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Research report

Neonatal Borna disease virus infection (BDV)-induced damage to the cerebellum is associated with sensorimotor deficits in developing Lewis rats

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Abstract

Neonatal Borna disease virus (BDV) infection of the brain produces developmental damage to the cerebellum in Lewis rats, with minimal classical inflammatory responses. In the present study, we assessed the consequences of this damage by measuring motor coordination and postural skills in developing (postnatal days 4 to 30) Lewis rats that were neonatally infected with BDV. Neonatal BDV infection-induced motor impairments were selective and correlated with the time course of BDV damage to cerebellar development. BDV-induced motor deficits were not seen until the end of postnatal week 2. By postnatal week 3, BDV-infected rats had deficits in negative geotropism, fore- and hind limb placing and grasping. BDV-infected rats also exhibited deficits in the ability to hold on to a bar and to cross a suspended bar. Neonatal BDV infection induced impairments in the acoustic startle response. Compared to controls, neonatally BDV-infected rats exhibited attenuated habituation of the acoustic startle at postnatal day (PND) 23 and decreased startle responsiveness at PND 30. Prepulse inhibition of the acoustic startle remained unaltered in BDV-infected rats. The data demonstrate that neonatal BDV brain infection of rats can be a valuable animal model system for studying the relationship between abnormal brain development and resultant behavioral deficits. Further studies of this model may elucidate specific pathogenic mechanisms that that may have implications in the study of neurodevelopmental human disorders.

Theme: Disorders of the nervous system

Topic: Developmental disorders

Keywords: Borna; Cerebellum; Motor coordination; Development; Rat

1. Introduction

A number of human psychiatric diseases are believed to result from early brain injury following exposure to various teratogens [20]. Perinatal virus infections, as teratogens, have been long associated with abnormal brain development and resultant neurological and behavioral disorders [21,57]. In most cases, different physiological and behavioral alterations were associated with global brain damage produced by inflammation reactions, encephalitis and/or meningitis [21]. In a few cases of virus infection, behavioral abnormalities have been linked to selective brain injuries, allowing the investigators to suggest causative links between localization of the brain damage and observed behavioral disorders. For example, herpes simplex virus infection of the temporal lobe of the brain has been found to result in personality disorders [34], or vesicular stomatitis virus infection of rat neurons has been associated with motor abnormalities [30].

Borna disease virus (BDV) infection of the neonatal rat

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brain is one of those rare viruses that is able to cause abnormal brain development with minimal signs of classical inflammatory reactions in the central nervous system (CNS) [7,8,22,33]. Thus, BDV infection of neonatal rats provides a valuable model for investigations of the causative relationship between virus-induced localized brain damage and associated behavioral deficits [7,8,23].

BDV is a 8.9-kb atypical, neurotropic, non-segmented, negative-strand, enveloped RNA virus in the unique Bornaviridae family [11,29,45]. In tissue culture, BDV replicates persistently and noncytopathically, i.e., non-lytic infection. Natural hosts of BDV include horses, sheep, dogs, cats, rabbits, and ostriches [45]. BDV has been associated with several psychiatric diseases and recently has been recovered from human blood cells and human brain [3,40,47,52].

As an experimental teratogen, BDV is most commonly studied in intracranially inoculated Lewis rats, where a persistent infection of neurons and astrocytes ensues [7,18,22,33]. Lewis rats infected with BDV as neonates appear grossly normal, yet selective, measurable and reproducible signs of behavioral abnormalities, including locomotor hyperactivity, abnormalities in spatial learning and memory, and social interactions have been reported [4,5,12,23,41,42]. These behavioral abnormalities may be linked to virus-induced developmental damage to the cerebellum (hypoplasia, loss of the granule and Purkinje cells) [5,13], hippocampus (degeneration of the dentate gyrus granule cells) [8,23], and neocortex [54].

The cerebellum undergoes substantial postnatal maturation [2] and is particularly vulnerable to neonatal teratogens, including virus infection [16,32,36]. Although BDV-induced abnormal development of the cerebellum has been described [5,23], there is little information on resultant deficits in motor behaviors. Although horizontal and vertical locomotor hyperactivity [4,23,42], mild gait ataxia, mild hind paw spasticity and a deficient ability to hang on the dowel have been described in neonatally BDV-infected rats when tested as adults, i.e., 12–76 weeks of age [23], little is known about the developmental course of such deficits. We have undertaken a battery of behavioral tests to further characterize cerebellar behavioral abnormalities in neonatally BDV-infected and sham-inoculated Lewis rats throughout postnatal development of the cerebellum by repeatedly assessing performance on a standard test battery. Somatic development of neonatally BDV-infected rats was assessed by measuring body weight evolution. Acoustic startle response (ASR) was studied in neonatally BDV-infected rats to assess reflexic auditory–somatomotor development and integrity. Overall neuromuscular development and simple motor behaviors were examined by various tests, including righting, negative geotactic, placing, and grasping responses. These tests are thought to reveal development of exteroceptive, vestibular and proprioceptive systems modulated by the cerebellum [1,17,37]. Complex motor and postural skills were appraised in the bar crossing, twine climbing and bar holding tests. In addition, evidence that early viral infections could interfere with the development of sensorimotor gating has been demonstrated in rats subjected to early postnatal infection with cytomegalovirus [46], and herpes simplex virus type 1 [15]. Thus, possible sensorimotor gating deficits were studied with a prepulse inhibition (PPI) paradigm [49].

2. Material and methods

2.1. Animals

Pregnant Lewis rats (16–18 days of gestation) were used in these studies (Harlan, Indianapolis, IN, USA). All rat pups were born and reared in the animal vivariums at Johns Hopkins University School of Medicine, Baltimore, MD, USA, or at the Center for Biologics Evaluation and Research (CBER), FDA, Bethesda, MD, USA. Following weaning, rats were kept in groups of two to three in 45×26×23 cm pan-type polypropylene cages with woodchip bedding and an overhead wire grid supporting food pellets and a water bottle. Cages containing infected animals were kept in a DUO-FLOTM biosafety cabinet (Bio-Clean Lab Product Inc., NJ, USA). The sham-inoculated rats were kept in the same room. Rats were maintained on a 10/14-h light/dark cycle (lights on at 8 a.m.) and had free access to food and water. Room temperature was maintained at approximately 21°C.

2.2. Inoculation

Rat pups were inoculated intracranially within 24 h of birth either with 0.02 μl of CRP_3 BDV strain (BDV-infected rats) or uninfected inoculum (control rats), as described previously [4,8].

2.3. Startle apparatus

The experiments with the startle response were performed at CBER, Bethesda, MD, USA. Two identical startle chambers were used for measuring startle reactivity and plasticity (SR-LAB system, San Diego Instruments, San Diego, CA, USA). Within each chamber, there was a Plexiglas cylinder (9 cm in diameter) into which the rat was placed. A loudspeaker, mounted 24 cm above the cylinder, provided the broadband background noise and acoustic stimuli. Sudden movements by the rat were detected by a piezoelectric accelerometer attached below the cylinder. Stabilimeter readings were rectified, digitized on a 4095-relative unit scale, and recorded by a computer. An average of 100 1-ms readings, starting at stimulus onset, were used as the measure of startle amplitude. Sound level was measured inside the startle cabinets by means of the digital sound level meter (Realistic, Tandy

Corporation, Fort Worth, TX, USA). The accelerometer sensitivities within each startle chamber were calibrated regularly and were found to remain constant over the test period.

2.4. Procedure

2.4.1. Weighing

Somatic development was assessed by measuring body-weight gain. BDV-infected and normal rats were weighed daily when behavioral experiments were performed, i.e., PND 4–30. After weighing, rats were returned either to their dams (until PND 21), or to their home cages (PND 22–28).

2.4.2. Behavioral tests

Assessment of the integrity of somatosensory and vestibular systems and their proprioceptive and exteroceptive components was performed at postnatal days (PND) 4–20. This assessment consisted of the measures of body righting, negative geotropism, fore limb and hind limb placing, fore limb and hind limb grasping, vibrissae placing, locomotor pattern, and bar-holding tests. Complex motor skills and postural adjustments were examined at PND 25–28. These assessments included tests of twine climbing and bar crossing [1,37]. Eight BDV-infected and eight normal rats were used in these experiments. At PND 18, one BDV-infected rat died, therefore, from PND 19 to PND 28, only seven BDV-infected rats were tested.

All behavioral measures were done by a trained observer who was blind to group identity. The subset of tests performed after postnatal day 20, a time when size differences between infected and uninfected rats begin to become apparent, involved objective measures (twine climbing and bar crossing) or computer-software-determined results (startle response and prepulse inhibition), thereby reducing the likelihood of bias introduction. All data were coded prior to analysis.

For body righting, rats were gently placed on their backs and a score was given according to the following criteria: 0=rat does not turn over; 1=rat struggles with positioning, but does not turn over within 10 s; 2=rat turns over immediately, with little to no effort.

For negative geotropism, the rat was placed on a 45° slope with its head pointing down the incline. Scoring was based on (1) the number of rats that turned upwards before the 15-s cut-off time period, and on (2) the latency to turn upwards within the 15-s cut-off time period.

For fore limb and hind limb placing (dorsal surface), the rat was gently held by its body and the response (lifting a foot from under the object and putting it on top of the object) to a pencil touching the dorsal surface of all four paws was scored. Scoring was based on a five-point scale: 0=rat does not respond; 1=rat responded to touch on dorsal surface of one paw; 2=rat responded to touch on dorsal surface of two paws; 3=rat responded to touch on dorsal surface of three paws; 4=rat responded to touch on dorsal surface of four paws.

For fore limb and hind limb placing (lateral surface), the rat was gently held by its body and the response (the same as in the above test) to a touch on the lateral surface of all four paws was scored. Scoring was based on a five-point scale: 0=rat does not respond; 1=rat responded to touch on lateral surface of one paw; 2=rat responded to touch on lateral surface of two paws; 3=rat responded to touch on lateral surface of three paws; 4=rat responded to touch on lateral surface of four paws.

For grasping responses (fore paws), the rat was gently held by its body, and the response (grasping the pencil being used to gently strike the inside of one paw) was scored. Scoring was based on a three-point scale: 0=rat did not respond; 1=rat responded to touch on the ventral surface of one fore paw; 2=rat responded to touch on the ventral surface of two fore paws.

For grasping responses (hind paws), the rat was gently held by its body, and the response (the same as in fore paw grasping) to a touch on the ventral surface of hind paws was scored. Scoring was based on a three-point scale: 0=rat did not respond; 1=rat responded to touch on the ventral surface of one fore paw; 2=rat responded to touch on the ventral surface of two fore paws.

For vibrissae placing, the rat was gently suspended by the tail and lowered towards the tip of a pencil. When the vibrissae touched the pencil, the response (raising the head and extending the fore limbs for grasping the pencil) was scored. Scoring was based on a three-point scale: 0=rat did not respond; 1=rat either turned the head toward the pencil or extended fore paws toward the pencil; 2=rat both turned the head toward the pencil and extended fore paws toward the pencil.

For locomotor pattern, the rat was placed with its four paws on a flat surface, and the ability to stand for 10 s on all four feet was assessed. Scoring was based on an eleven-point scale: 0=rat lay on ventral surface of its body; 1=rat could stand on all four paws for 1 s; 2=rat could stand on all four paws for 2 s; 3=rat could stand on all four paws for 3 s, etc.

Bar holding test included placing the rat's fore paws on a round wooden bar (7 mm in diameter) and scoring the ability to hang for 10 s with or without placing its hind paws on the bar. Scoring was based on a three-point scale: 0=rat was unable to hang for at least 10 s; 1=rat was able to hang for 10 s without placing its hind paws on the bar; 2=rat was able to hang for 10 s and place its hind paws on the bar.

For the twine climbing test, the rat was held by the nape of its neck and its fore paws were placed against a rope. The cotton rope (diameter was 5 mm) was knotted, each knot being spaced 10 cm apart. The rope was 20 cm in length and positioned vertically so that the bottom end of the rope was 20 cm above the center of a container filled with sawdust to cushion the fall. The time (in seconds) that

elapsed between the moment a rat was positioned on the rope and the moment it fell was recorded, with the cut-off being 60 s.

The bar crossing test consisted of two elevated platforms connected by a plywood bridge (0.4 mm thick; 30 cm length, 2.5 cm width). A sawdust-filled box below the bridge served as protection for the falling rats. One rat at a time was placed at the start platform. We assessed three parameters in this test: (1) the percentage of rats in each group able to cross the bridge; (2) the time (in seconds, time cut-off=60 s) required by the rat to cross the bridge, and (3) the number of times the rat slipped while crossing the bridge. A slip was scored when one of paws slipped below the surface of the bridge.

2.4.3. Acoustic startle response (ASR) tests

The amplitude and the within-session habituation of the acoustic startle response (ASR) were assessed in experimentally naive rats at PND 17, 23, and 30. Ten control and ten neonatally BDV-infected rats were tested at each time point. At the beginning of the test, each rat was exposed to the experimental chamber for 5 min with no presentation of the acoustic startle stimulus and no background noise (the acclimatization period). Upon completion of the acclimatization period, a rat was given ten 100-ms 108-dB white noise stimuli at a 20-s inter-stimulus interval, with a background noise of 65 dB throughout the entire session.

Previous studies showed significant difference in body weights between BDV-infected and control rats [4,22,42]. Therefore, amplitudes of the ASR were analyzed and presented as the maximum value of the startle response in relation to the rat's body weight. These weight-corrected ASRs were determined by dividing the ASR value by the weight (in grams) of the test subject.

In a separate experiment, prepulse inhibition (PPI) of the ASR was tested in rats at PND 17 and 30. None of these rats had prior experience with the acoustic startle stimuli. Twelve neonatally BDV-infected and eleven sham-inoculated rats were tested in each age group. Each rat was tested once. The PPI session was designed to permit an analysis of effects of (i) different intervals between the prepulse and startle pulse, and (ii) different intensities of prepulse stimuli. A rat was placed in one of the startle chambers for a 5-min acclimation period with a 65-dB background noise. Upon completion of the acclimation period, a rat was exposed to ten types of trials: pulse-alone trial (a 108-dB, 100-ms, broadband burst); the omission of stimuli (no-stimulus trial); and four prepulse–pulse combinations (prepulse–pulse trials). A 50-ms broad-band burst was used as a prepulse. Prepulse–pulse combinations included two prepulse intensities (10 or 15 dB above the background noise) and two prepulse-to-pulse intervals (40 and 80 ms). Each session consisted of 31 trials: seven pulse-alone trials, six of each prepulse–pulse trials (combinations), and seven no-stimulus trials. All trials were presented in pseudorandom order. PPI was assessed as the percentage scores of PPI (%PPI):100 X (mean startle amplitude on pulse-alone trials−mean startle amplitude on prepulse–pulse trials/mean startle amplitude on pulse-alone trials).

2.4.4. Histological analysis

Upon completion of behavioral tests (PND 30), representative six BDV-infected and six sham-inoculated rats were sacrificed for histopathological examinations and immunohistochemical studies for BDV antigens. Rat were deeply anesthetized using Metofane (Pitman-Moore, Mundelein, IL, USA) and were perfused with phosphate-buffered saline (PBS, pH=7.4) followed by 4% paraformaldehyde. Brains were removed and postfixed for 24 h. Brains were paraffin embedded and cut sagittally into 10-μm-thick sections. To examine virus distribution in the brain, sections were stained by avidin–biotin immunohistochemistry (Vector, Burlingame CA, USA) using a polyclonal rabbit anti-BDV antibody followed by biotinylated anti-rabbit IgG (Vector), as described previously [8]. Duplicate sections were stained with cresyl violet and examined under a light microscope for cerebellar abnormalities.

2.4.5. Statistical analysis

The body-weight gain, twine climbing and bar crossing, and habituation of the acoustic startle response were analyzed by two-way repeated measures analyses of variance (ANOVAs). For the PPI experiments, three-way ANOVAs were used. Post hoc Tukey's test for pair-wise comparisons was used when applicable. Since scoring motor behaviors was based on relative units, and the data passed neither the Normality nor Equal Variance Tests, nonparametric statistical analyses were applied. Effect of age was analyzed by Friedman Repeated Measures Analyses of Variance on Ranks for control and BDV-infected group separately. The effect of the infection status was analyzed by the Mann–Whitney test for each time point separately. A $P<0.05$ was considered as the criterion for statistical significance.

3. Results

3.1. Histology

Compared to the cerebella of control rats, the cerebella from neonatally BDV-infected rats were hypoplastic. Microscopic examinations of the cresyl violet sections revealed typical BDV-induced abnormalities in irregular cerebellar cortical layers, and thinning of the molecular and internal granule cell layers (Fig. 1). Immunostaining with anti-BDV antibodies showed BDV protein expression in brain sections of all BDV-infected rats in the previously reported distribution. BDV protein expression was mainly

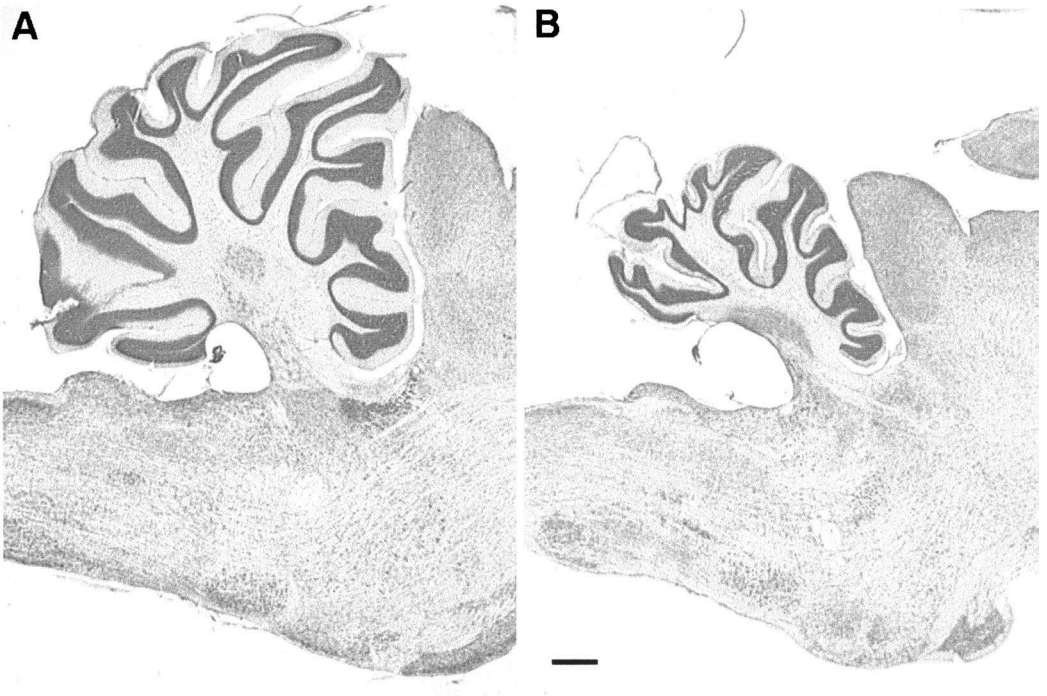

Fig. 1. Neonatal BDV-infection-induced damage to the cerebellum. Representative sagittal cerebellar sections from control (A) and neonatally BDV-infected (B) Lewis rats at postnatal day 30, stained with cresyl violet. Note reduced folia size and cerebellar size in the BDV-infected rat (B). Bar=500 μm.

observed in Purkinje cells, while no BDV-infected neurons were seen in the internal granule cell layer. No specific staining was observed in any of the brain sections in the control rats (data not shown).

3.2. Body weight

Neonatal BDV infection had a profound influence on body-weight gain in developing rats. As shown in Fig. 2, neonatally BDV-infected and control rats had similar body weights until PND 12. From PND 14 to PND 28, the mean body weight of control rats was higher than the mean body weight of the neonatally BDV-infected rats. There were significant effects of the infection status, $F(1,182)=564.3$, $P<0.00001$, of the age, $F(14,182)=401.2$, $P<0.00001$, and the infection status by age interaction, $F(14,182)=$

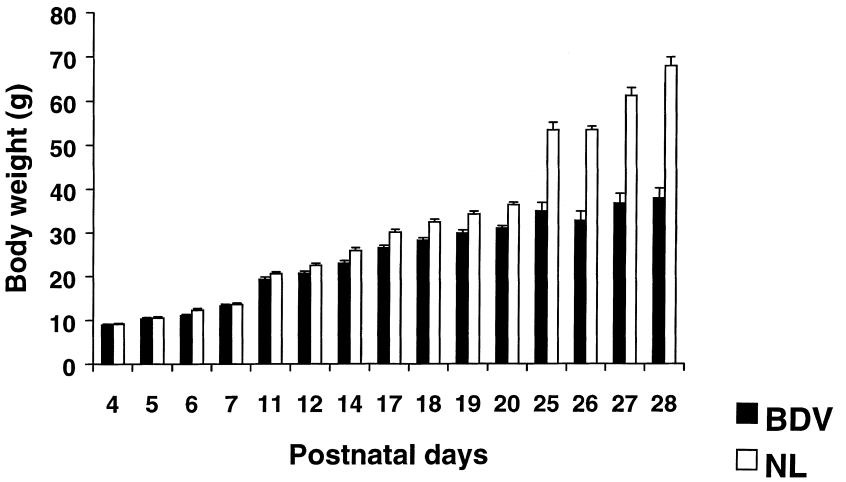

Fig. 2. Body-weight gain in control and neonatally BDV-infected Lewis rats. Open bars represent control animals; solid bars represent neonatally BDV-infected rats. *$P<0.05$ vs. neonatally BDV-infected rats.

Table 1
Motor co-ordination in neonatally BDV-infected and control Lewis rats at PND 4–20[a]

Age	Infection status	Righting	Negative geotropism	D-Place	L-Place	V/F-grasping	V/H-grasping	Vibrissae	Locomotor pattern	Bar holding
4	NL	1.1±0.1	15.0±1.2	0	0	0	0	0	0	0
	BDV	0.9±0.1	15.0±1.2	0	0	0	0	0	0	0
5	NL	1.5±0.2	15.0±1.2	0	0	0	0	0	0	0
	BDV	1.4±0.2	15.0±1.2	0	0	0	0	0	0	0
6	NL	1.3±0.2	12.8±1.2	0	0	0	0	0.5±0.2	0	0.1±0.1
	BDV	1.6±0.2	15.0±1.2	0	0	0	0	0.9±0.1	0	0
7	NL	1.9±0.1	13.1±1.2	0	0	0	0	0.9±0.1	0	0.3±0.3
	BDV	1.6±0.2	15.0±1.2	0	0	0	0	0.9±0.1	0	0.4±0.2
11	NL	1.8±0.2	9.9±1.2	0	0	0	0	1.4±0.2	0	0.6±0.3
	BDV	1.4±0.2	11.4±1.2	0	0	0	0	0.9±0.1	0	0.1±0.1
12	NL	1.8±0.2	10.4±1.2	0	0	0	0	1.1±0.1	0	0.5±0.3
	BDV	1.4±0.2	10.5±1.2	0	0	0	0	1.5±0.2	0	0
14	NL	1.9±0.1	4.1±1.2	0	0	0.25±0.3	0	1.0±0.3	7.5±1.7	1.1±0.2
	BDV	2.0±0	7.3±1.2	0	0	0	0	1.5±0.2	0	0
17	NL	2.0±0	5.4±1.2	3.5±0.3	2.0±0	2.0±0	0	1.6±0.2	10±0	1.8±0.3
	BDV	2.0±0	8.9±1.2	0	0	0	0.25±0.27	1.4±0.2	10±0	0
18	NL	2.0±0	4.3±1.2	3.8±0.3	1.5±0.3	2.0±0	0	1.9±0.1	10±0	1.5±0.3
	BDV	2.0±0	7.8±1.2	0.3±0.2	0	0.3±0.3	0	1.8±0.2	10±0	0
19	NL	2.0±0	3.9±1.2	3.4±0.3	1.6±0.3	2.0±0	0	1.6±0.3	10±0	1.8±0.3
	BDV	2.0±0	5.3±1.2	1.1±0.3	1.2±0.3	0.9±0.3	0	1.9±0.1	10±0	0
20	NL	2.0±0	4.8±1.2	2.8±0.4	1.8±0.3	1.5±0.3	0	1.3±0.2	10±0	1.8±0.3
	BDV	2.0±0	4.8±1.2	1.4±0.3	1.3±0.4	0.1±0.1	0	1.9±0.1	10±0	0

[a] Data are presented as the mean±S.E.M. Abbreviations: D-Place, forelimb and hindlimb placing (touch on dorsal surface); L-Place, forelimb and hindlimb placing (touch on lateral surface); V/F-Grasping, grasping responses to touch on ventral surface of fore paws; V/H-Grasping, grasping responses to touch on ventral surface of hind paws.

49.5, $P<0.00001$. Post-hoc tests showed that, compared to BDV-infected rats, control rats had greater body weights at PNDs 14–28 (all P values<0.01, Tukey).

3.3. Sensorimotor tests

Table 1 shows the results of the motor behaviors tests for the sham-inoculated and neonatally BDV-infected rats at PND 4–20. Table 2 shows the results of the complex motor co-ordination skills for the two groups of rats at PND 25–28.

3.3.1. Body-righting response

Righting response improved significantly from PND 4 to PND 20 for both control (Chi-square=42.2, $P<0.001$) and BDV-infected rats (Chi-square=39, $P<0.001$). There were no significant differences between the groups at any age.

3.3.2. Negative geotropism

Control rats began to demonstrate negative geotropism as early as PND 6 (two of eight rats), while BDV-infected rats first showed this response at PND 11 (three of eight). Despite this observation, differences between the two groups in terms of the numbers of rats successfully performing the test did not reach significance at any time point ($P>0.05$). The latency to upward turning significantly decreased throughout the postnatal period for both groups ($P<0.05$, Mann–Whitney). When the latencies were compared between groups at each time point separately, control rats had significantly shorter latencies at

Table 2
Complex locomotor skills in neonatally BDV-infected and control Lewis rats at PND 25–28[a]

Age	Infection status	Twine climbing (s)	Bar crossing (% of rats)	Bar crossing (s)	Bar crossing (slips)
25	NL	12.8±2.7	75±23	19.4±7.2	0.8±0.3
	BDV	4.4±1.4	86±14	17.8±7.7	4.1±0.3
26	NL	8.1±1.3	100±13	6.8±7.2	0.9±0.3
	BDV	14.6±2.2	100±14	13.1±7.7	2.4±0.3
27	NL	8.1±2.4	86±13	12.6±7.2	0.3±0.3
	BDV	16.9±3.5	71±14	20.4±7.7	1.5±0.4
28	NL	5.8±1.0	75±13	17.1±7.2	0.2±0.3
	BDV	9.8±1.3	86±14	11.9±7.7	2.0±0.3

[a] Data are presented as the mean±S.E.M.

PND 14 and 18 ($P<0.05$, Mann–Whitney), compared to BDV-infected rats.

3.3.3. Fore limb and hind limb placing tests

Responses to dorsal surface touching of four paws increased throughout the postnatal period in control, (Chi-square=69.5, $P<0.001$), and BDV-infected (Chi-square=55.3, $P<0.001$) rats. However, BDV-infected rats were significantly less responsive to dorsal touch than the control rats at PNDs 17–20 (all P values<0.05). Similar to dorsal touch, responses to lateral touch rose significantly for control rats (Chi-square=66.7, $P<0.001$), and BDV-infected rats (Chi-square=53.4, $P<0.001$). There were significant differences between the groups at PNDs 17–18 ($P<0.05$). At PNDs 19–20, both groups demonstrated similar performances on this test (all P values>0.05).

3.3.4. Fore limb and hind limb grasping tests

There was a significant effect of age for both groups (all Ps<0.05). From PNDs 17–20, BDV-infected rats showed attenuated responses compared to control rats (all P values<0.05). With the exception of one BDV-infected rat (PND 17), neither group responded to touch of the ventral surface of hind paws throughout the entire period of the observation.

3.3.5. Vibrissae placing

The responses to vibrissae touch increased with age for control (Chi-square=54.5, $P<0.001$), and BDV-infected (Chi-square=54, $P<0.001$) rats. There was a borderline difference between the two groups at PND 20 ($P=0.054$), when BDV-infected rats were hyper-responsive to touching vibrissae compared to control rats.

3.3.6. Locomotor pattern

There were significant effects of age for control (Chi-square=44.3, $P<0.001$) and BDV-infected (Chi-square=70, $P<0.01$) rats. At PND 14, control rats performed significantly better on this test ($P<0.05$), compared to BDV-infected rats.

3.3.7. Bar holding

BDV-infected rats were unable to hold onto the bar. Control rats were able to hang on the bar for 10 s from PND 14 (Chi-square=44.3, $P<0.01$). Significant differences between two groups began at PND 14 and persisted throughout the duration of the experiment (all P values<0.05).

3.3.8. Twine climbing

Neither effect of infection status [$F(1,39)=3.34$, $P=0.091$], nor effect of age [$F(3,39)=2.65$, $P=0.062$] were significant, while the interaction between the two factors was [$F(3,39)=7.71$, $P=0.0004$]. Compared to control animals, BDV-infected rats hung for a shorter period of time at PND 25, and for longer periods of time at PND 26 and 27 ($P<0.05$). There was no difference between BDV-infected and control rats at PND 28, $P>0.05$ (Table 2).

3.3.9. Bar crossing

When the number of rats successfully crossing the bar was analyzed, there were no differences between the two groups at any time point (all P values>0.05; Table 2). Analyses of time required to cross the bar showed no significant effects. Comparing the numbers of slips revealed significant effects of infection status [$F(1,39)=67.71$, $P<0.001$], and age [$F(3,39)=9.82$, $P=0.001$]. Compared to control animals, BDV-infected rats made significantly more slips at all time points tested ($P<0.05$; Table 2).

3.4. Acoustic startle response (ASR)

3.4.1. Amplitude and habituation of the ASR

Fig. 3 shows the mean startle amplitudes for BDV-infected and control rats at PNDs 17, 23, and 30. At PND 17, there were no differences between the two groups. At

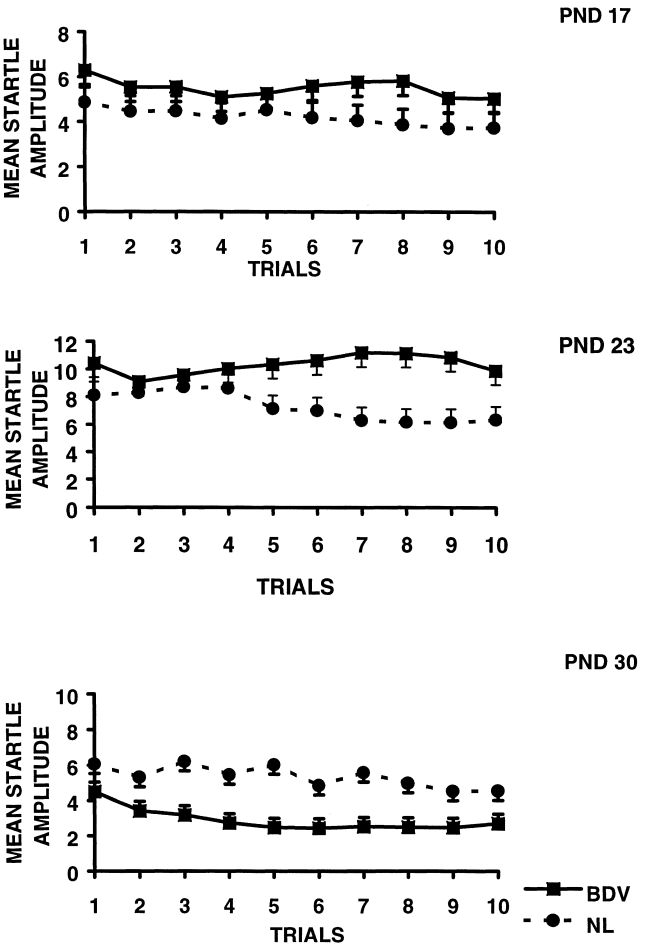

Fig. 3. Acoustic startle responses (ASR) and within-session habituation of the ASR in control and neonatally BDV-infected Lewis rats. Circles represent control animals. Squares represent neonatally BDV-infected rats. PND, postnatal day.

PND 23, there was the significant effect of infection status [$F(1,171)=6.84$, $P=0.017$], and a significant infection status by trial interaction [$F(9,171)=3.76$, $P<0.001$]. Control rats exhibited habituation of the ASR as was confirmed by the significant decrease of the mean amplitude of the startle response throughout the session ($P<0.05$). In contrast, BDV-infected rats did not show habituation of the ASR, and the mean amplitude of the startle response remained high across the trials ($P>0.05$). At PND 30, startle responsiveness was profoundly decreased in BDV-infected rats compared to control animals. There were significant effects of the infection status [$F(1,252)=24.6$, $P<0.001$], and the trial $F(9,252)=3.6$, $P<0.001$]. The infection status by trial interaction did not reach the significance ($P=0.3$).

3.4.2. Pre-pulse inhibition (PPI) of the ASR

At PND 17, mean amplitudes of the ASR during pulse-alone trials were 4.5±0.8 for control rats, and 5.1±0.7 for BDV-infected rats. Fig. 4 depicts the PPI of the ASR for BDV-infected and control animals. At PND 17, an effect of the infection status was borderline [$F(1,84)=4.0$, $P=0.058$]. There were significant effects of prepulse-to-pulse interval [$F(1,84)=4.8$, $P=0.03$], and intensity of prepulse [$F(1,84)=101.1$, $P<0.001$]. Interactions between factors did not reach significance (all P values>0.05). Post-hoc comparisons showed that PPI was significantly greater for the 40-ms prepulse-to-pulse intervals compared to the 80-ms prepulse-to-pulse intervals ($P<0.05$).

At PND 30, mean amplitudes of the ASR during pulse-alone trials were 6.1±0.6 for control rats, and 3.7±0.4 for BDV-infected rats. Three-way ANOVA showed a borderline effect of the infection status [$F(1,84)=2.1$, $P=0.067$]. There were significant effects of the intensity of prepulse [$F(1,84)=17.9$, $P<0.001$]; and prepulse-to-pulse interval [$F(1,84)=8.0$, $P=0.006$]. Interactions between the factors

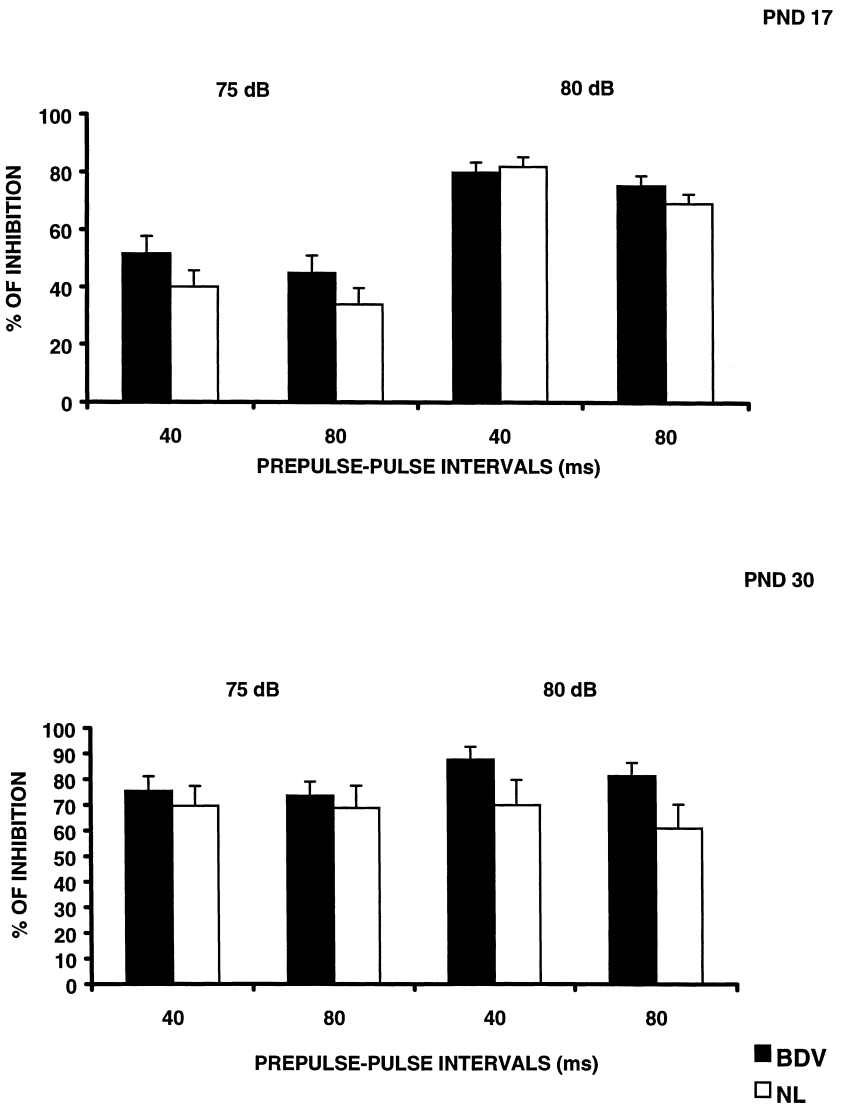

Fig. 4. Prepulse inhibition of the acoustic startle in control and neonatally BDV-infected Lewis rats. Open bars represent control animals; solid bars represent neonatally BDV-infected rats. 75-dB and 80-dB represent the intensities of the prepulses. PND, postnatal day.

were not significant ($P>0.05$). Significantly more PPI was observed when the intensity of prepulse stimuli was 15dB above the background noise compared to 10 dB ($P<0.05$, Tukey).

4. Discussion

We have found previously that as early as PND 14, BDV-infected rats showed irregular cerebellar cortical layers, thinning of the molecular and internal granule cell layer, and premature loss of the external granule cell layer [5,8]. Recently, a loss of Purkinje cells in cerebella of neonatally BDV-infected rats has been also reported [13,54]. However, until now, there have not been detailed studies of possible behavioral deficits associated with BDV-induced developmental damage to the cerebellum. Thus, the present work was undertaken to study the effects of neonatal BDV infection on the development of sensorimotor functions thought to be under the control of the cerebellum [1,2].

Somatic development, as indicated by weight gain, was significantly delayed in neonatally BDV-infected rats compared to control rats. These data are consistent with previous reports regarding the effects of neonatal BDV infection on body weights in 1-month-old and 3-month-old Lewis rats, and showed that body-weight differences started to emerge by the end of the second postnatal week [4]. Poor weight gain has been also reported for various mouse and rat mutants [6,56] as well as for rodents with early postnatal cerebellar damage, e.g., following X-irradiation or methylazoxymethanol treatment [16].

Significant differences in motor behaviors between BDV-infected and uninfected rats were not observed until the end of the second postnatal week, the time when infected rat cerebella begin to show typical BDV-induced developmental arrest [5,23]. Since, in the present study, we performed only qualitative microscopic examinations of BDV-induced cerebellar abnormalities, we were unable to make a regression analysis to statistically characterize a relationship between motor deficits and cerebellar pathology in BDV-infected rats. It should be pointed out that, for the most part, the cerebella of neonatally BDV-infected rats are severely hypoplastic. Infrequently though, moderately hypoplastic cerebella are encountered. We are in the process of evaluating the relationship between motor deficits and cerebellar pathology by quantitatively assessing the severity of BDV-induced neuropathology.

BDV-associated sensorimotor impairments were somewhat specific in that they 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, indicating that some aspects of exteroceptive sensation seem to be unaffected by neonatal BDV infection [24,56]. On the other hand, neonatal BDV infection induced motor deficits in a number of other sensorimotor tasks, including placing, grasping and the bar-holding tests. This pattern of motor deficit may suggest BDV-induced abnormalities in the proprioceptive system [39]. In addition, developmental deficits in righting responses and negative geotropism indicate impairments in the maturation of the integration of tactile and vestibular stimuli and/or damage to the central vestibular system [38]. Developmental abnormalities in the auditory–somatosensory integration are also evidenced by decreased habituation of startle responses in BDV-infected rats compared to uninfected animals [56]. Finally, important deficits occurred in those tests requiring more complex motor skills and balance. Neonatally BDV-infected rats performed worse than control rats in the bar-holding- and bar-crossing tests, which involve extensive use of hind limbs and balance. Hind-limb weakness and lack of hind-limb coordination has been reported to be one of the most pronounced deficits with cerebellar growth retardation after neonatal cerebellar injury [6].

BDV-induced motor deficits are reminiscent of the effects of other types of early postnatal cerebellar insults on motor behavior development in rodents [6,16]. Similar to effects of neonatal BDV infection, neonatal cerebellectomy produced deficits in righting and geotaxis responses. In contrast, placing reactions were unaffected after neonatal removal of a cerebellar hemisphere, and were significantly impaired in neonatally BDV-infected rats [31,39]. Deficient placing and grasping responses observed in neonatally BDV-infected rats were reminiscent of similar motor disabilities reported for shaker mutant rats with degeneration of Purkinje cells [56]. While significant loss of Purkinje cells has been reported in neonatally infected rats at 7 months of age [13], a putative loss of Purkinje cells in developing rats remains to be quantitated. Since the majority of Purkinje cells are infected with BDV as early as PND 14 [5], functional deficits in Purkinje cells could account for abnormal development of some sensorimotor behaviors, long before Purkinje cell death occurs [35].

In spite of the fact that abnormal development of the cerebellum is associated with motor disabilities, a number of motor skills, if impaired, can be restored with training. It has been suggested that abnormal development of the cerebellum caused by mutation or neonatal teratogens may be accompanied by a compensatory rewiring of spared brain circuitry [6]. The nature of these hypothetical compensations remains obscure, although lesions-induced neurochemical alterations have been suggested to underlie compensatory changes [6]. We did not examine the effects of training on the postnatal motor development in BDV-infected rats. The same rats were tested repeatedly and it is possible that the nature of the results would be different if separate groups of rats performed the tests at each age level. For example, if the training process did not take place in the present study, we might observe more severe deficits in some tests. On the other hand, since persistent virus infections may interfere with possible compensatory

responses, neonatally BDV-infected rats would continue to exhibit motor disabilities even after extensive training.

Persistent virus infections may also produce a delay in the expression of sensorimotor abnormalities. Some motor behaviors develop normally despite damage but become defective during subsequent development (a phenomenon known as 'growing into a deficit') [28]. For example, compared to control animals, startle responsiveness in BDV-infected rats was similar at PNDs 17 and 23 and became significantly lower at PND 30 and onwards. In a similar vein, more behavioral deficits may be seen as rats age, since the persistent neonatal BDV infection induces continuing degeneration of a number of cells in the rat brain (e.g., Purkinje cells in the cerebellum, and granule cells in the hippocampus) [8,13,54].

Impaired habituation of the acoustic startle response at PND 23, and decreased startle responsiveness at PND 30, in neonatally BDV-infected rats seems to be consistent with previous results indicating a role of the cerebellum in modulation of both amplitude and habituation of acoustic startle [27,48].

The fact that neonatal BDV infection may affect several brain regions in addition to the cerebellum (e.g., hippocampus and neocortex) could also account for the abnormal motor development observed in BDV-infected rats [8,19,54]. For example, virus-induced damage to the neocortex could affect startle responsiveness as functional inactivation of the neocortex has been shown to modulate startle responsiveness in adult rats [50]. In addition to developmental damage, the replication of the BDV throughout the brain might contribute to motor deficits in infected rats. Functional alterations induced by BDV in the neuronal circuitry of the ASR at the brainstem and/or spinal levels could produce decreased ASR [10]. Thus, it cannot be ruled out that extracerebellar damage by BDV might be responsible for some sensorimotor deficits observed in infected rats. Nevertheless, given that the cerebellar abnormalities are grossly more profound than neocortex damage and take place much earlier than the dentate gyrus degeneration appears to occur [8,54], observed motor deficits are more likely to be associated with developmental damage to the cerebellum in neonatally BDV-infected rats.

Neonatal BDV infection did not impair the PPI of the ASR in Lewis rats when tested at PNDs 17 and 30. On the one hand, these observations appear to be consistent with previous results indicating that PPI is resistant to gross insults. For example, the PPI has been spared after acute decerebration [9], and lesions of the hippocampus, septum, and amygdala [25] in adult rats. On the other hand, more recent studies have demonstrated disruption of the PPI after manipulations of limbic cortical structures, including the prefrontal cortex, hippocampus and amygdala [26,43,53]. Even if the cerebellum has not been clearly implicated in modulation of the PPI of acoustic startle in rats, disruption of the PPI in neonatally BDV-infected rats could have been expected because the virus infects neurons in most limbic regions in the rat's brain [8,54]. The lack of disruptive effects of neonatal BDV infection on the PPI in Lewis rats could be explained from a methodological standpoint. Putative impairments of the PPI in neonatally BDV-infected rats may have been detected if weaker prepulse intensities had been used, as demonstrated by others [14]. However, our unpublished observations indicated that the use of weaker prepulse intensities was unsuccessful (data not shown). Also, Lewis rats have been shown to be more resistant to isolation- and apomorphine-induced disruption of the PPI compared to Sprague–Dawley rats, a strain commonly used in PPI studies [51,55]. Therefore, it would be interesting to repeat these experiments using other strains of rats that were neonatally infected with BDV. Moreover, why some neonatally BDV-infected rats exhibited increased PPI remains unclear and warrants further investigation.

In conclusion, BDV-induced abnormal development of the cerebellum is associated with a variety of normal and impaired sensorimotor and postural skills in Lewis rats. Further investigations may shed more light on whether or not persistent neonatal BDV infection prevents or allows compensatory changes to occur, and if the virus-induced progressive loss of the Purkinje cells beyond PND 30 would have further impairing effects on sensorimotor skills in rats. In the broader context, many neuroanatomical, neurochemical and behavioral abnormalities following neonatal BDV infection appear to be similar to those reported for a number of developmental behavior disorders, including autism spectrum disorders [44]. Thus, neonatal rat brain infection with BDV may be a valuable animal model system for further exploration of the causal links between neurodevelopmental damage and associated behavioral deficits.

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References

[1] J. Altman, K. Sudarshan, Postnatal development of locomotion in the laboratory rat, Anim. Behav. 23 (1975) 896–920.
[2] J. Altman, S.A. Bayer, Development of the Cerebellar System in Relation To Its Evolution, Structure, and Functions, CRC Press, Boca Raton, FL, 1997, 783 pp.
[3] J.D. Amsterdam, A. Winokur, W. Dyson, S. Herzog, F. Gonzalez, R. Rott, H. Koprowski, Borna disease virus. A possible etiologic factor in human affective disorders?, Arch. Gen. Psychiatry 42 (1985) 1093–1096.
[4] J.R. Bautista, S.A. Rubin, T.H. Moran, G.J. Schwartz, K.M.

Carbone, Early and persistent abnormalities in rats with neonatally acquired Borna disease virus infection, Brain Res. Bull. 34 (1994) 31–40.
[5] J.R. Bautista, S.A. Rubin, T.H. Moran, G.J. Schwartz, K.M. Carbone, Developmental injury to the cerebellum following perinatal Borna disease virus infection, Dev. Brain Res. 90 (1995) 45–53.
[6] J. Caston, R. LaLonde, N. Delhaye-Bouchaud, J. Mariani, The cerebellum and postural sensorimotor learning in mice and rats, Behav. Brain Res. 95 (1998) 17–22.
[7] K.M. Carbone, B.D. Trapp, J.W. Griffin, C.S. Duchala, O. Narayan, Astrocytes and Schwann cells are virus-host cells in the nervous systems of rats with borna disease, J. Neuropathol. Exp. Neurol. 48 (1989) 631–644.
[8] K.M. Carbone, S.W. Park, S.A. Rubin, R.W. Waltrip II, G.B. Vogelsang, Borna disease: association with a maturation defect in the cellular immune response, J. Virol. 65 (1991) 6154–6164.
[9] M. Davis, P.M. Gendelman, Plasticity of the acoustic startle response in the acutely decerebrate rat, J. Comp. Physiol. Psychol. 91 (1977) 549–563.
[10] M. Davis, The mammalian startle response, in: J.M. Eaton (Ed.), Neural Mechanisms of the Startle Behavior, Plenum, New York, 1984, 361 pp.
[11] J.C. de la Torre, Molecular biology of borna disease virus: prototype of a new group of animal viruses, J. Virol. 68 (1994) 7669–7675.
[12] W. Dittrich, L. Bode, M. Kao, K. Schneider, Learning deficiencies in Borna disease virus-infected but clinically healthy rats, Biol. Psychiatry 26 (1989) 818–828.
[13] L.M. Eisenman, R. Brothers, M.H. Tran, R.B. Kean, G.M. Dickson, B. Dietzschold, D.C. Hooper, Neonatal Borna disease virus infection in the rat causes a loss of Purkinje cells in the cerebellum, J. Neurovirol. 5 (1999) 181–189.
[14] B.A. Ellenbroek, M.A. Geyer, A.R. Cools, The behavior of APOSUS rats in animal models with construct validity for schizophrenia, J. Neurosci. 15 (1995) 7604–7611.
[15] J.A. Engel, J. Zhang, T. Bergstrom, N. Conradi, C. Forkstam, A. Liljeroth, L. Svensson, Neonatal herpes simplex virus type 1 brain infection affects the development of sensorimotor gating in rats, Brain Res. 863 (2000) 233–240.
[16] S.A. Ferguson, Neuroanatomical and functional alterations resulting from early postnatal cerebellar insults in rodents, Pharmacol. Biochem. Behav. 55 (1996) 663–671.
[17] H.C. Geisler, J. Westerga, A. Gramsbergen, Development of posture in the rat, Acta Neurobiol Exp., (Warsz) 53 (1993) 517–523.
[18] D. Gonzalez-Dunia, C. Sauder, J.C. De la Torre, Borna disease virus and the brain, Brain Res. Bull. 44 (1997) 647–664.
[19] D. Gonzalez-Dunia, M. Watanabe, S. Syan, M. Mallory, E. Masliah, J.C. De La Torre, Synaptic pathology in Borna disease virus persistent infection, J. Virol. 74 (2000) 3441–3448.
[20] R.M. Greestein, E.M. Dykens, Genes, behavior, and development, in: M.D. Levine, W.B. Carey, A.C. Crocker (Eds.), Developmental–Behavioral Pediatrics, Third edition, W.B. Saunders, Philadelphia, 1999, pp. 209–229.
[21] R.T. Johnson, Viral Infections of the Nervous System, Lippincott–Raven, Philadelphia, 1998, 527 pp.
[22] N. Hirano, M. Kao, H. Ludwig, Persistent, tolerant or subacute infection in Borna disease virus-infected rats, J. Gen. Virol. 64 (1983) 1521–1530.
[23] M. Hornig, H. Weissenbock, N. Horscroft, W.I. Lipkin, An infected-based model of neurodevelopmental damage, Proc. Natl. Acad. Sci. USA 96 (1999) 12102–12107.
[24] R.E. Hruska, S. Kennedy, E.K. Silbergeld, Quantitative aspects of normal locomotion in rats, Life Sci. 25 (1979) 171–180.
[25] E.D. Kemble, J.R. Ison, Limbic lesions and the inhibition of startle reactions in the rat by conditions of preliminary stimulation, Physiol. Behav. 7 (1971) 925–928.
[26] M. Koch, H.-U. Schnitzler, The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation, Behav. Brain Res. 89 (1997) 35–49.
[27] R.N. Leaton, W.F. Supple Jr., Medial cerebellum and long-term habituation, Behav. Neurosci. 105 (1991) 804–816.
[28] C.T. Leonard, M.E. Goldberger, Consequences of damage of the sensorimotor cortex in neonatal and adult cats. I. Sparing and recovery of function, Dev. Brain Res. 32 (1987) 1–14.
[29] H. Ludwig, H.L. Bode, G. Gostozni, Borna disease: a persistent virus infection of the central nervous system, Prog. Med. Virol. 35 (1988) 107–151.
[30] A.K. Mohammed, O. Magnusson, J. Maehlen, F. Fonnum, E. Norrby, M. Schultzberg, K. Kristensson, Behavioral deficits and serotonin depletion in adult rats after transient infant nasal viral infection, Neuroscience 35 (1990) 355–363.
[31] M. Molinari, L. Petrosini, T. Gremoli, Hemicerebellectomy and motor behaviour in rats. II. Effects of cerebellar lesion performed at different developmental stages, Exp. Brain Res. 82 (1990) 483–492.
[32] A.A. Monjan, D.H. Gilden, G.A. Cole, N. Nathnson, Cerebellar hypoplasia in neonatal rats caused by lymphocytic choriomeningitis virus, Science 171 (1971) 194–196.
[33] O. Narayan, S. Herzog, K. Frese, H. Scheefers, R. Rott, Behavioral disease in rats caused by immunopathological responses to persistent borna virus in the brain, Science 220 (1983) 1401–1403.
[34] S.P. Neeley, A.J. Cross, T.J. Crow, J.A. Johnson, G.R. Taylor, Herpes simplex virus encephalitis. Neuroanatomical and neurochemical selectivity, J. Neurol. Sci. 71 (1985) 325–337.
[35] M.B. Oldstone, An old nemesis in new clothing: viruses playing new tricks by causing cytopathology in the absence of cytolysis, J. Infect. Dis. 152 (1985) 665–667.
[36] M.L. Oster-Granite, R.M. Herndon, The pathogenesis of parvovirus-induced cerebellar hypoplasia in the Syrian hamster, *Mesocricetus auratus*. Fluorescent antibody, foliation, cytoarchitectonic, golgi and electron microscopic studies, J. Comp. Neurol. 169 (1985) 481–522.
[37] L.J. Pellegrino, J. Altman, Effects of differential interference with postnatal cerebellar neurogenesis on motor performance, activity level, and maze learning of rats: a developmental study, J. Comp. Physiol. Psychol. 93 (1979) 1–33.
[38] S. Pellis, V. Pellis, Development of righting when falling from a bipedal standing posture: evidence for the dissociation of dynamic and static righting reflexes in rats, Physiol. Behav. 56 (1994) 659–663.
[39] L. Petrosini, M. Molinari, T. Gremoli, Hemicerebellectomy and motor behaviour in rats. I. Development of motor function after neonatal lesion, Exp. Brain Res. 82 (1990) 472–482.
[40] O. Planz, C. Rentzsch, A. Batra, T. Winkler, M. Buttner, H.J. Rziha, L. Stitz, Pathogenesis of borna disease virus: granulocyte fractions of psychiatric patients harbor infectious virus in the absence of antiviral antibodies, J. Virol. 73 (1999) 6251–6256.
[41] M.V. Pletnikov, S.A. Rubin, K. Vasudevan, T.H. Moran, K.M. Carbone, Developmental brain injury associated with abnormal play behavior in neonatally Borna Disease Virus (BDV)-infected Lewis rats: A model of autism, Behav. Brain Res. 100 (1999) 30–45.
[42] M.V. Pletnikov, S.A. Rubin, G.J. Schwartz, T.H. Moran, T.J. Sobotka, K.M. Carbone, Persistent neonatal Borna disease virus (BDV) infection of the brain causes chronic emotional abnormalities in adult rats, Physiol. Behav. 65 (1999) 823–831.
[43] B. Pouzet, J. Feldon, C.L. Veenman, B.K. Yee, M. Richmond, J. Nicholas, P. Rawlins, I. Weiner, The effects of hippocampal and fimbria–fornix lesions on prepulse inhibition, Behav. Neurosci. 113 (1999) 968–981.
[44] I. Rapin, R. Katzman, Neurobiology of autism, Ann. Neurol. 43 (1998) 7–14.
[45] J.A. Richt, S. Herzog, J. Pyper et al., Borna disease virus: Nature of the etiologic agent and significance of the infection in man, Arch. Virol. Suppl. 7 (1993) 101–109.
[46] D.M. Rothschild, M. O'Grady, L. Wecker, Neonatal cytomegalovirus exposure decreases prepulse inhibition in adult rats: implications for schizophrenia, J. Neurosci. Res. 57 (1999) 429–434.

[47] C. Sauder, A. Muller, B. Cubitt, J. Mayer, J. Steinmetz, W. Trabert, B. Ziegler, K. Wanke, N. Mueller-Lantzsch, J.C. de la Torre, F.A. Grasser, Detection of Borna disease virus (BDV) antibodies and BDV RNA in psychiatric patients: evidence for high sequence conservation of human blood-derived BDV RNA, J. Virol. 70 (1996) 7713–7724.

[48] Z.I. Storozheva, M.V. Pletnikov, Habituation of acoustic startle in rats—a functional ablation study, Neuroreport 16 (1994) 2065–2068.

[49] N.R. Swerdlow, D.L. Braff, N. Taaid, M.A. Geyer, Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenia patients, Arch. Gen. Psychiatry 51 (1994) 139–154.

[50] C. Van der Staak, Habituation and sensitization of the acoustic startle response during cortical spreading depression in rats, Physiol. Behav. 6 (1976) 681–687.

[51] G.B. Varty, M.A. Geyer, Effects of isolation rearing on startle reactivity, habituation, and prepulse inhibition in male Lewis, Sprague–Dawley, and Fisher F344 rats, Behav. Neurosci. 112 (1998) 1450–1457.

[52] R.W. Waltrip II, R.W. Buchanan, A. Summerfelt, A. Breier, W.T. Carpenter, N. Bryant, S.A. Rubin, K.M. Carbone, Borna disease virus and schizophrenia, Psychiatry Res. 56 (1995) 33–44.

[53] F.J. Wan, N.R. Swerdlow, The basolateral amygdala regulates sensorimotor gating of acoustic startle in the rat, Neuroscience 76 (1997) 715–724.

[54] H. Weissenbock, M. Hornig, W.F. Hickey, W.I. Lipkin, Microglial activation and neuronal apoptosis in Bornavirus infected neonatal Lewis rats, Brain Pathol. 10 (2000) 260–272.

[55] L.S. Wilkinson, S.S. Killcross, T. Humby, F.S. Hall, M.A. Geyer, T.W. Robbins, Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition, Neuropsychopharmacology 10 (1994) 61–72.

[56] L.W. Wolf, M.C. LaRegina, D.L. Tolbert, A behavioral study of the development of hereditary cerebellar ataxia in the shaker rat mutant, Behav. Brain Res. 75 (1996) 67–81.

[57] R.H. Yolken, E.F. Torrey, Viruses, schizophrenia and bipolar disorders, Clin. Microbiol. Rev. 8 (1995) 131–145.