Available online at www.sciencedirect.com
ScienceDirect
Behavioural Brain Research 176 (2007) 141–148
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

# Abnormal social behaviors in young and adult rats neonatally infected with Borna disease virus

Karen Lancaster, David M. Dietz, Timothy H. Moran, Mikhail V. Pletnikov *

*Division of Neurobiology, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 600 North Wolfe Street, CMSC 8-121, Baltimore, MD 21287, USA*

Received 10 April 2006; received in revised form 29 May 2006; accepted 12 June 2006
Available online 21 July 2006

**Abstract**

Autism spectrum disorders (ASD) have been the focus of a great deal of research and clinical speculation. This intense interest relates to both the perplexing pathogenesis and devastating consequences of these disorders. One of the obstacles to understanding the pathogenesis of autism and to developing efficient treatment has been the paucity of animal models that could be used for hypotheses-driven mechanistic studies of abnormal brain and behavior development and for the pre-clinical testing novel pharmacological treatments. In this report, we briefly review our animal model of ASD based on neonatal Borna disease virus (BDV) infection and present new data about abnormal social interaction in adult BDV-infected rats. We found that neonatal BDV infection profoundly affected social behaviors in adult rats. Compared to the control rats, both 90- and 180-day-old infected rats spent less time in active social interaction and more time in following their partners. In the intruder–resident test, the BDV-infected resident rats exhibited less aggression towards the intruders and showed more the following-the-intruder behavior. The following-the-partner behavior may be an example of "stereotypic" activity due to BDV-induced abnormal social communication between rats. The previously published results and present findings indicate that neonatal BDV infection significantly altered the normal pattern of social interaction in rats. Co-localization of activated microglia and dying Purkinje cells in BDV-infected rats suggests that the BDV model could be used to study a pathogenic link of Purkinje cell dropout and neuroinflammation to abnormal social behaviors.


*Keywords:* Autism; Borna disease virus; Social behavior; Cerebellum; Rat

## 1. Introduction

Autism is a neurodevelopmental disorder that is characterized by abnormal social interaction, communication ability, patterns of interests, and patterns of behavior [23,27]. The etiology and physiological basis for autism are unknown, and the psychiatric criteria for the diagnosis are based on behavioral attributes rather than clinical tests. Evidence firmly links autism with abnormalities in the cerebellum, the medial temporal lobe, and the frontal lobe [35,36]. Specifically, there is an emerging pattern of increased cell packing in the limbic system, reduced numbers of Purkinje cells in the cerebellum, age-related changes in cerebellar nuclei and inferior olives, cortical dysgenesis, and increased brain size, especially in the *young* autistic child, as measured by head circumference, magnetic resonance image (MRI) brain volume, and postmortem brain weight [10,35,36].

These morphologic changes allude to complex neurodevelopmental mechanisms operating in autism. The paucity of relevant animal models significantly inhibits experimental studies of the developmental neurobiology of ASD. Developing animal models for psychiatric conditions is an extremely difficult undertaking [14,18,26]. As has been suggested, an "ideal" animal model should resemble the disease in its symptomology, etiology, biochemistry, and treatment [30,61]. Unfortunately, with autism as with other developmental neuropsychiatric illnesses, the etiology and the pathogenic mechanisms remain poorly understood [10,27,36]. Furthermore, since key symptoms of human psychiatric conditions are of a cognitive and language nature, it appears impossible to generate a relevant animal model that would mimic those abnormalities. However, if one assumes that animal models are not supposed to exactly

* Corresponding author. Tel.: +1 410 502 3760; fax: +1 410 614 0013.
*E-mail address:* mpletnik@jhmi.edu (M.V. Pletnikov).

0166-4328/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2006.06.013

mimic every feature of complex human diseases, our experimental goal of generating an animal model becomes achievable. A good animal model serves to answer a specific question(s) relevant to the disease. For example, if a genetic mutation for a neurodevelopmental disorder has been identified, genetically engineered mouse models can be produced to evaluate effects of the mutant gene on brain development. Similarly, if an environmental agent has been found to cause the disease, laboratory animals can be exposed to that factor to study the pathogenesis of the disease and/or to test potential therapeutic compounds.

Since ASD can be caused by different etiologic agents, multiple animal models are needed to better appreciate the complex pathogenic mechanisms. Current animal models of abnormal neurodevelopment utilize various genetic approaches, physical ad chemical insults, endocrine dysfunction [2,16,17,29,50,51]. Unfortunately, there are few animal models of neurodevelopmental injury using virus infections [34,42,56] despite the evidence that prenatal and early postnatal viral infections have been associated with a number of human psychiatric disorders, including schizophrenia and autism [10,64]. The best association to date has been made between congenital rubella and autism [9]; however, cytomegalovirus and other members of the herpes virus family have been also associated with autism [28,57,63].

For several years, we have been studying our animal model of ASD based on early brain injury following persistent central nervous system (CNS) infection of neonatal rats with an experimental teratogen, a 8.9 kb, non-segmented, negative-strand, round, enveloped RNA virus, Borna disease virus (BDV) [6,11]. Neonatal BDV infection was first described in the early 1980s by Narayan et al., and Hirano et al., and was further characterized by Carbone et al. [8,24,32]. Recently, interest in neonatal BDV infection has significantly grown when many groups recognized that this model provides new insights into the pathogenesis of neurodevelopmental abnormalities that have been associated with perinatal viral insult [19,25,52].

Neonatal BDV infection is produced in newborn Lewis rats via intracranial BDV inoculation within 24 h of birth. A few days post infection (p.i.), viral antigens and RNA can be found in the olfactory bulb, hippocampus, frontal cortex and the deep cerebellar nuclei [4,19,20,52]. At about 3 weeks p.i., viral antigen can be seen in neurons throughout the brain [20,31]. Both a reduced body weight and a smaller body length have been documented in BDV-infected rats [3,25,41]. A simultaneous and proportional BDV-induced decrease in body weight and length seems to indicate growth retardation.

Neonatal BDV infection induces selective damage to the cerebellum. Early infection of Purkinje cells (PC) on days 3–5 p.i. [4] leads to a gradual loss of these cells over a protracted period of time, with about 70% of cells missing by 7 months p.i. [13,43]. A continuing degeneration of granule cells of the hippocampal dentate gyrus (DG) is another hallmark of BDV infection [8,25,43,60,65]. Degenerating neurons are replaced by astrocytes and microglia cells [8,65]. Neonatal BDV has been shown to also be associated with thinning of the cortex [19,43]. Thus, neonatal BDV infection causes damage to brain regions that continue to develop after birth, and may be vulnerable to environmental insults [1].

The mechanisms of neuronal loss due to neonatal BDV infection are unknown. In addition to direct effects of BDV on neuronal functioning and survival [21,22], a role of soluble neurotoxins secreted by activated microglia and astrocytes has been proposed [25,37,46,52,53,60]. The latter mechanism is particularly intriguing given a recently described association between PC loss and microglia activation in autistic brains [59].

Effects of neonatal BDV infection on development of brain monoamine system have been also studied [40,44]. Specifically, elevated tissue content and increased numbers of post-synaptic receptors for serotonin were found in the cortex and hippocampus of BDV-infected rats. These results, along with findings of accumulation of synaptic vesicles in the pre-synaptic terminals in BDV-infected neuronal cultures [22] suggest that BDV may affect neuronal transmission by exploiting the vesicular transport for spreading [7].

Virus-associated structural and neurochemical alterations could be responsible for abnormalities in the rat's behavioral repertoire. Neonatal BDV infection has been found to be associated with emotional disturbances characterized by hyperreactivity to novel and/or aversive environment [25,39]. Since the hippocampus plays a major role in the brain mechanisms of learning and memory [15,48], effects of BDV-induced hippocampal injury on learning and memory have been a focus of several studies. Deficient performance in the Y-maze and the hole board tests, poor performance in the Morris water maze as well as attenuated fear conditioning have been documented in BDV-infected rats [12,39,49].

Since BDV affects the brain regions that are involved in the neuronal mechanisms of social activity in rats (e.g., cortex and cerebellum) [33], Pletnikov et al. have assessed social behaviors of young BDV-infected rats [38]. We found that the effects of BDV infection on different social behaviors in young rats were variable. For example, compared to control animals, BDV-infected rats exhibited attenuated play activity but elevated social non-play interaction, suggesting alterations rather than a general inhibition of social behaviors [38].

Here, we sought to further characterize effects of neonatal BDV infection on social behaviors in rats. Specifically, we evaluated non-aggressive and aggressive social activities in young adult and adult rats neonatally infected with BDV. We found that neonatal BDV infection affected social behaviors in 90- and 180-day-old rats. In the open field test, neonatal BDV infection significantly decreased the total time of social interaction. In the resident–intruder paradigm, neonatal BDV infection was associated with significant attenuation of aggressive responses in the resident rats and freezing behavior in the intruder rats. In contrast, in both tests, the following-the-partner behavior was significantly increased in the BDV-infected rats compared to the control animals. BDV-associated alterations in social activity were unlikely due to a non-specific decrease in general exploratory activity since we found no differences between the groups in the number of approaches to or time spent in exploring an inanimate object.

## 2. Materials and methods

### 2.1. Animals

Pregnant Lewis rats (16–18 days of gestation) were used in the present study (Harlan, Indianapolis, IN). All rat pups were born and reared in the animal vivarium at Johns Hopkins University School of Medicine, Baltimore, MD. Following weaning, rats were kept in groups of two or three in 45 cm × 26 cm × 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 Inc., NJ). The sham-inoculated rats were kept in the same room. Rats were maintained on a 12/12 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

BDV stock was prepared from homogenized BDV-infected rat brain tissue as described elsewhere [8]. Pups were inoculated via 26G needle intracranially within 24 h of birth either with 20 μL of infected brain homogenate (the titer was $10^4$ $TCID_{50}$/g of brain tissue, $CRP_3$ (He-80) BDV strain) (BDV-infected rats) or uninfected brain homogenate (control rats, NL). For intracranial inoculation, a rat pup was taken out of the home cage and was anesthetized by placing on ice until the pup ceased to move. After an injection, the pup was warmed with a warm cloth, and returned to the home cage.

### 2.3. The schedule of the experiments

For all experiments described in this study, male rats were tested at postnatal days (PND) 90 or 180. Upon completion of the behavioral experiments, the rats were euthanized for histological experiments as described below.

- *PND 90*. The habituation test for 4 days; the object exploration test (1 day); a brief social isolation (4 days); the social interaction test (1 day); euthanasia (four rats from each group). All other rats were re-distributed and left in three per cage until PND 180 without additional tests.
- *PND 180*. A brief social isolation (4 days); the social interaction tests: rats were tested either in the open field arena (1 day) or in the intruder–resident test (2 days); euthanasia.

### 2.4. Habituation test

At PND 90, BDV-infected ($n = 9$) and control ($n = 8$) rats were habituated to the open field arena. Habituation was carried out at 16:00–18:00 h and took place in a square open field, 50 cm × 50 cm, with 20 cm high opaque plastic walls. The floor of the open field was divided into 36 sections of equal area by a series of solid lines forming small squares. A blowing generator produced 60 dB white noise, as measured in the center of the open field. The open field was illuminated by a 25 W red light bulb mounted 80 cm above the center of the open field. Each rat was placed in the center of the open filed box and left there for 3 min daily for four consecutive days. The rat's behavior was videotaped and later locomotion (ambulation) was scored by the number of sections crossed by all four paws. Scoring was performed by an experienced observer blind to the conditions of the experiments.

### 2.5. Object exploration test

On the day after completion of habituation, a wooden cylinder was placed in the center of the arena for 5 min. Investigation of the novel object was videotaped and was assessed by a trained observer blind to the experimental group of the subject. Novel object contacts were measured as "approaches" (examination of the object with the nose and/or paws) and "exploration time" (time spent in active sniffing of the object).

### 2.6. Social behavior tests

For the social tests below, we used rats that were not used in the habituation and object exploration tests.

#### 2.6.1. Social interaction in the open field

BDV-infected and control rats were individually housed for 4 days to increase their social motivation. On the 5th day, two rats from the different groups were placed in the open field box used in the habituation test, and social and non-social activities of the rats were videotaped for 10 min. The following pairs of the rats were used—control: control rats (NL–NL: 6 pairs at PND 90 and 4 pairs at PND 180); control: BDV-infected rats (NL–BDV: 11 pairs at PND 90 and 8 pairs at PND 180); BDV-infected: BDV-infected rats (BDV–BDV: 5 pairs at PND 90 and 4 pairs at PND 180). At either PND, each rat was tested only once in either pair. The social activities measured included sniffing (time spent actively sniffing the partner), following (moving to or after the partner), the number of times paws were on the head of the partner, and the total time the rats engaged in positive social behaviors including sniffing, grooming, following, standing, sitting or lying down next to each other. In addition, aggressive behavior was evaluated by counting the number of the attacks or bites.

#### 2.6.2. Intruder–resident test

For the intruder–resident test, control and BDV-infected rats (residents) were individually housed in the standard cages for 4 days. On the 5th day, an unfamiliar rat (intruder) was taken from the regular home cage containing two to three rats and was placed in the resident's cage for 10 min, and all behaviors of both rats were videotaped. The test was repeated on next day using the same resident and intruder rats. The following pairs of rats were tested: control resident–control intruder (NL–NL, $n = 5$); control resident–BDV-infected intruder (NL–BDV, $n = 3$); BDV-infected resident–control intruder (BDV–NL, $n = 2$); BDV-infected resident–BDV-infected intruder (BDV–BDV, $n = 6$). The social behaviors scored were: sniffing (time spent actively sniffing the partner), following (moving to or after the partner), the number of times paws were on the head of the partner, and the total time the rats engaged in positive social behaviors including sniffing, grooming, following, or standing, sitting and lying down next to each other. The number of the aggressive attacks and bites were measured to evaluate aggressiveness in rats. In addition, the time spent freezing was scored for each intruder rat. For each resident, a separate intruder was used for the 2-day test.

#### 2.6.3. Histopathological examination and anti-BDV immunohistochemistry

Control ($n = 6$) and BDV-infected ($n = 6$) Lewis were randomly selected from the pool of rats used in behavioral experiments described in this study. Upon completion of behavioral tests, selected rats were deeply anesthetized with EUTHASOL (Diamond Animal Health Inc., IA) and perfused with phosphate buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde. Brains were removed, postfixed for 4 h, embedded in paraffin and cut sagittally into 12 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 anti-horse IgG (Vector, Burlingame, CA) as described previously [43].

### 2.7. Statistical analyses

Analyses of variance were used to evaluate effects of neonatal BDV infection on the social behaviors in rats. The pair type and/or the test day were used as independent variables. The threshold for significance was set at $p < 0.05$.

## 3. Results

### 3.1. Habituation in the open field arena

Neonatal BDV infection impaired habituation of ambulation in the open field. While uninfected control animals exhibited a decrease in locomotion over the 4-day period, $p < 0.05$, BDV-infected rats significantly increased locomotion by the last day of testing, $p < 0.05$ (Fig. 1).

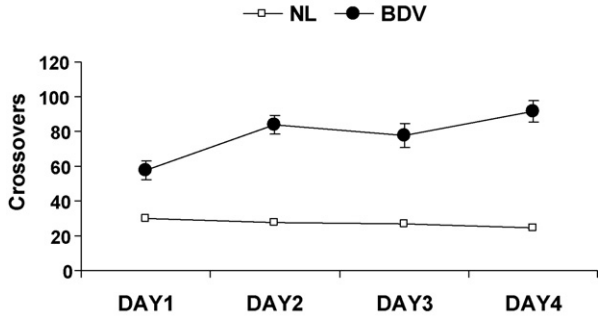


Fig. 1. Neonatal BDV infection impaired habituation of ambulation in the open field. The mean numbers of crossovers for the control (NL) and BDV-infected (BDV) rats are presented over 4 days of testing. Note habituation of horizontal locomotor activity in the control rats and the absence of habituation in the BDV-infected rats. The values are mean ± S.E.M.

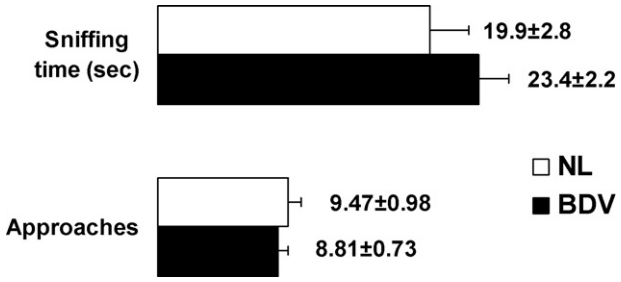


Fig. 2. Neonatal BDV infection did not affect exploration of the inanimate object. The mean time of sniffing the object (the upper panel) and the number of approaches to the object (the bottom panel) are presented for the control (NL) and BDV-infected (BDV) rats. Note comparable levels of exploration of the inanimate object in both groups of rats. The values represent mean ± S.E.M.

### 3.2. Object exploration test

Neonatal BDV infection did not significantly affect the object exploration in rats (Fig. 2). An analysis of the data for the number of approaches to the object showed no effect of the infection status, $p > 0.05$. Similarly, there was no effect of BDV infection on the time spent sniffing the object, $p > 0.05$.

### 3.3. Social interaction

#### 3.3.1. Social interaction in the open field

Neonatal BDV infection significantly attenuated social behaviors in young adult and adult rats (Fig. 3 and Table 1). For 90-day-old rats, the time spent sniffing the partner and the total time of social interaction were significantly more in the NL–NL pairs compared to all other pairs, $p < 0.05$ (Table 1). In contrast, the time spent following the partner was significantly more in the BDV–BDV pairs compared to the NL–NL or the NL–BDV pairs, $p < 0.05$. No difference between the groups was found in the number of paws on head (Table 1).

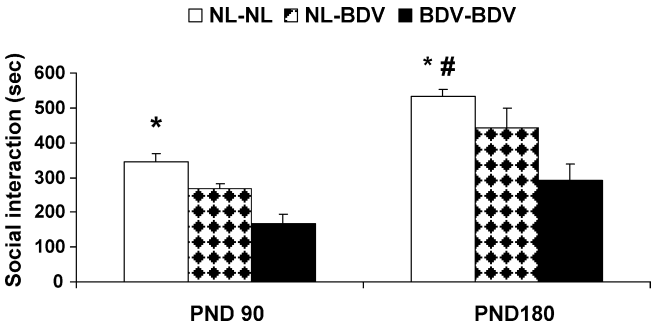


Fig. 3. Neonatal BDV infection decreased the total time of social interaction. The mean values of the total time of social interaction in the control–control pairs (NL–NL), the control–BDV-infected pairs (NL–BDV), and the BDV-infected–BDV-infected pairs (BDV–BDV) at postnatal day (PND) 90 and 180. Note significantly more time of social interaction in the NL–NL pairs compared to the BDV–BDV pairs. $^{*}p < 0.01$ vs. the BDV–BDV pairs; $^{\#}p < 0.05$ vs. PND 90. The values represent mean ± S.E.M.

For 180-day-old rats, the time spent sniffing the partner and the total time of social interaction were significantly greater in the NL–NL pairs compared to the other pairs (Fig. 3 and Table 1). Similar to the 90-day-old rats, the time spent following the partner was significantly greater in the BDV–BDV pairs compared to the NL–NL or the NL–BDV pairs, $p < 0.05$.

Comparing the time of social interaction between the NL–NL pairs of the two age showed that 180-day-old control rats spent significantly more time sniffing the partner than 90-day-old control rats did, $p < 0.05$ (Table 1). Similarly, the total time of social interaction was significantly more in the older control rats compared to the younger control rats, $p < 0.05$ (Fig. 3). In contrast, no differences in the total time of social activity were found between the two age groups within the NL–BDV or the BDV–BDV pairs, $p > 0.05$. No difference between the age groups was found for BDV-infected rats or for the rat pairs consisted of control and BDV-infected animals, $p > 0.05$.

#### 3.3.2. The intruder–resident test

In the intruder–resident test, we found no effects of neonatal BDV infection on the following non-aggressive social behaviors:

Table 1
Effects of neonatal BDV infection on the rat's social behaviors in the open field

| | PND 90 | | | PND 180 | | |
|---|---|---|---|---|---|---|
| | NL–NL | NL–BDV | BDV–BDV | NL–NL | NL–BDV | BDV–BDV |
| Sniffing (s) | 291 ± 28* | 228 ± 10 | 141 ± 22 | 441 ± 36^ | 282 ± 21 | 171 ± 29 |
| Following (s) | 5.3 ± 2.0 | 6.8 ± 0.8 | 24.6 ± 6.2# | 2.5 ± 0.9 | 8.2 ± 1.7 | 16.0 ± 4.7# |
| Paws on head | 1.3 ± 0.5 | 3.6 ± 0.5 | 5.2 ± 1.5 | 0 ± 0 | 1.5 ± 0.4 | 4.2 ± 2.9 |

Abbreviations. NL–NL: control–control pairs; NL–BDV: control–BDV pairs; BDV–BDV: BDV-infected–BDV-infected pairs. The data are presented as mean ± S.E.M.

* $p < 0.05$ vs. all other pairs for this type of social activity.

# $p < 0.05$ vs. the NL–NL or NL–BDV pairs for this type of social activity.

^ $p < 0.05$ vs. the NL-NL pair at PND 90.

Table 2
Effects of neonatal BDV infection on the rat's social behaviors in the resident–intruder test

| | NL–NL | NL–BDV | BDV–NL | BDV–BDV |
|---|---|---|---|---|
| Sniffing (s) | | | | |
| Day 1 | 148.8 ± 47.1 | 236.5 ± 33.5 | 227.0 ± 64.0 | 170.4 ± 19.1 |
| Day 2 | 110.8 ± 20.5 | 157.0 ± 55.3 | 190.0 ± 55.0 | 164.2 ± 16.1 |
| Paws on head | | | | |
| Day 1 | 9.2 ± 1.3 | 7.5 ± 4.5 | 5.0 ± 2.0 | 8.7 ± 2.4 |
| Day 2 | 8.4 ± 1.4 | 11.7 ± 1.2 | 26.0 ± 7.0 | 9.8 ± 1.8 |
| Total social (s) | | | | |
| Day 1 | 165.2 ± 51.7 | 279.5 ± 21.5 | 288.5 ± 24.5 | 277.0 ± 28.5 |
| Day 2 | 147.0 ± 45.7 | 182.0 ± 52.0 | 230.0 ± 70.0 | 217.8 ± 18.3 |
| Bites | | | | |
| Day 1 | 4.4 ± 2.0 | 2.0 ± 1.0 | 0 | 1.4 ± 0.9 |
| Day 2 | 3.8 ± 1.2 | 4.3 ± 1.3 | 4.0 ± 1.0 | 2.3 ± 1.1 |

Abbreviations. NL–NL: control resident–control intruder; NL–BDV: control resident–BDV-infected intruder; BDV–NL: BDV-infected intruder–control resident; BDV–BDV: BDV-infected resident–BDV-infected intruder. The data are presented as mean ± S.E.M.

the sniffing time, the total time spent in social interaction and the numbers of paws on heads (Table 2). Neonatal BDV infection significantly decreased aggressive behavior in the BDV–BDV pairs compared to the NL–NL pairs as assessed by the number of attacks, $F(3,31) = 5.1$, $p < 0.05$ (Fig. 4). There was a significant decrease in the freezing behavior in the intruders from the BDV–BDV, NL–BDV and BDV–NL pairs compared to the intruders from the NL–NL pair, all $p < 0.05$ (Fig. 5). In contrast, the BDV-infected residents spent significantly more time in following the intruder than the control residents did, $F(3,31) = 5.66$, $p = 0.004$ (Fig. 6).

### 3.4. Histopathological findings

At PND 90 and 180, overall histopathological examination of the H&E stained brain sections showed that neonatal BDV infection produced characteristic brain damage, i.e. the complete degeneration of the dentate gyrus of the hippocampus, thinning of the neocortex and dramatic loss of the Purkinje cells in the cerebellum (data not shown). Anti-BDV staining of the brain sections revealed the presence of BDV-specific staining throughout the brain, with particularly strong staining being noted in the cortex, striatum, olfactory bulbs, pyramidal layers of the hippocampus, brainstem, and the nuclei of the cerebellum (data not shown).

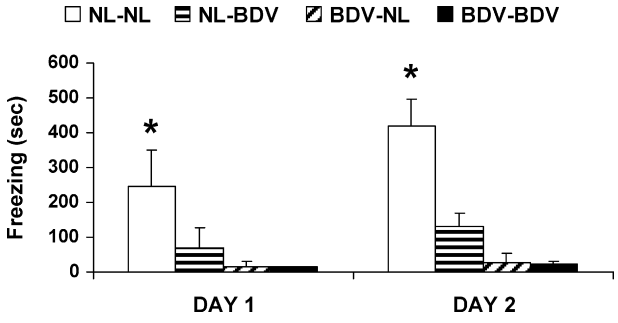


Fig. 5. Neonatal BDV infection attenuated the freezing behavior in rats. The mean values of time of freezing in the control resident–control intruder pairs (NL–NL), the control resident–BDV-infected intruder pairs (NL–BDV), the BDV-infected resident–control intruder pairs (BVD–NL) and the BDV-infected resident–BDV-infected intruder pairs (BDV–BDV). Note significantly more freezing in the NL–NL pairs compared to all other pairs. $^{*}p < 0.01$ vs. all other pairs. The values represent mean ± S.E.M.

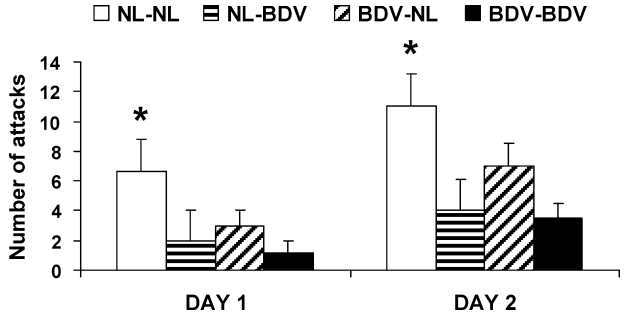


Fig. 4. Neonatal BDV infection decreased aggressiveness in adult rats. The mean values of the number of attacks on the intruders in the control resident–control intruder pairs (NL–NL), the control resident–BDV-infected intruder pairs (NL–BDV), the BDV-infected resident–control intruder pairs (BVD-NL) and the BDV-infected resident–BDV-infected intruder pairs (BDV–BDV). Note significantly more attacks in the NL–NL pairs compared to the BDV–BDV pairs. $^{*}p < 0.01$ vs. the BDV–BDV pairs. The values represent mean ± S.E.M.

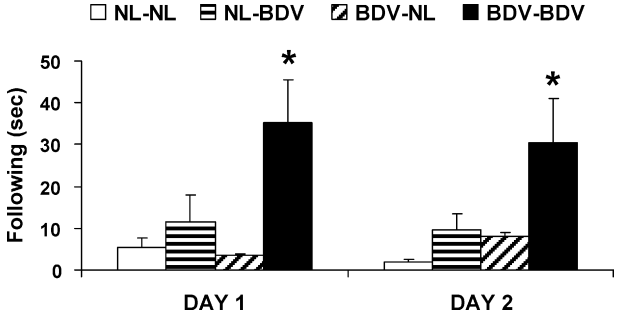


Fig. 6. Neonatal BDV infection increased the following-the-partner behavior in rats. The mean values of time of following in the control resident–control intruder pairs (NL–NL), the control resident–BDV-infected intruder pairs (NL–BDV), the BDV-infected resident–control intruder pairs (BVD–NL) and the BDV-infected resident–BDV-infected intruder pairs (BDV–BDV). Note significantly greater time spent following the intruder in the BDV–BDV pairs compared to all other pairs. $^{*}p < 0.01$ vs. all other pairs. The values represent mean ± S.E.M.

## 4. Discussion

Although BDV has not been implicated in the causation of autism, neonatal BDV infection is a valuable animal model of neurodevelopmental damage with relevance to some neuropathological and behavioral features of ASD such as developmental injury of the cortex, hippocampus and cerebellum and associated alterations in emotional, cognitive and social behaviors [27,36].

One of the key features of autism is abnormal social interaction [27]. Our previous study demonstrated that neonatal BDV infection significantly decreased play activity in post-weaning rats. The observed attenuation of play in BDV-infected rats was associated with decreased play solicitation and was not related to a general decline in locomotion or non-social behaviors that were, in fact, elevated in BDV-infected animals [38]. The present work further evaluated effects of neonatal BDV infection on social behaviors in rats by assessing different types of social interaction in adult BDV-infected rats. Neonatal BDV infection profoundly affected social behaviors in adult rats. Both 90- and 180-day-old infected rats spent significantly less time in social exploration of the partner compared to control uninfected rats. Attenuated social activity in BDV-infected rats was unlikely due to a non-specific decrease in their general exploratory activity since control and BDV-infected rats spent the comparable amount of time exploring the inanimate object and had a greater locomotor activity in a novel environment. We hypothesize that neonatal BDV infection affected the pattern of social activity in adult rats. For example, although the sniffing time and the total time of social interaction were significantly greater in the NL–NL pairs compared to the BDV–BDV pairs, the latter spent significantly more time following their partners. These results suggest altered rather than decreased social behaviors in BDV-infected rats. The hypothesis is consistent with the above data for play behavior, indicating that abnormal social interaction in developing and mature BDV-infected rats was not a result of a general decrease in social motivation.

The data from the resident–intruder test seem to provide more evidence for BDV-associated alterations in the social behaviors in adult rats. The test is based on a brief social isolation that increases social motivation and can facilitate territorial aggressiveness in isolated male rats [45]. For example, this treatment often elevates the number of aggressive postures; attacks and/or bites in resident rats towards intruders [62]. In contrast, the amount of non-aggressive social behaviors exhibited by resident rats can be decreased. For an intruder, encountering threats and aggressive postures by the resident usually evokes freezing to minimize likelihood of attacks [54]. The present results demonstrate that neonatal BDV infection dramatically changed the social behaviors in the resident and the intruders. Compared to the control residents, the BDV-infected resident rats exhibited less aggression towards the intruders and, instead, spent more time in following the intruder. As a result, the intruder's freezing behavior was significantly decreased in all pairs with the BDV-infected residents. Thus, neonatal BDV infection appeared to change the pattern rather than level of social activity in rats.

In this context, following-the-partner behavior is a good example of virus-increased social activity. Indeed, the two different tests used in the study consistently demonstrated a BDV-associated increase in following the partner. Given that this behavior was significantly elevated in the BDV–BDV pairs compared to all other pairs, one could speculate that this "stereotypic" activity could result from abnormalities in social communication between two infected animals. Notably, when the BDV-infected rats were paired with the control rats, the amount of following significantly decreased, suggesting that responses of the control partners might modulate social activity in the BDV-infected rats. It is in concordance with our previous investigation of play in young BDV-infected rats that increased the number of pinning (i.e., a play activity) after pairing with uninfected partners [38]. Thus, our pervious and present data demonstrate that neonatal BDV infection significantly affects social activity in developing and adult rats by altering the normal pattern of social interaction.

Decreased social communication and an abnormal pattern of social activity (e.g., stereotypy) observed in autism and the BDV model raise a question about putative common underlying mechanisms. In this context, the available neuropathological data for autism provide some clues as to where to look for this common pathology. A gradual loss of Purkinje cells (PC) in the cerebellum in some groups of autistic patients [5] and a protracted elimination of those cells in neonatally BDV-infected rats might be one of the hypothetical common neurobiological substrates underlying behavioral deficits in BDV-infected rats and autistic patients.

Recently, the role of the cerebellum in the brain mechanism of emotion, attention, language and social behavior has been increasingly recognized [55]. Given that PC are the only output of the cerebellum, it is not inconceivable that damage to PC will lead to major alterations in the neuronal mechanisms of emotional, cognitive or social behavior. We speculate that a continuing loss of PC in neonatally BDV-infected rats mimics the cerebellar pathology in autism and represents a major brain substrate of altered social activity. We further hypothesize that some pathogenic mechanisms of PC dropout in neonatally BDV-infected rats and autistic brains might also be similar. Specifically, a recent publication has demonstrated activated microglia and astrocytes along with elevated production of pro-inflammatory soluble factors in cerebella of the autistic patients [59]. These findings are strikingly similar to what has been found in the cerebellum of neonatally BDV-infected rats [13,25,65]. It remains unknown if microglia activation plays a causative role in PC dropout or microgliosis is a secondary response to neuronal death. We think that neonatal BDV infection is a good experimental system to address this question. If death of PC in autism and BDV-infected animals is caused by microglia activation and ensuing secretion of neurotoxic factors, treatments that inhibit microglia activation should have ameliorative effects on a loss of PC. In contrast, if PC death is primarily due to non-inflammatory factors (i.e., genetic in case of autism and viral replication in the neurons in case of BDV infection), anti-inflammatory treatments would not significantly improve survival of PC in the cerebellum [47,58]. In addition, if anti-inflammatory treatment

has beneficial effects on PC death, the BDV model can be used to test a hypothesis that a gradual elimination of PC may be in part responsible for abnormalities in social interaction in BDV-infected rats. Experiments to test the above hypotheses are in progress in our laboratory and represent a good example of how animal models can be used to address the specific mechanistic hypotheses that cannot be tested in patients.

In conclusion, neonatal BDV infection is associated with abnormalities in social behavior in young and adult rats. Alterations in social activity along with changes in emotional and cognitive domains can be attributed to developmental brain damage, particularly degeneration of the dentate gyrus of the hippocampus and a gradual loss of Purkinje cells in the cerebellum. We believe that the BDV model provides new opportunities in studying a role of early brain injury and neuro-inflammation in the pathogenesis of autism-like developmental abnormalities.

## References


[1] Altman J. Morphological and behavioral markers of environmentally induced retardation of brain development: an animal model. Environ Health Perspect 1987;74:153–68.

[2] Bachevalier J. Medial temporal lobe structures and autism: a review of clinical and experimental findings. Neuropsychologia 1994;32:627–48.

[3] Bautista JR, Schwartz GJ, de la Torre JC, Moran TH, Carbone KM. Early and persistent abnormalities in rats with neonatally acquired Borna disease virus infection. Brain Res Bull 1994;34:31–6.

[4] Bautista JR, Rubin SA, Moran TH, Schwartz GJ, Carbone KM. Developmental injury to the cerebellum following perinatal Borna disease virus infection. Dev Brain Res 1995;90:45–53.

[5] Blatt GJ. GABAergic cerebellar system in autism: a neuropathological and developmental perspective. Int Rev Neurobiol 2005;71:167–78.

[6] Briese T, Schneemann A, Lewis AJ, Park YS, Kim S, Ludwig H, et al. Genomic organization of Borna disease virus. Proc Natl Acad Sci USA 1994;91:4362–6.

[7] Carbone KM, Duchala CS, Griffin JW, Kincaid AL, Narayan O. Pathogenesis of Borna disease in rats: evidence that intra-axonal spread is the major route for virus dissemination and the determinant for disease incubation. J Virol 1987;61:3431–40.

[8] Carbone KM, Park SW, Rubin SA, Waltrip Jr RW, Vogelsang GB. Borna disease: association with a maturation defect in the cellular immune response. J Virol 1991;65:6154–64.

[9] Chess S. Autism in children with congenital rubella. J Autism Child Schizophr 1971;1(1):33–47.

[10] Ciaranello AL, Ciaranello RD. The neurobiology of infantile autism. Ann Rev Neurosci 1995;18:101–28.

[11] Cubitt B, de la Torre JC. Borna disease virus (BDV), a nonsegmented RNA virus, replicates in the nuclei of infected cells where infectious BDV ribonucleoproteins are present. J Virol 1994;68:1371–81.

[12] Dittrich W, Bode L, Ludwig H, Kao M, Schneider K. Learning deficiencies in Borna disease virus-infected but clinically healthy rats. Biol Psychiatry 1989;20:818–28.

[13] Eisenman LM, Brothers R, Tran MH, Kean RB, Dickson GM, Dietzschold B, et al. Neonatal Borna disease virus infection in the rat causes a loss of Purkinje cells in the cerebellum. J Neurovirol 1999;5:181–9.

[14] Ellenbroek BA, Cools AR. Animal models with construct validity for schizophrenia. Behav Pharmacol 1990;1:469–90.

[15] Fanselow MS. Contextual fear, gestalt memories, and the hippocampus. Behav Brain Res 2000;110:73–81.

[16] Ferguson SA. Neuroanatomical and functional alterations resulting from early postnatal cerebellar insults in rodents. Pharmacol Biochem Behav 1996;55:663–71.

[17] Gainetdinov RR, Mohn AR, Caron MG. Genetic animal models: focus on schizophrenia. Trends Neurosci 2001;24:527–33.

[18] Geyer MA, Braff DL. Startle habituation and sensorimotor gating in schizophrenia and related animal models. Schizophr Bull 1987;13: 643–68.

[19] Gonzalez-Dunia D, Watanabe M, Syan S, Mallory M, Masliah E, de la Torre JC. Synaptic pathology in Borna disease virus persistent infection. J Virol 2000;74:3441–8.

[20] Gosztonyi G, Ludwig H. Borna disease—neuropathology and pathogenesis. Curr Top Microbiol Immunol 1995;190:39–73.

[21] Hans A, Syan S, Crosio C, Sassone-Corsi P, Brahic M, Gonzalez-Dunia D. Borna disease virus persistent infection activates mitogen-activated protein kinase and blocks neuronal differentiation of pc12 cells. J Biol Chem 2001;276:7258–65.

[22] Hans A, Bajramovic JJ, Syan S, Perret E, Dunia I, Brahic M, et al. Persistent, noncytolytic infection of neurons by Borna disease virus interferes with ERK 1/2 signaling and abrogates BDNF-induced synaptogenesis. FASEB J 2004;18(7):863–5.

[23] Hill EL, Frith U. Understanding autism: insights from mind and brain. Philos Trans R Soc Lond B: Biol Sci 2003;358(1430):281–9.

[24] Hirano N, Kao M, Ludwig H. Persistent, tolerant or subacute infection in Borna disease virus-infected rats. J Gen Virol 1983;64:1521–30.

[25] Hornig M, Weissenbock H, Horscroft N, Lipkin WI. An infection-based model of neurodevelopmental damage. Proc Natl Acad Sci USA 1999;96:12102–7.

[26] Kilts CD. The changing roles and targets for animal models of schizophrenia. Biol Psychiatry 2001;50:845–55.

[27] Klin A, Jones W, Schultz R, Volkmar F, Cohen D. Defining and quantifying the social phenotype in autism. Am J Psychiatry 2002;159(6): 895–908.

[28] Libbey JE, Sweeten TL, McMahon WM, Fujinami RS. Autistic disorder and viral infections. J Neurovirol 2005;11(1):1–10.

[29] Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, Herrup K, et al. Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. Cell 1997;90:895–905.

[30] Lipska BK, Weinberger DR. To model a psychiatric disorder in animals: schizophrenia as a reality test. Neuropsychopharmacology 2000;23:223–39.

[31] Ludwig H, Bode L. Borna disease virus: new aspects on infection, disease, diagnosis and epidemiology. Rev Sci Technol 2001;19:259–88.

[32] Narayan O, Herzog S, Frese K, Scheefers H, Rott R. Behavioral disease in rats caused by immunopathological responses to persistent borna virus in the brain. Science 1983;220:1401–3.

[33] Pankseep J, Siviy S, Normansell L. The psychobiology of play: theoretical and methodological perspectives. Neurosci Biobehav Rev 1984;8:465–92.

[34] Pearce BD. Schizophrenia and viral infection during neurodevelopment: a focus on mechanisms. Mol Psychiatry 2001;6:634–46.

[35] Penn HE. Neurobiological correlates of autism: a review of recent research. Child Neuropsychol 2006;12(1):57–79.

[36] Pickett J, London E. The neuropathology of autism: a review. J Neuropathol Exp Neurol 2005;64(11):925–35.

[37] Plata-Salaman CR, Ilyin SE, Gayle D, Romanovitch A, Carbone KM. Persistent Borna disease virus infection of neonatal rats causes brain regional changes of mRNAs for cytokines, cytokine receptor components and neuropeptides. Brain Res Bull 1999;49:441–51.

[38] Pletnikov M, Rubin S, Vasudevan K, Moran T, Carbone KM. Developmental brain injury associated with abnormal play behavior in neonatally Borna disease virus-infected Lewis rats: a model of autism. Behav Brain Res 1999;100:43–50.

[39] Pletnikov M, Rubin S, Schwartz G, Moran T, Sobotka T, Carbone KM. Persistent neonatal Borna disease virus (BDV) infection of the brain causes chronic emotional abnormalities in adult rats. Physiol Behav 1999;66:823–31.

[40] Pletnikov M, Rubin S, Schwartz G, Carbone K, Moran TH. Effects of neonatal rat Borna disease virus (BDV) infection on the postnatal development of brain monoaminergic systems. Dev Brain Res 2000;119:179–85.

[41] Pletnikov M, Rubin S, Carbone K, Moran T, Schwartz GJ. Neonatal Borna disease virus infection (BDV)-induced damage to the cerebellum is associated with sensorimotor deficits in developing Lewis rats. Dev Brain Res 2001;126:1–12.

[42] Pletnikov MV, Moran TH, Carbone KM. Borna disease virus infection of the neonatal rat: developmental brain injury model of autism spectrum disorders. Front Biosci 2002;7:d593–607.
[43] Pletnikov MV, Rubin SA, Vogel MW, Moran TH, Carbone KM. Effects of genetic background on neonatal Borna disease virus infection-induced neurodevelopmental damage. I. Brain pathology and behavioral deficits. Brain Res 2002;944:97–107.
[44] Pletnikov MV, Rubin SA, Vogel MW, Moran TH, Carbone KM. Effects of genetic background on neonatal Borna disease virus infection-induced neurodevelopmental damage. II. Neurochemical alterations and responses to pharmacological treatments. Brain Res 2002;944:108–23.
[45] Potegal M, Einon D. Aggressive behaviors in adult rats deprived of playfighting experience as juveniles. Dev Psychobiol 1989;22(2):159–72.
[46] Rauer M, Pagenstecher A, Schulte-Monting J, Sauder C. Upregulation of chemokine receptor gene expression in brains of Borna disease virus (BDV)-infected rats in the absence and presence of inflammation. J Neurovirol 2002;8:168–79.
[47] Rausch DM, Stover ES. Neuroscience research in AIDS. Progr Neuropsychopharmacol Biol Psychiatry 2001;25:231–57.
[48] Rolls E. Memory systems in the brain. Ann Rev Psychol 2000;51: 599–630.
[49] Rubin SA, Sylves P, Vogel MW, Pletnikov M, Moran TH, Schwartz GJ, et al. Borna disease virus-induced hippocampal dentate gyrus damage is associated with spatial learning and memory deficits. Brain Res Bull 1999;48:23–30.
[50] Sadamatsu M, Kanai H, Xu X, Liu Y, Kato N. Review of animal models for autism: implication of thyroid hormone. Congenit Anom Kyoto 2006;46(1):1–9.
[51] Sanberg PR, Moran TH, Coyle JT. Animal models of dementia. In: Coyle JT, editor. Microencephaly: cortical hypoplasia induced by methylazoxymethanol. New York: Alan R. Liss Inc.; 1987. p. 253–78.
[52] Sauder C, de la Torre JC. Cytokine expression in the rat central nervous system following perinatal Borna disease virus infection. J Neuroimmunol 1999;96:29–45.
[53] Sauder C, Hallensleben W, Pagenstecher A, Schneckenburger S, Biro L, Pertlik D, et al. Chemokine gene expression in astrocytes of Borna disease virus-infected rats and mice in the absence of inflammation. J Virol 2000;74:9267–80.
[54] Schenberg LC, Povoa RM, Costa AL, Caldellas AV, Tufik S, Bittencourt AS. Functional specializations within the tectum defense systems of the rat. Neurosci Biobehav Rev 2005;29(8):1279–98.
[55] Schmahmann JD, Caplan D. Cognition, emotion and the cerebellum. Brain 2006;129(Pt 2):290–2.
[56] Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J Neurosci 2003;23(1):297–302.
[57] Sweeten TL, Posey DJ, McDougle CJ. Brief report: autistic disorder in three children with cytomegalovirus infection. J Autism Dev Disord 2004;34(5):583–6.
[58] van Gent T, Heijnen CJ, Treffers PD. Autism and the immune system. J Child Psychol Psychiatry 1997;38:337–49.
[59] Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 2005;57(1):67–81.
[60] Weissenbock H, Hornig M, Hickey WF, Lipkin WI. Microglial activation and neuronal apoptosis in Bornavirus infected neonatal Lewis rats. Brain Pathol 2000;10:260–72.
[61] Willner P. Animal models of depression: validity and applications. Adv Biochem Psychopharmacol 1995;49:19–41.
[62] Wongwitdecha N, Marsden CA. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 1996;75(1/2):27–32.
[63] Yamashita Y, Fujimoto C, Nakajima E, Isagai T, Matsuishi T. Possible association between congenital cytomegalovirus infection and autistic disorder. J Autism Dev Disord 2003;33(4):455–9.
[64] Yolken RH, Karlsson H, Yee F, Johnston-Wilson NL, Torrey EF. Endogenous retroviruses and schizophrenia. Brain Res Rev 2000;31:93–9.
[65] Zocher M, Czub S, Schulte-Monting J, de La Torre JC, Sauder C. Alterations in neurotrophin and neurotrophin receptor gene expression patterns in the rat central nervous system following perinatal Borna disease virus infection. J Neurovirol 2000;6:462–77.