Cerebellum volume and eyeblink conditioning in schizophrenia

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Abstract

Although accumulating evidence suggests that cerebellar abnormalities may be linked to the symptoms and course of schizophrenia, few studies have related structural and functional indices of cerebellar integrity. The present study examined the relationship between the volume of specific subregions of the cerebellum and cerebellar function, as measured by eyeblink conditioning (EBC). Nine individuals with schizophrenia and six healthy comparison participants completed structural magnetic resonance imaging of the brain and a delay EBC procedure. Volumetric measurements were taken for the whole brain, whole cerebellum, cerebellar anterior lobules I–V and posterior lobules VI–VII. The schizophrenia group had smaller cerebellar anterior lobes and exhibited impaired EBC relative to the comparison group. In the comparison group, larger anterior volume correlated with earlier conditioned response onset latencies and increased amplitudes of the unconditioned blink response during paired trials (i.e., when the conditioned and unconditioned stimuli co-occurred). The findings that smaller anterior cerebellar volumes and EBC impairments were associated with schizophrenia are consistent with non-human studies showing that anterior cerebellar abnormalities are associated with deficits in delay EBC. The lack of a significant correlation between indices of EBC and cerebellar volume within the schizophrenia group suggests an aberrant relationship between cerebellar structure and function.

Keywords: EBC; Magnetic resonance imaging; MRI

1. Introduction

Accumulating evidence indicates that the cerebellum plays a crucial role in higher order psychological processes (Katz and Steinmetz, 2002), including associative learning (e.g., Woodruff-Pak and Steinmetz, 2000) and temporal processing (e.g., Gerwig et al., 2005). The cerebellum also has been implicated in psychiatric disorders including schizophrenia (Andreasen et al., 1998; Andreasen, 1999), bipolar disorder (Lauterbach, 1996), and autism (Carper and Coughesne, 2000). In schizophrenia, abnormalities in the cerebellar node of the cortico-cerebellar-thalamic-cortical (CCTC) circuit are indicated by both structural and functional findings (e.g., Stephan et al., 2001; Ho et al., 2003; Keller et al., 2003).
Furthermore, cognitive disturbances associated with neurologic lesions affecting the cerebellum are similar to cognitive deficits reported in schizophrenia, including executive functioning deficits (Gimenez et al., 2003) and affective deficits (Earnst and Kring, 1997) (c.f. Schmahmann and Sherman, 1998). However, the relationship between structural and functional components of the cerebellum in schizophrenia remains unclear. The purpose of the current study was to explore the hypothesis that eyelid conditioning (EBC) deficits in schizophrenia (see Brown et al., 2005) are associated with structural abnormalities of the cerebellum. Accordingly, individuals with schizophrenia and healthy comparison subjects completed a single-cue delay EBC procedure and structural magnetic resonance imaging (MRI) to measure the volume of subregions of the cerebellum. In the subsequent paragraphs, the theoretical and empirical support for the present study is briefly reviewed.

Converging lines of evidence point to an important role of the cerebellum in schizophrenia. Theoretically, the “cognitive dysmetria” model (Andreasen et al., 1998; Andreasen, 1999) posits that disturbances in a cortico-cerebellar-thalamic-cortical circuit underlie important features of the disorder and its course. As a node in the this circuit, the influence of the cerebellum is underscored by feedback and feedforward loops connecting it with areas of the brain implicated in schizophrenia such as the thalamus and the limbic system (Kalil, 1981; Orioli and Strick, 1989), and the prefrontal cortex (Schmahmann and Pandya, 1995).

Evidence of impairments in the cerebellar node of the cortico-cerebellar-thalamic-cortical circuit in schizophrenia has been found at the cellular level. For example, cerebellar synaptic pathology is suggested by reduced synaptophysin and complexin II mRNA in schizophrenics (Eastwood et al., 2001). A post-mortem study of schizophrenia demonstrated an up-regulation of cerebellar extracellular signal-regulated kinase (ERK), a protein involved in synaptic development, dendritic growth, and cell death (Kyoschva, 2004). Schizophrenia also has been associated with smaller whole cerebellum (Keller et al., 2003) and vermal volume (Nopoulos et al., 1999; Ichimiya et al., 2001; Loeb et al., 2001). Interestingly, cerebellar volume may be associated with poor long-term outcome (Wassink et al., 1999) and cognitive dysfunction (Levitt et al., 1999; Wassink et al., 1999; Ho et al., 2003). However, findings from studies of gross cerebellum volume have not gone unchallenged and often vary both within and between studies (e.g., Ichimiya et al., 2001; Szeszko et al., 2003a,b; Keller et al., 2003; James et al., 2004; Shin et al., 2005). Since information-processing functions of the cerebellum may be characterized by each subregion’s distinct connectivity (O’Hearn and Molliver, 2001), the greatest weakness of these volumetric investigations is that subregions of the cerebellum, specifically, the anterior and posterior lobules, have not been adequately studied (see Loeber et al., 2001, for an exception).

Although few studies have examined the functional integrity of the cerebellum in schizophrenia, there is evidence of abnormalities from both functional MRI (Whalley et al., 2004; Takahashi et al., 2004) and positron emission tomography (Andreasen et al., 1996; Potkin et al., 2002). These functional imaging studies indicate that individuals with schizophrenia exhibit atypical cerebellar activation during sentence completion, affective processing, visual attention and verbal memory tasks.

Cerebellar function has also been assessed using EBC methodology. EBC is an associative learning task that yields time-dependent indices of motor learning, which have been shown in non-human and human research to be sensitive to the integrity of the cerebellum. Eyeblink conditioning involves pairing the presentation of a neutral tone (conditioned stimulus; CS) with an aversive corneal airpuff (unconditioned stimulus; US), which evokes a reflexive eyelid response (unconditioned response; UR). In the delay conditioning procedure, the onset of the CS precedes the onset of the US (e.g., by 350 ms), but the two stimuli co-terminate. With repeated CS-US paired presentations, a conditioned blink response (CR) develops prior to the onset of the airpuff and, in healthy individuals, the conditioned response is timed so that peak eyelid closure occurs just prior to the onset of the US (Gormezano et al., 1983).

Cellular recording and lesion techniques overwhelmingly support the role of the cerebellum in modulating the acquisition and timing of the CR in EBC. Specifically, non-human experiments have determined that activation of the CR is principally controlled by the cerebellar deep nuclei, specifically the interpositus nucleus (McCormick and Thompson, 1984; Woodruff-Pak et al., 1985; Steinmetz et al., 1991; Sears and Steinmetz, 1991). Inhibitory projections from Purkinje cortical neurons modulate the activity of the interpositus, effectively manipulating the timing of the CR (Mamounas et al., 1987; Bao et al., 2002). Evidence suggests that one locus of CR timing modification is the anterior region of the cerebellar cortex (Green and Steinmetz, 2005), an area wherein lesions disrupt CR onset and peak latencies (McCormick and Thompson, 1984; Perrett et al., 1993; Perrett and Mauk, 1995; Garcia et al., 1999) and, perhaps, CR acquisition (Lavond and Steinmetz, 1989; Garcia et al., 1999). Other studies have implicated the posterior cerebellar cortex in CR acquisition, but with a limited role.
Table 1
Characteristics of participants with schizophrenia

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of Dx</th>
<th>Medication</th>
<th>PANSS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
<td>f</td>
<td>18</td>
<td>Haloperidol</td>
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<td>12</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>m</td>
<td>10</td>
<td>Olanzapine</td>
<td>20 mg</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>m</td>
<td>19</td>
<td>–</td>
<td>–</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>m</td>
<td>n/a</td>
<td>–</td>
<td>–</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>f</td>
<td>28</td>
<td>Haloperidol</td>
<td>150 mg</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>30 mg</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paroxetine</td>
<td>20 mg</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Topiramate</td>
<td>25 mg</td>
<td></td>
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<tr>
<td>6</td>
<td>41</td>
<td>m</td>
<td>22</td>
<td>Divalproex Sodium</td>
<td>250 mg</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Risperidone</td>
<td>4 mg</td>
<td></td>
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<tr>
<td>7</td>
<td>46</td>
<td>f</td>
<td>29</td>
<td>Buspirone</td>
<td>150 mg</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>m</td>
<td>7</td>
<td>–</td>
<td>–</td>
<td>15</td>
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<tr>
<td>9</td>
<td>44</td>
<td>f</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>22</td>
</tr>
</tbody>
</table>

Unless otherwise noted, the dose schedule was once a day (QD), twice a day (BID), or three times a day (TID).

in the timing of the CR (e.g., Attwell et al., 2001). These findings in rabbits have been supported in humans using positron emission tomography (Logan and Grafton, 1995; Blaxton et al., 1996) and functional MRI (Rammani et al., 2000; Dimitrova et al., 2002). Clinically, insults to the cerebellum have been shown to markedly impair acquisition of the conditioned eyelink response (Daum et al., 1993; Woodruff-Pak et al., 1996).

In a prior study of the relationship between cerebellum volume and EBC in humans, a positive correlation was observed between CR acquisition and whole cerebellar volume in eight elderly patients (mean age 83; Woodruff-Pak et al., 2000). This finding held when additional younger participants (N=8) were added to the sample (Woodruff-Pak et al., 2001). In terms of the involvement of cerebellar lobules in EBC, whereas lesions to the posterior cerebellar lobes in humans have been associated with the occurrence of CRs (Gerwig et al., 2003, 2005), anterior lobe lesions have been associated with disturbances in the timing of the CR (Gerwig et al., 2005).

Given the evidence of structural and functional cerebellar abnormalities in schizophrenia, it is not surprising that this clinical group has also been found to exhibit various abnormalities in cerebellum-mediated EBC (Spain, 1966; Sears et al., 2000; Hofer et al., 2001; Marencio et al., 2003; Brown et al., 2005). Higher rates of conditioning have been reported in schizophrenia in two delay EBC studies (e.g., to a visual cue in Spain (1966) and auditory cues in Sears et al., 2000), impaired conditioning in another two studies (e.g., to auditory cues in Hofer et al., 2001), and no differences in delay conditioning in a final study (Marencio et al., 2003). A fairly consistent finding among recent studies has been earlier CR onset and peak latencies (Sears et al., 2000; Brown et al., 2005; see Marencio et al., 2003, for an exception). In the non-human literature, CR onset and CR peak latencies have been associated with anterior cerebellum (Perrett and Mauk, 1995; Garcia et al., 1999; Green and Steinmetz, 2005). Although additional research will be necessary to determine the impact of clinical state, medication status, and methodological manipulations on EBC in schizophrenia, there is evidence suggesting that schizophrenia may be associated with cerebellar dysfunction.

The purpose of the present study was to determine the relationships between cerebellar volume (determined by structural MRI) and EBC in individuals with schizophrenia and healthy comparison subjects. Two primary hypotheses were tested. First, given prior evidence that schizophrenia is associated with impaired EBC, it was predicted that the clinical group would exhibit EBC deficits, including impaired learning of the conditioned response, compared with the healthy control group. Second, based on animal research suggesting that anterior cerebellar lobules may mediate acquisition and timing of the conditioned response, as well as previous evidence of a relationship between human cerebellum volume and EBC, it was predicted that individuals with schizophrenia would have smaller anterior lobules than healthy controls.

2. Method

2.1. Subjects

Participants were 10 individuals (6 men and 4 women, mean age 40.0, SD=6.77) who met the Diagnostic and
Statistical Manual of Mental Disorders (DSM-IV) criteria for schizophrenia (using the Structured Clinical Interview for the DSM-IV; First et al., 2001), and seven comparison participants (4 men, 3 women, mean age 43.5, SD=6.2). Two participants were excluded from analysis because they were statistical outliers (see Section 3), and this yielded a final sample of nine schizophrenia patients and six healthy controls (HC). The groups did not significantly differ in age ($t(13)=1.113$, $P=0.29$). The Human Subjects Institutional Review Board at Indiana University School of Medicine approved of this study. All participants provided written informed consent prior to MRI and the EBC procedure, and all were paid for their participation. Additional patient characteristics, including medication status, and duration of disorder are provided in Table 1.

2.2. Delay EBC procedure, recording and processing

The EBC and data processing procedures followed those reported by Brown et al. (2005). All participants completed a 40-min, 108-trial single-euce tone delay EBC task. At the onset, eight US alone trials were presented with an intertrial interval (ITI) of 15 s, followed by 10 blocks of trials (mean ITI = 15 s; range = 10 to 20 s). Each trial block contained nine CS-US paired trials and one CS-alone trial, which was randomly presented within the last five trials of each block. The CS-US paired trials consisted of a 400-ms tone (1000 Hz; 10-ms rise and fall time; 80-dB SPL peak), which co-terminated with a 50-ms airpuff (10 psi at the source). The US airpuff was presented to the left eye via copper tubing (1/16 in. diameter) affixed to eye-glass rims and placed 1 cm away from the inner canthus of eye. A plastic tube (120 in.) connected the copper tubing on the glasses to a regulator receiving medical grade compressed air. Foam ear inserts (E-A-RLINK, Aero Company Auditory Systems, Indianapolis, IN) were used to present the tone CS by auditory transducers (10 $\Omega$ resistance; Neuroscan, El Paso, TX).

In order to maintain the attention of the participants throughout the experiment, neutral pictures selected from the International Affective Picture System (Lang et al., 1999) were presented for 2 s between each of the experimental trials. Participants rated the pleasantness of the pictures on a scale of 1 to 4 using a button response pad. In addition, participants were observed via a closed circuit monitor to ensure that their eyes remained open. The experiment was briefly suspended if signs of fatigue were observed so that the examiner could interact with the participant.

Eye blinks were recorded using pairs of bipolar EMG electrodes (8 mm AG/AG-CI; Model TD-23; MedAssociates, St. Albans, VT) placed on the orbicularis palpebrarum muscle below each eye, with a ground electrode on the forehead. EMG data were recorded continuously at 2.5 kHz with a SynAmps bioamplifier (highpass filter = 1 Hz, 12-dB/octave; lowpass filter = 300 Hz, 12 dB/octave; gain = 1000) using the Acquire data acquisition program (NeuroScan [v. 4.1], El Paso, TX).

The EMG data were processed in MATLAB (version 6.1, The MathWorks Inc., 2001) and the MATLAB-based program, DataMunch (King and Tracy, 1999), as described in Brown et al. (2005). For analysis of eight baseline URs (i.e., those occurring to solitary airpuffs presented prior to the first CS), the following occurred: (1) EMG from the stimulated left eye was segmented from the continuous recording from −100 to 225 post-stimulus; (2) high-pass-filtered at 28 Hz (12 dB/Octave); (3) baseline corrected from −50 to 0 ms; (4) rectified; (5) averaged; and then (6) baseline corrected again. Maximum UR blink amplitude was determined as the greatest amplitude between 30 ms and 90 ms post-stimulus. For analysis of the conditioning data, described completely by Brown et al. (2005), conditioned blink responses were determined by EMG activity occurring between 100 and 350 ms after CS onset which exceeded the baseline voltage by 5 S.D. Average baseline activity for a given trial was defined by a window extending 225 ms before CS onset. Trials were excluded from analysis if blinks were detected which exceeded 5 S.D. of baseline activity in a window from −75 ms to 25 ms post-CS onset.

2.3. Magnetic resonance imaging procedures

Whole-brain imaging was performed on a 1.5T GE Signa LX Horizon scanner (Waukesha, WI) with a birdcage head coil. T1-weighted anatomical images were acquired in 124 contiguous axial slices using a 3D Spoiled-Grass sequence (1.2 mm slice thickness; 35 ms TR; 8 ms TE; 1 excitation; 30° FA; 256×256 matrix; 24 cm FOV). Head motion was minimized by using a head-neck pad and dental bite bar. Considering FOV and matrix size, each voxel was 0.94 mm by 0.94 by 1.2 mm (in-plane).

Measurements of whole brain, whole cerebellum, and anterior (I–V) and posterior cerebellum lobules (VI–VII) were completed manually in the axial plane using the AFNI software (Analysis of Functional NeuroImages, Medical College of Wisconsin, Milwaukee, WI). These measures and subsequent primary analyses were taken by

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1 The current study was concerned with proportional change in volume across brain regions and diagnosis. Thus, because whole brain volume is sensitive to age-related, and possibly medication-related, changes in brain volume, it was preferred over intracranial volume.
the first author while he was blind to diagnostic assignment. Tracings were made by identifying the boundaries of interest and then tracing along those boundaries. This method yielded the total numbers of voxels within the traced boundaries. Volumetric measures corresponded directly to the number of traced voxels. Tracings of whole brain boundaries included both hemispheres from the frontal to occipital lobe whose lower boundary was represented by the inferior most regions of the cerebellum. For example, whole brain volumetrics included the basilar pons, lateral ventricles, and areas of the medulla at, or superior to, the bottom most edge of the cerebellum (including the cerebellum and cerebral spinal fluid within the identified boundaries). The volumetric tracings of the whole cerebellum included all lobules, vermal regions, and white and gray matter. The anterior lobe of the cerebellum was defined as only the grey matter anterior to the primary fissure, while the posterior lobe was represented by only the grey matter between the primary fissure and the prepymidal fissure (Schmahmann et al., 2000).

For each participant, measures of anterior and posterior volume were taken twice at least 14 days apart by CRE who was blind to groups. Pearson correlation coefficients were 0.953 and 0.951, respectively, indicating a high level of intrarater reliability. Interrater reliability was established by a second researcher (PDS), blind to groups, who also measured anterior cerebellar volume in all participants. The intraclass correlation coefficient, comparing CRE’s measurement with PDS’ measurement, was 0.96, indicating a high degree of consistency on cerebellar cortical measurement. The initial tracings by CRE were used for statistical analyses.

Finally, regional ratios (e.g., ratio of cerebellum to whole brain volume) were used to control for differences in brain size between the groups because structural MRI images were not submitted to a standardized space. Therefore, the ratios of interest were as follows: cerebellum/whole brain; anterior cerebellar lobules/whole cerebellum; and posterior cerebellar lobules/whole cerebellum. Because ratios are widely known to be skewed, as was the case in this data set (anterior/whole cerebellum = -1.254 [SE = 0.580], posterior/whole cerebellum = -0.843 [SE = 0.580]), a square-root transform was applied to all ratios and these were used for statistical analysis. For within- and between-group correlations the alpha level was set to $P < 0.05$.

3. Results

3.1. Volumetric analysis

Volumetric data from two participants (1 schizophrenic, 1 HC) met SPSS "outlier" criteria on volume measures of interest (i.e., greater than 3 times the interquartile range from the 75th percentile or 25th percentile, respectively) and, therefore, were excluded from analysis.

The raw volumetric measurements and ratios are presented in Table 2. Consistent with our hypothesis, the schizophrenia group had smaller anterior cerebellar tissue volume ($t(9.81)^2 = 1.91$, $P < 0.05$, $d = 0.92$, one-tailed) compared with healthy comparison subjects. There were no differences between the groups in posterior volume ratios ($t(13) = -1.10$, $P = 0.29$) or whole cerebellum volume ratios ($t(13) = -1.20$, $P = 0.25$).

3.2. Eyeblink conditioning analysis

Percent CRs to paired trials were assessed. Learning across the task was quantified by subtracting the mean percentage of CRs in the last two blocks from the mean percentage of CRs in the first two blocks. The groups differed on this measure of relative learning across the experiment ($t(13) = 2.16$, $P = 0.05$, $d = 1.10$). Consistent with this difference score, the clinical group had significantly fewer percent CRs during the latter part of the task in block 9 ($t(13) = 2.87$, $P < 0.05$, $d = 1.52$; see Fig. 1). The peak UR amplitudes to the first eight US stimuli (all presented prior to a CS tone) were used to examine group differences in baseline airpuff-induced startle reactivity. No group differences in peak UR amplitudes were observed ($t(13) = 0.77$, $P = 0.46$) and Pearson correlations yielded no significant relationship.

Table 2

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Age- and sex-matched controls</th>
<th>Schizophrenics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td>$1,339,494.0$ (45374.06)</td>
<td>$1,229,928.3$ (51594.50)</td>
</tr>
<tr>
<td>Whole cerebellum</td>
<td>$112,899.0$ (5766.83)</td>
<td>$110,789.3$ (4272.35)</td>
</tr>
<tr>
<td>CB lobules I–V</td>
<td>$14,407.0$ (634.07)</td>
<td>$12,952.4$ (668.89)</td>
</tr>
<tr>
<td>CB lobules VI–VII</td>
<td>$71,464.5$ (4897.74)</td>
<td>$71,498.7$ (2844.06)</td>
</tr>
<tr>
<td>Square-root ratios</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB:brain</td>
<td>$0.290$ (0.008)</td>
<td>$0.301$ (0.005)</td>
</tr>
<tr>
<td>Lobules I–V/CB</td>
<td>$0.358$ (0.003)</td>
<td>$0.342$ (0.008)</td>
</tr>
<tr>
<td>Lobules VI–VII/CB</td>
<td>$0.794$ (0.009)</td>
<td>$0.803$ (0.004)</td>
</tr>
</tbody>
</table>

Volumes expressed as voxels (0.94 mm by 0.94 by 1.2 mm [in-plane]). Controls ($N = 6$) and Schizophrenics ($N = 9$). Standard error of the mean in parenthesis.

2 The degrees of freedom for the independent samples $t$-test have been adjusted because of unusual variances typical of small samples (Levene's test for equality of variances, SPSS; $P = 0.028$).
between baseline UR amplitude and mean CR amplitudes ($r(13) = -0.010$, $P = 0.97$). Finally, the groups did not differ on UR amplitudes to paired stimuli during conditioning, ($t(13) = 2.16, P = 0.40$) or in CR peak latency ($t(13) = 1.12, P = 0.28$).

3.3. Eyeblink conditioning and volumetrics

There were no statistically significant correlations between EBC and volume measures when the patient and control groups were collapsed. However, when the control group was analyzed separately in a post-hoc analysis, the amplitude of the UR to paired CS-US stimuli negatively correlated with the relative anterior volume ($r(6) = -0.90, P < 0.05$; Fig. 2). There also was a significant positive correlation between the relative anterior volumes and CR onset latency ($r(6) = 0.82, P < 0.05$; Fig. 3). There were no significant correlations between cerebellar volumes and EBC when the schizophrenia group was considered separately (see also Fig. 3). These exploratory correlations were not corrected for multiple comparisons.

4. Discussion

Individuals with schizophrenia had smaller anterior cerebellar volume and impaired acquisition of the conditioned eyeblink response, compared with healthy comparison subjects. Further, within the healthy comparison sample, larger anterior cerebellar volume predicted an adaptive increase in CR onset latency and a decrease in UR amplitude during paired CS-US trials. This is the first study to report a structural deficit in the anterior lobe of the cerebellum in schizophrenia and to demonstrate in healthy individuals a significant relationship between anterior cerebellar volume and functional measures of cerebellar integrity, as assessed by EBC. The lack of group differences in baseline UR amplitudes suggests that differences between the groups in CRs and the correlation between anterior volumes and UR amplitudes to paired-clicks within the control group cannot be explained by group differences in basal reactivity to the US.

The observed CR acquisition deficit in schizophrenia is consistent with previous studies of auditory-cue EBC in schizophrenia (Hofer et al., 2001; Brown et al., 2005). The present results suggest that this acquisition deficit may be associated with structural abnormalities of the anterior cerebellum. This is in agreement with non-human studies showing that lesions to anterior cerebellum disrupt CR acquisition (Lavond and Steinmetz, 1989; Garcia et al., 1999). In fact, Garcia et al. (1999) contend that the anterior cortex is necessary for the acquisition/learning of novel paired CS-US relationships. The present results support this notion. Although the present findings do not directly replicate previous reports of a positive correlation between whole cerebellum volume and percent CRs (Woodruff-Pak et al., 2000, 2001), the present data are generally consistent with that finding and further substantiate the role of the cerebellum in associative learning. It should be noted that differences between the current data and results from the studies by Woodruff-Pak and colleagues could have been due to sample (e.g., age) and methodological differences (e.g., US intensity and duration). It must be noted, however, that despite the fact that the observed relationships between EBC and cerebellar volume appear
consistent with theoretical models of the cerebellum and extant empirical observations, acquisition deficits in the delay form of the task have not consistently been observed in schizophrenia (see Marencet al., 2003; Sears et al., 2000).

Data from the current study also provide empirical support for the position that the anterior cortices of the cerebellum have a modulatory influence on the timing of the CR and the magnitude of the UR during paired trials in healthy controls. Importantly, because the UR was assessed during paired CS-US trials, the magnitude of the UR may partially index associative learning for two reasons. First, the UR measured via EMG at the orbicularis oculi muscle also includes orbicularis activity associated with the CR on the analyzed trials in which the CS was paired with the US. Secondly, interpositus nucleus inhibition of brain stem nuclei responding to the US is initiated after execution of the CR (Hesslow and Ivarsson, 1996). Thus, reduction in UR amplitude over the course of the experiment is associated, in part, with the acquisition of the CR.

In terms of anterior cerebellum influences on CR timing, these data are in agreement with non-human studies showing that Purkinje cells in the anterior cerebellum, whose efferents target the interpositus nucleus, have a distinct pattern of activation followed by inhibition after the onset of a CS (Green and Steinmetz, 2005) and ablation of these cells causes timing disruptions of the CR (McCormick and Thompson, 1984b; Perrett et al., 1993; Perrett and Mauk, 1995; Garcia et al., 1999). The findings unique to the control group data also are in accord with a study by Gerwig et al. (2005) showing that anterior cerebellar damage is associated with short CR onset latencies. The results in this study also support a modulatory role for the cerebellar cortex with regards to UR amplitude. Increased anterior volume was associated with decreased UR amplitude during CS-US paired trials in healthy controls but not in the schizophrenia group, which exhibited evidence of EBC acquisition deficits. Conditioned reflex modification of the UR has been shown to be a consequence of CR acquisition (e.g., Sears and Steinmetz, 1991; Schreurs et al., 2000). Prior reports suggested that the neural mediators of this phenomenon may reside in the amygdala (Whalen and Knapp, 1991; Weiss et al., 1992) or the cerebellum via interpositus nucleus inhibition of the dorsal accessory olive (Canli et al., 1992). Data from the current report are the first to suggest the involvement of the cerebellum in the mediation of UR amplitudes during paired trials in healthy human participants and complement earlier literature demonstrating a structure–function relationship between cerebellar volumes and eye movements, specifically, prosaccade and antisaccade motor responses (Ettinger et al., 2005).

Finally, the present results point to the loss in the schizophrenia group of a normal structure–function relationship that was identified in the HC group. The absence of structure–function relationship in schizophrenia patients, in fact, has been demonstrated in prior literature. For example, Ettinger et al. (2004) showed that, although no volumetric differences were found between patient and control groups, different brain region volumes related differently to antisaccadic eye movements between the groups; suggesting abnormal structure–function relationships within the patient group. In addition, a plethora of research has found specific structural abnormalities to be associated with the cognitive/behavioral diagnostic criteria for schizophrenia, which are not found in healthy controls (e.g., Ho et al., 2003; Szeszko et al., 2003c).
Taken together, the data presented here are consistent with the EBC animal literature and are consistent with Andreasen’s cognitive dysmetria model (Andreasen, 1999). Nevertheless, there are limitations to the inferences that can be drawn from the present findings. Principally, small sample sizes and low statistical power hampered the statistical comparisons between the groups and between conditioned responses and volumetric measures. The small sample also did not permit examination of possible medication effects on conditioning or volume estimates. Further, methodological differences in CR measurement between the present and prior studies made direct comparisons of results difficult and undermined the extent to which the present findings could be generalized. Additional studies with larger samples of schizophrenics and healthy control participants will be useful in further elucidating the relationships between structural and functional indices of cerebellar integrity in humans.

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