THE CEREBELLUM IN AUTISM

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Summary

The cerebellum is the most consistent site of neural abnormality in autism. Postmortem studies report a variety of anomalies, most notably a reduction in the number of Purkinje neurons. Additionally, structural neuroimaging investigations have shown both decreases in cerebellar gray matter and increases in cerebellar white matter. For many years, controversy surrounded these findings. However, the confluence of evidence, coupled with a revolution in views of cerebellar function, seems to be moving us past the controversy toward an acceptance of abnormalities of the cerebellum as an important component of the pathophysiology of autism. Now, investigations into the functional impact of such abnormalities are crucial, and functional neuroimaging studies are just beginning to reveal the possible role(s) of cerebellar dysfunction in autism. This review discusses the implications of such dysfunction, considering how abnormal cerebellar functioning might impact behavior and the symptoms of autism. Dysfunction of the cerebellum in the context of a developing central nervous system is also considered, particularly how cerebellar dysfunction might influence the establishment of neural circuitry and thereby impact the development and functioning of other brain systems. The paper concludes with a discussion of how cerebellar abnormality can inform our understanding of the causes and potential treatments for autism spectrum disorders.

Key Words: Autism – Cerebellum – Connectivity – Development – FMRI – MRI – Purkinje cells

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A crucial step in determining the cause of autism is understanding its underlying neurobiology. Abnormalities in nearly every brain system have been proposed to contribute to the pathophysiology of this disorder (e.g., Anderson 2005, Bachevalier 2005, Bailey et al. 1996, Baron-Cohen et al. 2000, Blatt 2005, Ciaranello and Ciaranello 1995, Courchesne 1997, DeLong 1992, Holden and Liu 2005, Lee et al. 2002, Minshew 1994), and many of these brain systems are likely involved. However, this review will focus on the most consistent site of neuroanatomic abnormality in autism—the cerebellum. Emerging evidence for cerebellar abnormality in autism has been paralleled by a revolution in our understanding of normal cerebellar function, such that the importance of elucidating the contributions of the cerebellum to autism is now clear. Thus, the purpose of this review is to delineate and discuss cerebellar findings in autism and their implications for understanding the disorder.

Cerebellar abnormality in autism: Postmortem evidence

In postmortem studies of the brains of individuals with autism to date, the cerebellum has been the most consistently observed site of pathology, with cerebellar abnormalities reported in over 95% of cases in which the cerebellum was examined. The most frequently reported pathology in these studies was a reduction in the normal number of Purkinje neurons (Bailey et al. 1998, Fehlow et al. 1993, Kemper and Bauman 1998, Lee et al. 2002, Ritvo et al. 1986, Vargas et al. 2005, Williams et al. 1980). The precise anatomic distribution of Purkinje neuron reduction has yet to be decisively determined. While some studies described it as diffuse or widespread (Lee et al. 2002, Ritvo et al. 1986, Williams et al. 1980), others reported the reduction to be exclusive to the posterolateral neocerebellar cortex, with sparing of the vermis and anterior hemispheres.
(Bauman and Kemper 1985, Kemper and Bauman 1998). Variability in the localization of Purkinje cell reduction was further shown by studies that provided more detailed descriptions of their individual cases. For instance, Bailey et al. (1998) reported Purkinje neuron reduction in five cases. Of these, three demonstrated diffuse reduction, while two showed greater reduction in the hemispheres than the vermis, consistent with the reports of Bauman and Kemper. In contrast, Lee and colleagues reported on two cases with Purkinje neuron reduction, one of which showed greater reduction in the vermis than the hemispheres (Lee et al. 2002).

Clearly, more studies are required to determine the anatomic distribution of this pathology. Localized reduction exclusive to the posterior neocerebellar hemispheres is developmentally plausible, given that Purkinje cells in the vermis and anterior and posterior hemispheres have different times of peak neurogenesis, different migratory patterns, and different times of settling (Altman and Bayer 1997). Alternatively, variable localization of Purkinje neuron reduction may be yet another manifestation of the neurobiological heterogeneity that seems to characterize autism.

Autopsy studies have also provided interesting clues regarding the time during development when Purkinje cell reduction occurs. The absence of reactive gliosis in most reports (Kemper and Bauman 1998, Ritvo et al. 1986, Williams et al. 1980) and the lack of empty basket cells, which normally ensheathe the Purkinje neuron cell bodies (Bailey et al. 1998), have provided perhaps the strongest evidence for an early developmental (i.e., prenatal) reduction of Purkinje neurons. This is also supported by the absence of atrophy of the cerebellar folia (Bailey et al. 1998). However, Bailey and colleagues also reported increased numbers of Bergmann glia in some of their autopsy cases, in addition to increased glial fibrillary acidic protein (GFAP), both of which typically point to later acquired pathology (Bailey et al. 1998). More recently, Vargas and colleagues found increased expression of GFAP in the cerebellum of cases with Purkinje neuron reduction, and GFAP immunostaining showed increased astroglial reactions, including a marked reactivity of Bergmann glia in the Purkinje cell layer (Vargas et al. 2005). Moreover, Bailey et al. noted that Purkinje neuron reduction in the presence of a relatively normal appearing cerebellar cortex is atypical for pathology occurring early in development (Bailey et al. 1998). In other words, an early reduction of Purkinje neurons, which are known to play a central role in cerebellar development, would be thought to have more profound effects on the overall development of cerebellar cortex. Thus, this leaves open the possibility of a later reduction (or loss) of Purkinje neurons.

Of course, it is important to note two additional possibilities regarding the timing of Purkinje neuron reduction that are based on more recent findings. One is that autism may involve both an early developmental reduction and a later acquired loss of these cells. The study by Lee and colleagues (2002), who conducted a histological examination of the cerebellum in two cases mentioned above, lends support to this notion. One case, with a history of epilepsy demonstrated severe Purkinje neuron reduction, with Bergmann gliosis in the Purkinje cell layer and additional glial clusters in the molecular layer. In the other case, with no history of epilepsy, the reduction was less severe and not accompanied by gliosis. These cases suggest that both developmental reduction and acquired loss of Purkinje neurons may indeed co-exist in autism. A second intriguing possibility is that pathological changes in the cerebellum involve a chronic, ongoing process of neuroinflammation and neurodegeneration that extends well beyond early neurodevelopment and may actually continue into the later stages of life in individuals with autism (Vargas et al. 2005). This notion is based on Vargas and colleagues’ findings of marked immune responses in the cerebellum that were closely associated with degenerating Purkinje cells, granule cells, and axons. These findings, which were reportedly similar to those seen in neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis, may open the door to a new way of conceptualizing autism (i.e., as a neurodegenerative disorder of development).

While it remains the single most consistent finding in postmortem autism studies, Purkinje cell reduction is not the only cerebellar pathology to be reported. For instance, reduced numbers of granule cells have also been found (Bauman and Kemper 1994, Vargas et al. 2005), though much less frequently. Bauman and Kemper also reported reduced numbers of cells in the fastigial, globose, and emboliform nuclei, but not the dentate nucleus (Kemper and Bauman 1998). Postmortem studies have additionally revealed glutamatergic (Purcell et al. 2001a) and cholinergic (Lee et al. 2002, Martin-Ruiz et al. 2004) receptor abnormalities, as well as reduced levels of glutamic acid decarboxylase (Fatem et al. 2002), which is critical for synthesis of the neurotransmitter GABA.

Several abnormalities that may leave the cerebellum susceptible to aberrant development or neuronal loss have been discovered in recent years. These include reduced levels of several important neuroregulatory proteins in the cerebellum, such as Bcl-2, an inhibitor of apoptosis (Araghi-Niknam and Fatemi 2003, Fatemi et al. 2001a, Fatemi et al. 2001b), Reelin, which is involved in correct layering of the developing brain as well as cell signaling and synaptic plasticity in the adult brain (Fatemi et al. 2001b), and neuronal cell adhesion molecule (NCAM), a protein thought to be involved in several aspects of nervous system development (Purcell et al. 2001b), in addition to the abnormal immune system responses described above (Vargas et al. 2005). Other neuroregulatory or neuromodulatory substances appear to be abnormally increased in autism, and these too may impact cerebellar development. For instance, in an exploratory analysis of archived neonatal blood samples from children with autism, Nelson and colleagues found concentrations of vasoactive intestinal peptide (VIP), neurotrophin 4/5 (NT4/5), calcitonin gene-related peptide (CGRP), and brain-derived neurotrophic factor (BDNF) that exceeded control levels (Nelson et al. 2001). While some of these substances may lead to abnormally enhanced neuronal growth (Akshoomoff et al. 2002), elevated levels of BDNF and NT4 can actually lead to a loss of Purkinje neurons (Morrison and Mason 1998). Thus, abnormal levels of several substances may contribute to cerebellar pathology. A key goal of future investigations should
be to examine the relationships between these molecular markers and neuroanatomic differences in individuals with autism.

Cerebellar anatomic abnormality in autism: In vivo evidence

The anatomic study of the cerebellum in vivo using magnetic resonance imaging (MRI) has also provided a wealth of evidence for cerebellar abnormality in autism (Carper et al. submitted). In very young individuals with autism (2-3 years of age), gray matter volumes in the whole cerebellum did not differ significantly from normal (Courchesne et al. 2001), but cerebellar white matter volumes were significantly and substantially (i.e., nearly 40%) increased (Courchesne et al. 2001). Studies that did not separate gray and white matter also reported significant increases in total cerebellar volume. This has been shown in 3-to-4-year-olds (Sparks et al. 2002), 7-to-11-year-olds (Herbert et al. 2003), and 6-to-14-year-olds (Palmen et al. 2004) with autism spectrum disorders. In light of the findings of Courchesne and colleagues these increases in overall volume may be attributable to increased white matter.

Courchesne and colleagues also showed that early adolescence appears to be the time when differences in cerebellar white matter are diminishing (Figure 1A), while gray matter differences are becoming evident (Figure 1B), such that in older adolescents with autism, cerebellar gray matter volume was reduced relative to normal (Courchesne et al. 2001). This discrepancy between gray and white matter effects calls for the separate analysis of gray and white matter in future studies and highlights the need for very careful tissue characterization in MRI studies of cerebellar volume in autism. Furthermore, data showing the time course of specific gray matter changes in the cerebellum add further support to the notion that Purkinje neuron reduction may occur later in development than previously thought.

A newer approach to examining group differences in brain volume is voxel-based morphometry (VBM). VBM, which departs from traditional methods of studying brain volumes, is an anatomical derivative of the statistical parametric mapping approach to analyzing functional brain imaging data that involves performing voxel-wise statistics on grouped MRI data. VBM analyses report localized differences in tissue concentration, but do not provide information regarding the

Figure 1. Volumes of cerebellar white matter (A) and gray matter (B) in 2-to-16-year-old boys plotted with their best-fit curves (Adapted from Courchesne et al. 2001).
Figure 2. Midsagittal T1-weighted MR images from a representative normal control subject and an individual with autism. Superimposed tracings highlight the difference in midsagittal vermis area between these two individuals (Images courtesy of Eric Courchesne, Ph.D.).

The actual volume of a structure or the amount of volume difference between groups. The technique may be problematic when data are imperfectly registered across a group (Bookstein 2001), and perfect registration is very difficult to achieve, particularly in a population with known neuroanatomic heterogeneity such as individuals with autism. Thus, VBM may produce some results that are difficult to interpret (Carper et al. submitted). A few studies have applied VBM to the study of brain volume in autism, including three that reported differences in the cerebellum. Abell and colleagues (1999) demonstrated focal areas of increased tissue concentration in the cerebellar hemispheres and vermis in young adults with Asperger’s disorder. Another group, which examined children and adolescents with autism, reported decreased white matter concentration in the left cerebellar hemisphere (Boddaert et al. 2004). Similarly, McAlonan and colleagues (2005) reported bilateral reductions in white matter concentration in the cerebella of high-functioning adolescents with autism. These latter two studies stand in contrast to a study using more traditional methods of cerebellar volume quantification that reported dramatic increases in cerebellar white matter volume in autism (Courchesne et al. 2001). Further investigations are required to clarify the status of white matter in the cerebellum in autism and how it might differ across the autism spectrum.

In terms of cerebellar sub-regions, reduced size of one or more regions in the cerebellar vermis, which is predominantly gray matter, is frequently reported in autism MRI studies (Figure 2) (Carper and Courchesne 2000, Ciesielski et al. 1997, Courchesne et al. 1987, Courchesne et al. 1988, Courchesne et al. 1994b, Courchesne et al. 1994a, Courchesne et al. 1994d, Courchesne et al. 2001, Hashimoto et al. 1995, Kaufmann et al. 2003, Levitt et al. 1999, Piven et al. 1992, Saitoh et al. 1995), and in some cases, the reduction may even be substantial enough to be identified in a clinical neuroradiologic exam (Miles and Hillman 2000). In the largest MRI study of autism yet conducted, Hashimoto and colleagues studied over 100 patients ranging from 6 months to 20 years of age. These investigators showed that vermis measures were smaller than normal at all ages (Hashimoto et al. 1995). A localized reduction in the size of cerebellar vermis lobules VI-VII has also been reported in several studies, including an investigation of sixty 2-to-16-year-old boys with autism (Courchesne et al. 2001). Furthermore, in a study of 3-to-9-year-old children, this finding was specific to autism when compared to normal children and children with fragile X or Down syndrome (Kaufmann et al. 2003).

In addition to the findings in the cerebellar vermis, reduced size of the cerebellar hemispheres has been reported as well (Gaffney et al. 1987, Murakami et al. 1989). In contrast, a study that only examined subjects with high-functioning autism reported that the total volume (gray and white matter combined) of the cerebellar hemispheres was significantly increased (Hardan et al. 2001). Again, the overall volume increases may have been due to the specific increases in white matter previously noted.

While reduced size of posterior vermis lobules VI-VII is frequently reported, a similar reduction in anterior vermis lobules I-V is much less common. This discrepancy was noted in the earliest study to report cerebellar vermis differences in autism (Courchesne et al. 1988), and subsequent studies have confirmed it. Only one investigation reported lobules I-V to be reduced in size (Hashimoto et al. 1995), while two showed the size of these lobules to be increased (Akshoomoff et al. 2004, Piven et al. 1992). This discrepancy between the anterior and posterior vermis somewhat parallels the reported anterior-posterior difference in Purkinje neuron reduction described by others (Bauman and Kemper 1985, Kemper and Bauman 1998). It should be noted, however, that these latter studies described this discrepancy as a feature of the cerebellar hemispheres and not the vermis. Nevertheless, as the anterior and posterior lobes of the cerebellum develop from different portions of the rhombencephalon and have different times of neurogenesis (Altman and Bayer 1997), such a discrepancy is developmentally plausible. In fact, it might be an important detail regarding cerebellar abnormality in autism, and may have implications for other aspects of brain development in this disorder (see below, The
Implications of Cerebellar Pathology in Autism: Disruption of Connectivity.

Controversy Surrounding the Cerebellum in Autism

Despite all of the supporting data, the issue of cerebellar involvement in autism has long been steeped in controversy. This controversy exists for several reasons, a major one being the fact that several studies failed to replicate the finding of cerebellar vermis size reduction in autism (Garber and Ritvo 1992, Holttum et al. 1992, Kleiman et al. 1992, Manes et al. 1999, Piven et al. 1997). The reasons for this are many, and they have been reviewed extensively elsewhere (Akshoomoff et al. 2002, Courchesne et al. 1994d). For a full discussion, the interested reader is referred to those papers. However, one factor contributing to the lack of replication of vermis findings will be discussed here, and that is the use of IQ as a matching variable or covariate.

IQ and Autism Specificity

Several autism researchers have argued that controlling for IQ, either through matching subjects based on their intellectual abilities or using IQ as a covariate, is an essential step in the investigation of potential neuroanatomic differences in autism (Filipek 1995, Holttum et al. 1992, Kleiman et al. 1992, Manes et al. 1999, Piven et al. 1992, Piven et al. 1997). When these methods are employed, cerebellar differences are not always seen. The purpose of IQ matching is to control for possible group differences in general cognitive functioning. However, in autism, IQ is not a valid measure of such functioning due to the atypical profile of subtest performance (Lincoln et al. 1988). Moreover, for studies with anatomical or physiological dependent variables, ability measures are not always appropriate matching variables. Factors chosen as matching variables are typically those that may influence the dependent variable in a manner that is not of interest to the experiment at hand; age and sex are classic examples in studies of brain anatomy. Although IQ and other behavioral variables may certainly impact brain structure through processes of activity-dependent development, one must also consider the well-established and likely stronger impact that brain structure has on IQ. Depending on the goal of a study, controlling for a variable that is influenced by the dependent variable in this manner can negatively impact the study’s results. Consider an investigation that examines whether two groups differ on a measure of brain anatomy. The anatomical region of interest also happens to be involved in a wide range of cognitive functions, including many of those indexed by IQ. In these two groups, there is a true difference in the size of this brain region. However, the variability in the region is also correlated with variability in IQ. Thus, removing the so-called “IQ effect” also removes true differences from normal that also happen to influence IQ. The cerebellum is just such a brain region. It is involved in a wide range of cognitive functions, including those indexed by IQ (see below, Modern Views of Cerebellar Function), and its anatomic size is known to be correlated with IQ (Andreasen et al. 1993, Paradiso et al. 1997). Thus, rather than removing the influence of IQ on cerebellar size, controlling for IQ may lead to the removal of differences that are important features of autism and also happen to influence IQ.

The relationship between cerebellar anatomy and IQ in autism is certainly interesting and important. Therefore, rather than remove this relationship from the analyses, one may employ a different approach and directly examine the interrelationship among anatomy, ability, and autism severity. For instance, a reanalysis of vermis measures from multiple studies (Courchesne et al. 1994d) showed that indeed, individuals who were the most impaired in terms of IQ showed the greatest reduction in vermis size. At the other end of the spectrum, Hardan and colleagues (2001) showed no differences in cerebellar vermis area in individuals with high-functioning autism. And, in a study directly comparing low- and high-functioning children with autism, the area of vermis lobules VI-VII was significantly smaller in children with low-functioning autism, while the measures in normal control children were intermediate to these two groups (Akshoomoff et al. 2004).

Thus, evidence does support an important relationship between cerebellar anatomy and IQ in autism, and controlling for IQ is not incorrect per se. Rather, whether or not it is done simply reflects fundamentally different views regarding the purpose of a comparison group and the goals of such comparisons. On the one hand, investigators who are primarily concerned with understanding how the brains of individuals with autism differ from normal will not be inclined to control for IQ, while investigators concerned with identifying abnormalities that are specific to autism will. Those who take the specificity approach have argued that because cerebellar abnormalities are also seen in other disorders of childhood, both developmental and acquired, such abnormalities may be less relevant to our understanding of the neurobiological basis of autism (Ciesielski et al. 1997, Ciesielski and Knight 1994, Schaefer et al. 1996). This view has fostered, at least in part, the use of IQ matching, which in some cases leads to the inclusion of individuals with mental retardation or borderline intelligence in the comparison group (Holttum et al. 1992, Manes et al. 1999, Piven et al. 1992, Piven et al. 1997). Clearly, comparing such disparate groups can limit our ability to understand how the brains of individuals with autism differ from normal. Furthermore, this type of matching may also cloud our ability to understand autism, for although multiple developmental disorders of different type and etiology may show abnormalities of the cerebellum, the morphologic pattern of these abnormalities can differ drastically (Kaufmann et al. 2003), as do the actual cellular bases for such abnormalities (Courchesne et al. 1994). Macropscopic deviations from normal can serve as important guides for subsequent microscopic studies, and limiting our ability to identify these deviations will surely slow our progress in identifying the true nature of neuroanatomic differences in autism.

Disagreements about the use of IQ as a matching variable or covariate have a rich tradition and are thus bound to continue. However, until it is determined precisely how the brains of individuals with autism differ
from normal, attempts to identify those abnormalities that are specific to autism may be premature. In other words, until we understand clearly how autism differs from normal, methods that limit our ability to achieve such an understanding may be throwing the baby out with the bathwater.

**Modern Views of Cerebellar Function**

Another source of controversy surrounding the cerebellum in autism was the longstanding, traditional view of the cerebellum as an exclusively motor structure. The dominance of this view caused many to question the relevance of cerebellar abnormality to a disorder characterized by predominantly non-motor deficits. However, evidence in recent years renders the traditional view of cerebellar function obsolete. For instance, the cerebellum is one of the most widely connected brain structures, receiving projections from and/or sending projections to all major divisions of the central nervous system (Allen et al. 2005, Andan et al. 1959, Clower et al. 2001, Dietrichs et al. 1994, Harper and Heath 1973, Heath and Harper 1974, Middleton and Strick 2001, Schmahmann 1996). Furthermore, acquired cerebellar lesions can lead to a variety of cognitive deficits and affective changes (Courchesne et al. 1994c, Fiez et al. 1992, Gottwald et al. 2003, Gottwald et al. 2004, Ivry et al. 2002, Leisohn et al. 2000, Molinari et al. 1997b, Molinari et al. 1997a, Riva and Giorgi 2000, Schmahmann and Sherman 1998, Townsend et al. 1999, Trillenberg et al. 2004). This led Schmahmann and Sherman to outline a “cerebellar cognitive affective syndrome” characterized by language, visuospatial, and executive function deficits in addition to personality changes (e.g., inappropriate behavior and flattening of affect) (Schmahmann and Sherman 1998). Interestingly, a similar syndrome has been reported in children following surgical resection of cerebellar tumors (Leisohn et al. 2000, Riva and Giorgi 2000), and in at least one of these pediatric cases, certain behavioral changes resembled classical symptoms of autism (e.g., gaze aversion, social withdrawal, and stereotyped movements) (Riva and Giorgi 2000).

In addition to the clinical findings, functional neuroimaging studies revealed that the cerebellum is involved in a variety of cognitive, social, and emotional functions (Allen et al. 1997, Desmond et al. 1997, Doyon et al. 2002, Flament et al. 1996, Gao et al. 1996, Jenkins et al. 1994, Kim et al. 1994, Le et al. 1998, Ploghaus et al. 1999, Raichle et al. 1994, Rao et al. 1997, Reiman et al. 1989, Toni et al. 1998, Xiang et al. 2003). Together, these findings not only significantly revised the traditional view of cerebellar function, but also indicated that developmental cerebellar pathology can affect a wide range of cognitive and affective deficits (Allen and Courchesne 1998). In concert with evidence for cerebellar anatomic abnormality in autism, this revised view of the cerebellum as a structure involved in a broad range of cognitive, social, and emotional functions indicates that it is not only reasonable, but imperative to examine the potential role(s) of cerebellar dysfunction in autism.

**Imaging Cerebellar Function in Autism**

Functional neuroimaging data on the cerebellum in individuals with autism are relatively limited, with few studies designed specifically to address cerebellar function. Early investigations using positron emission tomography (PET) to measure resting glucose metabolism showed no difference from normal in the cerebellar vermis and hemispheres (Heh et al. 1989, Rumsey et al. 1985). In contrast, serotonergic abnormalities in the cerebella of boys with autism were revealed using PET (Chugani et al. 1997), and activation studies using the PET-15O method demonstrated less activity than normal while listening to sentences (Müller et al. 1998) or tones (Müller et al. 1999), and greater activity than normal while repeating sentences (Müller et al. 1998). Abnormal cerebellar function was further supported by magnetic resonance spectroscopy studies, which exhibited lower levels of N-acetyl-aspartate, a putative marker of general neuronal function, in the cerebella of individuals with autism (Chugani et al. 1999, Otsuka et al. 1999).

Early neurobehavioral studies established that the cerebellar anatomic abnormalities in autism are associated with deficits in specific attention operations. For instance, individuals with autism have difficulty rapidly reallocating attentional resources to new sensory modalities (Akshoomoff and Courchesne 1992, Courchesne et al. 1994c) and to new spatial locations (Harris et al. 1999, Townsend et al. 1996a, Townsend et al. 1996b, Townsend et al. 1999). Such findings are more prominent in those with greater abnormality of the cerebellum (i.e., hypoplasia), and they are also seen in patients with acquired neocerebellar lesions (Akshoomoff and Courchesne 1992, Akshoomoff and Courchesne 1994, Townsend et al. 1999). These findings inspired a more direct investigation of cerebellar function in individuals with autism using functional magnetic resonance imaging (fMRI). This study examined functional activation in the cerebella of patients with autism and normal comparison subjects performing a non-spatial visual selective attention task (Allen and Courchesne 2003). Participants were eight patients with high-functioning autism (age 14 to 38 years) and eight healthy, normal comparison subjects matched for age, sex, handedness, and non-verbal intellectual ability. The attention task had been previously used to demonstrate attentional activation of the cerebellum in normal subjects (Allen et al. 1997). During this task, circles, squares, or triangles in red, green, or blue were presented randomly one at a time at fixation. The task tested the ability to selectively attend and respond to targets (squares or red shapes) by pressing a button with the dominant thumb. To control for the effects of visual sensory stimulation, this task was alternated with a passive visual stimulation condition, during which subjects observed the same stimuli, but did not selectively attend or respond. Multiple versions of the attention task were used. These varied in terms of the rate of stimulus presentation so that subjects could later be matched separately according to accuracy (“performance match”) or task version (“task match”).

An anatomical region-of-interest (ROI) approach to analyzing fMRI data allowed a detailed examination of functional integrity in regions of cerebellar cor-
Figure 3. Functional maps demonstrating sites of attention activation in normal subjects and individuals with autism. Activation is overlaid on averaged coronal anatomical images. Anterior-posterior (i.e., Y) Talairach coordinates are shown. Ipsilateral (to the moving hand) cerebellum is to the reader’s left. Color scale represents the number of subjects overlapping at any single voxel. Motor activation effects have been removed (Adapted from Allen and Courchesne 2003).

Figure 4. Correlation between cerebellar anatomy and cerebellar attention activation. Percent ROI active in the right superior posterior cerebellar hemisphere (lobule V/VI) during the attention task (performance-matched) plotted against summed anatomical areas (mm²) in that same ROI for normal subjects \( r = 0.72; t = 2.54, df = 6, p < 0.05, \) two-tailed \( t \) test) and subjects with autism \( r = 0.88; t = 4.54, df = 5, p < 0.05, \) two-tailed \( t \) test) (Adapted from Allen and Courchesne 2003).
Figure 5. Cerebellar motor activation in normal subjects and individuals with autism overlaid on averaged coronal anatomical images of cerebellum. Ipsilateral (to the moving hand) cerebellum is to the reader’s left. Anterior-posterior (i.e., Y) Talairach coordinates are shown. Images show the greater expanse of motor activation in the autism relative to normal. Color scale represents the number of subjects overlapping at any single voxel (Adapted from Allen et al. 2004).

text with known involvement in attention operations. ROIs were manually traced on the high-resolution MR image corresponding to each functional slice location in each subject. These ROIs, all located in cerebellar cortex (i.e., tracing excluded white matter and cerebrospinal fluid), were hemisphere lobule VI (posterior quadrangular lobule) ipsilateral to the moving hand (iVI), contralateral lobule VI (cVI), and right and left superior hemisphere lobules VIIA (RVIIA and LVIIA; superior semilunar lobule). These areas were chosen because they are consistently active in normal subjects during attention tasks (Allen et al. 1997, Casey et al. 2000, Corbetta et al. 1998, Coull and Nobre 1998, Le et al. 1998, Rees et al. 1997). Within each ROI, both the anatomical area and the activation area (i.e., the total area of all significantly active voxels) were calculated and collapsed across slices to create anatomical and activation “volumes.” The ratio between these volumes was then calculated to determine the percent volume active, a measure of “activation extent.” The mean percent signal change within each ROI, a measure of “activation magnitude,” was also calculated.

Figure 3 shows the attention activation results. Similar to previous findings (Allen et al. 1997), normal subjects showed bilateral superior posterior cerebellar hemisphere activation during the attention task. However, unlike their normal counterparts, subjects with autism showed minimal activation during the attention task, and this was reflected in the quantitative ROI effects. Whether subjects were matched for performance or task, activation in all ROIs was reduced in individuals with autism. For the performance match, activation extent was significantly reduced in iVI and RVIIA, with the difference in cVI approaching significance. Activation magnitude was significantly lower in iVI and cVI. For the task match, activation extent was significantly reduced in iVI, cVI, and RVIIA, and this difference approached significance in LVIIA. Activation magnitude was significantly lower in iVI, with the difference in cVI and RVIIA approaching significance. In addition to these group differences, a strong positive correlation between the size of cerebellar hemisphere lobule VIIA and activation extent in that same region was also observed in both groups (Figure 4). Similar to these findings of reduced cerebellar attention activation, two other studies reported reduced FMRI activation of the cerebellum in autism. One of these studies investigated brain activity during a task involving judgments of facial expression (Critchley et al. 2000), while the other was a study of motor learning (Müller et al. 2003).

In addition to examining cerebellar functioning in the context of attention, Allen and colleagues also studied patterns of activation in the cerebellum during a simple motor task (Allen et al. 2004). For this task, each subject held a joystick in the dominant hand and pressed a button with the thumb repeatedly at a comfortable pace. Subjects alternated between this task and
rest in a blocked FMRI design. The mean number of button presses across the motor task blocks was used as an index of button press frequency. Again, FMRI data were analyzed using an anatomic ROI approach. For this study, the approach had two stages. In the primary analysis, activation was examined in cerebellar regions normally involved in the motor task (Allen et al. 1997, Desmond et al. 1997, Stephan et al. 1995). In the secondary stage, we explored beyond these areas. In this manner, we were able to investigate possible functional abnormalities in cerebellar motor areas, in addition to potential abnormal involvement of cerebellar tissue outside of these regions. Primary ROIs were the anterior vermis (Av), the anterior hemisphere (central and anterior quadrangular lobules) ipsilateral to the moving hand (Iah), and iVI. ROIs for the secondary analysis were the posterior vermis (Pv), the anterior hemisphere contralateral to the moving hand (cAh), iVI, RVIIA and LVIIA. In the primary, hypothesis-driven analysis, Bonferroni correction protected against Type I error (i.e., alpha of .05 adjusted for 4 ROIs = .05/4 = .0125). In the secondary analysis, which was more exploratory in nature, such protection procedures were not employed.

As shown in Figure 5, individuals with autism showed substantially increased cerebellar activation relative to normal controls during the simple motor task. Furthermore, primary ROI analyses revealed that activation extent and magnitude in Iah were significantly increased, with the differences in iVI and Av approaching significance. Moreover, secondary analyses showed that activation extent was significantly greater than normal within RVIIA. Similar to these findings, Müller and colleagues (2001) showed greater than normal cerebellar activity in subjects with autism during a finger movement task.

Overall, the results from functional neuroimaging studies are just beginning to reveal the role of cerebellar dysfunction in autism. However, a pattern seems to be emerging from the findings. This pattern suggests that decreased cerebellar activation in autism occurs with greater frequency during higher-level sensory and cognitive tasks (e.g., attention, learning, decision making) (Allen and Courchesne 2003, Crichtley et al. 2000, Müller et al. 2003), while abnormal increases in cerebellar activation appear more common during basic motor and speech processes (Allen et al. 2004, Müller et al. 1998, Müller et al. 2001). The reasons for this dichotomy are not clear. However, an intriguing possibility is that it may be a functional reflection of a regional difference in Purkinje neuron reduction. Cognitive operation typically engage the posterior neocerebellar hemisphere, while motor tasks typically engage the anterior hemisphere and vermis. As noted above, the posterior neocerebellar hemispheres are reported to be the location of more prominent Purkinje neuron reduction, while the anterior hemispheres and vermis show less reduction. Reduced activation in the posterior cerebellar hemispheres correlates significantly with reduced size of these same regions (Allen and Courchesne 2003), and it is likely that this relationship between function and structure extends to the microscopic level (i.e., to a reduction in Purkinje neuron number). Thus, PET and FMRI findings may be a functional manifestation of the anterior-posterior discrepancy seen in both postmortem and in vivo structural MRI studies. The functional imaging findings also call for a more detailed inquiry into the functional implications of cerebellar pathology.

The Implications of Cerebellar Pathology in Autism

When considering the implications of cerebellar pathology in a developmental disorder such as autism, it is necessary to consider two separate questions about cerebellar dysfunction: 1) how might dysfunction of the cerebellum alone impact behavior and the symptoms of autism? and 2) how might cerebellar dysfunction in the context of a developing central nervous system influence the establishment of circuits involving the cerebellum and thereby impact the development and functioning of other brain systems?

Disruption of Function

The implications of a dysfunctional cerebellum obviously depend on the exact function of the cerebellum, which is not completely understood. However, several theories describe the fundamental role of the cerebellum as anticipatory or predictive (e.g., Coenen et al. 2001, Courchesne and Allen 1997, Darlot 1993, Miall et al. 1993, Nixon 2003, Paulin 1993, Tesche and Karhu 2000). It is well known that the cerebellum is involved in learning (for review, see Courchesne and Allen 1997). Furthermore, the cerebellum seems to have a special role in detecting signals in temporal sequences (Braitenberg et al. 1997) that arrive at the cerebellum as both exogenous (e.g., sensory events) and endogenous (e.g., from frontal cortex, hippocampus, hypothalamus, etc.) neural activities. Several papers by Courchesne and colleagues propose that when presented with such sequences, the cerebellum predicts - based on prior learning - what is about to happen and initiates preparatory actions that alter neural responsiveness in whichever neural systems are to be needed in upcoming moments (Akshoomoff et al. 1997, Allen et al. 1997, Courchesne et al. 1994c, Courchesne and Allen 1997). The cerebellum is thought to perform this fundamental function for all systems with which it maintains connections (e.g., sensory, motor, autonomic, memory, attention, affect, and language systems).

When preparatory functioning is impaired, other neural systems can continue to perform, but will do so sub-optimally in situations where prediction and preparation might aid performance. This can apply to any context in which successful performance requires an individual to rapidly and efficiently process or produce coordinated sequences of events or actions (e.g., following the natural, sequential flow of conversations and other social interactions or understanding the causal relationship between behaviors and their consequences). So, if we consider the three broad categories of autism symptoms (American Psychiatric Association 1994), we see that many can be conceptualized as impairments of preparatory functioning. Symptoms that fall under the categories of impaired social interaction and impaired communication may be due, at least in

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part, to difficulty processing and responding to unpredictable sequences of actions or sensory events (e.g., social overtures or conversations). Furthermore, restricted and repetitive interests and behaviors may emerge from attempts to adapt to such unpredictable situations by reducing the environment to something more predictable, for in a world where the ability to prepare for change is impaired, repetition may be particularly reinforcing. Therefore, through its proposed role in preparatory functioning, cerebellar abnormality may not only be a common feature of autism, but a key contributor to some of its hallmark symptoms. In fact, this contribution may be evidenced by a few recent investigations. For instance, in a study of children with autism exploring a structured environment (Pierce and Courchesne 2001), reduced exploration and repetitive behaviors were significantly correlated with the size of cerebellar vermis lobules VI-VII (but not with total brain or frontal lobe volumes). Also, using discriminant function analysis, Akshoomoff and colleagues recently showed that cerebellar white matter volume and cerebellar vermis size were particularly strong predictors of diagnosis in the autism spectrum (Akshoomoff et al. 2004). And in another recent MRI investigation that compared monozygotic twin pairs clinically concordant for autism to clinically discordant pairs, only the clinically concordant pairs showed concordance in cerebellar volume (Kates et al. 2004). Thus, the degree of cerebellar abnormality may well be of critical importance to the behavioral manifestations of autism.

Disruption of Connectivity

An emerging theoretical view in the autism field considers the abnormal development of neural connectivity to be a characterizing feature of the brains of individuals with autism (Belmonte et al. 2004a, Belmonte et al. 2004b, Courchesne et al. 2004, Just et al. 2004). Cerebellar pathology is a likely contributor to aspects of this aberrant connectivity. Purkinje neuron reduction will lead to a disruption of activity leaving the cerebellum for other brain regions. Normally, Purkinje neurons provide inhibitory modulation of excitatory output from the deep cerebellar nuclei. A reduction of Purkinje neurons will release the deep nuclei from inhibition, leading to aberrant activity along the cerebellum-thalamus-cerebral cortex circuit. Such activity may lead to the abnormal strengthening of anatomic connections between the cerebellum and other brain regions and the emergence of aberrant patterning of functional connectivity with cerebral sites (Belmonte et al. 2004a). (Figure 6)

Abnormalities of cerebellar connectivity may prove even more complex if autopsy studies to date have accurately reflected cerebellar neuropathology. Recall that Purkinje neuron reduction has been reported as being more prominent in the posterior cerebellar hemispheres (Bailey et al. 1998, Kemper and Bauman 1998), while the anterior hemispheres and vermis are reportedly less affected. Add to this the finding of reduced neurons in the fastigial, globose and emboliform nuclei, but not the dentate (Kemper and Bauman 1998), and a very interesting picture emerges with regard to the impact of pathology on connectivity. A reduction of Purkinje neurons in the posterior neocerebellar hemi-

Figure 6. Hypothetical increases in cerebellar connectivity in autism shown on an oblique T1-weighted MR image passing through cerebellum, thalamus, and frontal cortex. A reduction in Purkinje neurons is thought to result in the disinhibition of deep cerebellar nuclei projections (represented by the increased weight of the cerebello-thalamic connection) and the emergence of aberrant patterning of functional connectivity with cerebral sites.
spheres, which project primarily to the intact dentate nuclei, implies an increase in output from these nuclei (Figure 7). The dentate is the primary source of cerebellar output to cerebral cortex via the thalamus, including projections to dorsolateral prefrontal cortex and posterior parietal cortex in addition to motor and premotor cortices (Clower et al. 2001, Middleton and Strick 2001). In contrast, reduced numbers of neurons in the fastigial nucleus, which receives input from the vermis where Purkinje cells are reportedly less affected, implies a decrease in output from this nucleus (Figure 7). Primary projection sites for the fastigial nucleus include several structures in the limbic system (e.g., hypothalamus, hippocampus, amygdala, and other temporal lobe sites; Anand et al. 1959, Dietrichs et al. 1994, Harper and Heath 1973, Heath and Harper 1974). Thus, cerebellar connectivity with limbic regions may be reduced, while connectivity with cerebrocortical sites may be abnormally increased.

The implications of reduced versus increased connectivity obviously depend on the exact purpose of cerebellar output to these brain systems, which as already discussed is not completely understood. However, although its precise function is not known, there is growing consensus that the cerebellum performs some form of general modulatory function for all of the brain systems to which it sends projections (e.g., Akshoomoff et al. 1997, Andreasen et al. 1996, Bower 1997, Courchesne and Allen 1997, Ito 1997, Ivry et al. 2002, Leiner et al. 1991, Schmahmann 1996, Snider 1950). A lack of limbic modulation could lead to a dysregulation of emotional functions, thus contributing to the prominent socio-emotional deficits that characterize autism. In contrast, whereas selective long-range connections between the cerebellum and cerebral cortex are thought to facilitate efficient information processing (Belmonte et al. 2004a), aberrant formation of connections could lead to a variety of abnormalities, including variable functional maps in cerebral cortex (Müller et al. 2001, Pierce et al. 2001), excessive growth of cerebrocortical areas (Carper and Courchesne 2000, Carper and Courchesne 2005), impaired modulation of frontal event-related potentials (Townsend et al. 2001), and overexcitation of thalamocortical projections (Belmonte et al. 2004a, Belmonte et al. 2004b), increasing central nervous system “noise” and decreasing efficient information processing. A major goal of our future research is to examine how abnormalities of cerebellar connectivity alone may affect overall brain functioning and how it may interact with structural and functional changes in other brain systems to ultimately impact behavior in autism spectrum disorders.
The Implications of Cerebellar Pathology for Understanding the Etiology of Autism

The etiology of autism is unknown. Family and twin studies suggest a strong yet complex genetic component (Veenstra-VanderWeele and Cook, Jr. 2004), and linkage and association studies have pointed to an extensive list of candidate genes (Wassink et al. 2004). The confluence of evidence for cerebellar abnormality in autism suggests that investigators attempting to determine the genetic basis of autism consider genes that are involved in cerebellar development.

Mouse studies have identified a number of genes that play a role in cerebellar development (Millen et al. 1999). One of these is Engrailed 2 (En2). Misexpression or loss of function of the En2 gene in mice results in hypoplastic cerebella with reduced numbers of Purkinje neurons (Kuemerle et al. 1997), suggesting a possible connection with autism. Gharani and colleagues thus examined En2 as a susceptibility locus for autism spectrum disorders and found a significant family-based association (Gharani et al. 2004). This finding suggests that genes that play a role in cerebellar development indeed may be contributing factors to the etiology of autism, and examination of other genes involved in cerebellar development seems warranted.

Despite strong evidence for genetic contributions to autism, environmental factors are still a growing concern, particularly in response to apparent increases in autism prevalence observed over the past decade (Fombonne 2005). These increases are at least partially attributable to increased awareness of autism spectrum disorders, evolving diagnostic criteria, and more complete ascertainment. Nevertheless, they have also raised suspicion that exposures to chemical or biological agents during critical periods of development, either prenatally or postnatally, may too have contributed to the apparent rise of autism. Agents that have raised particular concern include prenatal viral infection (Fatemi et al. 2003), mercury and other heavy metals (Lawler et al. 2004), and pharmaceuticals such as thalidomide and valproic acid (Arndt et al. 2005, Lawler et al. 2004). The agent that has probably received the most attention in this context is mercury, given its presence in vaccines containing the preservative thimerosal. The idea of a relationship between thimerosal and autism has remained an attractive hypothesis to many, despite a number of studies that have failed to support this association (e.g., Andrews et al. 2004, Heron and Golding 2004, Madsen et al. 2003, Parker et al. 2004).

Industrial waste is yet another source of potential mercury exposure, and a recent study examining the association between environmentally released mercury, special education and autism rates in the state of Texas suggested a 61% increase in the rate of autism for each 1000 pounds of environmentally released mercury (Palmer et al. 2006). Although a causal association cannot be drawn from this study, the intriguing findings certainly suggest the need for further research.

A full discussion of the data supporting or refuting the various environmental agents that may be involved in autism is beyond the scope of this review. Furthermore, as noted by Lawler et al. (2004), our ability to understand the role of environmental factors in the etiology of autism is likely to be advanced if we transcend the gene-versus-environment argument and move toward an understanding of how genetic aberrations may leave an individual susceptible to environmental factors thought to be related to autism. In that context, it should be noted that Purkinje cells are highly vulnerable neurons (Fonn and Lock 2000, Kern 2003), and a wide range of abnormal physical conditions (e.g., hypoxia and ischemia; Brorson et al. 1995, Cervos-Navarro and Diemer 1991) and environmental insults (e.g., diseases, deficiencies, and toxic exposures; Bedi et al. 1980, Hua et al. 1995, Ingram et al. 2000, McDonald et al. 1998, Pletnikov et al. 2003, Ross et al. 1996, Sorensen et al. 2000) can bring about the death of these cells. Although the cerebellum is not the only brain region impacted by these factors, it is still interesting to recognize the overlap between the agents that have raised concern due their apparent association with autism and those that have been shown to be toxic to the cerebellum; viral infection, mercury exposure, and valproic acid exposure have all been associated with Purkinje neuron reduction in animal studies (Hua et al. 1995, Ingram et al. 2000, Pletnikov et al. 2003, Sorensen et al. 2000). Coupled with the accumulating evidence that at least some Purkinje neuron reduction in autism may be occurring later in development than previously thought, these findings call for further studies exploring the role of specific environmental factors in the development of autism, and how this relationship may be mediated by a loss of Purkinje neurons.

Implications for the Treatment of Autism

In the absence of a more complete understanding of the nature of cerebellar abnormality in autism, it may be somewhat premature to derive implications for treatment. However, new data suggesting that at least some of the reduction in Purkinje neurons may occur later in development than previously thought might open a window of opportunity to intervene in the process that is causing this reduction. Pair these data with reports that the degree of cerebellar pathology correlates with autism severity, and the paramount importance of understanding the causes and potential remedies for this pathology becomes even more evident.

To the extent that we understand the function of the cerebellum and the role of cerebellar dysfunction in autism, another goal of future research should be to identify those strategies that can assist individuals with autism in compensating for impaired cerebellar functioning. As already mentioned, much more work is needed before we fully understand normal functioning of the cerebellum and the implications of its dysfunction. However, current thinking regarding its role in learning and predictive and preparatory functions does highlight certain interventional approaches that may be helpful in developing compensatory strategies. For instance, compensation for difficulty learning material in a sequence, such as the required steps to complete a particular task, may be achieved by reducing the number of elements in a sequence as well as the rate of change from one element to the next. In addition, assistance in processing novel and unpredictable sequences of actions or events might be implemented in the form of auxiliary cues that better prepare an individual for im-
minent change. Also, in the context of repetitive and stereotyped behaviors, rewarding novel responses to unpredictable situations, when appropriate, may help individuals broaden their behavioral repertoire.

Future studies of cerebellar connectivity should identify those functional connections that are lacking or otherwise aberrant in autism. By linking this information with knowledge of those operations that are subserved by the pathways in question, neuroscience can inform the development of behavioral interventions that are designed to stimulate and help re-establish normal neurofunctional connections. Of course, this emphasizes the need for improved early identification of individuals with autism, for such interventions are certain to be more effective early in development when activity-dependent changes are more likely to occur. Meanwhile, our most pressing need concerning the cerebellum in autism is a fuller understanding of the nature of cerebellar abnormalities and their relevance to behavior in this common and devastating neurodevelopmental disorder.

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