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Postnatal weight gain inhibition does not account for neurobehavioral consequences of neonatal Borna disease virus infection

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Abstract

Neonatal Borna disease virus (BDV) infection of the rat's brain produces neurodevelopmental damage similar to some pathological and clinical features of human developmental disorders, e.g., autism and schizophrenia. Since BDV-infected rats exhibited an inhibition of postnatal weight gain, the present study sought to evaluate a contribution of nutritional status to virus-induced neurodevelopmental injury. We compared neuroanatomical, neurochemical, and behavioral alterations following neonatal BDV infection and rearing in the oversized litters in Fischer344 rats on postnatal day (PND) 26. Despite a comparable weight gain inhibition, different patterns of brain pathology, alterations in brain monoamine systems, and behavioral deficits were observed in the BDV-infected rats compared to the malnourished rats. While no appreciable cell injury was noted in the brains of the malnourished rats, a significant loss of Purkinje cells (PC) and early signs of degeneration of the hippocampal dentate gyrus were found in the BDV-infected rats. Both neonatal BDV infection and postnatal malnourishment increased tissue concentrations of serotonin [5-hydroxytryptamine (5-HT)] in the hippocampus. In contrast, increased turnover of 5-HT in the cortex and hippocampus and elevated turnover of dopamine (DA) in the striatum were found in the malnourished rats only, suggesting that different pathogenic mechanisms might underlie monoamine disturbances in virus-infected and malnourished rats. The observed dissimilar neuroanatomical and neurochemical abnormalities might explain the different responses to novelty in the BDV-infected and malnourished rats. Compared to the control rats, the BDV-infected rats exhibited novelty-induced hyperactivity, while no differences in locomotion were noted between the control and malnourished rats. Taken together, the present data indicate that virus-associated inhibition of postnatal weight gain is unlikely to account for the major BDV-associated neurodevelopmental alterations that seem to be due to specific effects of neonatal BDV infection.

Keywords: Borna; Developmental disorders; Animal model; Malnourishment

1. Introduction

Several developmental behavioral disorders have been associated with early brain injury following exposure to environmental insults [1–3]. Since studying the complex mechanisms of developmental abnormalities in humans is very difficult, animal models are often used to identify pathogenic events and associated neurobehavioral consequences and to search for novel therapeutic regimens [4,5].

Neonatal Borna disease virus (BDV) infection is a valuable animal model of neurodevelopmental damage [6,7]. In Lewis and Fischer344 rats, neonatal intracranial inoculation with BDV, an 8.9-kb nonsegmented negative strand enveloped RNA virus [8,9], produces persistent infection without confounding encephalitis and meningitis [10,11]. Neonatally BDV-infected rats appear grossly normal; however, they exhibit distinct behavioral deficits similar to several symptoms of developmental behavioral disorders, e.g., autism. For example, locomotor hyperreactivity to novel/aversive stimuli [12,13], deficient learning and memory [12,14], and abnormal social (e.g., play) interaction [15] are all manifestations in BDV-infected rats. The observed behavioral alterations may be explained by BDV-induced selective developmental damage to the neocortex, hippocampus, and cerebellum [16–21], brain regions that have been implicated in pathology of developmental behavioral disorders [22–24]. Additionally, BDV-induced alterations in serotonin and norepinephrine (NE)

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neurotransmissions [25] may contribute to behavioral deficits observed in neonatally BDV-infected rats.

Neonatally BDV-infected rats are smaller than control sham-inoculated animals. Both a reduced body weight and a smaller body length have been documented in BDV-infected rats [26]. A simultaneous and proportional BDV-induced decrease in the both external parameters of development seems to indicate an overall inhibition in the growth, i.e., growth retardation. While virus infections during prenatal and perinatal periods have been associated with growth inhibition in animals and humans [27], the mechanisms of BDV-induced runting remain obscure. For example, no detectable BDV-associated disturbances in the biosynthesis of growth hormone and insulin-like growth factor-1 have been found [26]. In addition to effects of the virus infection per se, virus infection-associated malnourishment could be also responsible for growth retardation [28–30]. Although neonatally BDV-infected 10-week-old rats have been shown to consume a normal amount of food, when the amount of food was calculated in relation to the body weight, neonatally BDV-infected rats actually consumed more food than control animals, suggesting that BDV-induced growth inhibition might result from nutritional problems associated with decreased absorption in the gastrointestinal tract, increased energy expenditure, or virus-altered cellular metabolism [17,31].

Since even a transient state of malnourishment during development can produce long-term neurobehavioral consequences [31], we sought to directly compare neuroanatomical, neurochemical, and behavioral effects of neonatal BDV infection and postnatal malnourishment in rats otherwise reared under the same conditions. Our study has demonstrated that postnatal malnourishment induced by rearing in oversized litters and neonatal BDV infection produced different brain pathology, monoamine alterations, and behavioral responses to novelty, suggesting that the main features of BDV-associated neurodevelopmental injury are likely due to specific effects of viral infection.

2. Materials and methods

2.1. Animals

Pregnant Fischer344 rats (16–18 days of gestation) were purchased for the present study (Harlan, Indianapolis, IN). All rat pups were born and reared in the animal facility at Johns Hopkins University School of Medicine, Baltimore, MD. Mothers and their litters were housed in 45 × 26 × 23-cm pan-type polypropylene cages with paper chip bedding and an overhead wire grid supporting food pellets and a water bottle. Cages containing infected animals were kept in a DUO-FLOU biosafety cabinet (Bio-Clean Lab Product, NJ). The sham-inoculated rats and the rats reared in the oversized litters were kept in the same room. Rats of all the groups were maintained on a 12:12-h light/dark cycle (lights on at 8 a.m.). Pregnant rats of all the groups had free access to food and water. After weaning, on PND 24, all rat pups were given free access to food and water. Room temperature was maintained at approximately 21 °C. Animal cages were changed four times per week in order to avoid unsanitary conditions in the overpopulated cages.

Male and female rats were tested in the open field test on PND 26 and were sacrificed for histological and neurochemical evaluation on PND 28–30.

2.2. Inoculation

BDV stock was prepared from homogenized BDV-infected rat brain tissue as described earlier [16]. Pups were inoculated intracranially under hypothermia anesthesia with 26-gauge needles within 24 h of birth either with 0.02 ml (the titer was 10^4 $TICD_{50}$/g of brain tissue) of He-80 BDV strain (BDV-infected rats) or uninfected inoculum (control rats) [16]. For intracranial inoculation, a pup was taken out of the home cage and placed on ice. After an injection, a rat pup was warmed with a warm cloth and returned to the home cage.

2.3. Rearing in oversized litters

In order to produce malnourishment, we used rearing in oversized litters, an established nutritional model [32–34]. Specifically, 1 day after birth, 17–18 rat pups from two to three different litters were placed in the same cage with a single mother. Pups were left with the mother until weaning, with food and water available ad libidum. Although the previous investigations have shown that rearing in oversized litters is not associated with inadequate maternal care or neonatal stress [32,35,36] in accordance with the protocol approved by the Johns Hopkins University ACUC, oversized litters were given special attention and care, including constantly monitoring of any signs of degradation, lethargy, or neurological abnormalities. In the present study, none of rats from the oversized litters were excluded from the study because of poor health.

2.4. Behavioral experiments

Novelty-induced locomotor activity was assessed in sham-inoculated ($n = 11$), BDV-infected ($n = 11$), and malnourished ($n = 20$) male and female rats in an open-field arena that consisted of a Plexiglas square box (60 × 60 × 50 cm) with transparent walls. The floor was divided into 36 sections of equal area by a series of solid lines forming squares. A 100-W white spotlight brightly illuminated the apparatus and the rats were not habituated to the experimental box. After a rat was placed in the open field, behaviors were videotaped for 10 min and scored later for horizontal locomotor activity (number of squares crossed) and rearing activity.

2.5. Histopathological examination and anti-BDV immunohistochemistry

Since behavioral studies did not reveal any effects of sex on weight gain inhibition or locomotor activity in the BDV-infected and malnourished rats, male and female rats were randomly combined for neuroanatomical and neurochemical analyses, and effects of sex on brain pathology and monoamine alterations were not analyzed.

Control (three males and two females, $n=5$), BDV-infected (three males and two female, $n=5$), and malnourished (three males and two females, $n=5$) rats were randomly preselected from the pool of rats used in behavioral experiments described in this study. Upon completion of behavioral tests, rats were deeply anesthetized with ether (Pitman-Moore, Mundelein, IL), followed by euthasol, and perfused with phosphate-buffered saline (pH = 7.4) followed by 4% paraformaldehyde. Brains were removed and postfixed for 24 h, paraffin embedded, and cut sagittally into 8-μm thick sections. Tissue sections were stained with hematoxylin and eosin for histopathological evaluation. Adjacent sections were stained by avidin–biotin immunohistochemistry (Vector, Burlingame, CA) using polyclonal horse anti-BDV antibodies followed by biotinylated antihorse IgG (Vector) as described previously [16].

2.6. Neurochemical experiments

After the completion of behavioral experiments, control (three males and two females, $n=5$), BDV-infected (three males and two females, $n=5$), and malnourished rats (three males and two females, $n=5$) were randomly selected and sacrificed by decapitation. Their brains were removed and rapidly dissected on ice. The following brain regions were dissected for analyses: frontal cortex, hippocampus, and striatum. Samples were stored at −70 °C until assay. Regional concentrations of NE, DA and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-HT or serotonin and its metabolite, 5-hydroxyindole-3-acetic acid (5-HIAA), were measured in the dissected brain regions by high-performance liquid chromatography with electrochemical detection (HPLC-ED) technique [25,37]. Monoamine peaks in chromatograms of samples were identified by their retention times. Monoamine concentrations were expressed as picogram/milligram tissue.

2.7. Statistical analyses

The data are presented as mean ± S.E.M. Two-way ANOVA with sex and treatment as independent variables was used to compare effects of neonatal BDV infection and postnatal malnourishment on body weights and locomotor activity. One-way ANOVA with treatment as an independent variable was used to analyze effects of malnourishment and neonatal BDV infection on concentrations of monoamines and their metabolites for each brain region separately. Tukey's tests or planned t tests were used when applicable. When the data did not pass the normality test and/or equal variance test, the data were subjected to the rank transformation and ANOVAs were rerun on the transformed data. A $P<.05$ was considered as the criterion for statistical significance.

3. Results

3.1. Body weight

Both neonatal BDV infection and rearing in the oversized litters induced significant decreases in body weight as measured on PND 26 (Fig. 1). Two-way ANOVA showed a significant effect of treatment, $F(2,36)=8.5$, $P<.001$, and sex, $F(1,36)=5.4$, $P=.026$, with no Treatment × Sex interaction being significant, $P>.05$. Post hoc comparisons showed that the control animals had greater body weights compared to those of the BDV-infected and malnourished rats, $P<.05$, while there was no difference in body weight between the infected rats and malnourished animals, $P>.05$, indicating a comparable weight gain inhibition in the BDV-infected and malnourished groups.

3.2. Behavioral alterations

Neonatal BDV infection and malnourishment produced different behaviors of rats in the open field test (Fig. 2). Specifically, compared to the control animals, neonatal BDV infection induced elevated horizontal (Fig. 2, panel A) and vertical locomotor activity (Fig. 2, panel B), while

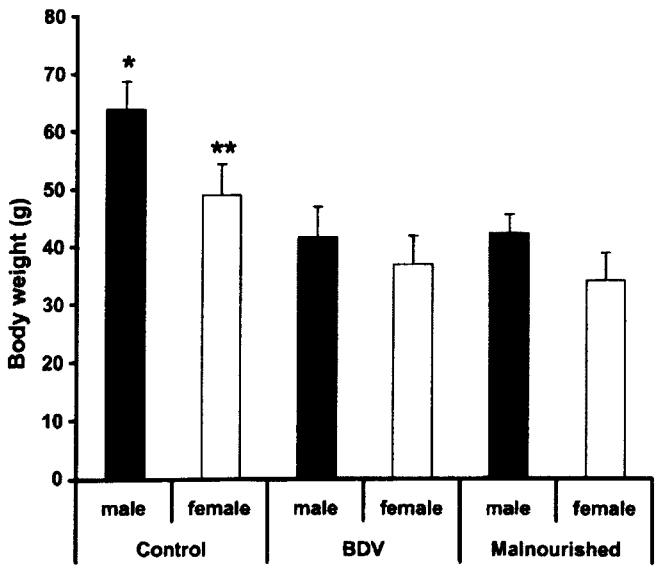

Fig. 1. Effects of neonatal BDV infection and rearing in the oversized litters on body weights in rats on PND 26. * $P<.05$ versus male rats of the BDV group and the malnourished group; * * $P<.05$ versus female rats of the BDV group and the malnourished group.

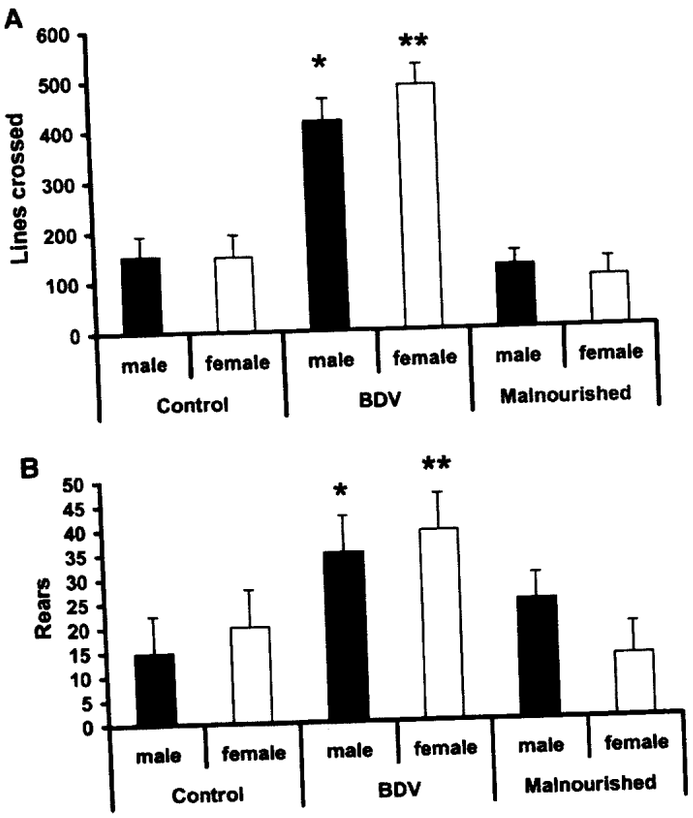

Fig. 2. Effects of neonatal BDV infection and rearing in the oversized litters on horizontal (A) and vertical (B) locomotor activity in rats on PND 26. * $P<.05$ versus male rats of the BDV group and the malnourished group; * * $P<.05$ versus female rats of the BDV group and the malnourished group.

postnatal malnourishment did not significantly affect locomotor activity. An analysis revealed a significant effect of treatment on horizontal activity, $F(2,36)=43.8$, $P<.001$, and rearing, $F(2,36)=4.4$, $P=.03$, while neither effects of sex nor Treatment × Sex interaction were significant, $P>.05$. Tukey's tests showed that horizontal activity of the BDV-infected rats was greater than that of the control and malnourished rats, $P<.05$. Rearing was greater in the BDV-infected rats compared to the control animals only, $P<.05$, with no significant difference being found between the control and malnourished rats, $P>.05$.

3.3. Histopathology

Anti-BDV immunostaining of the sagittal brain sections from the BDV-infected rats showed a typical regional distribution of BDV antigens in the brain, with most intense staining being seen in cortex, hippocampus (i.e., CA subfields and dentate gyrus), and cerebellum (i.e., PC). No viral antigens were found in sections from the brains of the control and malnourished rats (data not shown).

3.3.1. Hippocampus

There was a noticeable difference in effects of neonatal BDV infection and postnatal malnourishment on the hippocampus. In the BDV-infected rats, signs of a degeneration of the dentate gyrus were noted, e.g., thinning of granule cell layer, alterations in the shape of the dentate gyrus (Fig. 3). In contrast, qualitative examinations of the sections from the brains of the malnourished rats revealed little, if any, alterations in the dentate gyrus of the hippocampus.

3.3.2. Cerebellum

Neonatal BDV infection and malnourishment had also different effects on postnatal development of the cerebellum. While neonatal BDV infection produced a distinct dropout of PC, no appreciable loss of PC was noted in the

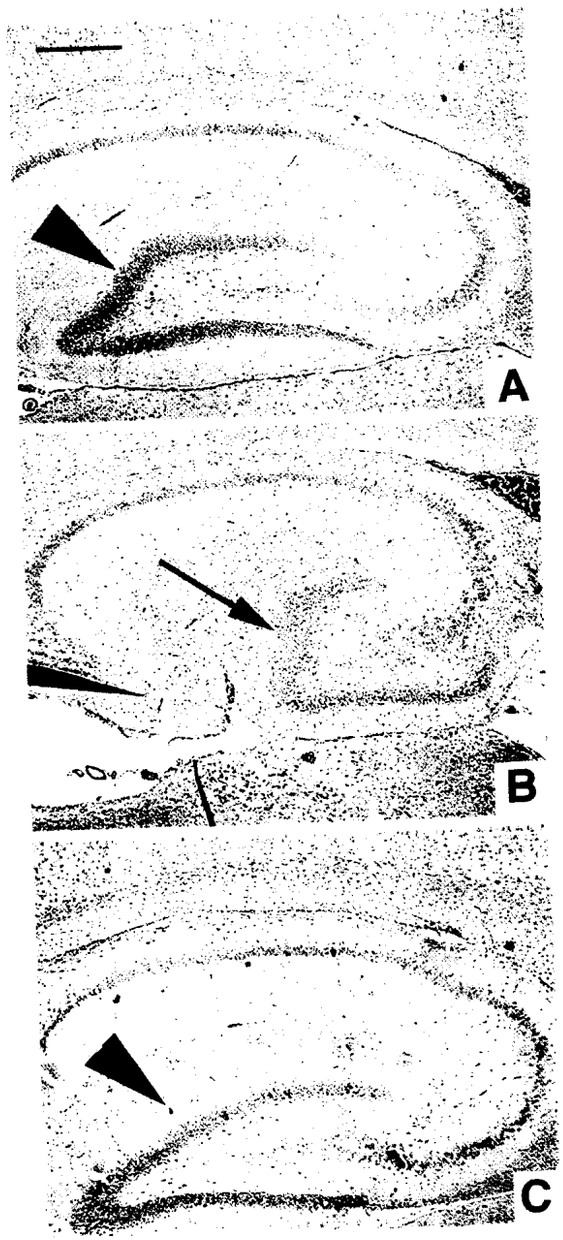

Fig. 3. Representative images of sagittal sections of the hippocampus from a sham-inoculated rat (A), a neonatally BDV-infected rat (B), and a malnourished rat (C) on PND 30. Note the well-developed dentate gyrus in A and C (arrowheads) compared to a degenerating dentate gyrus in B (arrow). Hematoxylin and eosine staining. Scale bar – 300 μm.

malnourished animals (Fig. 4). However, compared to the brain section from the control animals, some irregularity in the layer of PC (i.e., variable cell packing) was noticed in the sections from the malnourished group.

3.4. Regional monoamine concentrations

3.4.1. Cortex

5-HT and 5-HIAA/5-HT: Neither neonatal BDV infection nor postnatal malnourishment affected concentrations of 5-HT in cortex, $F(2,22)=1.7$, $P=.21$ (Table 1). In contrast, 5-HIAA/5-HT ratio, an indicator of 5-HT turnover, was significantly elevated in the malnourished group compared to the control and infected animals, $F(2,22)=11.2$, $P<.001$ (Table 1).

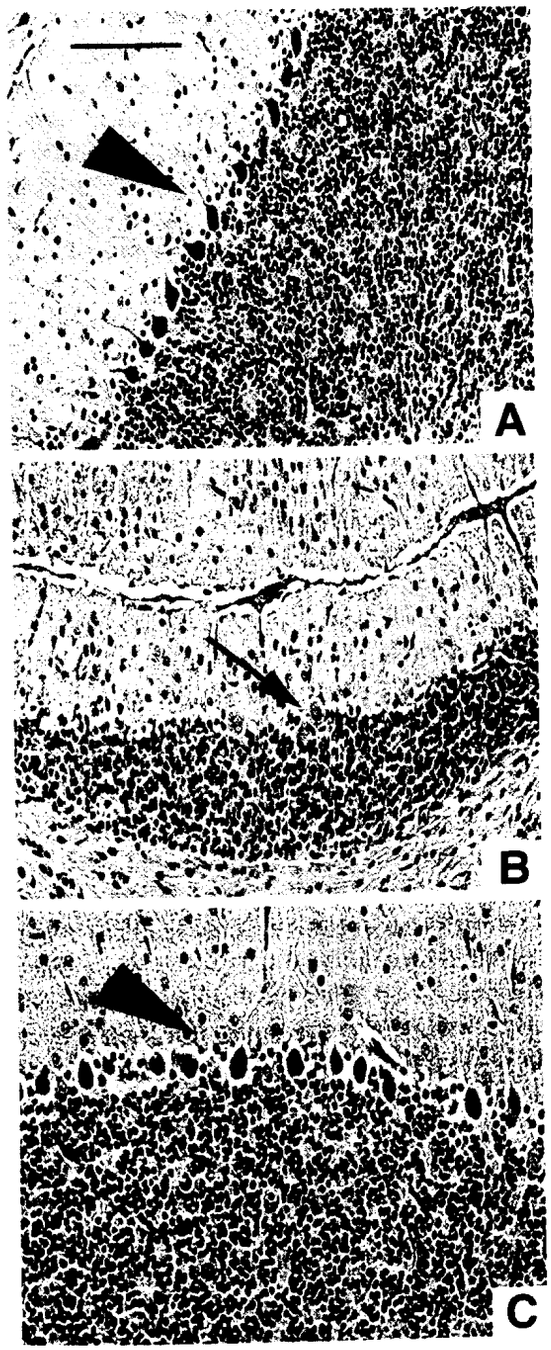

Fig. 4. Representative images of sagittal sections of the cerebellum from a sham-inoculated rat (A), a neonatally BDV-infected rat (B), and a malnourished rat (C) on PND 30. Note a distinct layer of PC in A and C (arrowheads) compared to single PC in B (arrow). Hematoxylin and eosine staining. Scale bar – 60 μm.

Table 1
Effects of neonatal BDV infection and malnourishment on monoamine concentrations in various brain regions in rat at PND 28

	Control	BDV	Malnourishment
Cortex			
5-HT	614 ± 56	769 ± 107	544 ± 63
5-HIAA/5-HT	0.58 ± 0.05	0.54 ± 0.08	1.14 ± 0.16 *
NE	429 ± 74	474 ± 51	423 ± 58
Hippocampus			
5-HT	647 ± 76	1035 ± 56 #	911 ± 64 #
5-HIAA/5-HT	0.73 ± 0.05	0.65 ± 0.08	0.96 ± 0.04 *
NE	526 ± 42	746 ± 55 #	592 ± 93
Striatum			
DA	5693 ± 805	4947 ± 630	4159 ± 251
DOPAC/DA	0.21 ± 0.02	0.18 ± 0.01	0.31 ± 0.04 *
5-HT	648 ± 67	732 ± 96	440 ± 33
5-HIAA/5-HT	0.93 ± 0.05	0.93 ± 0.09	1.19 ± 0.09

The data are presented as the means (pg/mg tissue) ± S.E.M.
* $P<.05$ versus control and BDV-infected rats.
\# $P<.05$ versus control rats.

Norepinephrine: Tissue content of NE remained unaltered in the BDV-infected and malnourished rats, $F(2,20)=0.2$, $P>.05$ (Table 1).

3.4.2. Hippocampus

5-HT and 5-HIAA/5-HT: Both neonatal BDV infection and malnourishment increased levels of 5-HT in hippocampus, $F(2,22)=9.7$, $P<.001$. Similar to the outcome in cortex, an analysis revealed a significant effect of treatment on 5-HIAA/5-HT ratio, $F(2,22)=4.5$, $P<.05$. While there was no difference in 5-HT turnover between the control and BDV-infected groups, 5-HIAA/5-HT ratio was significantly greater in the malnourished rats compared to BDV-infected rats, $P<.05$ (Table 1).

Norepinephrine: In contrast to malnourishment, neonatal BDV infection increased concentrations of NE in the hippocampus, $F(2,18)=4.0$, $P<.05$. Levels of NE were significantly greater in the hippocampus of the BDV-infected rats compared to the control animals, $P<.05$, whereas no difference in NE amounts was found between the control and malnourished groups, $P>.05$ (Table 1).

3.4.3. Striatum

DA and DOPAC/DA: Concentrations of DA in striatum remained unaffected by neonatal BDV infection and malnourishment, $F(2,21)=1.1$, $P>.05$ (Table 1). An analysis of variance showed a significant effect of treatment on DOPAC/DA ratio, an indicator of DA turnover, $F(2,21)=10.4$, $P<.05$. Compared to the BDV-infected and control

groups, DA turnover was significantly increased in the malnourished group, $P<.05$ (Table 1).

5-HT and 5-HIAA/5-HT: There were no significant alterations in the striatal levels of 5-HT due to neonatal BDV infection or malnourishment, $F(2,21)=3.0$, $P>.05$, with a small trend towards a decrease in 5-HT levels in the malnourished group (Table 1). An analysis did not reveal significant effects of neonatal BDV infection or malnourishment on 5-HIAA/5-HT ratio, $F(2,21)=3.3$, $P=.06$ (Table 1).

4. Discussion

The main objective of the present study was to evaluate a contribution of BDV-associated decrease in body weight on neurobehavioral alterations observed in infected rats. To this end, we have compared neuroanatomical, neurochemical, and behavioral abnormalities following neonatal BDV infection and rearing in oversized litters, a commonly used nutritional model [34]. Despite similar inhibition in the postnatal weight gain, the two treatments led to different brain pathology, neurochemical changes, and behavioral responses to novelty, supporting the notion about specificity of effects of neonatal BDV infection [38].

The neonatally BDV-infected and malnourished rats showed different behaviors in the open field test. Specifically, compared to the control animals, the infected rats exhibited hyperactivity in the open field. In contrast, there were no differences in locomotor activity between the malnourished rats and control rats. The present results are consistent with previous reports on behavioral effects of neonatal BDV infection [7,12,13], indicating that novelty-induced hyperactivity is a hallmark behavioral abnormality of neonatally BDV-infected rats. With respect to the responses of the malnourished rats to novelty, our results are in line with some observations [39,40] and disagree with others [41]. Since effects of malnourishment on locomotion in the open field strongly depend on the time of malnourishment (i.e., prenatal vs. postnatal) [42], the methods of producing malnourishment (low protein diet vs. oversized litters) [34,41], a strain of animals [43], and the occurrence of nutritional rehabilitation [44,45], it is difficult to directly compare the present results with earlier reports. Nonetheless, our data demonstrate the different responses to novelty in the BDV-infected and malnourished rats under the same test conditions.

The fact that locomotor hyperreactivity to novel/aversive stimulation are unlikely to be explained by decreased body weight has several implications. As a major behavioral BDV-associated alteration, hyperreactivity may also contribute to poor performances observed in BDV-infected rats when tested in several cognitive and social behavior paradigms. For example, abnormally elevated nonplay social interaction and impaired juvenile play in BDV-infected rats could be a result of an exaggerated response to a novel situation of meeting a different juvenile rat after a brief social isolation [15]. Similarly, elevated activity in a new environment could play a role in deficient functioning of BDV-infected rats in the T-maze and hole board tests [12]. Still, since other virus-associated behavioral alterations have not been directly evaluated in this study, a putative contribution of decreased body weight to BDV-associated cognitive and social dysfunctions remains to be studied.

The dissimilar behaviors of the BDV-infected and malnourished rats in the open field could be due to the different pattern of brain pathology. In particular, while neonatal BDV infection produced a marked dropout of PC in the cerebellum and degeneration of the dentate gyrus of the hippocampus, no gross alterations were noted in these brain areas in the malnourished rats. The present data are in agreement with the results of previous studies on effects of neonatal BDV infection, including a loss of neurons in the cerebellum (e.g., PC) [7,18], in the cortex (e.g., GABAergic neurons) [19], and in the hippocampus (e.g., granule cell of the dentate gyrus) [16,21].

The BDV-associated profile of brain pathology substantially differs from that found in the malnourished rats. In the hippocampus, noticeable virus-associated disturbances in the shape and cell density of the dentate gyrus of the hippocampus are readily seen, whereas little, if any, changes are observed in the hippocampus of the malnourished rats. The present effects of malnourishment on the hippocampus do not appear to be entirely consistent with published observations. Bedi [46,47] have shown a significant reduction in the number of hippocampal granule cells following a combination of prenatal and early postnatal malnourishment. One could suggest that a discrepancy between our results and the previous findings could be due to different approaches used to produce malnourishment. Indeed, a longer period of malnourishment could account for a more appreciable cell loss in the dentate gyrus as reported by Bedi [47]. Also, it cannot be completely ruled out that that if we had performed a quantitative measurement of neuronal dropout, we may have been able to find some indication of a neuronal loss in the hippocampus of the malnourished rats. Nonetheless, we think that compared to malnourishment, neonatal BDV infection produces a greater decrease in the number of granule cells. For example, a dropout of granule cells in neonatally BDV-infected rats was more than 80% by PND 120 [48] compared to a 40% decline in the number of granule neurons following the combination of pre- and postnatal malnourishment when its effects were assessed at PND 212 [47].

While a profound loss of PC was found in the cerebella of the BDV-infected rats, no dropout of PC was associated with malnourishment, consistent with previous negative findings [49–51]. In contrast, postnatal malnourishment has been shown to produce the increased density of PC [49,51,52]. Although our qualitative analysis was not able to evaluate the numerical density of PC, some irregularities in cell packing of the PC layer could be noted in the malnour-

ished group as compared with the evenly distributed PC in the control animals (Fig. 4). Thus, our qualitative analysis indicates a significant difference between the effects of postnatal malnourishment and neonatal BDV infection on survival of PC in the cerebellum.

Although not evaluated in the present study, different abnormalities following BDV infection and malnourishment have been also documented in the cortex. For example, neonatal BDV infection leads to a loss of up to 30% of cortical neurons by PND 45 [19], whereas early postnatal malnourishment does not change the number of cortical neurons [53]. Taken together, these data demonstrate that neonatal BDV infection and postnatal malnourishment produced different brain pathology in developing rats.

There were also several differences between neonatal BDV infection and malnourishment in the effects on brain monoamine systems. Although both BDV infection and malnourishment seemed to increase tissue concentrations of 5-HT in the hippocampus, a significant increase in 5-HT turnover was noted in the malnourished rats only. Similarly, despite the lack of changes in striatal levels of DA in the both experimental groups, turnover of DA was augmented by malnourishment and remained unaffected by the virus infection. Hippocampal concentrations of NE were increased in the BDV-infected rats and were unaltered in the malnourished animals.

Previous findings have shown a consistent elevation of 5-HT in malnourished rats in different brain regions [39,54,55]. While this alteration is similar to 5-HT changes observed in BDV-infected rats, the underlying mechanisms of increased 5-HT content might be different in the two groups of rats. For example, increased turnover of 5-HT was found in malnourished rats only. Elevated turnover of 5-HT may indicate an activation of 5-HT catabolism. 5-HT catabolism might be increased in order to reduce 5-HT neurotransmission augmented by high concentrations of presynaptic 5-HT. Indeed, attenuated responses to 5-HT agonists have been described in malnourished rats as a result of down-regulation of postsynaptic 5-HT receptors [56]. In contrast, our previous data have suggested that virus-induced elevation of 5-HT could be due to decreased rather than increased synaptic levels of as indicated by a simultaneous up-regulation of postsynaptic 5-HT1a and 5-HT2a receptors [57] and an enhanced responsivity to 5-HT agonists by BDV-infected rats (Pletnikov, unpublished observations).

Although the previous investigations have shown that oversized litters do not lead to the poor maternal care or neonatal stress [32,35,36], we cannot rule out that some of the neurochemical disturbances in the malnourished rats resulted from a brief stress of overcrowding during the last week before weaning and/or some alterations in maternal behavior associated with nursing oversized litters [58,59].

Taken together, the present study showed that the major neuroanatomical, neurochemical, and behavioral features of BDV-associated neurodevelopmental damage cannot be explained by postnatal weight gain inhibition and are likely produced by the virus infection per se.

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