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Exploring the cerebellum with a new tool: neonatal Borna disease virus (BDV) infection of the rat's brain

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Cerebellar pathology has been associated with a number of developmental behavioral disorders, including autism spectrum disorders. Despite the fact that perinatal virus infections have been implicated in neurodevelopmental damage, few animal models have been developed to study the pathogenesis involved. One of the most interesting *in vivo* models of virus-induced cerebellar damage is the neonatal Borna disease virus (BDV) infection of the rat brain. The present review describes molecular, cellular, neuroanatomical, neurochemical and behavioral features of the BDV model and also provides a basis for a new understanding of the pathogenic mechanisms of cerebellar malformation and associated behavioral deficits.

Keywords:
Borna – cerebellum – developmental disorders – animal models

Pletnikov MV, Rubin SA, Moran TH, Carbone KM.
Exploring the cerebellum with a new tool: neonatal Borna disease virus (BDV) infection of the rat's brain.
Cerebellum 2003; 2: 62–70

Introduction

The cerebellum has long been considered the motor/balance/posture coordinator. This view of the cerebellum has expanded over the past decade and we now know that it is a far more complex structure also involved in the regulation of emotions, language and cognition.[1,2] Cerebellar pathology has been described in patients with schizophrenia,[3,4] autism,[5,6] and attention deficit/hyperactivity disorder,[7] suggesting that some mood and cognition deficits could be due to cerebellar abnormalities.[2,8] However, it has been extremely difficult to attribute particular pathology of the cerebellum to distinct symptoms and syndromes of autism or schizophrenia since these disorders of neurodevelopment, make it impossible to sort out primary and secondary alterations in the abnormally developing human brain.[8]

Animal models are valuable to better understand the pathogenesis of abnormal maturation of the cerebellum and effects of cerebellar injury on the other areas of the brain, including structural and functional changes in brain regions intimately connected with the cerebellum.[9,10] Numerous animal models of early cerebellar damage have been developed using genetic, lesion and neurotoxic manipulations.[11–14] However, relatively few animal models of cerebellar damage have undergone substantial characterization despite the fact that perinatal virus infections have been associated with abnormal brain maturation and resultant neurological and behavioral deficits.[15–17]

Received 29 April 2002; Revised 8 August 2002; Accepted 21 August 2002

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Virus infections and the cerebellum

The cerebellum continues to develop postnatally in humans and animals.[9] During the early postnatal period, the normal cerebellum undergoes a dramatic increase in size and acquires its foliage and laminar organization.[9] This is accompanied by an intense proliferation and radial migration of cerebellar granule cells, from the external germinal layer (EGL) towards the internal granule layer (IGL). Although Purkinje cells (PCs) are generated between embryonic days 13 and 15, significant maturation of PCs (e.g., dendritic tree development) still occurs postnatally.[9] The postnatal maturation of the cerebellum renders it particularly vulnerable to harmful effects of perinatal virus infections.[9,15,18]

Many viruses are capable of infecting the cerebellum in both natural and experimental infection.[19–22] In many of these cases, little to no cerebellar pathology results. However, in cases of lymphocytic choriomeningitis virus (LCMV), parvovirus and neonatal Borna disease virus (BDV) infection, cerebellar pathology is clearly evident.[23–25]

The features and pathogenesis of the cerebellar damage caused by these viruses differs substantially. In mice or rats inoculated with LCMV,[23,26] the cerebellar astrocytes and Bergman glia are the first cells to become infected followed by secondary infection of the PCs and cells of the EGL and IGL. Within two weeks post

inoculation (p.i.), a rapidly progressive $CD8^+$ T-cell mediated destruction of the cerebellum ensues. In natural and experimental infection of numerous species with parvovirus,[15,24] there is a selective destruction of the EGL prior to postnatal migration, leading to abnormal foliation and a nearly total disappearance of the IGL. No other cell types become infected. Unlike the cerebellar damage induced by LCMV, that induced by parvovirus is due to direct virus mediated cell lysis.[15]

In contrast to LCMV and parvovirus infections, neither immune-mediated nor direct viral lysis appear to contribute to BDV-induced cerebellar injury.[25] In this review, we critically evaluate the existing data about possible mechanisms of cerebellar damage in BDV-infected rats and indicate future directions in studying the pathogenesis of BDV-induced cerebellar abnormalities.

Borna disease virus

BDV, the prototype of the family *Bornaviridae,* is an enveloped spherical virus carrying an 8.9 kb single-stranded, non-segmented RNA with negative polarity that replicates in the nucleus.[26] BDV is a natural pathogen in a wide range of mammalian and avian species, from rhesus monkeys to ostriches.[27] Although it is possible that in some hosts BDV infection may not be associated with inflammatory responses, in most naturally infected animals, BDV causes a sporadically occurring progressive immune-mediated viral encephalomyelitis with striking behavioral disturbances also known as Borna disease (BD). Despite a very robust host immune response, BDV causes persistent infection of neurons and astrocytes, i.e. no viral clearance.[27] *In vitro*, BDV also replicates persistently and noncytolytically.[28] While there have been reports on prevalence of anti-BDV antibodies in psychiatric patients and on the isolation of viral RNA from postmortem brain tissue, a putative role of BDV infection in the causation of human neuropsychiatric disorders remains controversial.[29]

Neonatal BDV infection

In contrast to classical BD, experimental intracerebral injections of BDV to newborn, immunologically immature Lewis rats leads to selective neurodevelopmental abnormalities with distinct behavior deficits in the absence of the destructive global inflammatory responses (encephalitis and meningitis) seen in rats inoculated as adults.[30,31]

Narayan et al. and Hirano et al. were the first to describe the non-inflammatory BDV brain infection in neonatal Lewis rats.[30,31] Later, Carbone et al. characterized several hallmark brain abnormalities of neonatal BDV infection.[32] Recently, neonatal BDV infection has been recognized as a model of human neurodevelopmental disorders[25,33] in which a perinatal injury to the developing CNS appears to play a leading pathogenic role.[34,35]

Intracranial injection of the infectious virus in a newborn rat pup within first 24–48 hours after birth is the most common way of inducing neonatal BDV infection in rats. Neonatally BDV-infected rats exhibit no overt signs of the CNS infection, such as malaise, paresis, aggressiveness, and anorexia.[30–32] Infected rats have normal body shape and proportion but are overall smaller than uninfected control pups.[32,36] Differences in the overall body size and weight begin to emerge as early as at day 12 p.i.[37] The basis for runting is unclear. Notably, levels of glucose, growth hormone, and insulin-like growth factor-1 have been found to be unaltered,[36] and the amount of food consumed by neonatally BDV-infected and uninfected rats over the 24-hour period were similar.[38]

BDV-induced neuropathology

Several days after infection, viral antigens and RNA can be found in neurons of discrete brain regions, most notably in the olfactory bulb, the hippocampus (CA3 and CA4 areas), the frontal cortex and the cerebellum (cortex and nuclei).[32,38,39] By the end of the third postnatal week, viral antigens are detected in neurons of most brain regions. Following infection of neurons, viral antigen is also found in glial cells. Astrocytes, oligodendrocytes, ependymal cells, and Schwann cells in the peripheral nervous system all express BDV markers.[32,40] The temporal dissociation between neuron and glial cell infection suggests that glial cells may become infected due to a release of progeny virus by infected neurons. For example, Bergmann glial cells in the cerebellum become infected only after prior infection of PCs.[38] Furthermore, the hypothesis that glial cells are secondarily targeted by BDV may also explain a greater number of infected neurons compared to that of infected glial cells.[33,41] Notably, no infection has been detected in brain microglial cells, epithelial cells of choroids plexus or endothelial cells, consistent with the demonstration that macrophage cell lines are apparently resistant to BDV infection.[41] In the late stages of neonatal infection, BDV spreads centrifugally by anterograde axonal transport and infects most inner organs innervated by peripheral or autonomic nerves.[27] This invasion of BDV in extraneural tissues of neonatally BDV-infected rats has been attributed to the absence of neutralizing antibodies that may limit spreading BDV infection outside the CNS.[42]

Although BDV exhibits a noncytolytic strategy of replication *in vitro*,[28] neonatal BDV infection selectively damages brain regions undergoing substantial postnatal development, including the cerebellum, the dentate gyrus (DG) of the hippocampus and the neocortex.[25]

Cerebellum

Cerebellar injury is one of the most salient morphological features of neonatal infection (Figure 1). Interestingly, despite an overall decrease in the size, BDV-infected cerebellum appears to retain normal foliation and laminar organization.[41,43] In the cerebellum, Purkinje cells (PCs) are the predominant, if not only, cells harboring the viral markers. Viral protein expression can be seen in PCs as early as day 3 p.i.[38,41,43] In contrast to LCMV and parvovirus infections, neonatal BDV infection induces a prominent loss of PCs, with up to 75% of PCs dropping out by seven months p.i.[43] Although the exact timing of PCs loss is still unclear, one study has estimated the onset to be between days 27 and 33 p.i.[44] A loss of PCs and their dendrites in the molecular layer (ML) has been suggested to play a major role in markedly reducing cerebellum size.[41,43]

Unlike LCMV and parvovirus, BDV does not appear to infect cerebellar granule cells. Nevertheless, BDV-associated loss of granule cells has been suggested.[38,41,44] Interestingly, granule cells death may depend on the time of infection. For example, BDV infection at postnatal day 15 did not cause damage to the IGL.[45] Since PCs still probably die under those conditions, less damage to the granule cells in rats infected with BDV at postnatal day 15 appears to be in line with findings that survival of granule cells depends on PCs integrity primarily during an early critical period.[46,47] Indeed, studies on mutant pcd mice, mutant shaker Sprague-Dawley rats and spastic Han-Wistar rats have demonstrated that late dropout of PCs has little impact on granule cell numbers.[14,48–50]

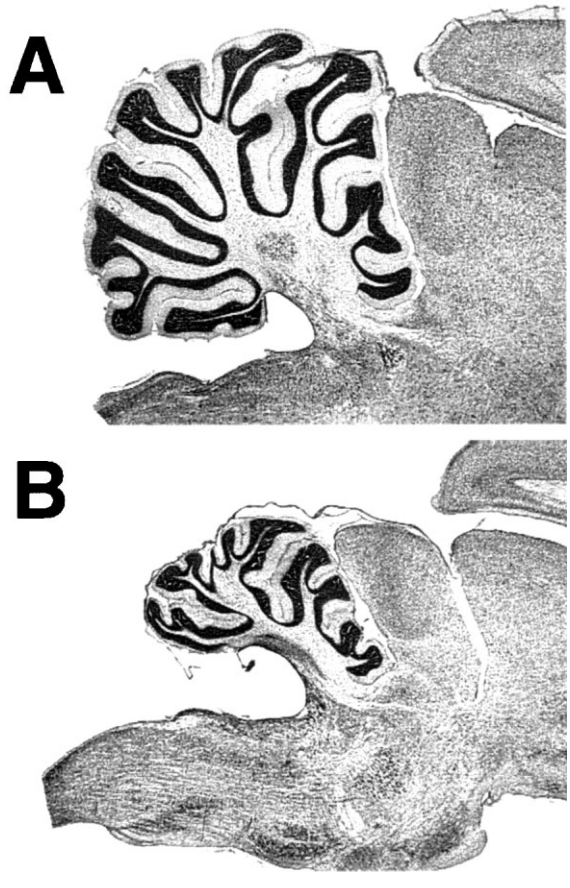

Figure 1
Neonatal BDV infection-induced damage to the cerebellum. Representative sagittal brain sections from sham-inoculated (A) and neonatally BDV-infected (B) rats at postnatal day 30, stained with cresyl violet. Note reduced cerebellar size in the BDV-infected rat (B). Original magnification ×12.5.

Other brain regions affected by BDV

In addition to damage to the cerebellum, neonatal BDV infection affects the postnatal maturation of the hippocampus. In the hippocampus, the virus primarily targets neurons of the CA3 and CA4 areas.[27] Curiously, despite this high replication of BDV, there is little overt damage to the neurons in the CA subfields. In contrast, BDV infection of dentate gyrus (DG) neurons is associated with their continuing loss and eventual complete disappearance by day 45–55 p.i.[32,33,44] Since the vast majority of granule neurons of DG are generated after birth, the proliferating activity of these cells may explain their selective vulnerability to the virus.[51]

Neonatal BDV infection also induces cortical shrinkage.[41,52] A recent report has shown that up to 30% of cortical neurons are lost in BDV-infected rats by 45 days p.i.[52] A selective decrease in neurons with a diameter over 100 μM and with positive immunostaining for parvalbumin suggest that both pyramidal and GABA-ergic neurons may be particularly vulnerable to neonatal BDV infection.

As neurogenesis in the olfactory bulb continues well into adulthood in rats,[53] and as BDV infects neurons of the olfactory bulb,[33,41] it is conceivable that an appreciable cell loss might be found upon a closer examination. Thus far, however, there have been no documented observations of BDV-induced damage to the olfactory bulb.

Putative mechanisms of BDV-induced cerebellar injury

The precise mechanisms of virus-induced neuronal loss in the cerebellum remain unclear. Both direct and indirect effects of virus infections could lead to neuronal death. Figure 2 presents a hypothetical view of pathogenic events mediating neuronal loss in BDV-infected cerebellum.

There have been several reports on possible mechanisms by which BDV could directly affect normal neuronal function and trigger cell death. For example, BDV may directly alter responses of neurons to trophic factors crucial for their survival.[54] BDV has been found to block neurite outgrowth in neuron-like PC12 cells in response to nerve growth factor (NGF). The lack of response to NGF has been associated with down-regulation of NGF

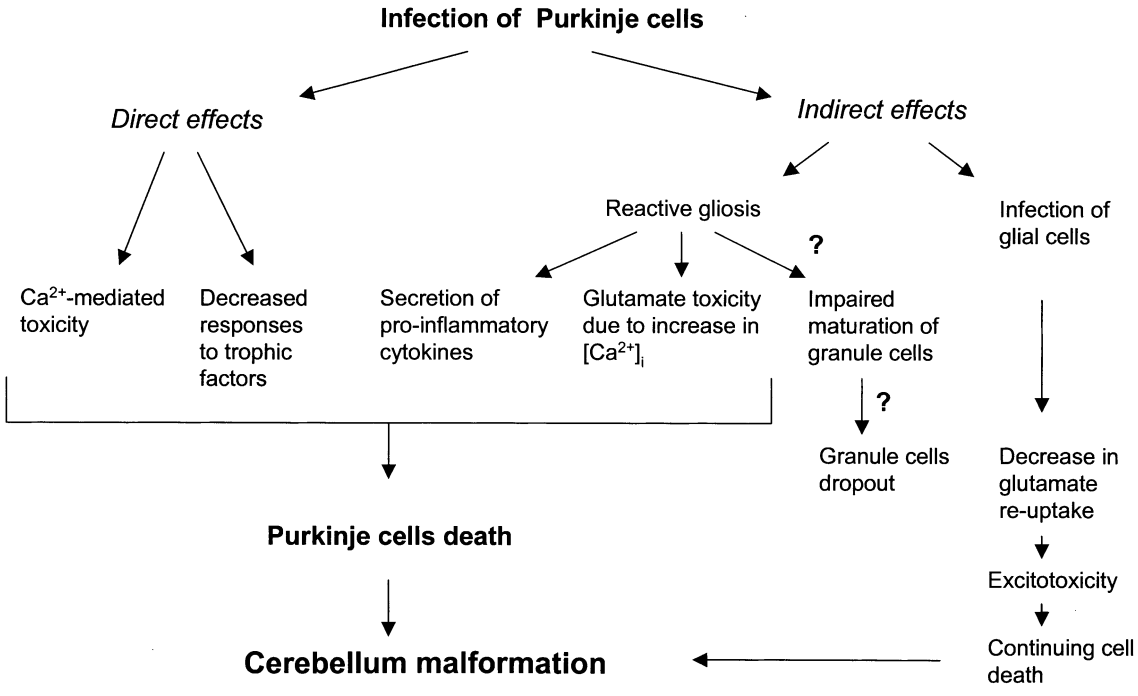

Figure 2
A proposed model outlines pathogenic processes involved in BDV-associated cerebellar injury. The observed cerebellar developmental abnormality is largely believed to be the result of both direct and indirect effects of BDV-infection of the Purkinje cells. In the case of direct effects, both calcium mediated toxicity and altered responses to trophic factors may occur and can lead to Purkinje cell death. Purkinje cell death can also be the consequence of indirect effects of BDV-infection. For example, BDV-infected Purkinje cell-mediated gliosis and glial infection can lead to secretion of pro-inflammatory cytokines, glutamate toxicity and excitotoxicity, all of which may be responsible for Purkinje cell loss. In addition, there is some evidence that granule cell loss resulting from impaired maturation (e.g., suppressed proliferation and/or migration) may also contribute to the cerebellar malformation.

receptors and inhibition of translocation of activated extracellular signal-regulated kinase (ERK) to the nucleus in cells.[54] Similarly, virus-associated reduction in expression of mRNAs coding for neurotrophin receptors, TrkB and TrkC, has been noted in BDV-infected cerebellum three weeks p.i. and later.[44] Interestingly, BDV-induced down-regulation of neurotrophin receptors precedes the onset of a PCs loss, suggesting a putative role of neurotrophin disturbances in BDV-induced cerebellar damage.[44] Additionally, BDV may directly interfere with normal brain maturation by binding of the virus phosphoprotein (P) to amphoterin, a 30-kDa neurite outgrowth factor abundantly present in the developing brain[55] and important in cell process outgrowth and migration activity.[55]

Other plausible targets of direct virus effects could include synaps and the axonal transport machinery because BDV appears to spread along nerve fibers and synaptic contacts.[56] In this way, BDV might directly affect supply and trafficking of physiologically active compounds, e.g., growth factors. For example, a progressive decline in expression of growth-associated protein 43 (GAP-43, a presynaptic membrane phosphoprotein that accumulates in neuronal growth cones) and synaptophysin (SYN, a 38 kDa calcium-binding protein present in the membranes of presynaptic vesicles) has been noted in the brains of BDV-infected rats.[52] Thus, these data suggest that BDV may directly impair crucial neuronal functions, and, in doing so, might either directly initiate cell death or render infected neurons highly susceptible to adverse effects of factors secreted by neighboring astrocytes and microglia cells.

Proliferation and activation of astrocytes and microglia, a common hallmark of neuronal damage, are seen in neonatally BDV-infected rats.[57] A significant increase in immunoreactivity of an astrocyte-specific marker, the glial fibrillary acidic protein, is seen as early as at day three p.i.[38] followed by continued astrocytosis in the areas of greatest neuronal damage, i.e., the cerebellum, DG of the hippocampus and the neocortex.[32,39] Intense microglial activation is also noted in BDV-infected brain.[41,58] Activated astrocytes and microglia are known to secrete soluble factors that may exert adverse effects on neurons.[57] BDV-associated activation of these cells, important participants in the CNS's resident immune system, leads to secretion of pro-inflammatory molecules, and exemplifies putative indirect mechanisms of virus-related brain injury.

Several groups have demonstrated alterations in the levels of cytokine gene expression in brains of neonatally BDV-infected rats.[33,58,59] Localization of increased expression of pro-inflammatory cytokine mRNAs to sites of major neuronal loss supported the hypothesis that pro-inflammatory soluble factors may be responsible for

BDV-associated neuronal damage. A sustained up-regulation in expression of RNAs for chemokines IP-10 and RANTES and chemokine receptors, CCR5 and CX_3CR1 has been also demonstrated in brains of BDV-infected rats as early as at day 14 p.i.[60,61] Elevated mRNA levels for IP-10 have been shown to be particularly conspicuous in cerebellar astrocytes and the Bergmann glial cells, suggesting that they may serve as an astrocytic alarm signal by which immune cells are recruited for the elimination of infected neurons (e.g., PCs).[60,61] Alternatively, recent reports have indicated that in addition to their participation in immune responses, chemokines could be also involved in the mechanisms of normal brain maturation.[62] Thus, by altering normal function of cytokines/chemokines pathways in the developing brain, BDV could produce neuronal damage.

A pathogenic role of astrocytosis and microgliosis in BDV-associated injury can be further highlighted by comparing two strikingly different outcomes of BDV infection in rats and mice. Unlike the profound neurodevelopmental damage in BDV-infected rats, BDV infection in some strains of mice is not associated with appreciable brain abnormalities.[63,64] Although the reasons for such discrepancies remain obscure, it is important to note that the absence of histopathological changes in C57BL/10J- β2m mice with a targeted disruption of the β2-microglobulin gene and the lack of CD8+T cells is associated with minimal, if any, up-regulation of pro-inflammatory cytokines (e.g., TNF-α and IL-1β).[63,64] These data suggest that an attenuated or absent cytokines response might explain the lack of frank brain alterations in some strains of BDV-infected mice.

Little is known about the exact mechanisms of PCs death. Although PCs have been proposed to undergo apoptotic elimination, these data are not universally accepted.[33,44] Also, there is no information on what particular features of PCs may be responsible for their enhanced sensitivity to effects of neonatal BDV infection. PCs are GABA-ergic neurons,[12,46] and GABA-ergic neurons of the neocortex and hippocampus have been shown to be particularly vulnerable to external insults, including virus infections.[65] Thus, it has been suggested that specific characteristics of GABA-ergic neurons as high concentrations of calcium-binding proteins predispose PCs to calcium-mediated toxicity.[41] BDV-associated calcium-mediated toxicity might be related to BDV-induced alterations in $[Ca^{2+}]_i$ in astrocytes. If BDV-stimulated increase in $[Ca^{2+}]_i$ propagates as Ca^{2+} waves through gap-junctionally coupled astrocytes,[66] it could lead to glutamate release and resultant excitotoxicity.[67]

While glia cells appear to become infected after neuron infection occurs (e.g., 30 days p.i.),[33,38,58] BDV infection of astrocytes might also play a role in a continuing neuronal loss, especially at later time points when expression of cytokines and chemokines appears to subside.[33,38] For example, BDV-associated decrease in astrocytic re-uptake of glutamate might contribute to processes of excitotoxicity.[68]

The mechanisms of granule cell loss in BDV-infected rats remain undetermined. A loss of trophic support from infected/dying PCs is one possible pathogenic factor,[46] aborting the development of normal numbers of granule cells within weeks of infection. In addition, there is a suggestion of continuing death of granule cells beyond initial cerebellar development, as one study reported a 2–6-fold increase in numbers of apoptotic granule cells between days 14 and 48 p.i. in BDV-infected rats compared to control animals.[44] Similar to PCs death, apoptotic elimination of granule cells might be a result of glutamate-mediated toxicity.[65,67]

Neurochemical alterations and cerebellar dysfunction

The cerebellum receives monoaminergic innervation from neurons within the reticular formation.[69,70] Serotonin (5-HT) and norepinephrine (NE) modulate the firing rate of PCs *in vivo* and *in vitro*.[69,70] Neonatal BDV infection has been shown to significantly increase concentrations of 5-HT and NE in the damaged cerebellum.[71] Increased tissue content for 5-HT and NE has been proposed to result from monoamine hyperinneravtion of the hypoplastic cerebellum.[71] A similar phenomenon has been described for neurochemical alterations induced by methylazoxymethanol acetate (MAM), a compound that kills proliferating neurons and leads to hypoplasia of a brain region, e.g., cortex.[72] Since MAM-induced hypoplasia of the cortex does not affect ingrowing monoamine afferents, relative hyperinnervation of the region with increased monoamine levels ensues, i.e., the same amount of monoamine fibers in a smaller region.[72] By the same token, smaller cerebella in BDV-infected rats may receive abnormally increased monoamine innervations as well. Functional outcomes of monoamine hyperinnervation remain unclear. Hyperinnervation might lead to over-stimulation of postsynaptic 5-HT and NE receptors and might affect the regulatory actions of the cerebellar cortex on neurons in the brainstem and spinal motor centers.[69,70]

Although hormones have been shown to exert powerful influence on postnatal development of the brain,[73] effects of neonatal BDV infection on different hormonal systems have not been investigated. One study reported no virus-associated changes in levels of growth hormone and insulin-like growth factor-1.[36] Weissenbock et al.[41] found no histological alterations in adrenals of neonatally BDV-infected rats, suggesting no overt adrenal damage.

BDV-associated behavioral deficits

In the rat, cerebellum-controlled behaviors have a discrete, organized pattern of evolution, paralleling the physical development of the cerebellum. For example,

the ability to maintain static quadruped posture precedes the ability to maintain quadruped posture during movement, which is followed by the acquisition of simple motor skills.[74] Thus, studying the development of motor and balance skills in neonatally BDV-infected rats across the postnatal period allows for an analysis of possible causative relationships between unfolding pathological events and the expression of particular behavioral alterations. Recently, a developmental time course of sensorimotor deficits in developing BDV-infected rats (postnatal days 4–30) has been described.[37]

Significant effects of neonatal BDV infection on the motor development were first observed at the end of the second postnatal week, a time when infected rat cerebella begin to show typical BDV-induced developmental injury.[33,38] Importantly, BDV-associated sensorimotor impairments were not observed across all tests in developing rats. For instance, there were no differences between the two groups in the vibrissae placing and twine climbing tests, suggesting that certain mechanisms of exteroceptive sensation remained unaffected by the virus infection.[14] The deficient performance of neonatally BDV-infected rats in placing, grasping, bar holding tests and in their ability to hang on a dowel may indicate developmental alterations in the proprioceptive system[75] Furthermore, observed deficits in righting and negative geotropism appear to be associated with abnormal maturation of the ability to integrate tactile and vestibular stimuli and/or damage to the central vestibular system.[76] Testing the acoustic startle responses in BDV-infected rats revealed signs of affected auditory-somatosensory integration as evidenced by decreased within-session habituation of the acoustic startle response.[14] Most dramatic BDV-associated deficits have been noted in the tests that required the extensive use of hind limbs and balance, i.e., the bar holding and bar crossing tests. Notably, hind limb weakness and the lack of hind limb coordination have been consistently found in animals with cerebellar growth retardation and/or cerebellar injury.[77,78]

When compared to other natural and experimental teratogens affecting the cerebellum development, neonatal BDV infection produced both similar and specific behavioral abnormalities.[10,18] Similar to effects of neonatal BDV infection, neonatal cerebellumectomy results in deficient righting and geotaxis responses. In contrast, placing reactions were significantly affected in BDV-infected rats but remained intact following hemicerebellumectomy.[11,73] With respect to impairments in placing and grasping, neonatally BDV-infected rats were similar to shaker mutant rats with degeneration of PCs.[14] It is tempting to speculate that a continuing loss of PCs in the both conditions might contribute to comparable disease outcomes. Alternatively, since PCs become infected with the virus as early as at day 3 p.i.,[38] impairments in some sensorimotor behaviors could be due to functional deficits in PCs long before those neurons die.[79]

Our data have indicated that neonatal BDV infection may be associated with a delayed expression of some sensorimotor abnormalities. The acoustic startle response is a case in point. While the magnitudes of the acoustic startle response have been found to be similar between uninfected and infected rats at day 17 and 23 p.i., later on, BDV-infected animals demonstrated significantly lower startle responsiveness compared to control rats. This late appearance of an abnormality may represent a phenomenon known as "growing into a deficit" wherein some motor behaviors develop normally despite damage but become defective during subsequent development.[80] Delayed alterations in the startle responses might be explained by continuing dropout of PCs.[41,43]

In addition to the motor and balance abnormalities, BDV-infected rats demonstrate various deficits in circadian rhythm and sleep cycle,[36] emotional responses,[81,82] learning and memory abilities,[82,83] and social behaviors.[84] It is tempting to speculate that locomotor hyper-reactivity to novelty and abnormal social (play) behavior in one-month-old infected rats might be largely associated with BDV-induced damage to the cerebellum .[38,84] However, even if histological signs of BDV-associated injury are not clearly present in one-month-old rats, one cannot completely rule out a contribution of functional abnormalities in hippocampal and cortical circuitries due to BDV infection, leading to some emotional and cognitive deficits.

Perspectives

Our understanding of the mechanisms underlying BDV-induced developmental damage to the cerebellum remains preliminary. There are several important issues that need to be addressed. The exact timing of PCs dropout as well as characterization of the types of neurons that are affected needs more rigorous quantitative analyses.

An observation that BDV-induced neuronal loss in the cerebellum is characterized by a regional pattern[38] is also worth exploring. One could hypothesize that the phenomenon of regional vulnerability may be associated with a differential connectivity of different cerebellar regions with the frontal brain regions where BDV infection first occurs that provides the virus with a variable access to cerebellar areas.

The molecular mechanisms of virus-induced neuronal death also remain unclear. Identification of glial cells-secreted toxic factors and the temporal analysis of their expression in relation to neuronal death appear to be a fruitful area for future research. In this context, reductionistic approaches in organotypic and/or cell culture systems, allowing for direct examinations of effects of chemokines and cytokines, will be especially fruitful.

Although PCs have been proposed to die via apoptosis, the available data are not conclusive. Notably, while apoptosis in BDV-infected brain significantly declines by the end of the first month,[33] PCs continue to die

thereafter,[43] suggesting that other processes may be involved. The mechanisms underlying the selective vulnerability in PCs require further study as well, including exploration of the role of calcium-binding proteins in BDV-induced PCs dropout. One of the promising approaches to address the complexity of virus-neuron interaction could include powerful tools of genomics and proteomics, helping identify genes and their protein products that mediate neurons' responses to virus invasion and neuronal adaptation to virus replication.

Given the important role of hormones in neurodevelopment,[73] future investigations are clearly required to explore effects of potential BDV-induced endocrine disturbances on survival of neurons in the cerebellum.

A putative role of alterations in nutritional conditions needs further clarifications as well. Albeit a cumulative 24-hour food intake remained unchanged in neonatally BDV-infected rats when tested as adults,[38] BDV infection is associated with the inhibition of the body weight gain in developing rats. There may be several reasons for inhibition of weight gain in infected rats. One could include increased energy expenditure as suggested by an elevated food intake in BDV-infected rats when their food intake was calculated in relation to their body weight.[38] Also, decreased nutrients absorption in infected gastrointestinal tract may be a factor. In addition, abnormal suckling behavior in BDV-infected rats may lead to attenuated weight gain as well. Thus, there is a possibility that neonatal BDV infection produces postnatal malnourishment that per se can lead to cerebellar damage and behavioral deficits,[85] indicating a need in direct comparisons of effects of postnatal malnourishment and BDV infection.

From a broader perspective, neonatal BDV infection can serve as a valuable model for addressing more general issues in the pathogenesis of neurodevelopmental damage. Specifically, a role of host factors in differential vulnerability to environmental insult could be evaluated by comparing extent and features of BDV-associated cerebellar abnormalities in different genetically characterized strains. For example, recent experiments in mice and gerbils have demonstrated great variability in the neurobehavioral disease in neonatally BDV-infected animals of other species, suggesting the species-specific pathogenic mechanisms.[86–88] BDV-induced cerebellar damage has been mostly studied in Lewis rats. However, cerebellar pathology could be also evaluated in other rat strains that are characterized by greater or less sensitivity to environmental teratogens. This approach may uncover particular traits that are responsible for different pathological and neurological outcomes of the same virus infection.[89]

In conclusion, neonatal BDV infection of the brain provides an important model for the investigation of multidisciplinary mechanisms of neurodevelopmental damage and its functional outcomes.

Acknowledgement

The study was supported by the NIH grant RO1 MH48948-08A1.

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