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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. Nine individuals with schizophrenia and six healthy comparison participants completed structural magnetic resonance imaging of the brain and a delay eyeblink conditioning 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 eyeblink conditioning compared 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 associative deficits in delay eyeblink conditioning. The lack of a significant correlation between indices of eyeblink conditioning and cerebellar volume within the schizophrenic group suggests an aberrant relationship between cerebellar structure and function.

Keywords

EBC; Magn Reson 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, Paradiso and O'Leary, 1998; Andreasen, 1999), bipolar disorder (Lauterbach, 1996), and autism (Carper and Courchesne, 2000). In schizophrenia abnormalities in the

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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 eyeblink 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 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 limbic system (Kalil, 1981; Orioli and Strick, 1989), and 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 (Kysosseva, 2003). Schizophrenia also has been associated with smaller whole cerebellum (Keller et al., 2003) and vermal volume (Nopoulos et al., 1999; Ichimiya et al., 2001; Loeber 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; Szesko et al., 2003a; Szesko et al., 2003b; 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 eyeblink conditioning methodology (EBC). 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 eyeblink 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, 1984a; 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 regions of cerebellar cortex (Green and Steinmetz, 2005), an area wherein lesions disrupt CR onset and peak latencies (McCormick and Thompson, 1984b; Logan, 1991; 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 in the timing of the CR (e.g., Attwell, Rahman, and Yeo, 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 (Ramnani et al., 2000; Dimitrova et al., 2002). Clinically, insults to the cerebellum have been shown to markedly impair acquisition of the conditioned eyeblink 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 8 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 CR's (Gerwig et al., 2003; Gerwig et al., 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 eyeblink conditioning (Spain, 1966; Sears et al., 2000; Hofer et al., 2001; Marengo, Weinberger, and Schreurs, 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], Brown et al. [2005]), and no differences in delay conditioning in a final study (Marengo 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 Marengo et al., 2003, for an exception). In the non-human literature, CR onset and CR peak latencies have been associated with anterior cerebellum dysfunction (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 cerebellum volume (determined by structural MRI), the presence or absence of schizophrenia, and eyeblink conditioning 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 to 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 a previous evidence of a relationship between human cerebellar volume and EBC, it was predicted that individuals with schizophrenia would have smaller anterior lobules compared to healthy controls.

2. Method

2.1. Subjects

Participants were ten individuals with (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 8 comparison participants (5 men, 3 women, mean age 43.5, SD = 6.2). Two participants were excluded from analysis because they were statistical outliers (see results section), this yielded a final sample of 9 schizophrenia patients and 6 healthy controls (HC). 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 eyeblink conditioning 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-cue tone delay EBC task. At the onset, 8 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 9 CS-US paired trials and 1 CS-alone trial, which was randomly presented within the last 5 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 coterminated 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, Aearo Company Auditory Systems, Indianapolis, IN) were used to present the tone CS by auditory transducers (10 Ω 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 and Greenwald, 1988) 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.

Eyeblinks were recorded using pairs of bipolar EMG electrodes (8 mm AG/AG-Cl; 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 = .05 Hz, 12 dB/octave; lowpass filter = 500 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 Tracey, 1999), as described in Brown et al. (2005). For analysis of 8 baseline URs (i.e., those occurring to solitary air puffs presented prior to the first CS) the following occurred: (1) EMG from under the stimulated left eye was segmented from the continuous recording from -100 to 225 post stimulus; (2) high pass filtered at 28Hz (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 standard deviations. 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 standard deviations 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 x 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 brain¹, whole cerebellum, and anterior (I-V) and posterior cerebellum lobules (VI-VII) were completed manually in the axial plane using AFNI software (Analysis of Functional NeuroImages, Medical College of Wisconsin, Milwaukee, WI). These measures and subsequent primary analyses were taken by 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. Using this method the total numbers of voxels within the traced boundaries were yielded. 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 lobule of the cerebellum was defined as only the grey matter anterior to the primary fissure, while the posterior lobule was represented by only the grey matter between the primary fissure and the prepyramidal fissure (Schmahmann et al., 2000).

For each participant, measures of anterior and posterior volume were taken twice at least 14 days apart by a trained researcher (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 trained 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 .96, indicating a high degree of consistency on cerebellar cortical measurement. The initial tracings by CRE were used for statistical analyses.

¹The 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.

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: cerebellum/whole brain; anterior cerebellar lobules/whole cerebellum; and posterior cerebellar lobules /whole cerebellum. Because ratios are widely known to be negatively skewed, as was the case in this data set (anterior/whole cerebellum = -1.254 [SE = .580], posterior/whole cerebellum = -.843 [SE = .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 $P < .05$.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 to healthy comparison subjects. There were no differences between the groups in posterior volumes ratios ($t(13) = -1.10$, $P = 0.29$) or whole cerebellum volumes 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 ($t(13) = 2.16$, $P = .05$, $d = 1.10$). Consistent with this difference score, the clinical group had significantly fewer percent CRs during the latter part of the task in block 9, ($t(13) = 2.87$, $P < .05$, $d = 1.52$, see Figure 1). The peak UR amplitudes to the first 8 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 ($t(13) = .77$, $P = 0.46$) and Pearson correlations yielded no significant relationship between baseline UR amplitude and mean CR amplitudes ($r(13) = -.010$, $P = 0.97$). 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 ($r(6) = -0.90$, $P < .05$; Figure 2). There also was a significant positive correlation between the relative anterior volumes and CR onset latency ($r(6) = .82$, $P < .05$; Figure 3). There were no significant correlations between cerebellar volumes and EBC when the schizophrenia group was considered separately (see also Figure 3). These exploratory correlations were not corrected for multiple comparisons.

²The 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; $P = .028$).

4. Discussion

In this study, individuals with schizophrenia had smaller anterior cerebellar volume and impaired acquisition of the conditioned eyeblink response, compared to 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 eyeblink conditioning. 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 and colleagues (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 and 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 Marengo et al., 2003, and 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; Parrett 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; Weiz et al., 1992) or the cerebellum via interpositus nucleus inhibition of the dorsal accessory olive (Canli et al., 1992). Data from the current paper is the first to suggest the involvement of the cerebellum in the mediation of UR amplitudes during paired trials in healthy human participants and compliments prior 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., 2003).

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 which 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 schizophrenia 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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References

- Andreasen NC. A unitary model of schizophrenia: Bleuler's "fragmented phrene" as schizencephaly. *Archives of General Psychiatry* 1999;56:781–787. [PubMed: 12884883]
- Andreasen NC, O'Leary DS, Cizadlo T, Arndt S, Rezai K, Boles LL, Watkins GL, Hichwa RD. Schizophrenia and cognitive dysmetria: a positron-emission tomography study of dysfunctional prefrontal—thalamic—cerebellar circuitry. *Proceedings of the National Academy of Sciences of the United States of America* 1996;93:9985–9990. [PubMed: 8790444]
- Andreasen NC, Paradiso S, O'Leary DS. Cognitive Dysmetria" as an integrative theory of schizophrenia: a dysfunction in cortical-subcortical-cerebellar circuitry? *Schizophrenia Bulletin* 1998;24:203–212. [PubMed: 9613621]
- Attwell PJE, Rahman S, Yeo CH. Acquisition of eyeblink conditioning is critically dependent on normal function in cerebellar cortical lobule HVI. *The Journal of Neuroscience* 2001;21:5715–5722. [PubMed: 11466443]

- Bao S, Chen L, Kim JJ, Thompson RF. Cerebellar cortical inhibition and classical eyeblink conditioning. *Proceedings of the National Academy of Sciences of the United States of America* 2002;99:1592. [PubMed: 11805298]
- Blaxton TA, Zeffiro TA, Gabrieli JDE, Bookheimer SY, Carrillo MC, Theodore WH, Disterhoft JF. Functional mapping of human learning: a positron emission tomography activation study of eyeblink conditioning. *Journal of Neuroscience* 1996;16:4032–4040. [PubMed: 8656296]
- Brown SM, Kieffaber PD, Vohs JL, Carroll CA, Tracy JA, Shekhar A, O'Donnell BF, Steinmetz JE, Hetrick WP. Eye-blink conditioning deficits indicate timing and cerebellar abnormalities in schizophrenia. *Brain and Cognition* 2005;58:94–108. [PubMed: 15878730]
- Canli T, Detmer WM, Donegan NH. Potentiation or diminution of discrete motor unconditioned responses (rabbit eyeblink) to an aversive Pavlovian unconditioned stimulus by two associative processes: Conditioned fear and a conditioned diminution of unconditioned stimulus processing. *Behavioral Neuroscience* 1992;106:498–508. [PubMed: 1616616]
- Carper RA, Courchesne E. Inverse correlation between frontal lobe and cerebellar sizes in children with autism. *Brain* 2000;123:836–844. [PubMed: 10734014]
- Christian KM, Thompson RF. Neural substrates of eyeblink conditioning: acquisition and retention. *Learning and Memory* 2003;11:427–455. [PubMed: 14657256]
- Cornia DP, Jose-Robertson LS, Guillemin A, High J, Braun AR. Language lateralization in a bimodal language. *Journal of Cognitive Neuroscience* 2003;15:718–730. [PubMed: 12965045]
- Daum I, Schugens MM, Ackermann H, Lutzenberger W, Dichgans H, Birbaumer N. Classical Conditioning after cerebellar lesions in humans. *Behavioral Neuroscience* 1993;107:748–756. [PubMed: 8280385]
- Dimitrova A, Weber J, Maschke M, Elles HG, Kolb FP, Forsting M, Diener HC, Trimmann D. Eyeblink-related areas in human cerebellum as shown by fMRI. *Human Brain Mapping* 2002;17:100–115. [PubMed: 12353244]
- Earnst KS, Kring AM. Construct validity of negative symptoms: an empirical and conceptual review. *Clinical Psychological Review* 1997;17:167–189.
- Eastwood SL, Cotter D, Harrison PJ. Cerebellar synaptic protein expression in schizophrenia. *Neuroscience* 2001;105:219–229. [PubMed: 11483314]
- Ettinger U, Antonova E, Crawford TJ, Mitterschiffthaler MT, Goswami S, Sharma T, Kumari V. Structural neural correlates of prosaccade and antisaccade eye movements in healthy humans. *Neuroimage* 2005;24:487–494. [PubMed: 15627590]
- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured clinical interview for DSM-IV-TR axis I disorders, research version, Patient Edition. (SCID-I/P). New York: Biometrics Research, New York State Psychiatric Institute; 2001.
- Garcia KS, Steele PM, Mauk MD. Cerebellar cortex lesions prevent acquisition of conditioned eyelid responses. *Journal of Neuroscience* 1999;19:10940–10947. [PubMed: 10594075]
- Gerwig M, Dimitrova A, Kolb FP, Maschke M, Brol B, Künne A, Böring D, Thilmann AF, Forsting M, Diener HC, Timmann D. Comparison of eyeblink conditioning in patients with superior and posterior inferior cerebellar lesions. *Brain* 2003;126:71–94. [PubMed: 12477698]
- Gerwig M, Hajjar K, Dimitrova A, Maschke M, Kolb FP, Frings M, Thilmann AF, Forsting M, Diener HC, Timmann D. Timing of conditioned eyeblink responses is impaired in cerebellar patients. *Journal of Neuroscience* 2005;25:3919–3931.
- Gimenez M, Junque C, Perez M, Vendrell P, Baeza I, Salamero M, Mercader JM, Bernardo M. Basal ganglia N-acetylaspartate correlates with the performance in the procedural task 'Tower of Hanoi' of neuroleptic-naïve schizophrenic patients. *Neuroscience Letters* 2003;347:97–100. [PubMed: 12873737]
- Gormezano, I.; Kehoe, EJ.; Marshall, BS. Twenty years of classical conditioning research with the rabbit. In: Sprague, JM.; Epstein, AN., editors. *Progress in Psychobiology and Physiological Psychology*. New York: Academic; 1983. p. 198–275.
- Green JT, Steinmetz JE. Purkinje cell activity in the cerebellar anterior lobe after rabbit eyeblink conditioning. *Learning and Memory* 2005;12:260–269. [PubMed: 15897252]
- Heath RG. Modulation of emotion with a brain pacemaker. Treatment for intractable psychiatric illness. *Journal of Nervous and Mental Disease* 1977;165:300–317. [PubMed: 303280]

- Heath RG, Dempsey CW, Fontana CJ, Fitzjarrell AT. Feedback loop between cerebellum and septal-hippocampal sites: its role in emotion and epilepsy. *Biological Psychiatry* 1980;15:541–546. [PubMed: 6772242]
- Hesslow G, Ivarsson M. Inhibition of the inferior olive during conditioned response in the decerebrate ferret. *Experimental Brain Research* 1996;110:34–46.
- Ho BC, Andreasen NC, Nopoulos P, Arndt S, Magnotta V, Flaum M. Progressive structural brain abnormalities and their relationship to clinical outcome. *Archives of General Psychiatry* 2003;60:585–594. [PubMed: 12796222]
- Hofer E, Doby D, Anderer P, Dantendorfer K. Impaired conditional discrimination learning in schizophrenia. *Schizophrenia Research* 2001;51:127–136. [PubMed: 11518633]
- Ichimiya T, Okubo Y, Suhara T, Sudo Y. Reduced volume of the cerebellar vermis in neuroleptic-naïve schizophrenia. *Biological Psychiatry* 2001;49:20–27. [PubMed: 11163776]
- James AC, James S, Smith DM, Javaloyes A. Cerebellar, prefrontal cortex, and thalamic volumes over two time points in adolescent-onset schizophrenia. *American Journal of Psychiatry* 2004;161:1023–1029. [PubMed: 15169690]
- Kalil K. Projections of the cerebellar and dorsal column nuclei upon the thalamus of the rhesus monkey. *Journal of Comparative Neurology* 1981;195:25–50. [PubMed: 7204651]
- Katz DB, Steinmetz JE. Psychological functions of the cerebellum. *Behavioral and Cognitive Neuroscience Reviews* 2002;1:229–241. [PubMed: 17715595]
- Keller A, Castellanos FX, Vaituzis AC, Jeffries NO, Giedd JN, Rapoport JL. Progressive loss of cerebellar volume in childhood-onset schizophrenia. *American Journal of Psychiatry* 2003;160:128–133. [PubMed: 12505811]
- King, DAT.; Tracy, J. DataMunch: A Matlab m-file collection for the analysis of trial-based spike and behavioral data. 1999. Available from: <http://www.novl.indiana.edu/~dmunch/>
- Kyosseva SV. The role of the extracellular signal-regulated kinase pathway in cerebellar abnormalities in schizophrenia. *Cerebellum* 2004;3:94–99. [PubMed: 15233576]
- Lauterbach EC. Bipolar disorders, dystonia, and compulsion after dysfunction of the cerebellum, dentatorubrothalamic tract, and substantia nigra. *Biol Psychiatry* 1996;40:726–730. [PubMed: 8894064]
- Lavond DG, Steinmetz JE. Acquisition of classical conditioning without cerebellar cortex. *Behavioral Brain Research* 1989;33:113–164.
- Levitt JL, Mccarley RW, Nestor PG, Petrescu C, Donnino R, Hirayasu Y, Kikinis R, Jolesz FA, Shenton ME. Quantitative volumetric MRI study of the cerebellum and vermis in schizophrenia: clinical and cognitive correlates. *American Journal of Psychiatry* 1999;156:1105–1107. [PubMed: 10401463]
- Loeber RT, Cintron CMB, Yurgelun-Todd DA. Morphometry of individual cerebellar lobules in schizophrenia. *American Journal of Psychiatry* 2001;158:952–954. [PubMed: 11384906]
- Logan CG, Grafton ST. Functional anatomy of human eyeblink conditioning determined with regional cerebral glucose metabolism and positron-emission tomography. *Proceedings of the National Academy of Sciences of the United States of America* 1995;92:7500–7504. [PubMed: 7638220]
- Mamounas LA, Thompson RF, Madden JT. Cerebellar GABAergic processes: evidence for critical involvement in a form of simple associative learning in the rabbit. *Proceedings of the National Academy of Sciences of the United States of America* 1987;84:2101–2105. [PubMed: 3470780]
- Marenco S, Weinberger DR, Schreurs BG. Single-cue delay and trace classical conditioning in schizophrenia. *Biological Psychiatry* 2003;53:390–402. [PubMed: 12614992]
- McCormick DA, Thompson RF. Cerebellum: Essential involvement in the classically conditioned eyelid response. *Science* 1984a;223:296–299. [PubMed: 6701513]
- McCormick DA, Thompson RF. Neuronal responses of the rabbit cerebellum during acquisition and performance of a classically conditioned nictitating membrane-eyelid response. *Journal of Neuroscience* 1984b;4:2811–2822. [PubMed: 6502205]
- Nopoulos PC, Ceilley JW, Gailis EA, Andreasen NC. An MRI study of cerebellar vermis morphology in patients with schizophrenia: evidence in support of the cognitive dysmetria concept - a controlled postmortem study. *Biological Psychiatry* 1999;46:703–711. [PubMed: 10472423]
- O'Hearn E, Molliver ME. Organizational principles and microcircuitry of the cerebellum. *International Review of Psychiatry* 2001;13:232–246.

- Orioli PJ, Strick PL. Cerebellar connections with the motor cortex and the arcuate premotor area: An analysis employing retrograde transneuronal transport of WGA-HRP. *Journal of Comparative Neurology* 1989;288:621–626.
- Perrett SP, Mauk MD. Extinction of conditioned eyelid responses requires the anterior lobe of the cerebellar cortex. *Journal of Neuroscience* 1995;15:2074–2080. [PubMed: 7891154]
- Perrett SP, Ruiz BP, Mauk MD. Cerebellar cortex lesions disrupt learning-dependent timing of conditioned eyelid responses. *Journal of Neuroscience* 1993;13:1708–1718. [PubMed: 8463846]
- Perrett SP, Mauk MD. Extinction of conditioned eyelid responses requires the anterior lobe of cerebellar cortex. *Journal of Neuroscience* 1995;15:2074–2080. [PubMed: 7891154]
- Potkin SG, Alva G, Fleming K, Anand R, Keator D, Carreon D, Doo M, Jin Y, Wu JC, Fallon JH. A PET Study of the Pathophysiology of Negative Symptoms in Schizophrenia. *American Journal of Psychiatry* 2002;159:227–237. [PubMed: 11823264]
- Ramnani N, Toni I, Josephs O, Ashburner J, Passingham RE. Learning- and expectation-related changes in the human brain during motor learning. *Journal of Neurophysiology* 2000;84:3026–3035. [PubMed: 11110829]
- Roskies AL, Fiez JA, Balota DA, Raichle ME, Petersen SE. Task- dependent modulation of regions in the left inferior frontal cortex during semantic processing. *Journal of Cognitive Neuroscience* 2001;13:829–843.
- Schmahmann, JD.; Doyon, J.; Toga, AW.; Petrides, M.; Evans, AC. *MRI Atlas of the Human Cerebellum*. San Diego, California: Academic Press; 2000.
- Schmahmann JD, Pandya DN. Prefrontal cortex projections to the basilar pons in rhesus monkey: implications for the cerebellar contribution to higher function. *Neuroscience Letters* 1995;199:175–178. [PubMed: 8577391]
- Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain* 1998;121:561–579. [PubMed: 9577385]
- Sears LL, Andreasen NC, O’Leary DS. Cerebellar functional abnormalities in schizophrenia are suggested by classical Eyeblink conditioning. *Biological Psychiatry* 2000;48:204–209. [PubMed: 10924663]
- Sears LL, Steinmetz JE. Dorsal accessory olive activity diminished during acquisition of the rabbit classically conditioned eyelid response. *Brain Research* 1991;545:114–122. [PubMed: 1860037]
- Shin S-E, Lee J-S, Kang M-H, Kim C-E, Bae J-N, Jung G. Segmented volumes of cerebellum in first episode schizophrenia with auditory hallucinations. *Psychiatry Research: Neuroimaging* 2005;138:33–42.
- Schreurs BG, Shi T, Pineda S, Buck DL. Conditioning the unconditioned response: modification of the rabbit’s (oryctolagus cuniculus) unconditioned nictitating membrane response. *Journal of Experimental Psychology: Animal Behavior Processes* 2000;26:144–156. [PubMed: 10782430]
- Spain B. Eyelid conditioning and arousal in schizophrenic and normal subjects. *Journal of Abnormal Psychology* 1966;71:260–266. [PubMed: 5941044]
- Steinmetz JE, Sears LL, Gabriel M, Kubota Y, Poremba A, Kang E. Cerebellar interpositus nucleus lesions disrupt classical nictitating membrane conditioning but not discriminative avoidance learning in rabbits. *Behavioral Brain Research* 1991;45:71–80.
- Stephan KE, Magnotta VA, White T, Arndt S, Flaum M, O’Leary DS, Andreasen NC. Effects of olanzapine on cerebellar functional connectivity in schizophrenia measured by fMRI during a simple motor task. *Psychological Medicine* 2001;31:1065–1078. [PubMed: 11513374]
- Szeszko PR, Goldberg E, Gunduz-Bruce H, Ashtari M, Malhotra AK, Lencz T, Bates J, Crandall DT, Kane JM, Bilder R. Smaller anterior hippocampal formation volume in antipsychotic-naïve patients with first-episode schizophrenia. *American Journal of Psychiatry* 2003;160:2190–2197. [PubMed: 14638589]
- Szeszko PR, Gunning-Dixon F, Ashtari M, Snyder PJ, Lieberman JA, Bilder RM. Reversed cerebellar asymmetry in men with first-episode schizophrenia. *Biological Psychiatry* 2003a;53:450–459. [PubMed: 12614998]
- Szeszko PR, Gunning-Dixon F, Goldman RS, Bates J, Ashtari M, Snyder PJ, Lieberman JA, Bilder RM. Lack of normal association between cerebellar volume and neuropsychological functions in first-episode schizophrenia. *American Journal of Psychiatry* 2003b;160:1884–1887. [PubMed: 14514506]

- Takahashi H, Koeda M, Oda K, Matsuda T, Matsushima E, Matsuura M, Asai K, Okubo Y. An fMRI study of differential neural response to affective pictures in schizophrenia. *Neuroimage* 2004;22:1247–1254. [PubMed: 15219596]
- Wassink TH, Andreasen ND, Nopoulos P, Flaum M. Cerebellar morphology as a predictor of symptom and psychosocial outcome in schizophrenia. *Biological Psychiatry* 1999;45:41–48. [PubMed: 9894574]
- Weisz DJ, Harden DG, Xiang A. Effects of amygdala lesions on reflex facilitation and conditioned response acquisition during nictitating membrane response conditioning in rabbits. *Behavioral Neuroscience* 1992;106:262–273. [PubMed: 1317182]
- Whalen PJ, Knapp BS. Contributions of the amygdaloid central nucleus to the modification of the nictitating membrane reflex in the rabbit. *Behavioral Neuroscience* 1991;105:141–153. [PubMed: 2025386]
- Whalley HC, Simonotto E, Flett S, Marshall I, Ebmeier KP, Owens DGC, Goddard NH, Johnstone EC, Lawrie SM. fMRI correlates of state and trait effects in subjects at genetically enhanced risk of schizophrenia. *Brain* 2004;127:478–490. [PubMed: 14749289]
- Woodruff-Pak DS, Goldenberg G, Downey-Lamb MM, Boyko BO, Lemieux SK. Cerebellar volume in humans relates to magnitude of classical conditioning. *Neuroreport* 2000;11:609–615. [PubMed: 10718323]
- Woodruff-Pak DS, Lavond DG, Thompson RF. Trace conditioning: Abolished by cerebellar nuclear lesions but not lateral cerebellar cortex aspirations. *Brain Research* 1985;348:249–260. [PubMed: 4075084]
- Woodruff-Pak DS, Papka M, Ivry RB. Cerebellar involvement in eyeblink classical conditioning in humans. *Neuropsychology* 1996;10:443–458.
- Woodruff-Pak, DS.; Steinmetz, JE. Past, present, and future of human eyeblink classical conditioning. In: Woodruff-Pak, DS.; Steinmetz, JE., editors. *Eyeblink classical conditioning: Applications in humans*. 1. Kluwer Academic Publishers; Boston: 2000. p. 1-17.
- Woodruff-Pak DS, Vogel RW, Ewers M, Coffey J, Boyko OB, Lemieux SK. MRI-assessed volume of cerebellum correlates with associative learning. *Neurobiology of Learning and Memory* 2001;76:342–357. [PubMed: 11726241]

Figure 1.
Percent conditioned responses across all 10 blocks between 8 schizophrenics and 6 healthy participants. Error bars represent the standard error of the mean.

Figure 2. Correlation between the mean unconditioned response amplitude and the square root of the anterior cerebellar volume/whole cerebellum volume ratio in 6 healthy participants with a linear best fit line.

Figure 3.

Correlation between mean conditioned response onset and the square root of the anterior cerebellar volume/whole cerebellum volume ratio in 6 healthy participants with a linear best fit line. Healthy normal image on left, image within the schizophrenia group on the right.

Table 1

Characteristics of participants with schizophrenia

Case	Age	Sex	Duration of Dx	Medication	Positive	PANSS Negative	General
1	42	f	18	Haloperidol	12	11	19
2	29	m	10	Olanzapine	11	24	30
3	43	m	19	--	10	10	16
4	42	m	n/a	--	16	22	30
5	46	f	28	Haloperidol Olanzapine Paroxetine Topiramate Divalproex Sodium	20	12	41
				150mg 4/wks 30mg QD 20mg QD 25mg BID 250mg TID			
6	41	m	22	Risperidone	15	13	24
7	46	f	29	Buspirone	22	19	38
8	26	m	7	--	15	18	27
9	44	f	20	--	22	15	40

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

Table 2

Menu volumes and mean square-root ratios by group

Brain region	Age- and Sex-Matched Controls	Schizophrenics
Whole Brain	1339494.0(45734.06)	1229928.3(51594.50)
Whole Cerebellum	112899.0(5766.83)	110789.3 (4272.35)
CB lobules I-V	14407.0 (634.07)	12952.4 (668 89)
CB lobules VI-VII	71464.5 (4897.74)	71498.7(2844.06)
Square-root ratios		
CB/Brain	.290 (.008)	.301 (.005)
Lobules I-V/CB	.358 (.003)	.342 (.008)
Lobules VI-VII/CB	.794 (.009)	.803 (.004)

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 parentheses.