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The startle reflex in schizophrenia: habituation and personality correlates

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Abstract

Schizophrenia has long been associated with abnormal patterns of arousal that are thought to reflect disturbances in the reticular-activating system of the brain. Psychophysiological investigations of sensory responsivity have repeatedly demonstrated reduced reactivity and habituation to moderately intense stimuli in patients with schizophrenia. While not traditionally used as a measure of physiological arousal, the startle reflex represents an alternative method for studying reactivity and habituation in schizophrenia. This study examined eye blink responsivity to a repeatedly presented intense acoustic startle probe in men with chronic schizophrenia and healthy normal controls. Subjects’ personality profiles were also measured, as increased reactivity and arousal have been traditionally implicated as a physiological component to the personality trait of neuroticism. Results indicated that schizophrenic subjects did demonstrate significantly reduced rates of habituation to the acoustic startle probe and higher scores on measures of neuroticism in comparison to normal controls. However, no correlation between habituation rate and neuroticism emerged. These studies replicate previous findings of habituation in schizophrenia and provide further evidence for sensory reactivity disturbances in schizophrenia. The relationship of these findings to cognitive disturbances in schizophrenia is considered and directions for future research are discussed.

Keywords

Acoustic Startle; Habituation; Schizophrenia; Neuroticism; Arousal; Psychophysiology

1. Introduction

Schizophrenia has long been associated with abnormal patterns of arousal to stimuli (Venables, 1966), which are thought to reflect disturbances in a well-described brain system ascending from the brain stem to midbrain regions (Steriade, 1996). Psychophysiological studies primarily using EEG or skin conductance orienting response (SCOR) paradigms have consistently demonstrated higher resting rates of arousal, but lowered responsivity to orienting and other moderately intense stimuli in patients with schizophrenia (Venables, 1966; Venables and Wing, 1962; Dawson et al., 1992, 1994, Olbrich et al., 2001). Consistent with these findings

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are studies that have demonstrated reduced autonomic habituation, as assessed by electrodermal activity, to a repeatedly presented sensory stimulus (e.g., Depue and Fowles, 1973; Gruzelier et al., 1981; Hollister et al., 1994). These findings have demonstrated that schizophrenic patients show irregular response patterns over time that result in slower rates of habituation.

Although not commonly used to quantify an organism’s level of activation, the startle reflex is considered a measure of reactivity to environmental stimuli, and as such follows a similar course of decreasing sensitivity to stimuli when repeatedly presented. Behaviorally, the startle reflex is best characterized as a sudden, involuntary reaction to an intense, unexpected sensory event that is characteristically manifested as a quick, involuntary eye blink (Lang et al., 1990). Animal studies of the startle response indicate that a primary brain stem circuit through the nucleus reticularis pontis caudalis mediates the reflex (Davis, 1986; Cadenhead et al., 2000). The startle reflex is most often measured using electromyographic (EMG) technology to record the strength of contraction of the orbicularis oculi muscle surrounding each eye as it blinks to the startle probe (Lang et al., 1990). Studies of the startle response in schizophrenia indicate a similar pattern of abnormal reactivity to that observed with autonomic measures, with schizophrenic subjects habituating more slowly than non-schizophrenic psychiatric patients or normal controls (Geyer and Braff, 1982; Braff et al., 1992).

Also of interest is that arousal has been consistently implicated as a physiological component of certain personality styles or profiles (Stelmack, 1990; Gray, 1982; Larsen and Ketelaar, 1991), which have demonstrated to be characteristic of schizophrenia (DiLalla and Gottesman, 1995; Berenbaum and Fujita, 1994). In particular, the personality trait of neuroticism has been repeatedly associated with a hypersensitivity to aversive sensory stimuli. Conceptualized according to J.A. Gray’s model of a Behavioral Inhibition System (BIS), neuroticism may be thought of as a biologically based susceptibility to negative stimuli that results in increased arousal and negative affect in response to aversive or mismatched sensory events (Gray, 1982). Previous study of this theory has shown that neurotic subjects demonstrate more reactivity to negative stimuli than non-neurotic subjects (Larsen and Ketelaar, 1991).

The current study uses the startle reflex paradigm to further elucidate the nature and extent of habituation disturbances in schizophrenia and its relationship to personality traits. We first examine habituation rates of startle response in male patients compared to matched control subjects. Slower rates of habituation are predicted to characterize schizophrenic patients, all of whom show a chronic disease course. In addition, the personality trait of neuroticism will be measured and correlated with startle response amplitudes for all subjects to examine the extent to which habituation rates may relate to individual difference in personality profiles.

2. Methods
2.1. Subjects
Twenty-three men with a DSM-IV diagnosis of schizophrenia were recruited from inpatient and out-patient populations of a Veterans Affairs Medical Center. Seventeen normal controls were recruited via newspaper advertisements from the communities surrounding the medical center. Of the 40 subjects recruited, 5 schizophrenic subjects and 1 normal control were excluded from final analyses due to missing data and/or noncompliance with research protocol, leaving 18 schizophrenic patients and 16 normal controls. All subjects were screened for the presence of active substance abuse, medical illnesses, neurological problems, and lifetime incidence of loss of consciousness, all of which served as exclusion criteria for this study.

Before participation in the study, schizophrenic subjects’ psychiatric diagnoses were confirmed through chart review and clinical interview with the Structured Clinical Interview for the
Diagnostic and Statistical Manual-Fourth Edition (SCID-IV) (First et al., 1997). In addition, current symptom severity was assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1986). All schizophrenic subjects in this study were medicated with an average chlorpromazine (CPZ) equivalent dosage of 561.5 mg/day (range 120–1333 mg/day; see Table 1). The average length of illness was 19.7 years and the average number of hospitalizations was 7.5. Calculations of total days of hospitalization since illness onset provide further evidence of this group’s illness severity and chronicity. Subjects spent a lifetime average of 1624 days in the hospital for psychiatric care since their initial diagnosis of schizophrenia (see Table 1).

Normal control subjects were screened before participation in this study using the non-patient edition of the SCID-IV to rule out any current or past psychiatric disorders. In addition, all subjects completed a mini-mental status exam and the Hollingshead Index of Socio-Economic Status (SES). Schizophrenic and normal control subjects were matched on age, mental status, and parental SES (see Table 1).

2.2. Stimuli and apparatus

2.2.1. Physiological measures—The acoustic startle stimuli consisted of a series of 50 103-dB, 1000-Hz tones, 40 ms in duration with 10-ms rise and 0-ms fall times, which were generated and presented by Neuroscan stimulus software. Inter-stimulus intervals (ISIs) of the probe ranged from 8 to 30 s and were randomly selected by the computer. The tones were presented binaurally via Telephonics headphones that were plugged directly into the stimulus computer.

The eyeblink component of the startle response was measured by recording EMG activity from the orbicularis oculi muscle beneath the left eye using 11-mm Beckman Ag–AgCl miniature skin electrodes. The reference, ground, and channel electrodes were attached to subjects’ faces as suggested by Fridlund and Cacioppo (1986) with the ground electrode placed in the center of the subject’s forehead and the reference and channel electrodes placed on the orbicularis oculi muscle parallel to the lower edge of the eyelid.

EMG activity was recorded at a bandpass range of 30–500 Hz using Neuroscan Synamps programmable digital amplifiers and acquisition software. EMG activity was then digitized at a 2-kHz rate for a sample interval of 100 ms prior and 600 ms after stimulus onset. Editing of data using Neuroscan editing programs included rectifying, filtering (0.01–20 Hz), and baseline correcting EMG activity. The amplitude of all eyeblinks occurring within a latency window of 40–200 ms was scored.

2.2.2. Personality measure—For the assessment of personality profiles, all subjects were asked to complete the NEO-Five Factor Personality Inventory (NEO-FFI) prior to participation in the physiological component of the experiment. The NEO-FFI is a brief self-report questionnaire that provides a comprehensive measure of the five domains of personality (Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness) (Costa and McCrae, 1992).

2.3. Data analysis

An EMG response score for each tone was calculated by subtracting the mean EMG level during the 1-s immediately preceding tone onset from the highest mean EMG level measured within 40 to 200 ms after tone onset. EMG amplitudes to the first and last tones of each subject’s trial were dropped from analysis due to lost data for many of the subjects due to technical difficulties. The remaining 48 EMG amplitudes for each subject (from tones 2–49) were then condensed into eight blocks of six events each. Group EMG amplitude means were calculated
for the schizophrenic and control groups across the eight blocks of events. In addition, overall EMG amplitude means were calculated for each group. EMG data were submitted to a repeated measures multivariate analysis of variance (MANOVA) with the two groups (schizophrenic and control) as the between subjects factor and the event block (1–8) as the within subjects factor. Further, as another measure of habituation, the mean microvolt decline in EMG amplitude from event block 1 to event block 8 was calculated for each group and then converted into an overall percentage. Paired samples t-tests were used to calculate if there was a significant decline in reactivity from event block 1 to event block 8 for each group. In addition, Pearson correlations of subjects’ scores on the N-factor score of the NEO-FFI and their mean EMG score for each of the eight event blocks and their overall mean EMG score were computed.

3. Results

3.1. Habituation of the startle response

Fig. 1 presents a graph of EMG amplitude data across the eight blocks of six trials each for both subject groups. MANOVA revealed no significant main effect for diagnosis with both control (mean = 12.4 mV, S.D. = 19.3) and schizophrenic (mean = 12.5 mV, S.D. = 18.3) subjects showing similar overall mean EMG amplitudes. As Fig. 1 also shows, controls exhibited greater initial reactivity to the startle probe (mean = 22.5 mV, S.D. = 33.3) than did patients with schizophrenia (mean = 15.5 mV, S.D. = 25.6), but these differences were far from statistically significant (p > 0.25). In fact, mean reactivity of the two subject groups did not differ significantly for any of the eight blocks of trials.

The MANOVA revealed a significant multivariate effect for the diagnosis × block interaction (F(7,26) = 2.533, p = 0.040), indicating that the schizophrenic and control groups showed different rates of habituation over the eight blocks of trials. As Fig. 1 shows, the control subjects habituated more quickly than the schizophrenic subjects.

To further understand these findings, additional analyses of the data were performed. As shown in Fig. 1 and presented in Table 2, the mean EMG amplitude for the first block of acoustic startle probes were markedly but not statistically different for the control (22.5 mV, S.D. = 33.3) and schizophrenic subjects (15.5 mV, S.D. = 25.6). As also can be seen in Fig. 1 and Table 2, the two groups showed different rates of changes in their mean EMG amplitude over time, especially for the first three blocks. The control subjects mean reduction in EMG amplitude from block 1 to block 8 was 13.9 mV (S.D. = 23.0), which translates to a 62% reduction over time. The schizophrenic subjects mean reduction in EMG amplitude from block 1 to block 8 was 6.0 mV (S.D. = 18.5), which is a 39% decline over time. Paired samples t-tests revealed that this change in EMG amplitude from the first to last block of acoustic startle probes was significant for the control subjects only (t = 2.314, p = 0.035). The lack of significant change in the schizophrenic subjects’ EMG amplitude from block 1 to block 8 suggests that they showed minimal habituation over the course of the eight blocks of trials.

In an attempt to specify the source of this multivariate effect, EMG amplitudes for the first half (blocks 1–4) of the experiment were analyzed separately from the last half of the experiment (blocks 5–8). A MANOVA for the first four blocks of trials again revealed no significant main effect for diagnosis or block, although the effect for block approached significance (F(3,30) = 2.677, p = 0.065). Again, a significant multivariate effect for diagnosis × block was observed (F(3,30) = 4.394, p = 0.011) for the first four blocks of trials. No significant main effects for diagnosis or block and no significant multivariate effect for diagnosis × block were observed for the second half (blocks 5–8) of the data. These results indicate that the source of the observed significant multivariate interaction in this data is, in fact, confined to the first four blocks of trials.
3.2. Personality correlates of the startle response

The mean scores for the schizophrenic and control subjects on each of the five personality factors of the NEO-FFI personality scale are presented in Table 3. Schizophrenic and control subjects had significantly different scores for three of the five personality factors: neuroticism ($t = -3.37, p = 0.002$), agreeableness ($t = 2.23, p = 0.033$), and conscientiousness ($t = -3.24, p = 0.003$).

To examine whether these differences in individual factor scores resulted in any significant overall differences in the schizophrenic and control subjects’ personality profile, a MANOVA, with one between subject factor of diagnosis (schizophrenic and control) and one within factor of personality factor (neuroticism, extraversion, openness, agreeableness, and conscientiousness) was performed. The MANOVA did not reveal a significant main effect for diagnosis, which suggests that each group had similar means when personality scores were collapsed across the five factors. The MANOVA revealed a significant diagnosis × personality factor interaction ($F(4,29) = 3.394, p = 21$), suggesting that schizophrenic and control subjects demonstrated significantly different personality profiles on the NEO-FFI.

Fig. 2 represents the personality profiles for each subject group. As the significant interaction of the MANOVA indicated, schizophrenic and control subjects differ in their overall personality make-up, with differences in the neuroticism factor being most significant. It was hypothesized that subjects’ responsivity to the startle probe would correlate with measures of neuroticism. Analysis of the relationship between the schizophrenic group’s mean startle response for each of the eight blocks of trials and their responses on all five factors of the NEO-FFI revealed no significant relationships. Analysis of the relationship between responsivity to the startle probe and personality for the control group yielded similar results. It was additionally hypothesized that subjects’ rate of habituation would negatively correlate with scores of neuroticism on the NEO-FFI. Pearson correlations of the absolute microvolt change in EMG amplitude from block 1 to block 8 and personality factor scores did not yield any significant relationships for either subject group. Likewise, analysis of the relationship between absolute microvolt change in EMG amplitude from block 1 to block 4 and personality data was not significant for either group as well. Thus, although the control group did demonstrate a faster rate of habituation to the startle probe than the schizophrenic group, and although the schizophrenic group scored higher on neuroticism than the control group, no apparent relationship between habituation and neuroticism exists in this data set.

4. Discussion

The present study examined habituation of the acoustic startle response, and NEO personality profiles in patients with schizophrenia and age-matched control subjects. We hypothesized that schizophrenic patients would show abnormal patterns of reactivity to a repeatedly presented startle probe which in turn would correspond with distinct patterns of scores on personality measures. The results of the study did provide evidence of abnormal regulation of EMG response to a sudden acoustic startle probe in patients with schizophrenia. In addition, the data also revealed a distinct NEO personality profile for schizophrenic subjects in relation to control subjects. However, contrary to our hypothesis, EMG data did not correlate with personality measures for either schizophrenic or control groups.

In particular, the principal psychophysiological finding of the current study revealed a significantly reduced rate of habituation in the EMG amplitude to repeatedly presented auditory startle probes in patients with schizophrenia in comparison to control subjects. Further analysis revealed that the source of the significant rate of habituation effect arose primarily from the first half of the experiments, as assessed by the first four blocks of trials. This dramatic
difference in initial stages of habituation to the startle probe drove the significant diagnosis × block interaction.

The current habituation findings parallel those of previous studies examining autonomic response habituation to less intense, orienting stimuli (Depue and Fowles, 1973; Gruzelier et al., 1981; Hollister et al., 1994), as well as previous measures of startle response habituation in schizophrenia using different methodologies (e.g., Geyer and Braff, 1982; Braff et al., 1992, Grillon et al., 1992). Sensorimotor gating and prepulse inhibition paradigms are by far the most common means of investigating the startle reflex in schizophrenia. In these paradigms, the startle reflex is examined as a measure of disinhibition and thought to reflect a breakdown in information processing and/or attention functions in schizophrenia. Studies examining the startle reflex in schizophrenia using this paradigm consistently demonstrate reduced gating and habituation (Geyer et al., 1990; Braff et al., 1991, 1995; Swerdlow et al., 1994). These independent sources of evidence have converged to provide support for the pervasiveness of reactivity and habituation deficits in schizophrenia across different methodologies.

Sensory reactivity is essentially mediated by the reticular formation of the brain stem. Neurobiological investigations of the reticular formation and its ascending pathways indicate that neural impulses from the reticular formation travel to the cerebral cortex via the intralaminar nucleus of the thalamus (Steriade, 1996). Activation of cholinergic neurons in the reticular formation sensitizes the thalamus and cerebral cortex and potentiates their responses to sensory stimuli (Pare and Steriade, 1990 in Steriade, 1996). In addition, or perhaps because of, its role in physiological and cortical arousal, the reticular formation also mediates the intensity of a startle response through contralateral projections to the facial motor muscles (Davis, 1986; Cadenhead et al., 2000). Given this pathway of innervation, the current study’s findings are limited by its examination of the startle response on the left eye only. Bilateral EMG recordings would have allowed for the examination of lateralized differences in responsivity or habituation. The consideration of lateralized differences in EMG responses is of particular interest for this patient population given the well-documented left hemisphere dysfunction associated with schizophrenia (e.g., Shenton et al., 1992) and evidence for lateralized differences in the startle response in prepulse inhibition studies (Cadenhead et al., 2000).

Schizophrenia is a heterogeneous disorder with several subclassifications that represent distinct symptom profiles. While the schizophrenic subjects of the present study were not categorized according to symptom clusters, previous investigations of autonomic habituation have demonstrated some differences in arousal across symptom presentations. For example, Venables and Wing (1962) found that autonomic hyperactivity, as measured by skin conductance, was positively correlated with negative symptoms. Several investigators (e.g., Dawson et al., 1994; Olbrich et al., 2001) found that resting rates of arousal were elevated in comparison to controls only with patients experiencing acute psychosis. Patients in the present study were chronic with a mix of both positive and negative symptoms. Further examination of the relationship of specific symptom profiles to sensory responsivity disturbances in schizophrenia using the acoustic startle would help elucidate physiological correlates of the different clinical presentations of schizophrenia.

The EMG results of this study must also be understood in the context of the effects of antipsychotic medication. Animal research suggests that the startle reflex can be facilitated or attenuated by psychoactive substances. In a study investigating a LSD model of schizophrenia, rats exposed to LSD demonstrated increased startle response amplitudes and reduced startle habituation (Geyer et al., 1978; Braff and Geyer, 1980). Further, in studies of long-term habituation of the startle response in rats exposed serotoninergic antagonists, startle habituation was facilitated by a reduction in serotonin (Geyer and Tapson, 1988). While there has been no...
direct investigation of the effects of neuroleptics on the startle response and habituation in schizophrenia, prepulse inhibition studies have demonstrated that dopaminergic agonists decreased prepulse inhibition and startle amplitude in rats and that treatment with antipsychotic medications reversed these effects (Mansbach et al., 1988; Swerdlow et al., 1994; Zhuang et al., 2001). The findings of these studies suggest that the habituation deficits observed in the current study may have been attenuated by the presence of antipsychotic medication. The subjects of the current study were medicated with extensive histories of long-term neuroleptic use and medications represented the range of neuroleptic therapies. Further investigation of the effects of long-term neuroleptic use on the acoustic startle response as well as the examination of unmedicated patients would help elucidate the potential effects of medication on startle reactivity and habituation.

As predicted, on personality measures, schizophrenic subjects scored significantly higher than control subjects on the Neuroticism factor of the NEO-FFI. This finding is consistent with the results of previous investigations of personality traits in schizophrenia, which have consistently shown higher rates of neuroticism in schizophrenic patients (Berenbaum and Fujita, 1994; DiLalla and Gottesman, 1995). However, no significant correlation between neuroticism and startle reactivity was found. This finding suggests that while neuroticism may be associated with increased responsivity, these findings do not generalize to a startle reflex paradigm. Further investigation using more traditional measures of arousal (heart rate and skin response) may be more effective in capturing this relationship.

In summary, the findings of this study provided further understanding of the neurophysiological disturbances that characterize chronic schizophrenia. Previous investigations of autonomic habituation have identified that schizophrenic patients are slower to habituate to repeatedly presented stimuli and previous startle studies have shown similar reactivity and habituation deficits despite differences in methodology. While the precise functional significance of abnormal reactivity and habituation patterns in schizophrenia has yet to be elucidated, it is known that physiological arousal and attention are intimately connected, with the former providing a general source of resource activation and energy for cognition and the latter exerting a more selective effect on perception and thought (Kahneman, 1973; Nestor et al., 1999). In fact, some models of schizophrenia suggest disease-related disturbances in arousal lead to modulation failures of attention, thus resulting in a wide range of cognitive deficits (Gjerde, 1983). Increased physiological arousal can interfere with an organism’s ability to attend to, process, and remember information. Thus, the abnormal sensory reactivity in schizophrenia observed in this study and others may therefore be conceptualized as contributing to many of the neurocognitive symptoms often associated with this disorder, including those related to attention, memory, and communication. The results of this study highlight that further investigation of the relationship of acoustic startle habituation and cognitive dysfunction in schizophrenia is needed.

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Fig. 1.
Mean EMG amplitude across eight blocks of trials.
Fig. 2.
Mean five-factor personality profile for schizophrenic and control subjects.
Table 1

Demographic and descriptive statistics for SZ and control groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SZ mean (S.D.)</th>
<th>Control mean (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>41.6 (± 7.9)</td>
<td>46.4 (± 6.2)</td>
</tr>
<tr>
<td>Parental SES</td>
<td>2.7 (± 1.1)</td>
<td>2.7 (± 1.1)</td>
</tr>
<tr>
<td>Mini-mental status</td>
<td>28.1 (± 1.7)</td>
<td>29.1 (± 1.4)</td>
</tr>
<tr>
<td>CPZ in mg/day</td>
<td>561.5 (± 401.7)</td>
<td>N/A</td>
</tr>
<tr>
<td>PANSS positive syndrome score</td>
<td>17.1 (± 7.1)</td>
<td>N/A</td>
</tr>
<tr>
<td>PANSS negative syndrome score</td>
<td>18.1 (± 5.7)</td>
<td>N/A</td>
</tr>
<tr>
<td>PANSS general syndrome score</td>
<td>35.7 (± 10.4)</td>
<td>N/A</td>
</tr>
<tr>
<td>Length of illness</td>
<td>19.7 (± 7.7)</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of hospitalizations</td>
<td>7.5 (± 3.5)</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of days in hospital</td>
<td>1624 (± 3110)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* No significant difference between the two groups on matching variables.
Table 2

Mean and absolute differences in EMG amplitude (in microvolts) for each subject group across eight blocks of trials and for the overall EMG mean

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenic</td>
<td>15.5</td>
<td>16.8</td>
<td>12.4</td>
<td>12.2</td>
<td>11.7</td>
<td>13.4</td>
<td>11.5</td>
<td>9.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Control</td>
<td>22.5</td>
<td>12.1</td>
<td>11.2</td>
<td>14.6</td>
<td>9.5</td>
<td>10.6</td>
<td>7.5</td>
<td>8.6</td>
<td>12.4</td>
</tr>
<tr>
<td>Difference</td>
<td>7.0</td>
<td>4.7</td>
<td>1.2</td>
<td>2.4</td>
<td>2.2</td>
<td>2.8</td>
<td>4.0</td>
<td>0.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Table 3
Schizophrenic and control groups’ mean scores for the NEO-FFI factors

<table>
<thead>
<tr>
<th>Personality factor</th>
<th>Schizophrenic (S.D.)</th>
<th>Control (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroticism*</td>
<td>24.2 (7.8)</td>
<td>15.2 (7.7)</td>
</tr>
<tr>
<td>Extraversion</td>
<td>27.5 (5.3)</td>
<td>31.2 (6.2)</td>
</tr>
<tr>
<td>Openness</td>
<td>24.6 (4.8)</td>
<td>27.1 (7.3)</td>
</tr>
<tr>
<td>Agreeableness*</td>
<td>30.5 (6.3)</td>
<td>35.0 (5.3)</td>
</tr>
<tr>
<td>Conscientiousness*</td>
<td>27.8 (6.3)</td>
<td>36.2 (5.0)</td>
</tr>
</tbody>
</table>

* Schizophrenic and control group scores significantly differ at the $p < 0.05$ level.