Both genetic and environmental factors contribute to the pathogenesis of a wide variety of neurodevelopmental disorders, including autism, mental retardation, and schizophrenia. Some heritable disorders approach 100% penetrance; nonetheless, even in these disorders, subtle aspects of clinical disease expression may be influenced by the environment. In other disorders with genetic influences, exogenous factors, and the timepoint(s) during nervous system development at which they are introduced, modulate expression of disease. Elucidation of the mechanisms guiding this intricate interplay between host response genes, environmental agents, and the neurodevelopmental context within which these interactions occur, is necessary to understand the continuum of clinical outcomes. This chapter will review the evidence that infectious and immune factors may contribute to the pathogenesis of neurodevelopmental disorders, describe an animal model of neurodevelopmental disorders based upon viral infection, identify processes by which neural circuitry may be compromised, and outline areas for future research.

Key Words: neurodevelopmental disorders; autism; virus; immune; Borna disease virus; animal models

No established animal model for autism exists that adequately addresses autism’s: 1) likely perinatal origins [Gillberg and Gillberg, 1983; Hoon and Reiss, 1992; Bauman and Kemper, 1994]; 2) linkage to viral and/or immune factors [Chess, 1971; Warren et al., 1986; Singh et al., 1991; 1993; 1997a,b; Singh, 1996; 1997; Warren, 1998]; 3) association with hippocampal, amygdalar, and cerebellar dysfunction [Bauman and Kemper, 1994]; 4) connection with dopamine and serotonin disturbances [Cook, 1990; Anderson, 1994; Cook and Leventhal, 1996; Cook et al., 1997]; and 5) wide spectrum of neurobehavioral derangements (motor, postural, and sensory deficits; hypotonia; stereotypies; poor eye contact; mental retardation; islands of normal to supernormal functioning) superimposed on impairments in social interaction, communication, and behavior [Wing, 1997]. Additionally, although perinatal exposure to infectious agents and toxins is linked to the pathogenesis of neuropsychiatric disorders, the mechanisms by which environmental triggers interact with developing immune and neural elements to create neurodevelopmental disturbances are poorly understood.

Perinatal central nervous system (CNS) infection [Chess, 1971; Gillberg and Gillberg, 1983; Hoon and Reiss, 1992; Barak et al., 1999] and disturbed neuroimmune networks [Warren et al., 1986; 1987; 1990; 1991; 1994; 1995; Singh et al., 1991; 1993; 1997a,b; 1998; Singh, 1996; 1997; Burger and Warren, 1998; Warren, 1998] have been proposed as factors in the pathogenesis of autism spectrum disorders. An immune or infectious basis for autism is supported by epidemiologic studies suggesting an increased rate of autism in particular geographic regions [Gillberg et al., 1991; Baron-Cohen et al., 1999; Department of Developmental Services, 1999; Gillberg and Wing, 1999], season-of-birth effects [Bartlik, 1981; Kostantareas et al., 1986; Burd, 1988; Tanoue et al., 1988; Atlas, 1989; Gillberg, 1990; Bolton et al., 1992; Mouridsen et al., 1994; Barak et al., 1995; 1999; Ticher et al., 1996; Torrey et al., 1997; Stevens et al., 2000], and linkage to viral and/or immune factors [Chess, 1971; Warren et al., 1986; 1987; 1990; 1991; 1994; 1995; Singh et al., 1991; 1993; 1997a,b; 1998; Singh, 1996; 1997; Burger and Warren, 1998; Warren, 1998; Stevens et al., 2000]. Heritable predisposing factors may include immunologically relevant influences such as linkages to major histocompatibility complex (MHC) genes [Warren et al., 1992; 1996a,b; Warren and Singh, 1996; Warren, 1998]; increased frequency of the null allele of the complement component 4b locus, located in the MHC complex [Warren et al., 1991]; and increased frequency of a family history of autoimmune disorders [Comi et al., 1999]. Animal models and the epidemiology of autism, then, suggest that genetic and environmental components work in concert to cause disease. Frustration with the ability to move beyond simple allelic associations to genetic causation has led to models based on interactions of multiple genes, and reintroduction of the environment into the nature-nurture equation. Although controversial, reports that the rate of autism spectrum disorders
may be increasing [Gillberg et al., 1991; Department of Developmental Services, 1999; Gillberg and Wing, 1999], and that this increase is restricted to certain geographic regions [Baron-Cohen et al., 1999; Department of Developmental Services, 1999; Gillberg and Wing, 1999], provide further support for environmental factors in the pathogenesis of these disorders.

These factors may include perinatal central nervous system (CNS) infection [Desmond et al., 1967; Chess, 1971; Blattner, 1974; Peterson and Torrey, 1976; Chess, 1977; DeLong et al., 1981; Schwab, 1982; Gillberg and Gillberg, 1983; Markowitz, 1983; Ivarsson et al., 1990; Mason-Brothers et al., 1990; Ritvo et al., 1990; Gillberg and Coleman, 1992; Hoon and Reiss 1992; Tepper et al., 1998; Barak et al., 1999] and toxins [Anonymous, 1999; Rice and Barone, 2000; Weiss and Landrigan, 2000].

EVIDENCE TO SUPPORT A ROLE FOR VIRAL INFECTION IN THE PATHOGENESIS OF NEURODEVELOPMENTAL DISORDERS

The implication of a role for prenatal and/or postnatal viral infections in the etiology of autism stems from retrospective epidemiologic studies of in utero or perinatal viral exposures, of seasonal and geographic factors, and, more indirectly, from studies of immune aberrations in the blood of children with autism [Mason-Brothers et al., 1990; Gillberg and Coleman, 1992; DelGiudice-Asch and Hollander, 1997; Warren, 1998; Trottier et al., 1999]. A number of congenital microbial exposures have been reported in association with autistic features, including rubella virus [Desmond et al., 1967; Chess, 1971; Deykin and MacMahon, 1979], cytomegalovirus [Blattner, 1974; Stubs and Crawford, 1977; Markowitz, 1983; Stubs et al., 1984; Ivarsson et al., 1990; Ritvo et al., 1990], herpes simplex virus [Peterson and Torrey 1976; DeLong et al., 1981; Ritvo et al., 1990], varicella zoster virus [Stubs and Crawford 1977; Deykin and MacMahon, 1979], enteroviruses [Sells et al., 1975], human immunodeficiency virus [Tepper et al., 1998], and syphilis [Stubs and Crawford, 1977; Schwab, 1982].

Establishing a causal relationship between infection with a microbial agent and a specific brain disease can be complex [Lipkin and Hornig, 1998]. In some instances, for example, herpes simplex encephalitis, the agent is readily implicated: the virus is present in brain and destroys infected tissue through replication. Alternatively, tissue damage and disease may be the indirect result of a host immune response to microbial gene products present in neural cells. Immune responses to microbial agents can also lead to breakdown of tolerance to host antigens and result in tissue damage [Zhao et al., 1998]. The agent responsible for induction of autoimmunity need not be present in CNS at the time of clinical presentation. Furthermore, the original infection may have been peripheral, as is the case in Sydenham’s chorea, or as is proposed for tics and obsessions following streptococcal infection. Yet another mechanism for brain disease is persistent nontyphoidal viral infection. Such infections can profoundly impact neurotransmitter function or brain development, yet remain cryptic unless specific reagents are used for detecting viral gene products [Lipkin et al., 1988a,b; Oldstone 1989a,b,c]. Diverse neuropsychiatric outcomes may even result from infection with a single agent, depending on: 1) timing of infection relative to the status of neural or immune systems (in utero, juvenile, adult); 2) genetic context (e.g., MHC antigens determining immune response and subsequent neuropathology); and/or 3) other environmental factors (infectious agents, toxins, psychosocial stress).

Despite a finding of season-of-birth effects in several studies [Bartlik, 1981; Burd, 1988; Gillberg, 1990; Barak et al., 1995; 1999; Ticher et al., 1996; Stevens et al., 2000]—many of which suggest a preponderance of March and/or August births or a second trimester pathogen exposure amongst children with autism—other studies have not confirmed this link [Landau et al., 1999]. Even if a microbial link is confirmed for a subset of children with autism, other factors may also vary seasonally, (e.g., nutritional differences) and influence expression of disease [Gillberg and Coleman, 1992].

Studies of immunologic function in children with autism reveal a wide array of abnormalities, including decreased cellular immune capacity [Warren et al., 1986; 1990; Wright et al., 1990; Yonk et al., 1990; Denney et al., 1996]; decreased plasma complement component C4b [Warren et al., 1994; 1995]; and increased humoral immune and autoantibody responses [Weizman et al., 1982; Singh et al., 1993]. These abnormalities provide general support for the hypothesis that children with autism may be predisposed to respond abnormally to viral infections either through the establishment of persistent infections or a virally-triggered autoimmune diathesis. Only limited conclusions may be drawn from these studies, as they are based on small, incompletely characterized, heterogeneous populations differing in etiology and course, and often diagnosed through superficial screening using Diagnostic and Statistical Manual (DSM) criteria alone, without use of standardized diagnostic instruments such as the Autism Diagnostic Interview-Revised (ADI-R) or Autism Diagnostic Observation Schedule (ADOS). Occasionally, similar immune disturbances are noted in first degree relatives or neurologic or healthy controls; however, in most studies, comparison groups are not screened for presence of autism spectrum disorder symptoms or disorder. Controls for such factors are time of blood collection, serum/plasma vs. intracerebral sources of cytokines, medications, age, IQ, psychosocial factors, immunization and infectious disease history, or family history of immune-based disorders. Findings of immune disturbances in peripheral blood specimens have generally not been assessed or corroborated in cerebrospinal fluid (CSF) samples and immune-mediated mechanisms have not been definitively supported by autopsy or brain imaging studies. Nonetheless, although the degree of convergence of epidemiologic, genetic, immunologic, and biochemical findings across or within subsets of children with autism spectrum disorders is as yet insufficient to warrant firm conclusions regarding pathogenesis, the data provide intriguing clues about potential mechanisms that may contribute to neural injury.

Several reports are consistent with diminished Th1 and increased Th2 responses in autism: 1) mitogen-stimulated T cell proliferation is decreased in some [Stubbs and Crawford 1977; Warren et al., 1986], but not all [Ferrari et al., 1988] studies; 2) Th1 (IFN-γ+ CD4+ and IL-2+ CD4+ cells) and Th2 (IL-4+ CD8+ cells) and TC1 (IFN-γ+ CD8+ and IL-2+ CD8+ cells) T cells are reduced, and Th2 (IL-4+ CD4+ cells) and TC2 (IL-4+ CD8+ cells) T cells are reportedly increased [Gupta et al., 1998]; 3) natural killer cell activity is reduced [Warren et al., 1987], despite unchanged numbers of natural killer cells [Warren et al., 1990]; and 4) increased levels of serum IgE [Gupta et al., 1996; Trottier et al., 1999]. Furthermore, as Th2 cells are implicated in systemic (nonorgan specific) autoimmune disorders [De Carli et al., 1994; Singh et al., 1999], the increased autoantibody production [Todd and Ciaranello, 1985; Todd et al., 1988; Plioplys et al., 1989a,b; Yuwiler et al., 2001].
1992; Singh et al., 1993, 1997a,b; 1998; Connolly et al., 1999) and family history of autoimmune disorders [Money et al., 1971; Raiten and Massaro, 1986; Gillberg et al., 1992; Comi et al., 1999] reported for children with autism lend further support to the idea that a Th2 predominant immune response may play a role in autism pathogenesis. In contrast, Singh reports increased plasma IL-12 and IFN-γ levels in autism [1996], findings more consistent with Th1 than Th2-weighted responses. Lastly, a preliminary report by Nelson and colleagues indicates a lower level of autoantibodies to MBP, GFAP, and NAFP at birth in children with autism or mental retardation than in normal controls [Nelson et al., 2000]. These apparent inconsistencies in levels of autoantibodies in children with autism might be explained by the difference in time of sampling, with later timepoints reflecting a break in immune tolerance.

It is conceivable that there is a link between susceptibility to infection with measles virus and HHV-6 and an autoimmune diathesis. The observation that the CD46 receptor binds complement proteins C3b and C4b is interesting in light of reports of decreased plasma levels of C4b [Warren et al., 1994; 1995] and increases in the null allele of the C4b gene [Warren et al., 1991] in autism. The C4b gene is located in the MHC on chromosome 6; partial C4 deficiency and the C4b null allele are associated with an increased susceptibility to a variety of autoimmune diseases [Brai et al., 1994; Ulgiati and Abraham, 1996; Naves et al., 1998; Kawano et al., 1999]. CD46 receptor, in addition to serving as the entry site for vaccine strains of measles [Tatsuo et al., 2000], is also the entry site for HHV-6 [Santoro et al., 1999; Clark, 2000]. Increased levels of antibodies to HHV-6 and measles are positively associated with peripheral autoantibodies to CNS antigens in children with autism [Singh et al., 1998]; studies have not yet been reported regarding such associations with autoantibodies in cerebrospinal fluid.

Alterations in T cell subsets may also fit with the hypothesis that increased susceptibility to specific types of viral infections may be mediated by regulation of virus-specific receptors and of Th1 vs. Th2 immunity, and may provide a link to development of CNS-directed autoimmune responses in autism. In conjunction with reports of an increase in T cells expressing a “late activated” pattern (i.e., positive for DR or class II MHC mole-

cules, but negative for IL-2 receptor or CD25) in autism [Phioply et al., 1994], consistent with the pattern seen in several autoimmune disorders [Burmester et al., 1984; Hafler et al., 1985; Bergroth et al., 1988], levels of DR+ IL2+ T cells in children with autism are inversely correlated with plasma levels of C4b [Warren et al., 1995]. The DR beta 1 gene is located in close proximity to the C4b gene on the HLA region of chromosome 6, and is also near to genes encoding IgA and 21-hydroxylase (class III region) [Wilton et al., 1985; Brai et al., 1994; Fiore et al., 1995; Reil et al., 1997; Schroeder et al., 1998]. IgA deficiency, also noted in autism [Warren et al., 1997], is associated with the presence of autoantibodies [Brai et al., 1994; Fiore et al., 1995] and an increased incidence of overt autoimmune disease [Barka et al., 1995]. Some DR beta 1 alleles reportedly have a very strong association with autism [Warren et al., 1992; Daniels et al., 1995; Warren et al., 1996a, b], although one larger study of multiplex sibships with autism did not confirm this result [Rogers et al., 1999]. Given the presumed heterogeneity of the disorder, and the possibility that genetic loading in families with multiple affected members may be less likely to require exposure to an environmental factor (e.g., virus, bacteria, toxin, or other agent) in order to lead to the autistic phenotype, the absence of linkage in this one study does not rule out a role for an HLA-linked immunogenetic vulnerability in a subset of children with autism. Immunogenetic studies that compare subpopulations with and without evidence of immune dysfunction will be required to address this possibility.

Expression of complex neuropsychiatric diseases such as autism may require the presence of specific genes, an environmental trigger, and exposure at a particular time during brain development; all factors, genetic and environmental, must be reconstructed within a neurodevelopmental context. Thus, developmental differences in host inflammatory and neuroendocrine capacities and in rates of maturation of neurons and immune system elements [Rubin et al., 1999a] are likely to contribute to the differential susceptibility of neuronal and glial populations to pre- or postnatal inflammatory stressors (infectious, immune) and impact the phenotypic expression of autism and other neurodevelopmental disorders [Briese et al., 1999; Hornig et al., 1999].

**NEONATAL BORNAVIRUS INFECTION OF LEWIS RATS: AN ENVIRONMENTAL MODEL OF NEURODEVELOPMENTAL DAMAGE**

Borna disease virus, an atypical, neurotropic, noncytolytic, negative-strand RNA virus, is tropic for limbic and cerebellar circuitry. Infection causes a spectrum of behavioral deficits depending on the age, immune status, central nervous system maturity, and genetics of the host. In immunocompetent adult Lewis rats, infection results in meningoencephalitis, diffuse central nervous system damage, dopamine neurotransmitter disturbances, and disorders of movement and behavior [Solbrig et al., 1994]. Neonatally infected Lewis rats have only minimal, transient inflammation [Hornig et al., 1999] but nonetheless have abnormalities of hippocampal and cerebellar development [Hornig et al., 1999], growth [Bautista et al., 1994], play behavior [Pletnikov et al., 1999a], emotional reactivity [Hornig et al., 1999], socioemotional communication (preliminary data, Hornig and Lipkin), spatial and aversive learning, [Dittrich et al., 1989], locomotor activity [Hornig et al., 1999; 2000], and taste preferences [Bautista et al., 1994]. These abnormalities bear obvious similarities to the impaired social interaction and atypical responses to sensory and emotional stimuli pathognomonic of autism.

Neonatal rat infection presents an intriguing model for neuropsychiatric illness; its immunopathologic correlates are more subtle and the cerebellar and hippocampal dysgenesis observed is consistent with the neurodevelopmental abnormalities reported in autism [Kemper and Bauman, 1993], schizophrenia [Altschuler et al., 1987; Fish et al., 1992], and affective disorders [Soares and Mann, 1997]. Close parallels exist amongst the core and associated features of these psychiatric disorders and the wide range of physiologic and neurobehavioral disturbances described in neonatally infected animals; in particular, the overlap of signs of autism spectrum disorders with elements of the neonatal syndrome is especially striking.

Abnormal growth and physiologic profiles have been noted, although the mechanisms contributing to these disturbances are not known. Neonatally infected animals are stunted in growth compared to uninfected littermates as early as day 4 postinfection [Bautista et al., 1994; Carbone et al., 1991; Hornig et al., 1999] without demonstrable alterations of glucose, growth hormone, in-
sulin–like growth factor–1 [Bautista et al., 1994], or amount of food ingested [Bautista et al., 1995]. They also display altered sleep–wake cycles and heightened taste preferences for salt-containing solutions [Bautista et al., 1994]. Given reported alterations in other neuropeptide systems following neonatal infection of Lewis rats [Plata-Salaman et al., 1999], it is intriguing to speculate that disturbed salt preferences might result from dysregulation of the neuropeptide arginine vasopressin, either through direct infection of magnocellular neurons of hypothalamus or indirect effects on the magnocellular division through perturbations of mineralocorticoid responses (including potential effects of BDV on mineralocorticoid release by the adrenal glands, or on mineralocorticoid receptors in hypothalamus). Alternatively, BDV could influence salt taste preferences through effects on gustatory neurocircuity, either at the level of primary gustatory transduction, sensory transmission via the chorda tympani branch of cranial nerve VII, or processing of taste signals in the rostral portions of the nucleus of the solitary tract, where primary gustatory nerves terminate for salt sensation [King et al., 1999].

A role for a higher order sensory processing defect may be a more likely explanation than salt depletion or aberrant taste discrimination capacity given that neonatally infected animals do not differ in the amount of salt solution consumed in single bottle taste acceptance experiments [Bautista et al., 1994]; nonetheless, the alternative hypotheses have yet to be examined by neurohormonal and serum osmolality assessments and detailed neuro-pathologic analysis of neural systems regulating salt intake, including the nucleus of the solitary tract.

Whether disturbances of circadian rhythms relate to direct or indirect effects of BDV infection is similarly unclear; however, a wide variety of inflammatory stressors are known to induce sleep–wake disturbances through shifts in cytokines, prostaglandins, and body temperature [Dunn, 1993; Dunn and Swiergel, 1998; Wong et al., 1997]. In studies of the retroviruses HIV [Opp et al., 1996] and feline immunodeficiency virus [Prospero-Garcia et al., 1994] in rats, sleep architecture changes are associated with the envelope glycoprotein (gp); in the case of HIV, intracerebroventricular administration of gp 120 also increases mRNA expression for the cytokines, interleukin (IL)-1β and IL-10 in hypothalamus, a brain region crucial for sleep regulation [Opp et al., 1996]. Intriguingly, increased brain levels of mRNA coding for IL-1β have been noted by several investigators following neonatal infection of Lewis rats [Hornig et al., 1999; Plata-Salaman et al., 1999; Sauder and de la Torre, 1999], suggesting the possibility that cytokines may contribute to sleep–wake cycle abnormalities in this model as well. The potential contribution of dysregulation of autocoids and temperature regulation to the pathogenesis of the neonatal syndrome remains to be studied. Of note, many children with autism demonstrate abnormal taste preferences and sleep disturbances [Wing, 1997].

Persistent disturbances are also reported in the cognition, socioemotional behavior and communication, and motor development of neonatally BDV-infected animals. Cognitive deficits and unusual emotional reactivity were first described in Wistar rats, in which spatial and aversive learning impairments were found along with increased motor activity and decreased anxiety responses in open field testing under bright light conditions from 15 weeks postinfection [Dittrich et al., 1989]. Similar deficits in spatial learning and memory were more recently confirmed in neonatally infected Lewis rats 43 to 72 days postinfection, in association with the peak of dentate gyrus granule cell loss [Rubin et al., 1999b]. Emotional responses of adult Lewis rats infected as neonates appear to depend both on testing conditions and age at testing. Dittrich et al. [1989] found locomotor hyperactivity and absence of freezing behavior in brightly lit open field testing of 4 month old Wistar rats infected with BDV as neonates, a finding which was interpreted as indicative of abnormally low anxiety responses. However, based on a comparison of the averageness of bright and dim illumination conditions in 3 to 4 month old, neonatally infected and sham-inoculated animals, Pletnikov et al. [1996b] noted that hyperactivity of infected animals was greater in bright light conditions than in dim light, whereas the opposite pattern pertained in the control animals. Although freezing and thigmotaxis (“wall-seeking” behavior consistent with an attempt to escape from the testing apparatus) behaviors were more evident in neonatally infected animals in bright light as opposed to dim light open field testing, the mean time spent in freezing behavior during the testing period was consistently lower in infected than in control animals. The authors concluded that the increased motor activity in bright light represented hyperreactivity of neonatally infected animals to aversive stimulation (i.e., the bright light). Other indices of disturbed emotional reactivity in infected rats, including increased defecation in the brightly lit open field, were consistent with this hypothesis of increased fearfulness and drive to avoid aversive stimuli. Indeed, the more traditional measures of anxiety that were measured in this study, freezing and thigmotaxis, were actually decreased in infected animals compared to controls [Pletnikov et al., 1999b]; however, these measures are less reliable as measures of anxiety at extremely high levels, where fearfulness and flight responses are induced [Davis and Shi, 1999; King, 1999].

Additionally, during acoustic startle testing of neonatally infected animals, Pletnikov and colleagues found a dissociation between the amplitude of the startle response and autonomic reactivity: whereas infected animals had a significantly lower startle response amplitude compared to sham-inoculated rats, and both habituation and footshock sensitization of their startle responses demonstrated a normal pattern, autonomic responses (defecation) were higher [Pletnikov et al., 1999b]. These findings point to a possible disturbance in integration of signals from neural circuits involved in higher order sensory processing (glutamatergic afferents to or efferents from a critical site in primary startle response neurocircuitry, the caudal pontine reticular nucleus), anxiety/fear responses, and autonomic system outputs. Although the role of amygdala in fear-potentiation of the classic motor aspects of startle responses of infected animals appears to be intact, exaggeration of responses of the central nucleus of the amygdala to no-adrenergic, autonomic signals from locus coeruleus may be faulty [Koch, 1999], possibly accounting for the opposite effects of neonatal infection on the motor and autonomic components of the startle response. The relationship of these abnormalities of anxiety and fear responses to developmental maturity and unfolding of CNS damage following neonatal infection was not reported. In this context, we have reported a transient, abnormal response to novelty (inhibition of locomotor activity responses upon introduction to novel environment) at 4 weeks postinfection, when brain levels of mRNAs for cytokines associated with decreased exploratory responses, such as IL-1, [Dantzer et al., 1998], were at their peak [Hornig et al., 1999]; this observation is also consistent with abnormal amygdaloid responses.

Disturbances of complex socioemotional behaviors and communication
known to be altered in conditions such as autism have also been reported. Play behavior is decreased with respect to both infected animals’ initiation of nondominance-related play interactions and response to initiation of play by noninfected, age- and gender-matched control animals or by infected littermates [Pletnikov et al., 1999a]. Given the selective effects of BDV on D2 receptors in the adult infection model [Solbrig et al., 1994; 1996a,b], it is interesting to note that this receptor subtype is thought to be the site of dopamine’s effects on social play behavior [Vanderschuren et al., 1997]. Other neuromodulators thought to be disrupted in adult BDV infection of Lewis rats, including monoamines, acetylcholine, and opioids [Solbrig et al., 1995], also play a role in regulating social play [Vanderschuren et al., 1997]. Studies of regional monoamine shifts following neonatal infections are underway to determine whether neurochemical aberrations similar to those in adult-infected animals may contribute to these social play abnormalities. Nucleus accumbens and striatum, two brain areas affected in both the adult and neonatal rat models, also impact initiation of play behaviors. Furthermore, these sites receive signals from the amygdaloid nuclei, areas involved in the selection of socially appropriate responses [Vanderschuren et al., 1997] and also observed to be heavily infected after adult and neonatal BDV infection. In another intriguing parallel with a core feature of autistic disorder, that of impaired social communication, infant-maternal communication appears altered following neonatal infections [Hornig et al., 1999]. Ultrasonic vocalization distress calls induced by maternal separation are one of the earliest and most universal social communication responses to develop in mammals [Hofer, 1996; Winslow and Insel, 1990], and are normally reduced by social signals (e.g., presence of an anesthetized mother or littermate in the testing chamber) and by serotonergic agents, but increased following administration of noradrenergic reuptake inhibitors [Hofer, 1996]. In neonatally infected Lewis rats, ultrasonic vocalizations are remarkable for increased call frequency and abnormal waveforms (Hornig and Lipkin, unpublished data). The efficacy of these calls in eliciting appropriate maternal responses and their responsibility to social cues are currently being explored. Even before neuronal subsets are observed to be depleted in cerebellum, the developmental progression of motor skills and activity levels is distorted. Neonatally infected rats show asymmetric protoambulatory (“pivoting”) responses, with an increased frequency of falls into a supine position between days 4 and 9 postinfection—an unusual movement never observed in control animals. In addition, they exhibit significant delay in righting themselves on a flat surface, and explore the open-field chamber less widely through the 9th postnatal day [Hornig et al., 1999]. Such sensorimotor processing disturbances suggest early functional damage to motor circuitry, including cerebellum and striatum, and to acetylcholine systems; in addition to known infection of cerebellum and striatum in the neonatal system, adult infections have been associated with losses of choline acetyltransferase-positive fibers before the onset of encephalitis. Thus, it is conceivable that similar damage may occur to cholinergic neurons in the neonatal system, where immune cell infiltration also occurs later and is only fleeting. Given a recent report by Teitelbaum and colleagues indicating early, often transient, locomotor abnormalities in children with autism, including abnormalities of the righting response, crawling, and ambulation, [Teitelbaum et al., 1998], further examination of the pathogenesis of neurodevelopmental disturbances in this infection-based animal model is warranted.

Because the cerebellum undergoes substantial postnatal development in many mammals, it is particularly vulnerable to injury from perinatal infection with any of several viruses including Borna disease virus, mumps virus, and lymphocytic choriomeningitis virus [Monjan et al., 1971, 1973; Oster-Granthite and Herdon, 1985; Rubin et al., 1998]. The observation that many agents may induce similar patterns of damage suggests that a nonspecific mechanism may be implicated, such as induction of soluble factors triggered by viral replication.

There is substantial evidence for the role of the cerebellum in motor behavior and motor learning (for reviews see [Llinas and Welsh, 1993; Roland, 1993; Thompson and Kim, 1996]). In neonatal BDV infection, overt cerebellar dysfunction appears late and is mild to moderate in severity [Hatalski, 1996; Hornig et al., 1999]. Only 5% of neonatally infected Lewis rats had mild gait ataxia between 12 and 24 weeks postinfection, but all were impaired in skills requiring more complex coordination and balance, such as that required to maintain balance while walking across a dowel [Hornig et al., 1999]. These mod-
Honigmann et al., 1993; Bautista et al., 1994; 1995; Gosztonyi and Ludwig, 1995; Plata-Salama et al., 1999; Rott and Becht, 1995; Rubin et al., 1999a, a phenomenon ascribed to the immaturity of the rat immune system in the postnatal period. Humoral immune responses to BDV in neonatally-infected animals are also reported to be restricted, with anti-BDV antibody titers remaining below 1:10 through 133 days postinfection. [Carbone et al., 1991]. In contrast to the many published reports of persistent infection without an inflammatory response, close serial analysis has revealed transient, regionally restricted inflammation [Hornig et al., 1999; Sauder and de la Torre, 1999]. These inflammatory infiltrates emerge about 4 weeks postinfection in perivascular areas of motor and parietal cortex, with less marked infiltrates in nonhippocampal portions of temporal cortex, thalamus, and basal ganglia, and disappear completely by 6 weeks postinfection. Despite nearly complete loss of the dentate gyrus, marked loss of cerebellar Purkinje cells, and thinning of granular cell layers in cerebellum by this timepoint, no such infiltrates are present in these areas at 2, 4, or 6 weeks postinfection. Areas of inflammation are also distinct from those areas showing the greatest amount of apoptosis (periventricular germinial layer, dentate gyrus, granular layer of cerebellum). Immunohistochemical analysis of infiltrates indicates that they are comprised primarily of T cells with approximately equal numbers of CD4+ and CD8+ cells [Weissenböck et al., 2000].

Curiously, brain lymphocytes from 4 week old, neonatally infected rats do not demonstrate cytotoxic activity against BDV antigens [Hornig, Briese, Planz, and Lipkin, unpublished data], suggesting that these T cells are not specific. Furthermore, neonatally thymectomized, infected animals do not differ from sham thymectomized, infected animals in degree of dentate gyrus damage, Purkinje cell loss, or cortical atrophy, despite successful ablation of inflammation at the 4 week timepoint by the neonatal thymectomy procedure [Hornig, Stitz, and Lipkin, unpublished data]. How these presumably activated, but nonspecific T cells are attracted to, and transiently retained in selected regions of CNS and not in others is an intriguing, but unanswered question. Likely candidates for direct viral effects include cell adhesion molecules and chemokines. Levels and distribution of the cell adhesion molecules PECAM and ICAM-1, as measured by immunohistochemistry in neonatally infected and sham-inoculated animals, suggest they cannot be implicated. Staining for PECAM on endothelial cells does not differ between infected and uninfected groups, and although levels of ICAM-1 increase on endothelial and other vascular and perivascular cells of neonatal BD rats at about 4 weeks postinfection, the most prominent staining appears in hippocampus and cerebellum in addition to cerebral cortex. Thus, the regional distribution of ICAM-1 does not explain the apparent exclusion of inflammatory infiltrates from hippocampus and cerebellum at 4 weeks postinfection.

Developmental maturity of the immune and nervous systems at the time of exposure to the viral agent are critical to determining inflammatory responses and ultimate neurodevelopmental outcomes [Hornig et al., 1999; Rubin et al., 1999a]. Animals infected within the first 12 hours of life demonstrate tolerance and do not have a robust immune response to the virus [Hornig et al., 1999]. In contrast, infected animals with low titer stocks or administered virus after the first 12 hours of life may have marked inflammatory cell infiltrates and enhanced morbidity and mortality.

The manner in which infection results in loss of select neuronal subsets in this model system is not yet known. Mechanisms may be both direct and indirect. Clearly, infection alone is an insufficient signal, as many neuronal populations remain persistently infected in neonatal BD without evident reduction of cell numbers. Cells that are lost due to neonatal infection, predominantly found in cerebral cortex, dentate gyrus, and the Purkinje cell layer of cerebellum, appear to be lost exclusively through apoptosis [Hornig et al., 1999; Weissenböck et al., 2000].

Apoptotic cells, characterized by shrinkage, hypereosinophilic cytoplasm, nuclear pyknosis and karyorrhexis, and signal on transferase dUTP-biotin nick end labeling (TUNEL) assay, peaked at four weeks p.i., with most marked pathology in dentate gyrus and cortical layers 5 and 6 of retrosplenial and cingulate cortex. Although apoptosis is described in hippocampus of developing animals as late as day 7 to 10 of postnatal life, it is not found at later timepoints [Toth et al., 1998]. It is intriguing to speculate that BDV may influence the expression of age-related programs associated with normal, development-associated apoptosis through the direct or indirect elaboration of soluble factors.

We observe reactivity and proliferation of microglial cells throughout the brain to be even more striking than astrocitosis. Interestingly, microglia remain activated even at 76 week postinfection, despite the absence of any evidence of infection; in contrast, a small proportion of astrocytes do become infected, although astrocytic activation subsides between 4 and 24 week postinfection. Cerebellar size has been reported to be reduced, with evidence of reactive astrocitosis as demonstrated by glial fibrillary acidic protein (GFAP) reactivity as early as 3 days postinoculation, preceding the identification of BDV proteins in the cerebellum. Furthermore, reactivity of cerebellar astrocytes and loss of cerebellar granule cells occur without signs of BDV infection in those cell populations at all timepoints through to 30 days postinfection. Curiously, Purkinje cells appear to be the predominant cerebellar cell population demonstrating BDV antigens, although these cells were previously reported to be retained through day 30 postinfection [Bautista et al., 1995]. However, our investigations indicate that by day 42 postinfection, Purkinje cell populations are selectively depleted. Similarly, at 7 months after neonatal infection, Eisenman and colleagues report 75% depletion of Purkinje cells [Eisenman et al., 1999].

Astrocitosis and microgliosis occur in all brain regions by 3 weeks postinfection. The onset and distribution of glial cell proliferation coincides with neuronal loss yet is sustained through 24 weeks postinfection, after neuronal cell death waves. Astrocyte and microglia morphology is consistent with activation (increased cytoplasm; short, thickened processes; additionally, in microglia, more intense staining with OX42). In hippocampus, gliosis is most evident in dentate gyrus. In cerebellum, gliosis is equally prominent in white and grey matter. Curiously, microglia express MHC Class I and II, CD4 and CD8 (OX8 and CD8b) molecules on their surface [Weissenböck et al. 2000].

Astrocytes and microglia are activated in the absence of infection, be it directly by BDV or indirectly through elaboration of soluble factors by other cell types. Given the role of astrocytes in guiding migration of granule cells during cerebellar development, an assessment of the frequency of astrocyte reactivity without viral infection in conjunction with studies of apoptosis in limbic structures would aid our understanding of the relative contributions of migrational failure and programmed cell death in the evolution of BD neuropathogenesis. Even more intriguing may be the mech-
anism by which microglia are activated in the absence of infection. In neonatal HIV-1 infection, for instance, diffuse microglial activation and reactive astrogliosis occur along with impaired brain growth and developmental delays [Epstein and Gelbard, 1999]. However, in contrast to neonatal BD, microglia internalize virus in neonatal HIV-1 infection and replication can occur upon chronic exposure to proinflammatory cytokines [Janabi et al., 1998]. Two other findings regarding microglia-related injury in HIV-1 infection may be relevant to an understanding of mechanisms in BD pathogenesis: 1) production of the neurotoxin, quinolinic acid, is substantially greater in uninfected than in HIV-1-infected monocytes following lipopolysaccharide or interferon-γ stimulation [Nottet et al., 1996]; and 2) the viral protein, gp41, may act indirectly to induce neurotoxicity by triggering IL1β production in microglia, thereby stimulating iNOS production and NO generation by astrocytes [Hu et al., 1999]. General mechanisms of CNS injury following microglial activation also appear to relate to differential regulation of MHC and other molecules on microglia [Stoll and Jander, 1999]. Whether the unusual pattern of upregulation of MHC, CD4, and CD8 molecules observed on uninfected, activated microglia in neonatal BDV infection might explain the pattern of damage observed is not known.

Higher levels of message for tissue factor (TF) have been found in infected hippocampus [Gonzalez-Dunia et al., 1996]. TF is a member of the class II cytokine receptor family primarily produced by astrocytes that plays important roles in cellular signal transduction, brain function, and neural development through its effects on coagulation protease cascades. Although this may be one mechanism by which BDV may alter CNS development [Gonzalez-Dunia et al., 1996], cerebellar changes cannot be explained by this mechanism if TF upregulation is not observed in cerebellum despite prominent astrocytosis. Furthermore, BDV infection of astrocytes appears to be required for TF upregulation [Gonzalez-Dunia et al., 1996], and cerebellar astrocytes are rarely infected [Hornig et al., 1999].

One means by which a virus might disrupt neural function and development in the absence of inflammation is through the induction of neuronotrophic cytokines. Neuronotrophic cytokines comprise a burgeoning set of immunoregulatory molecules, including the hematopoietic factors (e.g., interleukins, tumor necrosis factor family, interferons), the TGF-β superfamily factors (including TGF-β1, 2, 3; GDNF), and the classic neurotrophic factors (NGF, BDNF, NT3, NT4/5). A large subset of the neuronotropic, hematolymphephotic cytokines may be roughly categorized according to their origin from one of two types of T-helper cells: Th1 (cell-mediated immunity and stimulation of antigen-presenting cells) or Th2 (humoral or B-cell mediated immunity). The potential mechanisms of cytokine-mediated damage in the context of the developing brain include: direct effects on neuronal elements; activation or suppression of second messenger/intracellular signaling pathways; induction of shifts in excitotoxical elements such as quinolinic acid or acute phase proteins such as neopterin or β-2-microglobulin; direct alterations of neuronal function (e.g., inhibition of long-term potentiation in hippocampus); activation or suppression of glial cells; or alteration of glial cell proliferation or differentiation (including expression of adhesion molecules such as the integrins) [Benveniste, 1997; Mehler et al., 1996]. Given that the postnatal expression of neuronotrophic cytokine and cytokine receptor mRNAs in brain differs for each cytokine [Benveniste, 1997], and that the sensitivity of neuronal populations to the trophic or apoptosis-inducing effects of cytokines changes during development, wide variation in the patterns of virus-induced, cytokine-related damage would be expected, depending on the relative maturity of the evolving nervous system at the time of infection. In addition, cell loss induced by either BDV or developmentally-programmed changes may alter the capacity of resident CNS cells to both produce and respond to neuronotrophic cytokines.

One of the primary mechanisms of host defense following viral infection begins with the induction of interferon-γ (IFN-γ) and other cytokines, which in turn initiate a cascade of host responses in a wide variety of cell types. In the CNS, IFN-γ modulates oligodendrocyte, neuronal and glial cell functions, and is important in activating glial cells to produce mediators of cell damage or death, including toxic intermediates of nitrogen and oxygen, and complement components [St. Pierre et al., 1996]. Viral damage to neurodevelopmental circuitry may thus parallel the production of these downstream mediators following IFN-γ induction, and provide a means by which BDV might disrupt brain cell differentiation and function without inflammatory cell infiltration.

Recent studies concerning cytokine expression during neonatal infection provide a converging view of the potential importance of cytokines as mediators of BDV-related CNS injury in neonatally-infected rats [Hornig et al., 1999; Plata-Salaman et al., 1999; Sauder and de la Torre, 1999]. Cytokine expression changes over time in different brain regions, with maximal alteration occurring at 4 weeks. Higher levels of mRNAs for cytokine products of CNS macrophages/microglia (IL-1α, IL-1β, IL-6, TNF-α) are noted in hippocampus, amygdala, cerebellum, prefrontal cortex, and nucleus accumbens [Hornig et al., 1999]. Elevated levels of these proinflammatory cytokines were first apparent at 2 weeks, peaked at 4 weeks, and then declined at 6 and 12 weeks. No alterations in other proinflammatory cytokines, including IL-2, IL-3, TNF-β, and IFN-γ, were observed. Given that production of several of these proinflammatory cytokines is unique to T cells, B cells, mast cells, and bone marrow stromal cells, and not to macrophages or microglia, these data suggest that BDV may exert a selective effect on cells of microglial or macrophage lineage.

Following neonatal infection, BDV influences the expression of apoptosis-related products. Increased levels of mRNAs coding for FAS and ICE (caspase-1), two promoters of apoptosis, and decreased mRNA for bcl-x, a factor that inhibits apoptosis, were identified in hippocampus, amygdala, prefrontal cortex, nucleus accumbens, and cerebellum [Hornig et al., 1999]. These findings are consistent with promotion of apoptosis throughout the brains of rats neonatally infected with BDV by at least two strategies. A host of excitants or neurotoxins including arachidonic acid, platelet-activating factor, free radicals (NO, O2·−), glutamate, quinolinate, cysteine, cytokines (TNF-α, IL-1β, IL-6, amines, and as yet unidentified factors arising from stimulated macrophages and possibly reactive astrocytes may also influence apoptosis by excessive activation of N-methyl-D-aspartate (NMDA) receptors [Lipton, 1996]. Interestingly, Gosztonyi and Ludwig [1995] have proposed that the targeted pathology of BDV for two hippocampal cell layers, stratum oriens and stratum radiatum, may be due to their rich concentration of glutamate and aspartate receptors, and preliminary results from our laboratory indicate regional upregulation at 4 weeks postinfection of a non-NMDA glutamate receptor, the calcium-permeable AMPA receptor, GluR1 (Hornig and Lipkin,
unpublished data). Consistent with region-specific findings of apoptosis by TUNEL analysis, this upregulation is most evident on granule cells in dentate gyrus and at synapses of cells in molecular cell layer of cerebellum that terminate on Purkinje cells (presumably, Bergmann glia or basket cells).

Withdrawal of selected neurotrophic factors can also contribute to apoptotic losses [Takei et al., 1999]. In contrast to findings of diffuse alterations in gene expression for cytokines described above, changes in neurotrophic factor mRNAs are restricted to hippocampus. Decreased mRNA coding for BDNF and NT3 is prominent in hippocampus by 4 weeks, but is still evident by 12 weeks postinfection. Although decreased NT3 mRNA may reflect loss of the granule cell population in dentate gyrus, the role of BDNF in maintaining viability of cells suggests that its downregulation may be a more essential step in neonatal BDV pathogenesis. Nonetheless, if BDNF withdrawal is a potent influence on apoptosis, it is difficult to explain the abrupt dropoff in apoptotic losses in dentate gyrus after 5 to 6 weeks postinfection. Furthermore, this mechanism is unable to account for cell losses in cerebellum at any timepoint as BDNF is not expressed at substantive levels in normal cerebellum.

The epidemiology of Borna disease virus and its role in human disease remain controversial [Hataliski et al., 1997; Stae
deli et al., 2000]. Similaties between some behaviors observed in neonatally infected rats and in autistic children led to the hypothesis that the virus might be implicated in pathogenesis of autism. Although neither serologic nor molecular data support a role for Borna disease virus as an etiologic agent in autism [Hornig et al., 1999], multicenter studies are underway to assess whether it may be implicated in pathogenesis of autism. Additional research should focus on dissecting the specific neural cells and circuits. Future work should focus on dissecting the mechanisms of developmental neuropathology in animal models and using clues derived from these more simple systems to target infectious disease investigation.

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