findings has been interpreted to indicate that the negative symptoms of psychosis, such as social withdrawal, amotivation and blunted affect, are obstacles to the formation of social relations required to obtain drugs and alcohol.

Small doses of stimulants such as amphetamines and opioids such as benzodiazepines have been found to worsen psychotic symptoms (Dixon, Haas, Welden, Sweeney, & Frances, 1990). However, there is still relatively little literature on these specific drugs. There is some evidence indicating that stimulant use may precipitate chronic psychosis or schizophrenia (Gregg et al., 2007). Similar findings have been reported for cocaine (Gregg et al., 2007).

Research has been conducted on the association between schizophrenia and cigarette smoking. While this topic is not the focus of the current study, it is important to note that forty-two studies across twenty nations unfailingly found an association between schizophrenia and current smoking behaviors (de Leon & Diaz, 2005). Heavy smoking and high nicotine dependence were more frequent in smokers with schizophrenia compared with the general population (de Leon et al., 2005).

Taken together, it is clear that substance use, dependence, and abuse are a problem in the psychotic patient population. It is important to examine the possible causes and consequences of this conclusion. Because cannabis and alcohol are the drugs most frequently used by psychotic patients, and are the subject of this investigation, specific research will be reviewed about the effects of alcohol and cannabis on psychotic patients.

Alcohol Use by Schizophrenia Patients

It has repeatedly been shown that there is a relation between the use of alcohol and poorer clinical outcome in psychotic patients. Consistent with the notion that patients use substances for self-medication, some schizophrenia patients report that alcohol reduced discomfort caused by hallucinations (Alpert & Silvers, 1970; Dixon et al., 1990). AUDs are also associated with a poorer course and outcome of schizophrenia (Cuffel & Chase, 1994; Drake, Osher, & Wallach, 1989; Drake, Mueser, Clark, & Wallach, 1996; D'Souza et al., 2006).

It has been found that alcohol abuse can worsen the symptoms and outcome of those with schizophrenia and cause relapse, although there is no evidence that it can actually cause schizophrenia (Bernadt & Murray, 1986; Gregg et al., 2007; Hambrecht & Hafner, 1996). An additional study by Bloye and colleagues (2003) uncovered that 72% of their sample had an AUD or SUD, but there were more subjects with AUDs than SUDs. Cannabis was the second most abused drug in the study.

Cannabis Use by Schizophrenia Patients

It is important to understand the characteristics of CU in psychotic samples. A literature review in 2009 found that the median current rate of CU disorders (CUDs) in schizophrenia patients across 35 studies was 16%, and the median lifetime rate was 27.1% (Koskinen, Lohonen, Koponen, Isohanni, & Miettunen, 2009). The median rate of CUDs was significantly higher in first-episode psychotic patients compared to long-term patients and in studies where more than two-thirds of the participants were males. The reason for heightened CUD rates in first-episode psychotic patients may be due to the fact that patients who have not been hospitalized still have access to drugs in their social settings. CUDs were more prevalent in younger samples than in older samples (current 38.5%/16.0%, lifetime 45.0%/17.9%). The authors of this literature review concluded that approximately every fourth schizophrenia patient in the sample of studies had a diagnosis of a CUD **(Koskinen et al., 2009). These findings show the high prevalence of CUDs in psychotic patient populations.**

The use of cannabis is known to worsen psychotic symptoms. An early study on the topic found that schizophrenic patients who use cannabis reported a worsening of symptoms (Knudsen & Vilmar, 1984; Dixon et al., 1990). Another early study by Negrete, Knapp, Douglas, and Smith (1986) revealed that cannabis users had significantly more delusional and

hallucinatory activity than nonusers, with cannabis users showing the most symptoms (Dixon et al., 1990).

Research that has examined CU by psychotic patients generally confirms the assumption that users tend to have less severe negative symptoms, but more pronounced positive symptoms. Baeza and colleagues (2009) found that in adolescents diagnosed with first-episode psychosis, cannabis users had higher PANSS positive scores and lower PANSS negative symptom scores than nonusers. However, six months later, those who stopped using cannabis had lower symptoms than those who continued. Thus**,** CU may be related to higher positive symptom scores for patients, but there is greater improvement after six months for those who cease using cannabis. The same pattern of higher positive and lower negative symptoms in cannabis using psychotic patients is reported by other investigators (Baldacchino, et al., 2009; Compton, Furman, & Kaslow, 2004).

The Determinants of SU in Schizophrenia Patients: Self-Medication Hypothesis (SMH)

Khantzian (1985) was the first to propose the self-medication hypothesis of SU in psychiatric patients. He hypothesized that the drugs people with schizophrenia use reflect their efforts to cope with or reduce symptoms. There are two versions of the SMH. The first is that drugs are used to cope specifically with symptoms of mental disorders. The other definition is broader and assumes that the substances are used to enhance mood and cope with painful feelings in general. Henwood and colleagues (2007) classified the self-reported reasons for substance use from psychiatric patients in a qualitative study and found that only 11 out of 72 attributions involved using substances strictly to cope with symptoms of mental disorders. However, more than half of all attributions involved using substances to cope with painful feelings in general (Henwood & Padgett, 2007).

Other investigations have yielded similar results. One study found that many patients said they used drugs to get high and to relax or to increase pleasure, energy, and emotions (Dixon et al., 1990). A study by Goswami and colleagues found that most patients with both SU and psychosis report that their reasons for drug use were for pleasurable pursuit and for reduction in symptoms and distress. However, the reasons for drug use differed slightly by substance. CUrs reported use to get high, to relax, and to increase pleasure, while AUrs wanted to decrease their depression symptoms, decrease hallucinations, decrease suspiciousness, to relax, and increase sleep. According to patient reports, alcohol decreased anxiety significantly more than cannabis. Cannabis was used significantly more often to increase energy than alcohol. Overall, Goswami and colleagues concluded that there was only modest support for the SMH for some substances and some symptoms (Goswami et al., 2004). Thus, based on self-report, the broader view of the SMH appears to explain more SU among patients. While patients suffering from schizophrenia and other psychotic disorders certainly have more social problems than healthy individuals, their reasons for using drugs may be the same as the general population (Akerele et al., 2002).

Experimental studies are needed to obtain more conclusive evidence concerning the effects of substances on patients. For a host of reasons, including ethical concerns, there are few such investigations. There is only one experimental study of the effects of alcohol on symptoms in schizophrenia patients. D'Souza and colleagues (2006) found that subjects with schizophrenia reported greater euphoria and stimulatory effects in response to alcohol. The alcohol created small increases in positive symptoms and perceptual alterations, without affecting negative symptoms. The responses of the schizophrenic patients to alcohol were magnified compared to healthy controls, which may increase the risk for AUDs associated with schizophrenia (D'Souza

et al., 2006). Thus, the results do not support the SMH, in that there was no reduction in symptoms, although euphoria increased.

As noted above, it has been found that patients with a substance use and psychotic disorder diagnosis manifest fewer negative symptoms than those who did not use drugs (Akerele et al., 2002; Potvin, Sepehry, & Stip, 2005). Although this may indicate that substance abuse reduces the negative symptoms of schizophrenia, it is also plausible that the patients with fewer negative symptoms are more prone to use drugs. Thus, it is less likely that patients are able to obtain drugs if their negative symptoms are debilitating. A patient with severe negative symptoms will be less inclined to socialize and make the necessary social connections to obtain drugs.

In contrast to the SMH, Chambers, Krystal, and Self, (2001) argue that increased susceptibility to addictive behavior may be a result of the impact of the neuropathology of schizophrenia on the neural circuitry mediating drug reward and reinforcement. He suggests that the brain circuitry responsible for addictions may be very similar to those involved in schizophrenia. Therefore, patients with schizophrenia may not be able to control their cravings for drugs, and thus are not using drugs to self-medicate their symptoms.

In summary, although it is likely that many patients at-risk for or diagnosed with psychotic disorders utilize substances in an effort to reduce symptoms, there is little evidence that substance use does indeed ameliorate symptoms. In fact, it appears that most abused substances exacerbate symptom severity and potentially have the ability to trigger onset or relapse.

Relation of CU with Risk for Psychosis

As mentioned above, there is evidence that CU may reduce the schizophrenia vulnerability threshold, thereby increasing the likelihood of psychosis. An early study by Andreasson et al. (1987) was the first to report a dose response relationship between the amount of CU in adolescence and the subsequent risk of developing schizophrenia. The researchers followed forty-five thousand soldiers in the Swedish army for fifteen years. A strong correlation was found between history of CU at baseline and presence of schizophrenia at follow-up. The heavy CUrs were six times more likely to have a diagnosis of schizophrenia at follow-up than less frequent users or those who had never used cannabis (Andreason, Alleback, Engstrom, & Rydberg, 1987; Gregg et al., 2007).

Later studies have extended these earlier findings. A study by Weiser et al., (2002) examined a large cohort of 50413 adolescent males. The subjects who were later hospitalized for schizophrenia were more likely to have smoked cannabis at baseline than those who were not hospitalized (Weiser, Knobler, Noy, & Kaplan, 2002; Gregg et al., 2007). A study in Greece from a cohort of 3500 nineteen-year-olds revealed that CU was associated with both positive and negative dimensions of psychosis (Stefanis, Delespaul, Henquet, Bakoula, Stefanis, & Van Os, 2004). Another study found an increased risk of psychotic symptoms at follow-up in people who had used cannabis at baseline when compared to those who had not used cannabis at this time (Henquet, Krabbendam, Spauwen, Kaplan, Lieb, & Wittchen, 2005). It is clear that some CUrs are more vulnerable to developing schizophrenia than others, but researchers do not yet know how to identify those who are vulnerable.

Recently, it has been speculated that if schizophrenia patients did not use cannabis, 13% of cases of schizophrenia could be prevented. Arsenault et al. (2002) found that after controlling for pre-existing psychotic symptoms, CU increased the subsequent risk of developing

schizophrenia symptoms. This augmented risk was found to be the largest in users prior to fifteen years old. These findings suggest a heightened sensitivity in adolescents to drug use. Adolescence is a time of many important changes in the brain, especially in the frontal and limbic areas. Using drugs during this time may increase the chances of converting to psychosis. Thus, some CUrs may begin to see a period of functional decline in adolescence.

Research on individuals at-risk for psychosis has recently intensified its focus on a period referred to as the "prodrome." The prodrome is the period of functional decline that precedes the onset of psychosis (Larsen, McGlashan, & Moe, 1996). It is characterized by unusual sensory experiences and ideas, increasing social impairment, functional decline in occupational and academic domains, and mood symptoms such as anxiety and depression. Researchers often refer to these signs as "subclinical" symptoms. This is because the unusual sensory experiences and ideations are not at a level of severity that would meet criteria for hallucinations or delusions, yet they are troubling to the individual. These symptoms usually first appear in adolescence and become gradually worse until the individual meets criteria for a psychotic disorder in early adulthood. Using current procedures for characterizing the prodrome, individuals who meet prodromal criteria show a rate of conversion to psychosis in the range of 25 to 45% within a period of two years (Seeber & Cadenhead, 2005). Thus, youth who meet criteria for the prodrome are more likely than the general population to convert to a full-blown psychotic disorder. Prodromal individuals who use substances are increasingly likely to convert to a psychotic disorder (Kristensen & Cadenhead, 2007).

Experimental Studies of the Effects of Cannabis/THC

Given the accumulating evidence of a link between CU and risk for psychosis, investigators are now addressing the question of possible neural mechanisms. CU alters the

functioning of brain regions responsible for control of cognition and maintenance of intact perceptual functions. Cannabis causes changes in cerebral blood flow in the orbitofrontal and prefrontal cortices, the basal ganglia, the insula, the cingulate gyrus, and subcorticol areas of the brain (Cohen, Solowij, & Carr, 2008). Schizophrenia-like symptoms, such as suspiciousness and unusual sensory experiences, are often noted in cannabis smokers, implying that high doses of cannabis can induce a schizophrenia-like mental state (Talbot, 1969; Rathbone, Variend, & Mehta, 2008).

In an experimental study of the acute effects of cannabis smoking on cortisol, healthy male subjects smoked cannabis or cigarettes and plasma samples were obtained immediately after smoking (Cone, Johnson, Moore & Roache, 1986). Cannabis, but not cigarette smoking, was followed by a significant increase in cortisol secretion, as well as psychomotor impairments. Subsequent research has replicated these findings.

An experimental study by D'Souza (2004) conducted a three day, double-blind, randomized, and counterbalanced study of the behavioral, cognitive, and endocrine effects of 0, 2.5, and 5 milligram intravenous Delta-9-THC, the active ingredient in cannabis. Participants were 22 healthy subjects who had been exposed to cannabis, but had never been diagnosed with a cannabis abuse disorder. Delta-9-THC produced schizophrenia-like positive and negative symptoms, altered perception, increased anxiety, produced euphoria, disrupted immediate and delayed word recall, sparing recognition recall, impaired performance on tests of distractibility, verbal fluency, and working memory, but did not impair orientation. Most importantly for the purposes of the current study, researchers found increased plasma cortisol levels. These results indicate that Delta-9-THC produces brief psychotic symptoms, cognitive deficits, and increased cortisol secretion in healthy controls.

A later study by D'Souza et al. (2005) looked at a sample of schizophrenia patients who used cannabis and compared them to healthy controls. Delta-9-THC increased deficits in learning and recall, positive, negative, and general symptom severity, perceptual alterations, rigidity and dyskinesia, and deficits in vigilance. Plasma prolactin and cortisol levels also increased (D'Souza et al., 2005). Further, schizophrenia patients were affected more by the cannabis than healthy controls. Thus, Delta-9-THC worsens psychotic and cognitive problems in schizophrenia patients even more than in healthy controls subjects.

Another study by D'Souza et al. (2008) used the same study design to examine the effects of intravenous Delta-9-THC on healthy users and nonusers of cannabis. Twenty-two healthy controls who were nonusers of cannabis and 30 healthy frequent CUrs were recruited. The researchers found that Delta-9-THC produced brief effects that mimicked psychosis, including perceptual alterations, impaired memory and attention, increased subjective effects of being high, tachycardia, and increased cortisol. This was observed in both healthy controls and frequent CUrs. However, the frequent users showed blunted responses to the psychotomimetic properties of perceptual altering, cognitive impairment, anxiogenic, and cortisol increases, but not to its euphoric effects. The results suggest that frequent cannabis users are characterized by lower cortisol levels prior to use, have a less pronounced physical responses to cannabis, or that they develop a tolerance to the effects of cannabis. Taken together, the series of studies by D'Souza et al. show that cannabis does briefly mimic psychosis, produce cognitive deficits, and raise cortisol levels.

Consistent with the findings of D'Souza et al., a recent investigation by Ranganathan et al. (2009) showed a relation between moderate THC levels and cortisol release. The subjects were healthy nonusers and frequent CUrs. The researchers examined cortisol levels after

administering Delta-9-THC, the active ingredient in cannabis. The results indicated that at certain socially relevant doses, Delta-9-THC raised plasma cortisol levels in a dose-dependent curve.

Nonexperimental Studies of the Relation Between CU and Cortisol

Although the results of experimental studies are consistent in showing that cannabis leads to an acute increase in cortisol secretion, it appears that reduced cortisol levels are linked with CU in the general population. For example, Huizink and colleagues (2006) studied early (ages 9-12) and late (ages 13-14) onset CUrs. They found that the CUrs who began using at an earlier age had lower cortisol levels 30 minutes after waking up than those who started using later in pre-adolescence. However, when compared to non-users, the early and late onset CUrs had higher levels of cortisol at 8 p.m (Huizink, Ferdinand, Ormel, & Verhulst, 2006). These findings raise interesting questions about the relation of cortisol with cannabis. Did the lower waking cortisol levels precede the early CU, or is lower waking cortisol a consequence of longer term use of cannabis? Further, do the cannabis using boys have higher evening cortisol due to CU during the day? The results of another study shed light on the former question.

Evidence that lower cortisol levels precede CU is provided by a study that examined cortisol and CU in biological sons of fathers with SUD (Moss, Vanyukov, Yao, & Kirillova, 1999). They found that sons of fathers with SUDs manifested a decreased salivary cortisol response to an anticipated stressor. Further, sons of fathers with a history of SUD had lower anticipatory stress cortisol levels compared with sons of fathers without SUDs. Finally, addressing the issue of causality, lower preadolescent cortisol responses were associated with cigarette smoking and regular marijuana use during adolescence. Thus, it appears that males with lower cortisol levels are more likely to use cannabis.

In summary, although limited, the extant research findings point to three general conclusions 1) CU results in an increase in cortisol secretion, as well as various psychological and cognitive abnormalities, in both psychotic patients and healthy individuals, 2) lower cortisol levels, particularly waking cortisol, are associated with current CU and 3) lower cortisol levels are linked with an increased likelihood of subsequent CU. Thus, for some individuals, lower cortisol secretion may predispose to CU, which in turn, acutely raises cortisol levels.

Nonetheless, the extant data do not address the issue of individual differences. In other words, we do not know whether there are individual differences among people in both the determinants of CU and the biological and psychological consequences. However, one recent study suggests that there are probably such differences. In a longitudinal study of a representative birth cohort followed to adulthood, Caspi and colleagues (Caspi et. al., 2005) found that a functional polymorphism in the catechol-O-methyltransferase (COMT) gene moderated the influence of adolescent CU on developing adult psychosis. Carriers of the COMT valine158 allele were most likely to exhibit psychotic symptoms and to develop schizophreniform disorder if they used cannabis. CU had no such adverse influence on individuals with two copies of the methionine allele. These findings demonstrate a gene-environment interaction and suggest that there are genetic factors that determine the vulnerability to adverse effects of CU.

Cortisol Effects on Brain, Behavior, and Psychosis

It is well established that exposure to stress can worsen psychotic symptoms and hasten the onset of relapse in patients with schizophrenia (Goodyer, Park, Netherton, & Herbert, 2001). Recent theories have proposed that this effect is mediated, in part, by the release of cortisol. Cortisol is produced in reaction to stressful events, and sustained high levels of cortisol have

been shown to have adverse effects on brain structure and function, especially the hippocampus (Goodyer et al., 2001; Walker, Mittal, & Tessner, 2008). Because persistent high cortisol levels can impair brain function, it also has the potential to increase risk for cognitive deficits and psychopathology (Goodyer et al., 2001).

Elevated cortisol levels have been implicated in many psychiatric disorders, such as depression, anxiety disorders, as well as schizophrenia (Arborelius, Owens, Plotsky, & Nemeroff, 1999). Walker et al.'s review (2008) of HPA axis function in schizophrenia and other psychotic disorders emphasized several key trends in the literature. Specifically, schizophrenia and other psychotic disorders are associated with: (1) heightened baseline hypothalamic-pituitary-adrenal axis (HPA) activity, as indexed by cortisol release; (2) decreased cortisol release in response to antipsychotic drugs; and (3) reduced hippocampal volume. In fact, numerous studies have demonstrated that patients with schizophrenia have smaller hippocampi, and it is the most highly replicated brain abnormality in schizophrenia (Nelson, Saykin, Flashman, & Riordan, 1998). Smaller hippocampus volumes have also been linked to elevated basal cortisol levels (Huang, Lui, Chang, Lu, Wang, & Chang, 2009). Thus, the smaller hippocampal volumes in schizophrenia patients could be due to, or contribute to, elevated cortisol. Finally, Walker et al. (2008) reviews the extensive literature, which shows that there is a normal maturational increase in cortisol release during the course of adolescence. This increase appears to begin after the onset of puberty and extends through early adulthood. Thus, it occurs during the same period when the functional decline and subtle symptoms that precede psychotic disorders typically emerge.

Walker et al. (2008) also review experimental research demonstrating that cortisol release can increase dopamine activity in the brain. This is highly relevant to psychotic disorders, as

increased dopamine activity is hypothesized to be a component of the neuropatholgical process in psychosis (Volkow, 2009). It has been suggested that stress exposure and cortisol release can trigger the mesolimbic dopamine system, thereby increasing psychotic symptoms, and may also stimulate the desire for drugs in some individuals (Chambers et al., 2001; Walker et al., 2008).

Only a few studies to date have examined cortisol secretion in individuals at-risk for psychosis to determine whether it is associated with conversion to Axis I psychotic disorder. One research group conducted a study in which they administered the dexamethasone corticotrophin releasing hormone (DEX/CRH) test to 12 participants with prodromal symptoms (mean age was 19.4 years (SD = 3.6 years; range = 15–25)) at baseline, and 3 of the 12 developed psychosis within two years (Thompson et al., 2007). Due to the small sample size, statistical analyses were not conducted, but the authors reported that participants who did not develop psychosis showed a trend toward higher plasma cortisol levels in response to DEX/CRH at the latter stages of the test, when compared to the three participants who did develop psychosis. However, this study is limited by the small sample size, the absence of longitudinal data on cortisol secretion, and other methodological factors.

A more recent investigation directly measured cortisol in 56 adolescents who met prodromal criteria for psychosis (Walker, Brennan, Esterberg, Brasfield, Pearce, & Compton, In press). Of these, 14 subsequently met DSM-IV criteria for an Axis I psychotic disorder (schizophrenia, schizoaffective, or mood disorder with psychotic features). Participants were assessed at baseline, and then followed longitudinally. Salivary cortisol was sampled multiple times at initial assessment, interim follow-up, and 1-year follow-up. Area under the curve (AUC) was computed from the repeated cortisol measures. The findings indicate that at-risk subjects who subsequently developed psychosis showed significantly higher cortisol at the first

follow-up, a trend at the one-year follow-up, and a significantly larger AUC, when compared to those who did not convert. When analyses were conducted excluding those who may have converted prior to the 1-year follow-up, a similar pattern of group differences was observed. These findings converge with previous reports on HPA activity in psychosis, as well as theoretical assumptions concerning the effects of cortisol elevations on brain systems involved in psychotic symptoms.

Goals of the Present Study

Rates of SU, especially cannabis and alcohol, are higher in psychotic patients than in healthy individuals, and there is evidence that cannabis and AU are linked with poor prognosis. In the case of cannabis, there is a potential neural mechanism for this in that CU increases cortisol release. We know, based on the results of experimental studies, that cannabis can increase cortisol secretion. Further, we know that elevated cortisol is associated with risk for psychosis, heightened dopamine activity, and greater severity of psychotic symptoms. It has been hypothesized that increased cortisol secretion may mediate the relation between CU and conversion to psychosis in at-risk subjects (D'Souza, 2004; D'Souza, 2005; D'Souza, 2008; Ranganathan et al., 2009). Thus, it may enhance HPA axis sensitivity and increase dopamine activity.

To date, there are no studies that have tested the relationship between CU and cortisol levels in individuals at-risk for psychosis. The current study will address this question. Specifically, based on research findings as well as theoretical models, it is predicted that prodromal subjects who report CU will show elevated cortisol levels, as well as more severe positive symptoms. Thus, it is predicted that prodromal individuals will be more likely to show cortisol elevations in relation to CU. Further, it is predicted that cortisol elevations will mediate

the relation of CU with symptom severity and progression. It is also hypothesized that at-risk participants who are using drugs will be at higher risk of converting to Axis I psychosis. The relation of AU with cortisol and symptoms will also be examined. This is important, because CU and AU often co-occur. However, there is no empirical or theoretical basis for predicting a relation of AU with cortisol or symptom progression.

This research will contribute to our knowledge by testing hypotheses about the relation of cannabis with symptoms in patients who have not yet converted to psychosis, but rather, are still in the prodromal phase. Because alcohol and cannabis are the most commonly used drugs by psychotic patients, they are the focus of the present study. If it is found that CU is associated with both elevated cortisol and greater symptom severity and conversion to psychosis, then early intervention to prevent substance use in at-risk populations may prevent conversion to psychosis. This would certainly constitute a major advance in public health policy with regard to serious mental illness.

Method

Participants

Participants were recruited from the Atlanta area for a prospective study of risk for mental illness conducted at Emory University. Recruitment focused on youth with subclinical signs of risk for psychosis. Announcements that described prodromal symptoms in lay terminology were directed at parents and clinical practitioners.

This study sample is 33 healthy adolescent controls, 56 at-risk adolescents, and 40 adolescents with Axis II disorders (other than Schizotypal Personality Disorder) or conduct disorder. Healthy adolescent participants were screened to confirm the absence of any Axis I or II disorder. Participants ranged in age from 12 to 18 years. Data on salivary cortisol were

obtained at baseline and one year later at follow-up, and psychiatric outcome data were also obtained at these times. Assent and written consent was obtained from all participants and a parent, in accordance with guidelines of the Emory University Human Subjects Review Committee.

Subjects were designated as 'at-risk' if they met DSM-IV diagnostic criteria for Schizotypal Personality Disorder (SPD) (n=5), the Scale of Prodromal Symptoms (SOPS) criteria for Attenuated Positive symptom (APS) syndrome (n=17) (Miller et al., 2003), or both risk criteria (n=34). Exclusion criteria at baseline were a current Axis I disorder, mental retardation, substance addiction (DSM–IV criteria for a substance disorder), and neurological disorder.

Demographic characteristics are presented in Table 1. The sample was young. The mean ages were 14.09 years for the healthy controls, 14.11 years for the prodromal subjects, and 14.67 years for the psychiatric controls. There were no significant age differences among the groups. There were more males than females in the study. There were 16 healthy control males, 38 prodromal males, and 19 psychiatric control males for a total of 73 males in the study. There were 17 healthy control females, 18 prodromal females, and 21 psychiatric control females for a total of 56 females in the study.

There were multiple ethnic groups represented in the study. There were 46 African-Americans overall (14 healthy controls, 16 prodromal subjects, and 16 psychiatric controls). There were 76 Caucasians (19 healthy controls, 36 prodromal subjects, and 21 psychiatric controls). There were four Asian-Americans (3 prodromal subjects and 1 psychiatric control). There were three subjects who did not fit any ethnic category and were classified as other.

Information on substance use. Information on substance use was obtained for all participants at the baseline and each follow-up assessment using the Structured Interview of DSM-IV Personality Disorders (SIDP-IV) (Pfohl, Blum, and Zimmerman, 1997) and the Structured Clinical Interview for Axis I DSM-IV Disorders (SCID-I/P) (Steinberg, 1994). Additional supplementary information was obtained from parental reports on the Child Behavior Checklist (CBCL) and other screening interview measures (Achenbach, 1991). Thus, both self-report and informant report information was utilized. This is important, as participants are often hesitant to admit to self-report drug use.

Drug use information was collected retrospectively from subject files. Drug use information was obtained from the CBCL and SIDP-IV (Achenbach, 1991; Pfohl, Blum, & Zimmerman, 1997). Drug use was classified on the following scale: (0) no use, (1) occasional use, which was classified as once a month or less, (2) repeated use with problems, and (3) abuse/dependence. These categories are based on those utilized to classify substance use in the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association [*DSM-IV-TR*], 2000) and other diagnostic systems. Participants were given a separate score for alcohol, marijuana, amphetamines, opioids, hallucinogens, cigarettes, and other drugs that did not fit into any of these categories. Scores were given for drug use at the initial assessment and subsequent follow-ups. For each assessment, including baseline and annual follow-ups, participant records were checked for any evidence, from direct report or parent report, pertaining to substance use. Because the frequency of substance use other than cannabis and alcohol was so rare, these were combined into the "other" category and were not examined in the present study. Additionally, follow-up drug use frequencies were aggregated by combining drug use at all

follow-ups. In total, information on substance use was obtained in the records of 33 healthy controls, 56 prodromal subjects, and 40 subjects with other psychiatric disorders.

The rates of use for each substance by diagnostic group are presented in Table 2. As illustrated by Table 2, most subjects were classified as having no substance use at baseline, but there was an increase in drug use at follow-up. Because the rates of any alcohol and/or cannabis use were low, for further analyses all categories of use; namely, (1) occasional use, (2) repeated use with problems, and (3) abuse/dependence, were combined into one category.

Procedures

Diagnostic assessments were conducted at initial assessment and yearly follow-ups. The SCID-I/P (Steinberg, 1994) and the SIDP-IV (Pfohl et al., 1997) were administered to diagnose Axis I and II disorders, respectively. The SCID was administered during the initial evaluation and subsequent annual follow-up assessments for a four-year period.

The Structured Interview for Prodromal Symptoms (SIPS) was administered at initial assessment and annual follow-ups to measure prodromal symptoms (Miller et al., 2003). The SIPS contains the SOPS, which rates the severity of relevant symptoms with the following scale; absent (0), questionably present (1), mild (2), moderate (3), moderately severe (4), severe but not psychotic (5), and severe (6). The SOPS is comprised of four symptom domains that are classified as positive (e g., unusual thoughts or ideas, suspiciousness, perceptual abnormalities, disorganized communication); negative (e g., social isolation, avolition, decreased expression of emotion, decreased ideational richness, deteriorated role function); disorganized (eg., odd behavior, bizarre thinking, trouble with focus and attention); and general (sleep disturbance, dysphoric mood, and impaired stress tolerance). Following SIPS procedures, all subjects who were designated as prodromal received at least one rating of 3, 4, or 5 on a positive symptom,

and thus met the symptom severity criteria for the SOPS "attenuated positive symptom (APS) syndrome." However, the onset/duration criteria (i e., onset or 1-point worsening within past 12 months) could not be established for all participants.

Interviews were conducted by either a licensed clinical psychologist or an advanced doctoral candidate. Training of interviewers was conducted over a 2-month period, and interrater reliabilities for symptoms ratings exceeded the minimum criterion of .80 (Pearson correlation) and for diagnostic status mean Kappa was .85. All interviews were videotaped throughout the course of the study so that interrater reliability could be monitored. Videotapes were reviewed by a clinical psychologist and/or collaborating psychiatrist to confirm diagnostic reliability.

Salivary Cortisol collection and assay. Subjects and their parent/guardian were provided with written and verbal dietary instructions to observe the evening before and the morning of sampling. Instructions allowed a light breakfast, but instructed participants to refrain from caffeine, alcohol, dairy products, and nonprescription medications, as well as brushing teeth within 30 minutes prior to sampling. Subjects were questioned to confirm their compliance with the instructions.

Saliva samples for cortisol assay were obtained three times, on the hour, beginning at approximately 9:00am at each of two assessments; baseline and 12-14 month follow-up. Time of day for sampling is based on evidence that, when compared to afternoon and evening values, morning values are more consistent/reliable, and their variance reflects a higher proportion (60%) of trait as opposed to state variance. This is assumed to reflect the cumulative effects of situational factors (e g., diet, exercise, and daily events) on variance in cortisol measured later in

the day. Further, it should be noted that multiple saliva samples (n=3) were obtained so an average could be derived, as this increases the reliability of the cortisol estimate.

Saliva was stored in a -20°C freezer. In preparation for assay, samples were rapidly thawed and centrifuged at 300*g* for 10 minutes to remove coagulated protein and other insoluble material. Cortisol was assayed in duplicate 200 μL aliquots of the clear supernatant, using materials and procedures provided by Incstar Corporation (Stillwater, Minnesota). The assay was performed in tubes coated with an antiserum that shows significant cross-reactivity only with prednisone (83%), 11-deoxycortisol (6.4%), cortisone (3.6%), and corticosterone (2.3%). Standards in the range 1 to 30 ng/mL consisted of the serum standards provided with the kit materials diluted with 200 μL of phosphate buffered saline. Protein concentrations were equalized in standards and samples by adding cortisol-free serum to the samples. The mean coefficients of variation between duplicates and between assays were less than 5%. Compared with the serum standards, the mean recovery of cortisol from saliva has been indistinguishable from 100%. Using this method, the range (central 95%) of salivary cortisol concentrations in normal adults has been determined as 1.8 to 10.1 ng/mL. A more detailed description of methods for salivary collection and radioimmunoassay (RIA) of cortisol can be found in Mittal, Dhruva, Tessner, Walder, and Walker (2007). The current analyses used an average of the three cortisol samples obtained at each visit, which were collected in the morning, before lunch.

Results

Cannabis Use

Diagnostic Group Differences in Frequency of Use. Chi square analyses were conducted to determine whether there were diagnostic group differences in the frequency of <u>any</u> CU (i.e. baseline or follow-up). This analysis yielded a statistically significant diagnostic group

difference (x^2 (2) =13.426, p=.001). As shown in Table 2, the rate of any CU was higher in the other psychiatric disorders group than the other two diagnostic groups.

A separate chi-square analysis was conducted to determine whether there were diagnostic group differences in CU at baseline. The results showed a significant difference, with the other psychiatric disorders group showing more use than the healthy and prodromal groups (x^2(2) =10.41, p=.005). Follow-up rates of CU were also higher in the other psychiatric disorders group than in the other two diagnostic groups (x^2 (2) =12.399, p=.002) (See Table 2).

The Relation of CU with Cortisol. Based on research findings, as well as theoretical models, it was predicted that prodromal subjects who report CU will show elevated cortisol levels. Mean cortisol levels by CU at baseline and follow-up are presented in Table 3, Figure 1, and Figure 2.

In the first analysis, baseline cortisol levels in users and nonusers of cannabis were compared, combining all diagnostic groups. Two way tests (p-values) were used for all analyses. A one-way ANOVA was performed with <u>any</u> CU (baseline or follow-up) as the independent variable and cortisol levels at baseline as the dependent variable. There was no significant difference in baseline cortisol levels as a function of <u>any</u> CU (F(1, 100) =.852, p=.35). Similarly, for CU at baseline (F(1, 128) =.006, p=.94) and CU at follow-up (F(1, 96) =1.61, p=.21) there were no differences between users and nonusers in baseline cortisol. Thus, when diagnostic groups are combined, there is no evidence of differences in baseline cortisol levels as a function of current or future CU.

The same analyses were conducted using follow-up cortisol levels as the dependent variable. Using <u>any</u> CU as the independent variable, the ANOVA revealed a trend toward higher follow-up cortisol in those who <u>did not</u> use cannabis (F(1, 89) =3.37, p=.07) (See Figure 3). The

same trend was found when comparing baseline cannabis users to nonusers on follow-up cortisol levels ($F(1, 89) = 3.39$, $p = .07$), and when comparing follow-up users and nonusers ($F(1, 89) = 2.59$, $p = .11$) (See Figure 4). Thus, there was a trend toward an association between lower cortisol and use of cannabis.

In order to examine diagnostic group differences in the relation of cortisol with CU, as well as changes over time in cortisol in relation to CU and diagnostic group, a repeated measures ANOVA was performed. It should be noted that the repeated measures analysis included only those subjects who had cortisol data at both baseline and follow-up. Further, because the rate of CU was so low in the healthy control group, only the prodromal and personality disorders groups were included in these analyses.

In the first analysis, diagnostic group and any CU (baseline and/or follow-up) were the between-subjects independent variables, and time (baseline and follow-up cortisol) was the within-subjects variable. This analysis revealed no significant main effect of diagnosis, time or CU. However, there was a significant interaction between CU and time ($F(1, 62) = 5.97$, $p = .02$) (See Table 4) As illustrated in Figure 3, for both diagnostic groups, there was a trend for cortisol to increase over time for nonusers, but decrease over time for users. Posthoc tests were then conducted to compare baseline cortisol to follow-up cortisol in users and nonusers across diagnostic groups. The results showed no significant change in cortisol over time in the users of cannabis ($t(17) = 1.31$, $p = .21$), but a significant increase in cortisol over time in the nonusers ($t(48) = 2.11$, $p = .04$, two-tailed). Thus, the nonusers showed the normative rise in cortisol that has been observed in other longitudinal studies of cortisol during adolescence (Walker & Bollini, 2002). When the users and nonusers of cannabis were compared, there was not a significant difference in baseline cortisol ($t(64) = -.78$, $p = .45$, two-tailed), but follow-up cortisol levels were

different, with the users showing lower cortisol levels ($t(64) = 2.28$, $p = .03$, two-tailed). Thus, contrary to prediction, CU was linked with a decline, rather than an increase, in cortisol over time. These findings raise the question of whether cannabis is altering the trajectory of cortisol changes over time, with users showing a significant decline.

In order to explore this question, a repeated measures ANOVA of cortisol levels was conducted using baseline CU, diagnostic group, and time as the independent variables. This analysis revealed no significant main effects of diagnostic group, CU, or time. Interactions were also nonsignificant, although there was a trend toward a significant time x CU interaction ($F(1, 63) = 2.41$, $p = .13$). Again, the trend was toward decreased cortisol over time among baseline cannabis users, and an increase in nonusers. Thus, the results indicate that baseline CU is only marginally associated with a decline in cortisol over time.

Finally, the same analysis was conducted with a repeated measures ANOVA using follow-up CU, diagnostic group, and time as the independent variables. This analysis revealed no significant main effects of diagnostic group, CU, or time. There was a statistically significant interaction between time and CU ($F(1, 63) = 7.88$, $p = .007$), and a trend toward a significant time x diagnostic group interaction ($F(1, 63) = 2.95$, $p = .09$). The results for the combined diagnostic groups are illustrated in Figure 4.

Posthoc tests were conducted to explore the determinants of the significant two-way interaction between time and follow-up CU. In within subject analyses, baseline cortisol was compared to follow-up cortisol in users and nonusers across diagnostic groups. In this case, the results showed no significant change in cortisol over time in the users of cannabis ($t(16) = 1.48$, $p = .16$), but a significant increase in cortisol over time in the nonusers ($t(49) = 2.18$, $p = .03$, two-tailed). This parallels the results of the analysis of any CU, in that the nonusers of cannabis at

follow-up showed the normative rise in cortisol that has been observed in other longitudinal studies of cortisol during adolescence. When the users and nonusers of cannabis at follow-up were compared, there was not a significant difference in baseline cortisol ($t(64) = -.1.30$, $p = .20$, two-tailed), although follow-up cortisol levels showed a trend toward a difference, with the users showing lower cortisol levels ($t(64) = 1.93$, $p = .06$, two-tailed).

Because the above analyses did not differentiate between continuous CU versus CU at follow-up only, additional analyses were conducted to examine the relation of CU onset with cortisol. In order to explore the relation of CU with cortisol as a function of CU onset, subjects were divided into three groups; those with CU at follow-up but not baseline, subjects with CU at follow-up and baseline, and those with no CU at any time point. Because there was only one participant who used CU at baseline, but not follow-up, this CU category was not included in the analysis. A repeated measures ANOVA with CU onset category as the between-subjects factor and time as the repeated measure was conducted on cortisol values. The results yielded no significant main effects, but a marginally significant interaction of CU onset and time ($F(2, 85) = 2.48$, $p = .09$). This interaction is illustrated in Figure 5.

In order to explore the determinants of this marginally significant interaction, t-tests were conducted. The results revealed that the group with no CU manifested a significant increase in cortisol over time ($t(68) = -.2.46$, $p = .02$, two-tailed), whereas those who used only at follow-up or both baseline and follow-up showed no significant change in cortisol over time. However, as illustrated by Figure 5, both of the latter groups manifested a downward trend in cortisol levels, thus accounting for the marginally significant interaction. Also, between group comparisons revealed a significant difference between continuous CU (i.e. both baseline and follow-up) and

nonusers in cortisol level at follow-up ($t(75) = -.3.08$, $p < .01$, two-tailed). There were no significant differences in baseline cortisol among the groups.

The Relation of CU with Symptoms. The second hypothesis was that prodromal subjects who report CU will have more severe positive symptoms. First, ANOVAs were conducted combining the three diagnostic groups to compare levels of positive symptoms as a function of any, baseline and follow-up CU. These results yielded no significant differences, although the trends were all toward lower positive symptom scores in the users.

In order to examine diagnostic group differences in the relation of symptoms with CU, as well as changes over time in symptoms in relation to CU and diagnostic group, repeated measures ANOVA were performed. Again, it should be noted that the repeated measures analysis included only those subjects who had symptom data at both baseline and follow-up. Further, because the rate of CU was so low in the healthy control group, only the prodromal and personality disorders groups were included in these analyses.

In the first analysis, diagnostic group and <u>any</u> CU (baseline and follow-up) were the between subjects independent variables, and time (baseline and follow-up positive symptoms) was the within-subjects variable. As would be expected, there was a highly significant main effect of diagnostic group ($F(1, 67) = 53.03$, $p = .000$), with the prodromal group showing higher symptom scores (See Table 6). There were no other significant main effects or interactions.

When the repeated measures analysis was conducted using baseline CU and diagnostic group as the independent variables, the same pattern was observed; namely a significant main effect of diagnostic group ($F(1, 67) = 53.14$, $p = .000$), but no significant main effect of CU and no interaction of CU with diagnostic group or time.

The results were somewhat different, however, when follow-up CU was the independent variable in repeated measures ANOVA. Again, there were no significant main effects of CU or two-way interactions. In this case, there was a trend toward a significant three-way interaction of CU x time x diagnostic group ($F(1, 67) = 2.76$, $p = .10$). As shown in Figure 6, among the prodromal subjects, cannabis users showed a trend toward increased symptoms over time, whereas nonusers showed a decline. This was not apparent for the diagnostic group with other disorders (See Figure 7).

Cannabis use and conversion to psychosis. The final hypothesis was that at-risk participants who use cannabis will be at higher risk of converting to Axis I psychosis. Chi square analyses were performed to see if those using cannabis were more likely to convert to psychosis. Results for baseline and follow-up CU were not significant ($x^2(1) = .726$, $p = .394$ and $x^2(1) = .028$, $p = .866$) (See Table 7). Thus, contrary to prediction, there was no evidence of a relation between CU and conversion.

Alcohol Use

Diagnostic Group Differences in Frequency of Use. Chi square analysis of the frequencies of <u>any</u> AU (i.e. baseline or follow-up) by diagnostic group produced a statistically significant group difference ($x^2(2) = 7.421$, $p = .024$). Table 2 shows that the rate of AU was higher in the other psychiatric disorders group than the other two diagnostic groups. Baseline and follow-up rates of AU by diagnostic group showed the same trend, but did not reach statistical significance ($x^2(2) = 5.342$, $p = .069$ and $x^2(2) = 4.404$, $p = .111$ respectively) (See Figure 8 and Figure 9).

The Relation of AU with Cortisol. First one-way ANOVAs were conducted to test for relations between AU and cortisol across diagnostic groups. These analyses revealed no significant relation of baseline, follow-up, or any AU with cortisol.

Repeated measures ANOVAs were conducted on baseline and follow-up cortisol, with AU and diagnostic group as the between subjects factors, and time as the within subjects factor. Again there was no significant main effect of AU and no significant interactions (See Table 5, Figure 10, and Figure 11).

The Relation of AU with Symptoms. First, ANOVAs were conducted combining the three diagnostic groups to compare levels of positive symptom as a function of any, baseline and follow-up AU. These results yielded no significant differences, and no trends toward significance.

In order to examine diagnostic group differences in the relation of symptoms with AU, as well as changes over time in symptoms in relation to AU and diagnostic group, repeated measures ANOVA were performed. Again, it should be noted that the repeated measures analysis included only those subjects who had symptom data at both baseline and follow-up. Further, because the rate of AU was so low in the healthy control group, only the prodromal and personality disorders groups were included in these analyses.

The repeated measures ANOVAs revealed no significant main effect of AU and no significant interactions of AU with diagnostic group or time. Thus, AU appears to have no relation with symptoms.

Alcohol use and conversion to psychosis. A chi square analysis was conducted to see if participants who are using alcohol were more likely to convert to psychosis. Results at baseline and follow-up were not significant (x^2 (1) =.024, p=.878 and x^2 (1) =.830, p=.362) (See Table 7).

Discussion

Interpretation of Findings

The present study examined the relation of CU and AU with cortisol and symptoms in youth at-risk for psychosis as well as controls. Contrary to prediction, the results do not indicate that CU is associated with elevated cortisol in any of the diagnostic groups. In contrast, the present findings show longitudinal decreases in cortisol in subjects who use cannabis. Similarly, there is no relation of CU or AU with symptom severity or progression. In the discussion below, we consider the findings in light of past research on CU and AU.

Diagnostic group differences in CU. Diagnostic group differences in frequency of CU were significant. Consistent with previous reports, the rate of any CU was higher in the other psychiatric disorders group than in both the healthy controls and the prodromal subjects. The prodromal group fell between the other two groups. Diagnostic group differences were also found in CU at baseline and follow-up. Again, the other psychiatric disorders group had more use than the healthy controls and prodromal group. These findings are consistent with past research on adolescents with conduct disorder and other externalizing disorders. This research shows that adolescents with these disorders are more likely than age-matched controls to use illegal substances (Disney, Elkins, McGue, & Iacono, 1999). Thus, because the other psychiatric disorders group includes a large number of adolescents with these disorders, a higher rate of CU is expected in this group.

Relation of CU and cortisol. Across diagnostic groups, there were no significant differences in baseline cortisol levels as a function of overall, baseline, and follow-up CU. This is contrary to the hypothesis that cannabis users would have elevated cortisol levels. Moreover,

there was a trend toward higher follow-up cortisol levels in those who did not use cannabis and lower cortisol levels in those who did use cannabis.

The repeated measures analysis showed that youth who did not use cannabis displayed the normative increase in cortisol over time. However, this was not the case for those who were baseline or follow-up cannabis users. Thus, either lower cortisol increases the likelihood of CU, or CU lowers cortisol. In speculating on the plausibility of these interpretations, past research findings suggest several possibilities. First, as described above, experimental studies are consistent in showing that CU produces an acute increase in cortisol secretion that is associated with an acute increase in positive-like symptoms in both normal controls and schizophrenia patients. Thus, acute CU-induced cortisol increases may mediate the short-term effects of cortisol on symptoms.

On the other hand, nonexperimental studies indicate that low cortisol levels precipitate CU in the general population. Specifically, the Moss (2006) and Huizink (1999) studies also show that lower cortisol levels are linked with an increased likelihood of subsequent CU. Thus, lower cortisol levels appear to predate CU for some youth. The present findings do not provide significant support for the notion that lower cortisol levels predate CU. However, lower follow-up cortisol is linked with CU. It is, therefore, possible that those youth who did not show the normative age-related increase in cortisol during the period between baseline and follow-up were more likely to be drawn to CU during that period. Again, these findings are consistent with the Moss and Huizink finding that lower cortisol levels were associated with current CU. The present findings also suggest the absence of a longitudinal increase in cortisol levels for cannabis users. Thus, CU may also be linked with a dampening in the normative maturational rise in cortisol during adolescence.

Clearly, in the absence of controlled experimental studies, the causal mechanisms regarding the relation between CU and cortisol remains unclear. Nonetheless, taken together, the findings of the present and past research suggest that the relation between CU and cortisol is complex. As documented in the experimental studies, CU is associated with an acute rise in cortisol levels. However, in general population studies, future and current CU is linked with lower cortisol levels. Moreover, the present findings suggest that CU is linked with a dampening of the increase in cortisol typically observed in adolescence. There are several possible interpretations of the latter finding.

First, it is possible that persistent CU leads to a dampening of the HPA axis activity, and therefore, cortisol secretion in some youth. Thus, it may be that a subgroup of youth who experiment with CU find it pleasurable because it has a suppressive effect on their cortisol, and these are the youth who continue to use it because they are reinforced by its effects. This would be consistent with the notion of "self-medication," in that CU may reduce the increased levels of cortisol secretion that occur during adolescence. In contrast, individuals who experience an acute cortisol increase in response to CU may not try the drug again due to a negative experience with the drug. Their cannabis experimentation may go undetected and may not have been documented in the present or past studies of CU.

A second approach to interpreting the findings would be based on the assumption that the augmentation of cortisol induced by CU is actually reinforcing to some youth. Thus, for youth with low baseline levels of cortisol secretion, the acute increase in cortisol induced by CU may be appealing, and may sustain continued CU. This interpretation is consistent with past findings that lower cortisol precedes CU and that it is linked with subsequent lower cortisol levels.

Further, it suggests a "self-medication" process, in the sense that some individuals may seek CU to obtain an acute rise in cortisol.

Third, it may be that, for some, elevated basal cortisol levels cause the individual to try cannabis in order to calm themselves. Subsequently, over time, this prolonged CU may be reinforcing because it is lowering cortisol levels. The present data and past findings do not support this notion; however, in the absence of more detailed longitudinal studies involving repeated measurement of cortisol and CU, we cannot rule out the presence of such a subgroup.

It has been repeatedly demonstrated that elevated cortisol levels are associated with many psychiatric disorders, such as depression, and anxiety disorders, as well as schizophrenia (Arborelius, et al., 1999). Specifically, schizophrenia patients have an overactive HPA axis, which is evidenced by increased cortisol levels (Walker et al., 2008). When individuals with preexisting vulnerabilities to these disorders use cannabis, the results may be quite different from the effects observed in youth who are not at-risk. Obviously, it would not be ethical to expose at-risk youth to cannabis use for research purposes. However, future longitudinal studies are needed to track the developmental course of cortisol secretion in youth whose drug use is monitored repeatedly over time.

Cannabis use and symptoms. There was a significant diagnostic group difference of positive symptom scores. As expected, the prodromal group had the highest level of positive symptoms. This is because the diagnostic groups were defined on the basis of the number of positive symptoms.

Surprisingly, there was no significant relation between any CU and positive symptom levels at baseline, follow-up, or over time. However, there was a trend toward a significant three-way interaction of follow-up CU, time, and diagnostic group. Prodromal cannabis users

showed a trend toward increased symptoms over time, while nonusers showed a decline. This may indicate that as time passes, the CU increases positive symptoms. The fact that only the prodromals showed this trend may indicate that cannabis has a specific effect on people who are at-risk for psychotic disorders. This is consistent with earlier literature that demonstrates that CU is known to worsen psychotic symptoms. Studies by Knudsen et al. (1984), Dixon et al (1990) and Negrete et al. (1986) all show that cannabis users reported a worsening of symptoms and had more delusional and hallucinatory activity than nonusers. The sample size in the present study may have been too small to detect this effect, or the follow-up period may not have extended far enough into the risk period for psychosis onset.

Cannabis use and conversion to psychosis. Baseline and follow-up CU did not predict conversion to psychosis. These findings could be a result of sample size. Overall, only 16 subjects converted to a psychotic disorder by the end of the study. Disorders included schizophrenia, schizoaffective disorder, bipolar I with psychotic features, and major depressive disorder with psychotic features. While this conversion rate is consistent with previous reports on conversion rates for individuals who meet prodromal criteria, the subjects had not yet passed through the major risk period (ages 18 to 25) for conversion to psychosis at the most recent follow-up. Thus, more conversions would be anticipated in future follow-ups of the sample. It is possible that a relation between CU and conversion would be detected then.

Diagnostic group differences in AU. Diagnostic group differences in frequency of AU were also significant. As with CU, the rate of <u>any</u> AU in the other psychiatric disorders group was higher than both the healthy controls and the prodromal subjects. As noted above, the other psychiatric disorders group included adolescents who had conduct disorder and other

externalizing disorders, and adolescents with these disorders are more likely than age-matched controls to use illegal substances.

Relation of AU and cortisol. There were no significant differences in baseline cortisol levels as a function of overall, baseline, and follow-up AU. When the analyses of AU and cortisol were conducted over time, there was no significant main effect of diagnosis, time, or CU. Thus, there is no apparent effect of alcohol on cortisol levels. This finding is of interest because AU and CU often co-occur. This suggests the specificity of the relation of CU with cortisol.

Alcohol use and symptoms. Similarly to cannabis, there were no significant relations of AU with symptoms and no significant interactions of AU with diagnostic group or time. Thus, although it has been shown that AU is associated with a worsening of the course, symptoms, and outcome of patients with schizophrenia, it is not linked with the severity or course of prodromal symptoms (Cuffel and Chase, 1994; Drake, Osher, & Wallach, 1989; Drake, Mueser, Clark, & Wallach, 1996; D'Souza et al., 2006). It is also possible that AU shows no relation with prodromal symptoms because the individuals have not reached a level of symptom severity necessary to detect an effect of AU.

Alcohol use and conversion to psychosis. Baseline and follow-up AU did not predict conversion to psychosis. Again, these findings must be considered in light of the fact that the samples have not yet passed through the major developmental risk period for onset of psychosis.

Implications of the present findings

To date, there are no published reports on the relation of AU or CU with cortisol in subjects at-risk for pscyhosis. The findings indicate that there is no association of AU with cortisol or symptoms. In contrast, the present results show the normative longitudinal increases

in cortisol in subjects who do not use cannabis, but a trend toward a decline in users. These findings raise a variety of questions for psychosis research. In particular, it will be important to examine the possible determinants of individual differences in the relation of CU with cortisol levels. This may hold clues to the apparent ability of CU to induce or exacerbate psychosis in some individuals. If cannabis is found to be a true risk factor for psychosis in a subgroup of individuals, then individuals who are at-risk for psychosis can cease CU and possibly prevent conversion to full-blown psychosis.

Future research should delve into drug use in a more extensive way. Studies should be done specifically examining drug use patterns and changes in severity of use over time as a function of cortisol levels, symptom severity, and conversion.

Limitations

There are several limitations of the present study. First, although the number of prodromal subjects who converted to psychosis was similar to that reported in previous studies, it was nonetheless only 16 subjects. Further, the mean age of the sample is still at the lower end of the risk period for schizophrenia and other psychoses. Thus, more subjects are likely to develop psychotic disorders as time progresses. Ideally, in the future, the subjects from this study will be combined with the consortium of universities that are doing this type of research in order to obtain more statistical power and a larger sample. Future researchers should combine as many subject pools as possible to expand sample size and increase statistical power.

Next, with all longitudinal studies of at-risk populations, there was subject attrition. Thus, for most subjects, data were available for only the baseline and two follow-up assessments, although a subgroup had data from fourth and fifth follow-up assessments. In order to maximize sample size, data on AU and CU were combined across all of the follow-up periods. Because of

this, temporal condensing of follow-up data on CU and AU, combined with the declining numbers at each follow-up made it difficult to track the relation between CU and cortisol over the entire time period. Future studies with larger sample sizes are needed to more closely examine changes over time.

Additionally, in the data collection process, drug use was coded into no use, occasional use, repeated use with problems, and abuse/dependence. However, drug use was divided into use and no use for statistical analyses. This was done because the number of subjects in the latter two categories was too small for separate analysis. This facilitated data analyses, but reduced the ability to examine drug use severity in the sample population. Drug use severity may have changed as symptoms were worsening or as people were converting to psychosis. Future research can examine whether drug use severity has an effect on cortisol levels, positive symptom levels, and conversion.

Finally, the original focus of the larger study was not to examine drug use. In the present study, drug use was examined retrospectively by searching through patient files. If drug use was the main focus of the study, more detail could have been obtained about specific drug use patterns. For some subjects, it was hard to discern their exact drug use severity because the interviewers were not probing specifically for drug use information. They were asking about drug use as part of a larger clinical interview. On the other hand, as noted above, obtaining reliable data on illegal substance use is challenging in any study. In the absence of repeated urinary drug screens, there is no way to confirm the accuracy of subjects' reports about drug use. However, the use of drug screens often results in prospective subjects declining participation in the study. Moreover, even with urinary drug screens, only recent use can be determined.

Conclusions

In summary, the present study provided no evidence that CU or AU are linked with significant increases in prodromal symptoms or cortisol in youth. To the contrary, there is evidence that CU is linked with lower cortisol levels. These findings point to the likely complexity of the relation between CU and cortisol, and the possibility that cannabis has unique effects on youth at-risk for pscyhosis. More research is needed to replicate and extend the results.

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Table 1
Demographic and Clinical Characteristics of Sample

	Diagnostic Groups			
	Healthy Controls	Prodromal	Other Disorders	Total
Gender				
Male	16	38	19	73
Female	17	18	21	56
Baseline Age				
M (SD)	14.09 (1.974)	14.11 (1.670)	14.67 (1.789)	
Ethnicity				
African-American	14	16	16	46
Caucasian	19	36	21	76
Asian American	0	3	1	4
Other	0	1	2	3
Initial Drug Use				
Cannabis	1	4	10	15
Alcohol	3	5	9	17
Other	1	1	2	4
Follow-Up Drug Use				
Cannabis	3	4	13	20
Alcohol	9	10	16	35
Other	0	4	2	6

Table 2
Percentage of subjects using substances by diagnostic group

	Diagnostic Group			
	Healthy Controls	Prodromal	Other Disorders	Total
Overall Alcohol Use	36.0	28.6	58.8	40.6
Alcohol Use at Baseline	9.1	8.9	22.5	13.2
Alcohol Use at Follow-Up	36.0	25.0	51.6	36.5
Overall Cannabis Use	12.0	14.6	47.1	25.0
Cannabis Use at Baseline	3.0	7.1	25.0	11.6
Cannabis Use at Follow-Up	12.0	10.0	41.9	20.8

Table 3
Mean cortisol levels by CU at baseline and follow-up for all subjects

	Baseline Cannabis		Follow-Up Cannabis	
	Use	No Use	Use	No Use
Baseline Cortisol M (SD)	.46 (.21) (n=15)	.46 (.20) (n=114)	.51 (.23) (n=20)	.44 (.19) (n=76)
Cortisol Follow-Up M (SD)	.35 (.14) (n=10)	.57 (.37) (n=79)	.43 (.15) (n=19)	.55 (.34) (n=69)

Table 4
Mean cortisol levels by diagnostic group and use of ANY cannabis

	Prodromal Subjects		Other Psychiatric Disorders Subjects	
	Use	No Use	Use	No Use
Baseline Cortisol M (SD)	.49 (.29) (n=5)	.45 (.22) (n=32)	.48 (.24) (n=12)	.41 (.13) (n=17)
Lab Cortisol Follow-Up M (SD)	.30 (.12) (n=5)	.51 (.23) (n=32)	.45 (.15) (n=12)	.54 (.26) (n=17)

Table 5
Mean cortisol levels by diagnostic group use of ANY alcohol

	Prodromal Subjects		Other Psychiatric Disorders Subjects	
	Use	No Use	Use	No Use
Baseline Cortisol M (SD)	.47 (.27) (n=7)	.45 (.22) (n=30)	.47 (.18) (n=11)	.42 (.19) (n=18)
Lab Cortisol Follow-Up M (SD)	.43 (.36) (n=7)	.49 (.19) (n=30)	.47 (.24) (n=11)	.51 (.22) (n=18)

Table 6
Mean positive symptom levels by diagnostic group and any CU at baseline and follow-up

	Prodromal Subjects		Other Psychiatric Disorders Subjects	
	Use	No Use	Use	No Use
Baseline Positive Symptoms M (SD)	2.04 (.46) (n=5)	2.01 (.85) (n=35)	.46 (.39) (n=13)	.47 (.41) (n=18)
Follow-Up Positive Symptoms M (SD)	2.04 (1.18) (n=5)	1.67 (1.06) (n=35)	.49 (.57) (n=13)	.62 (.66) (n=18)

Table 7
Frequency of drug use and conversion status

	Use		No Use	
	Converted	Not Converted	Converted	Not Converted
Baseline Cannabis Use	3	13	13	102
Follow-Up Cannabis Use	3	18	12	64
Baseline Alcohol Use	2	16	14	99
Follow-Up Alcohol Use	4	32	11	50

Figure Captions

Figure 1. All subjects mean cortisol levels (SD) by baseline CU and diagnostic group over time.

Figure 2. All subjects mean cortisol levels (SD) by follow-up CU and diagnostic group over time.

Figure 3. Mean cortisol levels (SE) for prodromal and psychiatric control participants over time as a function of any CU.

Figure 4. Mean cortisol levels (SE) for prodromal and psychiatric control participants over time as a function of follow-up CU.

Figure 5. Longitudinal trends in mean cortisol levels (SE) for prodromal and psychiatric control participants as a function of CU.

Figure 6. Mean (SE) positive symptom scores over time as a function of follow-up CU in prodromal subjects.

Figure 7. Mean (SE) positive symptom scores over time as a function of follow-up CU in psychiatric controls.

Figure 8. All subjects mean cortisol levels (SD) by baseline AU and diagnostic group over time.

Figure 9. All subjects mean cortisol levels (SD) by follow-up AU and diagnostic group over time.

Figure 10. Mean cortisol levels (SE) over time as a function of any AU in prodromal subjects.

Figure 11. Mean cortisol levels (SE) over time as a function of any AU in psychiatric controls.

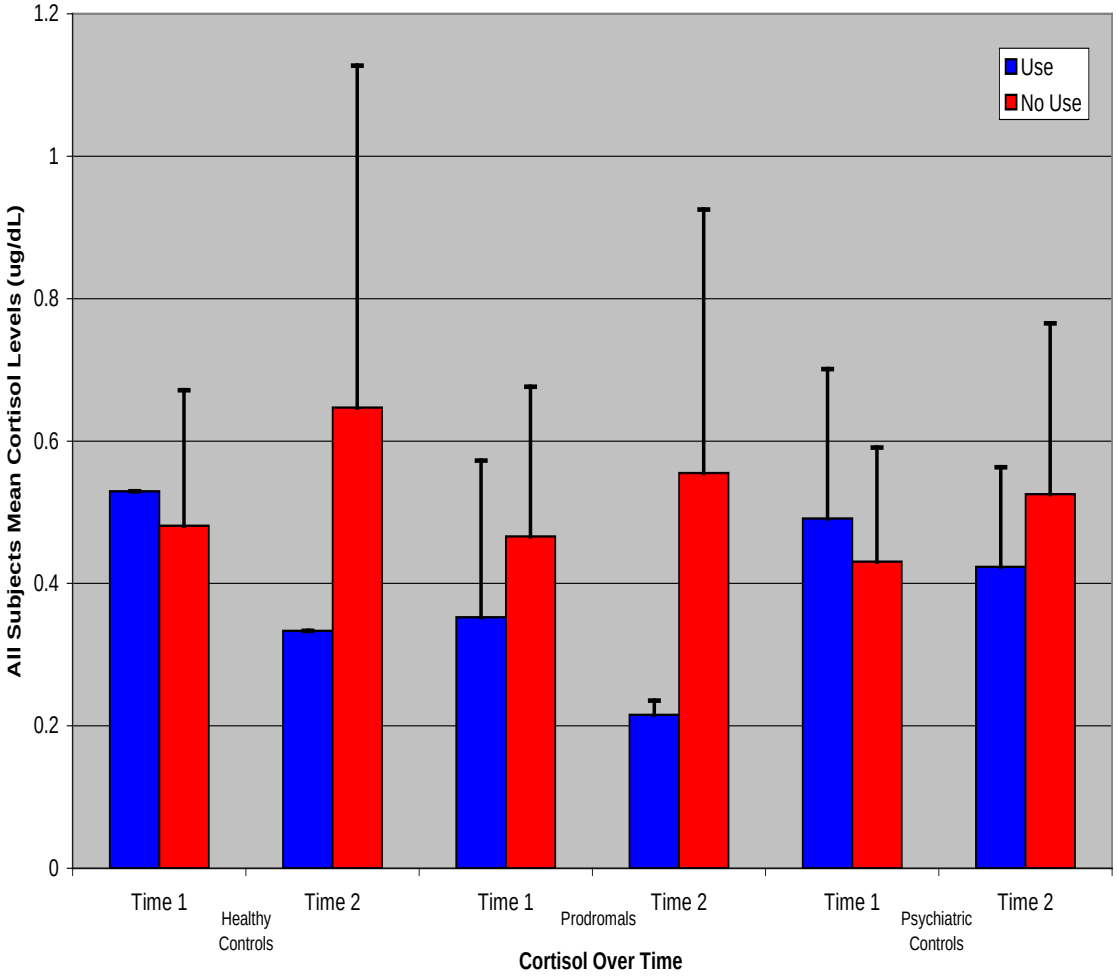

Figure 1. All subjects mean cortisol levels (SD) by baseline CU and diagnostic group over time.

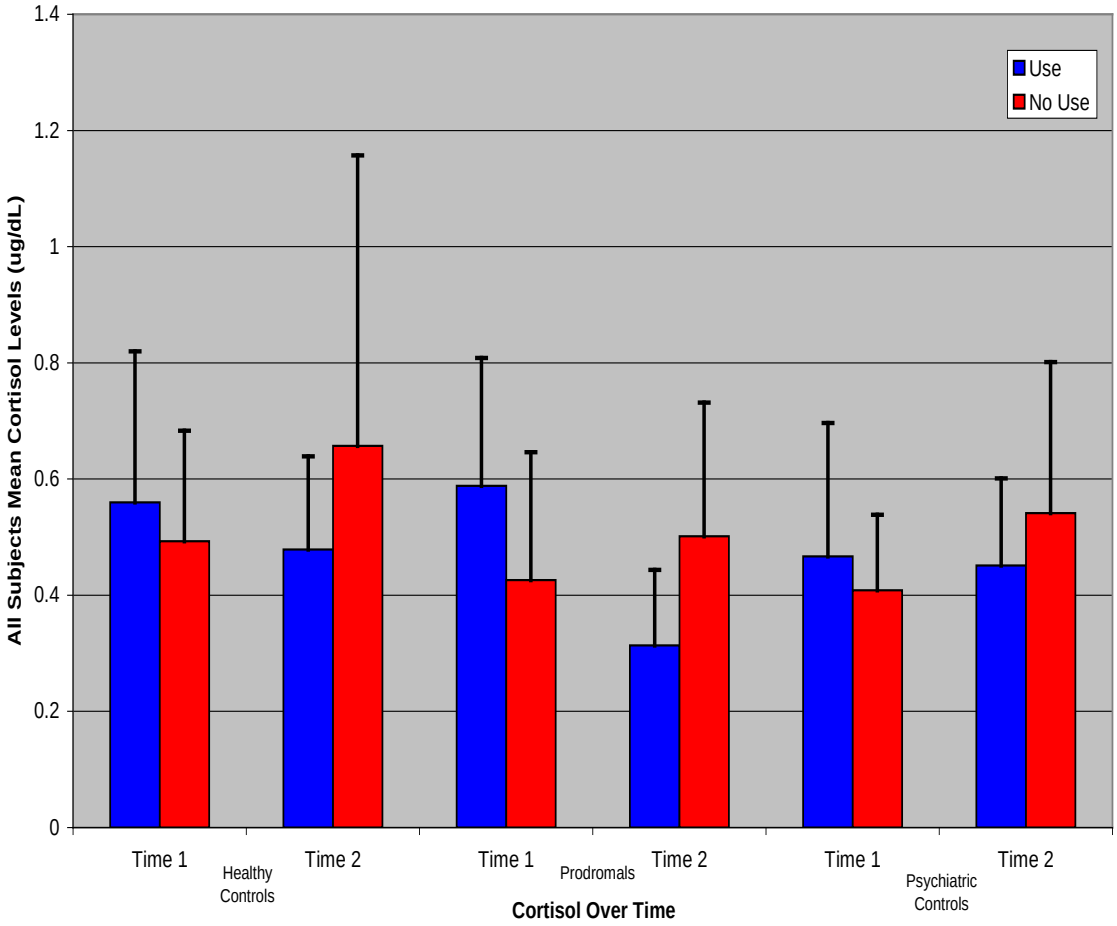

Figure 2. All subjects mean cortisol levels (SD) by follow-up CU and diagnostic group over time.

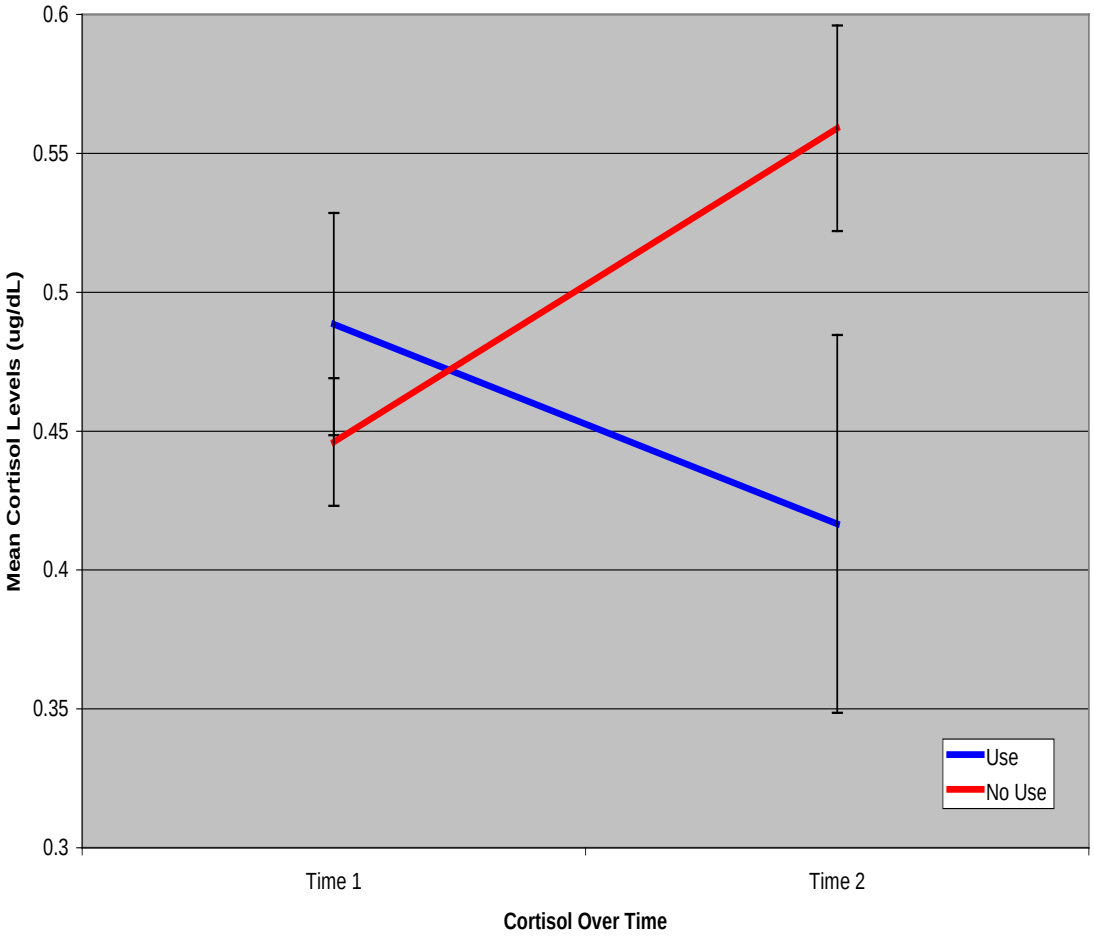

Figure 3. Mean cortisol levels (SE) for prodromal and psychiatric control participants over time as a function of any CU.

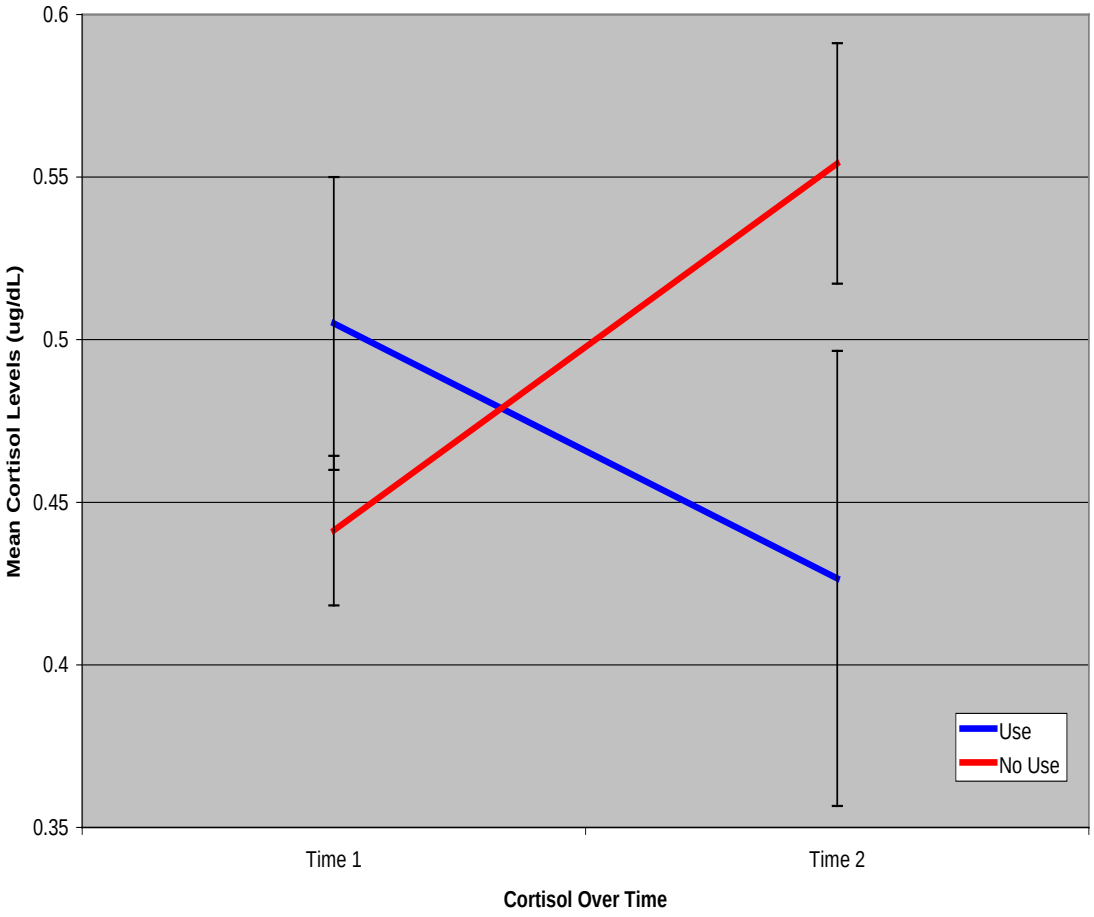

Figure 4. Mean cortisol levels (SE) for prodromal and psychiatric control participants over time as a function of follow-up CU.

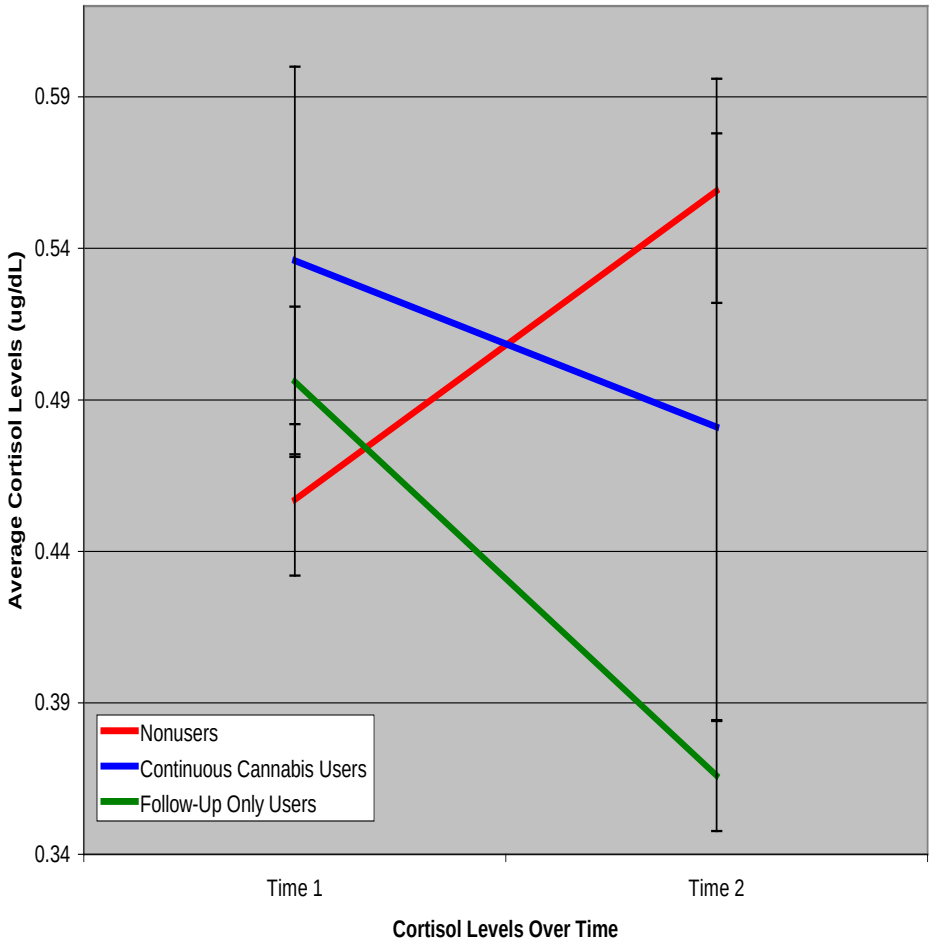

Figure 5. Longitudinal trends in mean cortisol levels (SE) for prodromal and psychiatric control participants as a function of CU.

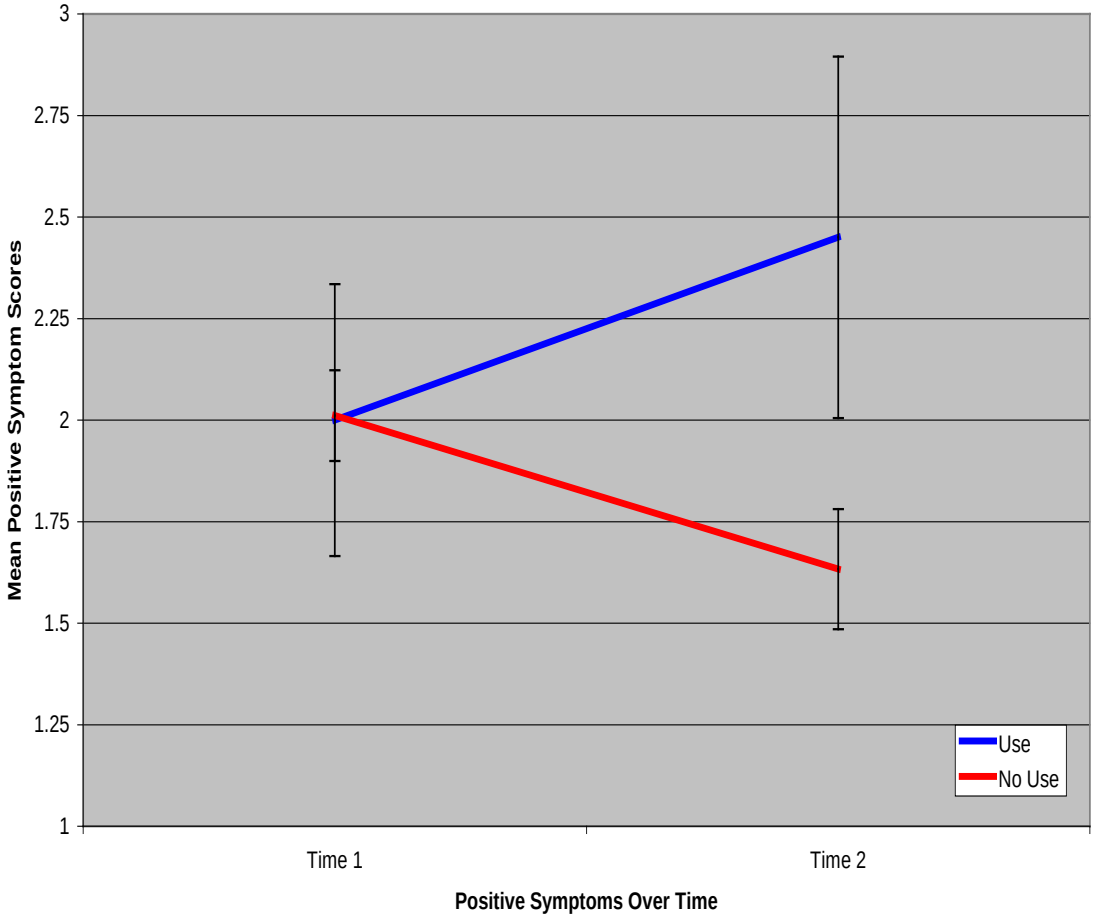

Figure 6. Mean (SE) positive symptom scores over time as a function of follow-up CU in prodromal subjects.

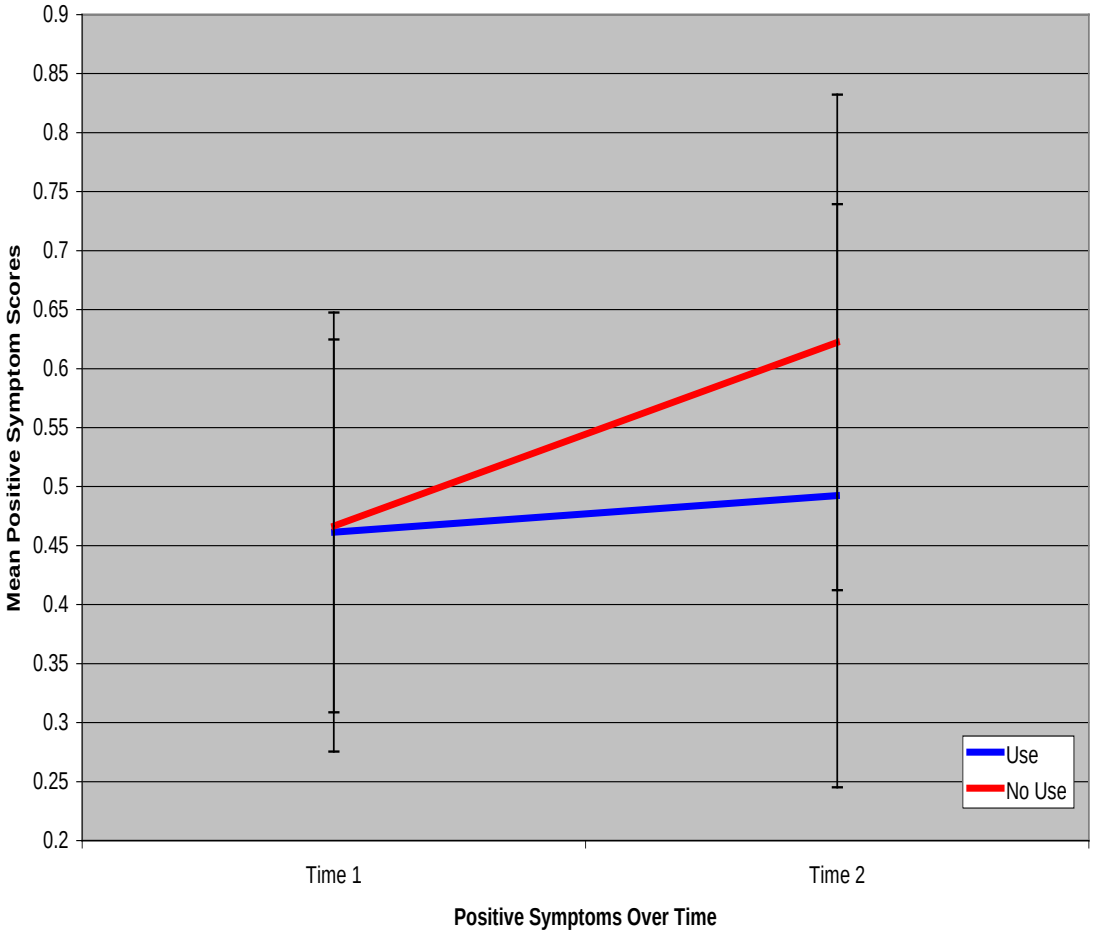

Figure 7. Mean (SE) positive symptom scores over time as a function of follow-up CU in psychiatric controls.

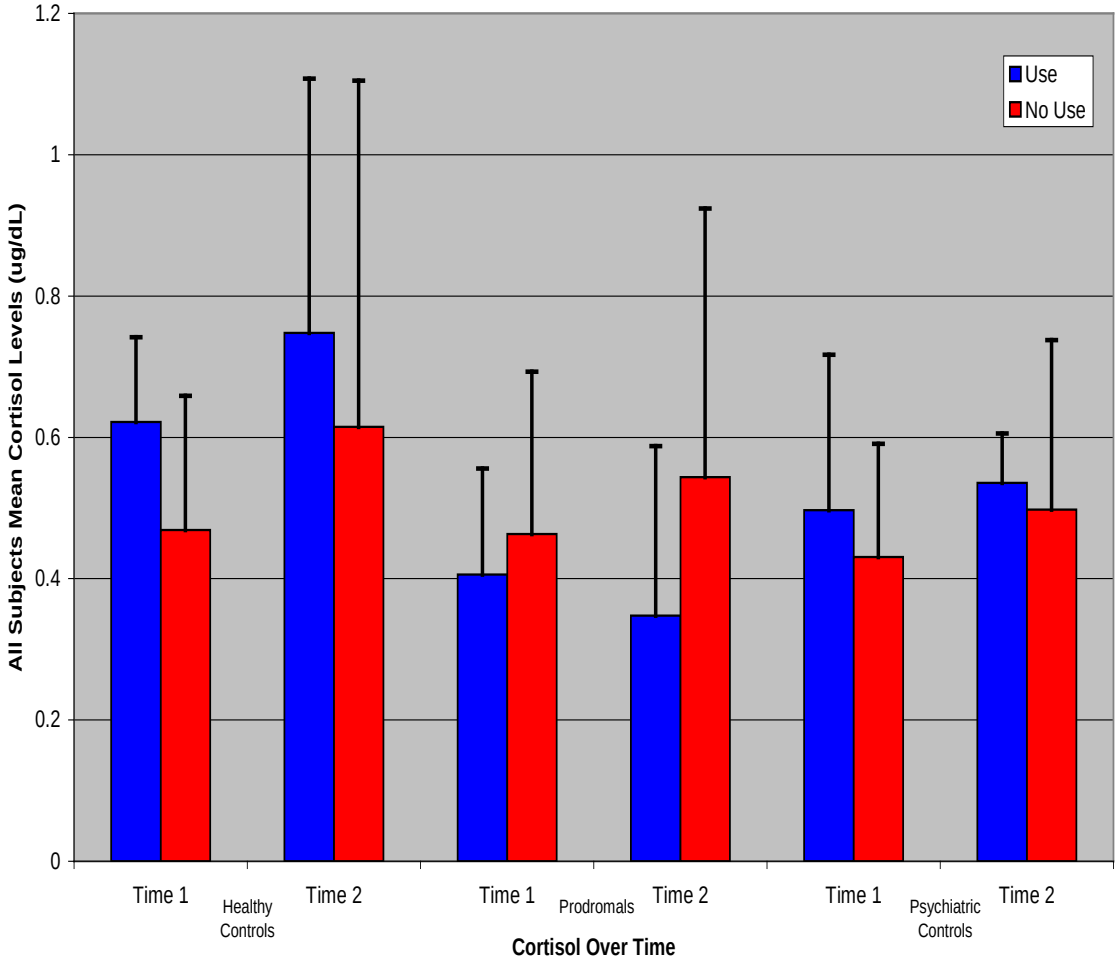

Figure 8. All subjects mean cortisol levels (SD) by baseline AU and diagnostic group over time.

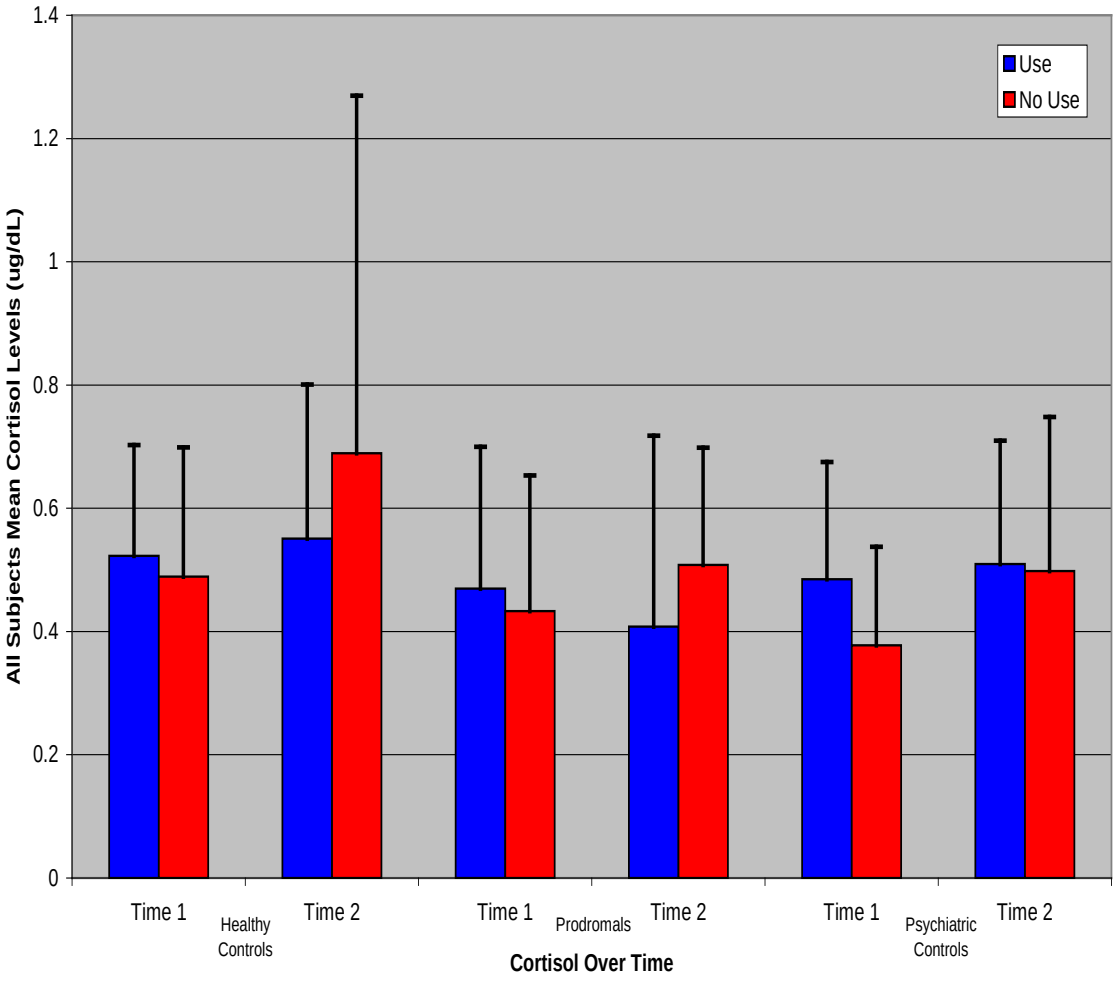

Figure 9. All subjects mean cortisol levels (SD) by follow-up AU and diagnostic group over time.

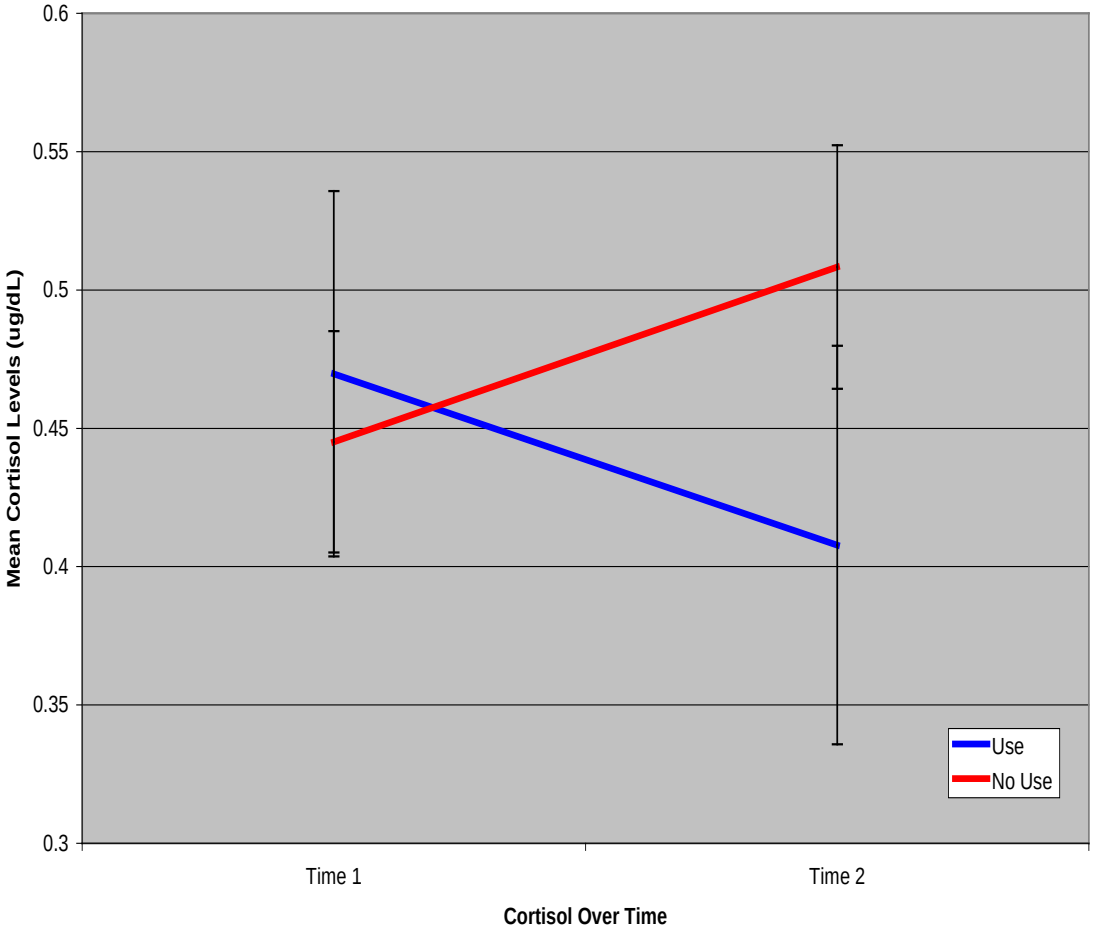

Figure 10. Mean cortisol levels (SE) over time as a function of any AU in prodromal subjects.

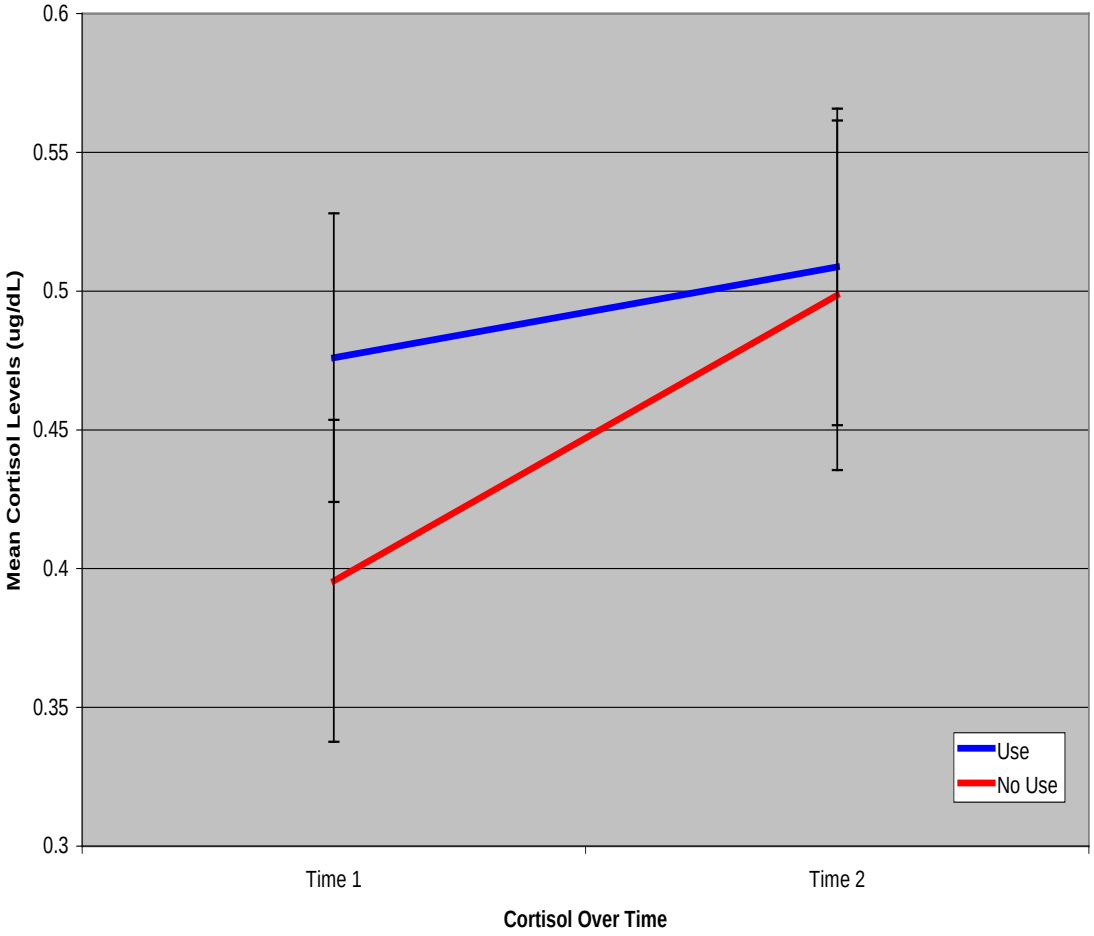

Figure 11. Mean cortisol levels (SE) over time as a function of any AU in psychiatric controls.