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Persistent Neonatal Borna Disease Virus (BDV) Infection of the Brain Causes Chronic Emotional Abnormalities in Adult Rats

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PLETNIKOV, M. V., S. A. RUBIN, G. J. SCHWARTZ, T. H. MORAN, T. J. SOBOTKA AND K. M. CARBONE. *Persistent neonatal Borna disease virus (BDV) infection of the brain causes chronic emotional abnormalities in adult rats.* PHYSIOL BEHAV **66**(5) 823–831, 1999.—Neonatal Borna disease virus (BDV) brain infection results in selective developmental damage to the hippocampal dentate gyrus and the cerebellum. When mature, neonatally BDV-infected rats show extreme locomotor hyperactivity and reduced freezing behavior in novel environments. Traditional interpretation of both of these behavioral abnormalities would suggest decreased anxiety in infected rats compared to normal animals. However, it also possible that the locomotor hyperactivity in infected rats reflects higher rather than reduced anxiety, and is the result of increased escape responses to aversive stimuli. The present experiments were undertaken to test a hypothesis about elevated anxiety in neonatally BDV-infected adult Lewis rats by studying their species-specific fear-related responses. Compared to normal subjects, BDV-infected rats exhibited locomotor hyperactivity and elevated defecation in a highly aversive, brightly lit open field. As expected, in a less aversive, dimly lit open field, uninfected controls increased ambulation, whereas infected rats significantly decreased locomotor activity and defecation. Unlike uninfected rats, BDV-infected rats exhibited an attenuated freezing response immediately after loud auditory stimuli. On the contrary, immediate freezing responses following footshock were comparable in the two groups of animals indicating an intact ability to freeze in BDV-infected rats. Despite a decreased baseline startle responsiveness, BDV-infected rats demonstrated increased sensitization of the startle response by preceding footshocks, suggesting a tendency toward elevated escape responses. Compared to normal subjects, BDV-infected rats showed decreased conditional freezing and elevated conditional defecation response in the context previously paired with aversive stimulation indicating sparing of an autonomic component of fear conditioning. The findings indicate that neonatally BDV-infected adult rats are hyperreactive to aversive stimuli, possibly as a result of chronic emotional abnormalities.

Borna Anxiety Fear Hippocampus Cerebellum Rat

BORNA disease virus (BDV) is an 8.9-kb negative strand RNA virus of the Bornaviridae class, in the Mononegavirales Order (23). BDV infection has been associated with some forms of human psychiatric diseases such as schizophrenia and affective disorders (23,48,51). In the Lewis rat model of BDV infection, neonatally inoculated rats develop a persistent infection without generalized meningitis or encephalitis (5,12,13). The minimal inflammatory response following neonatal BDV infection of the rat brain allows the study of the neuroanatomical and behavioral abnormalities associated with the pathogen in the absence of the complications of global inflammation-mediated brain damage (4,5).

The cerebellum and the hippocampus undergo substantial postnatal maturation in rats (1,36) and, therefore, are particu-

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larly susceptible to the peri- and early postnatal effects of various harmful agents such as X-irradiation, malnutrition, hypoxia, and viral infections (1). Notably, structural abnormalities in the cerebellum and hippocampal dentate gyrus have been documented in neonatally BDV-infected rats (5,13). Damage to the cerebellum and the hippocampus has been shown to result in cognitive and emotional deficits in animals (25,36), and distinctive sensorimotor, emotional, and cognitive deficits have been described in adult Wistar and Lewis rats neonatally infected with BDV (4,5,17).

BDV-infected rats exhibit extreme locomotor hyperactivity and show very little, if any, freezing in brightly lit open field (4,17). This pattern of behavioral results led Dittrich and associates (17) to suggest that BDV-infected rats have a lower-than-normal level of anxiety. However, another interpretation of these data is possible. The locomotor hyperactivity observed in the brightly lit open field might be the result of an increased drive to escape from the aversive illumination, indicating high rather than low anxiety (41,50). Moreover, the lack of freezing behavior might have been due to a subtle motor deficit associated with the cerebellar abnormalities, interfering with a proper expression of this species-specific fear response (36). To distinguish between these hypotheses, and to gain a better understanding of possible emotional abnormalities in neonatally BDV-infected rats, we conducted an extensive characterization of their fear-related species-specific behaviors by testing the animals in a variety of fear-eliciting situations. Specifically, we compared fear-related behaviors of neonatally BDV-infected and sham-inoculated adult Lewis rats by using (a) strong aversive stimulation (footshock and auditory) tests, (b) variable aversiveness of open field (bright vs. dim illumination), and (c) acoustic startle response paradigms (baseline startle responsiveness and sensitization of the acoustic startle by preceding footshock).

MATERIALS AND METHODS

Animals

Pregnant Lewis rats (16–18 days of gestation) were purchased (Harlan, Indianapolis, IN) for these studies. All rat pups were born and reared in the animal vivarium at CBER, FDA (Bethesda, MD). Following weaning, rats were kept in groups of two to three in 45 × 26 × 23-cm pan-type polypropylene cages with an overhead wire grid supporting food pellets and a water bottle, and containing a 1–2-cm layer of wood-chip bedding. Cages containing infected animals were kept in "DUO-FLO" biosafety cabinet (Bio-Clean Lab Product Inc., NJ). The sham-inoculated rats were kept in the same room. Rats were maintained on a 10:14-h light:dark cycle (lights on at 0800 h) and had free access to food and water. Room temperature was maintained at approximately 21°C.

Inoculation

Rat pups were inoculated intracranially within 24 h of birth either with 0.02 cm^3 of CRP3 BDV strain or uninfected inoculum, as described previously (4,5). For behavioral and histological experiments, two to three male rats were randomly selected from a single litter to make each experimental group. All experiments were performed on 100–120 day old rats.

Behavioral Tests

Open-field test. The test was carried out at 1600–1800 h. Testing took place in a square open field, 50 × 50 cm, with 20-cm high opaque plastic walls. The floor of the open field was divided into 36 sections of approximately 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. For the dim lighting condition, the open field was illuminated by a 25-W red light bulb mounted 80 cm above the center of the open field. For the bright lighting condition, the open field was illuminated by two 150-W white spotlights mounted 85 cm above the field, and positioned so as to eliminate shadows. In this experiment, 19 BDV-infected and 22 sham-inoculated male rats were used. Every rat was tested for 5 min in both the dark and bright open field. For half the rats, the dark test occurred 24 h prior to the bright test. The remaining rats were run in the opposite order. Video recording of rat behavior began 3–5 s after each rat was individually placed into the center of the field. Behavior was scored as belonging to one of the following categories: (a) locomotion (ambulation) scored by the number of sections crossed by all four paws; (b) rearing; (c) grooming; (d) freezing; (e) thigmotaxis (number of crossing the outermost sections in relation to total number of the sections crossed); and (f) other. Freezing was defined as a total immobility including vibrissae, except for movement necessitated by respiration. Fecal boli deposited were counted after each session.

Acoustic startle stimulation test. Startle chambers (San Diego Instruments Inc., San Diego, CA) consisted of a Plexiglas cylinder mounted on a Plexiglas base placed within a sound-attenuated chamber. A piezoelectric strain meter attached to the base transduced the startle response. Stabilimeter readings were rectified, digitized on a 4095 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 cabinet by means of the digital sound level meter ("Realistic," Tandy Corp., 1993).

One week following the completion of the open-field test, a subset of randomly selected nine BDV-infected and eight control rats were tested for fear-related behaviors following presentations of the startle stimulus. Pilot experiments showed that when an infected rat was placed into the experimental chamber there was a high probability of defecation, while no such a response was observed in normal rats. To reduce the high baseline rate of defecation in the BDV rats, BDV-infected and sham-inoculated rats were exposed to the experimental chamber for 3 min daily for 4 consecutive days before presentations of startle stimuli. On the fifth day, the initial freezing response and a baseline defecation were assessed for a 3-min period after placement in the startle chamber with no presentation of the acoustic stimulus (the prestimulation session). There was no background noise during the prestimulation session. Immediately thereafter, a rat was given 10 100-ms 108-dB white noise stimuli at a 20-s interstimulus interval with a background noise of 45 dB throughout the entire session. Immediately after startle stimulus presentations, freezing and defecation responses were scored again for another 3-min period (the training session), and then a rat was returned to its home cage. Twenty-four hours after the training session, each rat was again placed in the startle chamber and freezing and defecation responses were scored for a 3-min period with no background noise (the test session).

Freezing was scored by an experienced observer and defined as above. The amplitude of the acoustic startle response (ASR) and the habituation of the ASR across the startle session were assessed. Given significant difference in body weights between BDV-infected and sham-inoculated adult

rats (4), the startle amplitudes were analyzed and presented as the maximum value of the startle response in relation to the rat's body weight.

Shock test. Responses to footshock were assessed in some rats used in the open-field test but not utilized in the acoustic startle stimulation test ($n = 10$ for BDV-infected and $n = 8$ for uninfected). The startle chambers included shock grids and were connected to the programmable shockers (E13-10, Coulbourn Instruments, USA). Each rat was preexposed to the experimental chamber daily for 5 min for 4 consecutive days to reduce the baseline defecation response. On the fifth day, the rat was placed in the enclosure and the initial freezing behavior and a baseline defecation response were measured for 5 min (the preshock session). The rat was then given three unsignaled footshocks (500 ms of duration, 0.65 mA; 15-s intershock interval). Immediately after the last shock, the rat's freezing behavior and defecation were again scored for another 5 min (the training session). Twenty-four hours later, the rat was returned to the experimental enclosure, and freezing and defecation responses were assessed for 5 min (the test session).

Shock sensitization of the acoustic startle response (ASR). Experimentally naïve neonatally BDV-infected ($n = 16$) and sham-inoculated ($n = 12$) rats were used. To test for sensitization of the ASR amplitude, the rats were placed in the startle chamber for a 5-min period of acclimation without presentation of a background noise or startle stimuli. Immediately afterwards, rats were exposed to 20 startle stimuli (108 dB, 50 ms of duration) at a 20-s interstimulus interval. Immediately after the 20th startle stimulus, 10 foot shocks were administered. Ten footshocks (0.65 mA, 500 ms duration) were given at a rate of 1/s. After footshock presentations, the rat was left in the enclosure for another 5 min without any stimulation. This period was chosen because sensitizing effects are not usually detected until 4 min after shock presentation (15). Subsequently, a further 20 startle stimuli were presented to determine the change of the startle amplitude after the sensitization by footshock. The effect of sensitization by footshock was calculated as the mean change in the ASR amplitudes of the last five trials before and the first five trials after application of the footshock because sensitizing effect of preceding footshock on the startle response in rats is observed at best against a background of prior habituation (15,20).

Between each rat in all above behavioral experiments, the open field and the experimental enclosure (the startle chamber) were cleaned with antiseptic solution followed by tap water.

Immunohistochemistry

Upon completion of all the behavioral tests, a representative set of 10 BDV-infected and 10 sham-inoculated rats were deeply anesthetized by Metofane (Pitman–Moore, Mundelein, IL) and perfused with phosphate-buffered saline (PBS) solution (pH = 7.4). Their brains were removed and fixed in 4% paraformaldehyde, paraffin embedded, and cut sagittally into 8-μm thick sections. To examine virus distribution in the brain, sections were stained by avidin–biotin immunohistochemistry (Vector, Burlingame, CA) using a polyclonal rabbit anti-BDV antibody followed by biotinylated antirabbit IgG (Vector), as described previously (13). Alternate sections were stained with hematoxylin and eosin.

Statistics

For statistical analysis, analyses of variance (ANOVA) with post hoc Tukey's test for pair-wise comparisons, were applied. A $p < 0.05$ was considered as the criterion for statistical significance.

RESULTS

Body Weight

As previously reported (4), body weights of the BDV-infected rats (268.4 ± 4.4 g) were significantly ($p < 0.001$) less than those of the sham-inoculated animals (405.8 ± 6.0 g).

Histology

As previously found (5,12,13), the neonatally BDV-infected rats had profound destruction of hippocampal dentate gyrus and hypoplasia of the cerebellum. BDV protein expression was detected in brain sections of all rats in the BDV-infected group by immunohistochemistry. BDV proteins were not detected in any of the uninfected rats (not shown).

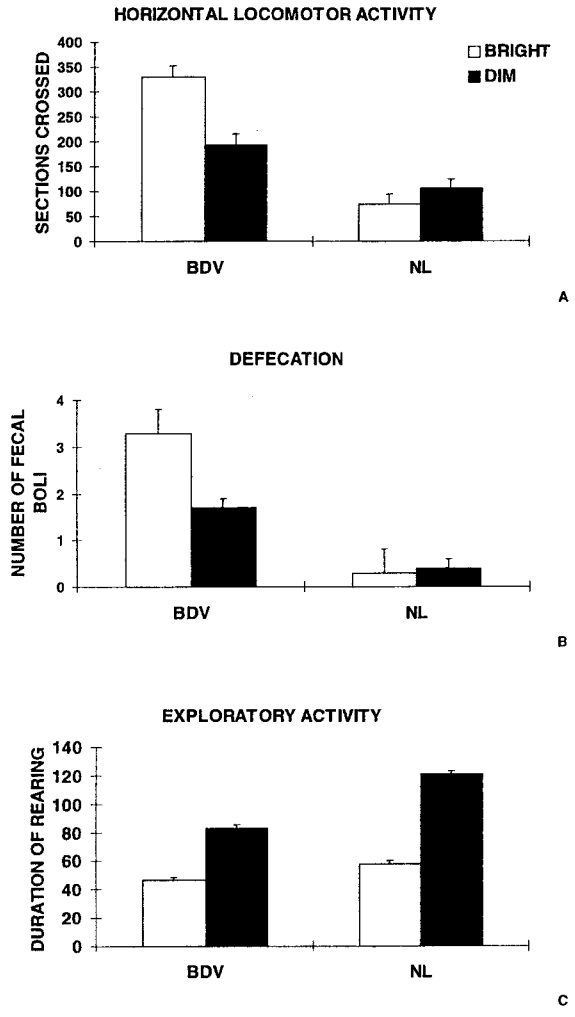

FIG. 1. Horizontal locomotor activity (A), defecation (B), and exploratory activity (C) observed in sham-inoculated (NL) and BDV-infected (BDV) adult Lewis rats in the brightly (bright) and dimly (dim) illuminated open fields. Bars and vertical lines represents the means and SEM.

TABLE 1

FEAR-RELATED BEHAVIORS IN NEONATALLY BDV-INFECTED AND SHAM-INOCULATED ADULT LEWIS RATS IN BRIGHTLY (BRIGHT) AND DIMLY (DIM) ILLUMINATED OPEN FIELDS

Groups	Freezing (SEC)	Thigmotaxis (% of Total Crossings)	Rearing (Frequency)
BDV			
Bright	5.1 ± 2.7	79.2 ± 7.2	31.8 ± 2.4
Dim	0	58.8 ± 7.3	50.8 ± 2.4
Sham-Inoculated			
Bright	12.3 ± 2.9	90.7 ± 7.5	18.6 ± 2.5
Dim	1.1 ± 2.6	70.7 ± 6.7	32.2 ± 2.2

The data are presented as means ± SEM.

Open-Field Test

Figure 1 and Table 1 summarize the results from the open field test. Analysis of variance (ANOVA) of total locomotor activity (i.e., the number of sections crossed) yielded a significant main effect of the infection status, $F(1, 77) = 137.18, p < 0.001$ (Fig. 1A). There was no significant overall effect of level of illumination, $F < 1$, but the interaction between infection status and illumination was significant, $F(1, 77) = 12, p < 0.001$. Post hoc comparison confirmed that BDV-infected rats showed more ambulation than normal rats in the both lighting conditions (both $ps < 0.05$, Tukey). Notably, BDV-infected rats were more active in the bright open field, while control animals crossed more sections in the dark open field (all $p < 0.05$, Tukey) (Fig.1A).

When thigmotaxic behavior was expressed as a percentage of total locomotor activity (total number of sections crossed), the amount of thigmotaxis was affected by both infection status, $F(1, 77) = 13.3, p < 0.001$, and level of illumination, $F(1, 77) = 11.82, p < 0.001$ (Table 1). Post hoc comparisons showed that control rats demonstrated more thigmotaxis than BDV-infected rats ($p < 0.05$). In addition, both groups of rats exhibited more thigmotaxic behavior in the bright open field compared to the dim (all $ps < 0.05$) (Table 1).

BDV-infected rats exhibited less freezing than controls, $F(1, 77) = 18.91, p < 0.001$, and the amount of freezing under the bright illumination was greater than under the dim for both groups, $F(1, 77) = 28, p < 0.001$ (Table 1).

An analysis of the amount of defecation revealed a significant main effect of infection, $F(1, 77) = 16.58, p < 0.001$,and a significant interaction between infection status × illumination level, $F(1, 77) = 5.25, p = 0.025$. Overall, BDV-infected rats showed higher levels of defecation than control rats and the amount of defecation, in BDV-infected but not control rats, was increased in the bright open field ($ps < 0.05$,Tukey) (Fig. 1B).

BDV-infected rats reared more often than control animals, $F(1, 77) = 45.17, p < 0.001$. Both groups reared more in the dimly lit than in the brightly lit open field, $F(1, 77) = 47.37, p < 0.01$. (Table 1). There was no significant interaction between infection status and illumination level, $F < 1$. To determine if the increase in rearing in BDV-infected rats might be associated with a shorter duration of each rear but not with exploratory activity per se, we assessed rearing in terms of the duration of rearing and found that control rats reared for a longer time than BDV-infected rats, $F(1, 77) = 11.5, p = 0.001$. Similar to the frequency score, the duration of rearing was greater under the dim illumination compared to the bright for the both groups of rats, $F(1, 77) = 47.8, p < 0.001$) (Fig. 1C).

Acoustic Startle Test

Two-way repeated measures ANOVA of freezing within the various phases of the acoustic startle test indicated signifi-

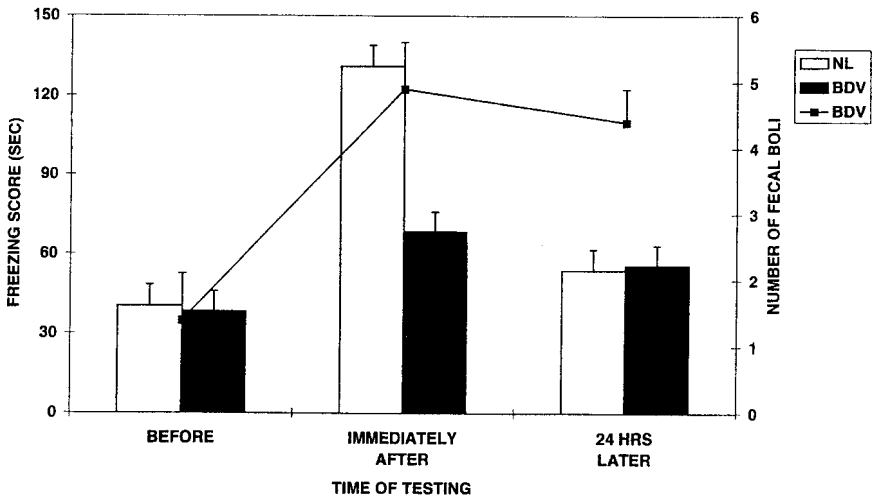

FIG. 2. Freezing behavior and defecation in sham-inoculated (NL) and BDV-infected (BDV) adult Lewis rats in the acoustic startle stimulation test. Bars and vertical lines represent the means and SEM for freezing behaviors before (BEFORE), immediately after (IMMEDIATELY AFTER), and 24 h after (24 HRS LATER) the acoustic startle stimulation. Horizontal solid line and small vertical lines represent the means and SEM of defecation responses of BDV-infected rats before (BEFORE), immediately after (IMMEDIATELY AFTER), and 24 h after (24 HRS LATER) the acoustic startle stimulation. Sham-inoculated rats did not show any defecation response following the acoustic startle stimulation.

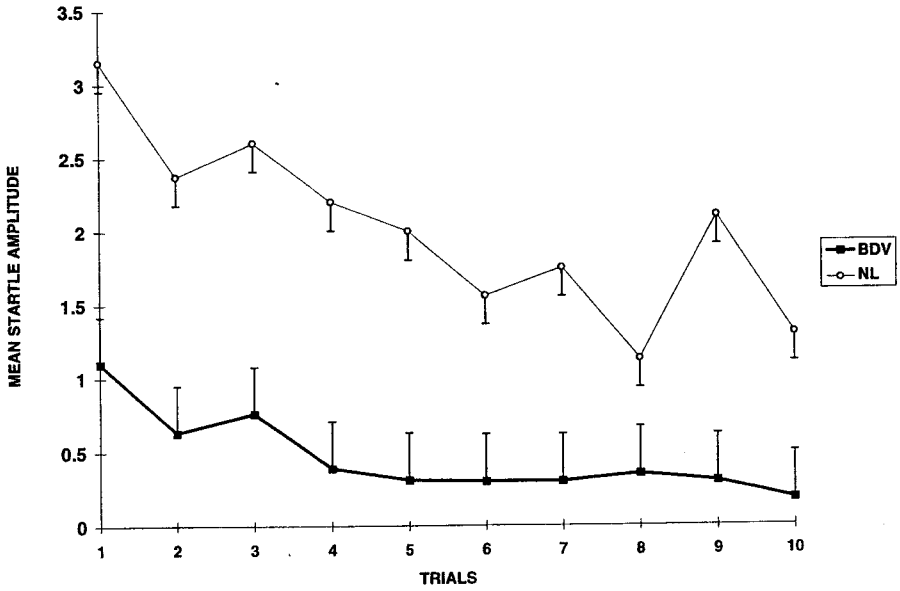

FIG. 3. The acoustic startle response (ASR) and the within-session habituation of the ASR in sham-inoculated (open circles) and BDV-infected (filled squares) adult Lewis rats. Shown is the mean startle amplitude at 10 consecutive acoustic startle stimulus presentations.

cant effects of infection status, $F(1, 50) = 6.94$, $p = 0.019$, session, $F(2, 50) = 44.88$, $p < 0.001$, and an infection status by session interaction, $F(2, 50) = 11.01$, $p < 0.001$. As shown in Fig. 2, both BDV-infected and normal rats increased freezing in response to the acoustic stimuli, although the increase in the controls in response to the acoustic stimuli was much greater than in the BDV-infected rats (all ps < 0.05, Tukey). In spite of showing less freezing behavior following startle stimulation, the autonomic response to acoustic stimulation was greater in BDV-infected rats. BDV rats produced more fecal boli following than before acoustic stimulation, and this remained elevated even when placed in the chamber 24 h later, $F(2, 16) = 7.5$, $p = 0.005$. In contrast, uninfected controls did not defecate in any phase of the test.

As indicated in Fig. 3, the mean amplitude of the acoustic startle response (ASR) was lower in BDV-infected rats than in normal animals, $F(1, 179) = 10.68$, $p = 0.005$. Within both groups, startle responses were stronger to the first stimulation

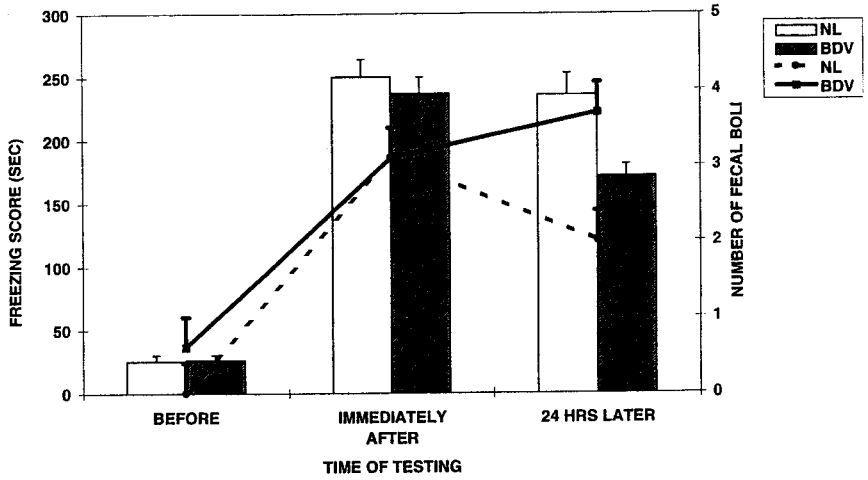

FIG. 4. Freezing behavior and defecation in sham-inoculated (NL) and BDV-infected (BDV) adult Lewis rats in the footshock test. Bars and vertical lines represent the means and SEM for freezing behaviors before (BEFORE), immediately after (IMMEDIATELY AFTER), and 24 h after (24 HRS LATER) three footshocks. Horizontal lines and small vertical lines represent the means and SEM of defecation responses of sham-inoculated (dotted) and BDV-infected (solid) adult Lewis rats before (BEFORE), immediately after (IMMEDIATELY AFTER), and 24 h after (24 HRS LATER) three footshocks.

than to the following, suggesting the occurrence of habituation of the response with repeated stimulation, $F(9, 179) = 3.43, p < 0.001$.

Shock Test

Figure 4 shows the mean percentage of freezing in the preshock session, and in the immediate and 24-h postshock sessions. Both sham-inoculated and infected rats increased comparable freezing immediately after and 24 h after footshock exposures. Repeated measures ANOVA yielded significant effects of infection status, $F(1, 53) = 6.93, p = 0.011$, session, $F(2, 53) = 189.45, p < 0.001$, and a significant interaction between infection status and session, $F(2, 53) = 4.2, p = 0.021$. Post hoc test revealed no difference between the groups in freezing behavior during the immediate session, $p > 0.05$ (Tukey), while uninfected rats froze significantly more than BDV-infected rats 24 h after shock stimulation, $p < 0.05$. Notably, BDV-infected rats showed more freezing behavior in the training session compared to the test session ($p < 0.05$), while for normal animals no oversession change in the amount of freezing behavior was found ($p > 0.05$, Tukey).

Shock elevated the amount of defecation in both groups immediately and 24 h following shock administration (Fig. 4). Friedman repeated-measures ANOVA of the data for the BDV-infected rats yielded the reliable effect of the session (chi-square = 12.067, $p = 0.004$), indicating more defecation in either of the both postshock sessions compared to the preshock baseline level (all $ps < 0.05$, Dunnett's). Analysis of the data for control rats also showed the significant effect of the session, $F(2, 23) = 23.45, p < 0.001$, and post hoc comparisons confirmed the increased defecation during both the postshock sessions compared to the preshock one (all $ps < 0.05$, Tukey). Notably, 24 h after footshock presentations, the defecation score was also different between the experimental groups, with greater autonomic response being observed in infected subjects ($p < 0.05$).

Foot Shock Sensitization of the Acoustic Startle Response

Consistent with the data from the acoustic startle habituation experiment, BDV-infected rats exhibited lower baseline startle responses than sham-inoculated rats, $F(1, 1100) = 966.82, p < 0.001$ (data not shown). Presentations of foot shock resulted in sensitization of the ASR in both groups of animals. The mean ASR amplitude of the last five trials before and the first five trials after the administration of footshocks to BDV-infected and normal rats is shown in Fig. 5. The change in the mean startle amplitude after presentations of footshock for BDV-infected rats was significantly greater than for normal rats (ANOVA on Ranks followed by Dunn's method, $p = 0.011$).

DISCUSSION

The present study demonstrates that neonatally BDV-infected rats exhibit hyperreactivity to aversive stimulation. Unlike normal animals, BDV-infected rats showed an elevated defecation response and a higher locomotor activity in the brightly illuminated open field, and exhibited a greater sensitization of the acoustic startle response by preceding footshock. These findings seem to indicate that hyperreactivity of BDV-infected rats may be associated with an elevated escape behavior and support the hypothesis that neonatally BDV-infected rats have an abnormal emotional reactivity when tested as adults.

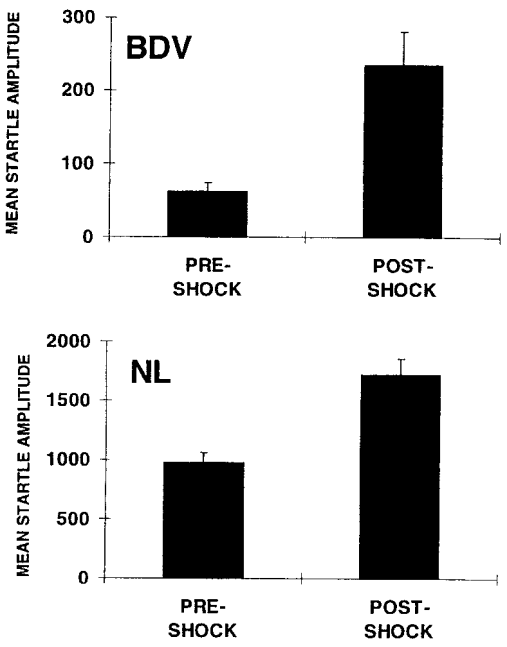

FIG. 5. The mean startle amplitude (SEM of the last five trials before presentations of footshocks (PRE-SHOCK) and the mean startle amplitude (SEM of the first five trials after presentations of footshocks (POST-SHOCK) in sham-inoculated (NL) and BDV-infected (BDV) adult Lewis rats.

Fear-Related Behaviors in BDV-Infected Rats in the Open Field

Dittrich et al. (17) and Bautista et al. (4) found that neonatally BDV-infected Wistar and Lewis rats displayed more ambulation and rearing than the controls in the brightly illuminated open field. The present work was aimed at providing a more complete characterization of locomotor hyperactivity of infected rats; specifically, we hypothesized that the hyperactivity might result from hyperreactivity to aversive stimulation, for example, the bright illumination. It was shown that manipulation of the level of aversiveness (illumination) of the open field changed fear-related responses in BDV-infected rats. Under the bright illumination, BDV-infected rats were hyperactive, while uninfected controls showed attenuated locomotor activity. Under the dim illumination (a less aversive setting), locomotor activity in BDV-infected rats remarkably declined, whereas ambulation in normal animals significantly increased. Similar to ambulation, BDV-infected rats' defecation responses, the other index of emotionality in rodents (31,46), were greater in the brightly lit open field compared to the dimly lit open field. Thus, hyperactivity of BDV-infected rats appears to be a result of hyperreactivity to aversive environment and unlikely to be an indication of reduced anxiety.

The number of rears was generally higher in BDV-infected rats than in the controls, whereas the duration of rearing was less in BDV-infected rats than in sham-inoculated animals. This dissociation between two measures of rearing activity could be due to the fact that BDV-infected rats showed rears with support only (wall rearing), with the duration of each rear being very short. Rearing is believed to reflect exploratory behavior in the open field (16,41) and, therefore, be negatively correlated with emotionality (3,37). Thus, less rearing activity (duration) in BDV-infected rats appears to be

also consistent with our hypothesis about hyperreactivity of BDV-infected rats to aversive stimuli.

Abnormally high locomotor activity observed in BDV-infected rats seemed to confound the results of their thigmotaxic and freezing behaviors. Indeed, BDV-infected rats exhibited less freezing and thigmotaxis than the control animals that might indicate less rather than more anxiety. A number of studies have shown that thigmotaxis and freezing behavior appear to be reliable indexes of fearfulness in rats (37,38,42). However, an extremely high locomotor activity in BDV-infected rats could interfere with expression of thigmotaxis and freezing activity. Moreover, because the walls of the open field were not high enough to completely prevent jumping out, BDV-infected rats often attempted to escape from the open field, possibly indicating an increased flight tendency (7,8). Notably, rats subjected to amygdala stimulation were also found to jump purposively from open arms of the maze, suggesting that they became sufficiently motivated by fear of the apparatus to escape from the maze by jumping (27). In this way, locomotor hyperactivity may make some traditional fear-related responses inappropriate for evaluation of anxiety in rats. Thus, the open-field test data appear to indicate that hyperreactivity in BDV-infected rats is associated with elevated tendency to escape aversive setting.

Neonatal damage to the cerebellum and/or the dentate gyrus of the hippocampus (e.g., X-irradiation, neurotoxicological treatments, malnutrition) produces a behavioral pattern, which is reminiscent of that we observed in neonatally BDV-infected rats (18,30,47). For example, early postnatal X-irradiation, methylazoxymethanol (MAM) treatment, and hyperthyroidism were found to produce cerebellar hypoplasia and result in hyperactivity and increased rearing frequency (1,22,40). Although the neural plasticity following neonatal damage to the brain may differ from the rewiring processes after adult lesions, some similar alterations in behaviors of animals may result from both experimental approaches. For example, hyperactivity was found to be one of the prominent features in adult animals with lesioned hippocampus or cerebellum (6,34,42,43). Moreover, the hyperactivity of hippocampus-lesioned animals has also been suggested to be due to a hyperreactivity to aversive stimulation, and to be relatively independent of general levels of motor behavior (2,7,9). The previous findings (4) and unpublished (Pletnikov) observations of BDV-infected rats in their home cages also suggest that a baseline activity of infected rats does not seem to differ from activity of uninfected controls. Thus, we hypothesize that the neuroanatomical abnormalities in BDV-infected rats may be responsible for hyperreactivity to environmental challenge and elevated escape behavior.

Pavlovian Fear Conditioning in BDV-Infected Rats

The present experiments revealed BDV-infected rats were able to exhibit freezing behavior following aversive stimulation. This fact is of importance because reduced freezing behavior in the open field was another reason for suggesting a low anxiety in BDV-infected rats (17). However, our data showed that freezing behavior in infected rats differed from that in sham-inoculated animals. For example, when tested 24 h after footshock, the amount of freezing was less in BDV-infected rats compared to uninfected subjects. It is typically assumed that freezing behavior following aversive stimulus presentations is a Pavlovian conditioned response. Foot shock or the startle stimulus are the unconditioned stimulus, while the context of the test chamber is the conditioned stimulus, and freezing is the conditioned response (10,19). Viewed in this light, the deficit in conditional freezing responses by BDV-infected rats may reflect a deficit in aversive Pavlovian conditioning. Notably, because the hippocampus and the lateral cerebellum were shown to be critically involved in aversive Pavlovian conditioning (19,28), attenuated conditional freezing behavior may be associated with neuroanatomical abnormalities observed in BDV-infected animals.

There may be, however, an alternative explanation for these results. The hippocampal and cerebellar abnormalities in infected rats may produce a deficit in behavioral inhibition that may interfere with expression of freezing (18,24,33), and, therefore, freezing behavior cannot be reliably used for assessing fear conditioning in rats in some situations. In fact, there is evidence of preserved contextual conditioning in animals with hippocampal lesions in behavioral paradigms that measured not only freezing but also other behavioral indexes of contextual conditioning (33). Similarly, our data show that defecation, an autonomic index of fear, profoundly increased in infected rats when tested immediately and 24 h later after footshocks or startle stimuli in the experimental chamber associated with past aversive stimulation. In this regard, the present findings are in line with suggestions stressing the importance of using multiple measures of conditioning to determine a behavioral specificity of a particular manipulation (33).

Startle Response in BDV-Infected Rats

Compared to the controls, BDV-infected rats showed lower responsiveness on the startle response measure, although, there was no suggestion of a group difference in habituation of the ASR. It remains unclear why BDV-infected rats exhibited fairly low startle responsiveness. They are unlikely to have hearing problems, because neonatal BDV infection did not appear to affect prepulse inhibition in developing and adult Lewis rats (Pletnikov, manuscript in preparation). Neonatally BDV-infected adult rats increased defecation after startle stimulus presentations, indicating that infected rats perceived stimuli as aversive. A body weight differential could not account for the differences because the startle data are presented in relation to the rat's body weight. One of the possible reasons for decreased startle amplitudes in infected rats may be an elevated level of movement in the startle chamber (11,49). In this regard, low startle responsiveness of BDV-infected rats seems to be similar to that found in spontaneously hypertensive and neonatally MAM-treated rats (21,26,29). Obviously, further investigations into possible mechanisms of low startle responsiveness in BDV-infected rats are needed.

Interestingly, low startle responsiveness in BDV-infected rats may be another contradictory issue in the light of the current hypothesis about their hyperreactivity. The acoustic startle response is viewed as unconditioned fear-related reflex, the magnitude of which is thought to be positively related to anxiety. However, in addition to the jump-like response, the startle stimulus evokes a set of different autonomic responses (heart beat acceleration, defecation, changed respiration) which can or cannot correlate with the whole body behavior (14). Notably, low startle responsiveness in spontaneously hypertensive rats was shown to be accompanied by enhanced cardiovascular responses to the same startle stimuli, indicating a dissociation between different components of emotional reaction of the same animal (26,44). In the present work, BDV-infected rats exhibited low startle responsiveness associated with increased defecation, a dissociation not seen in the controls. Thus, the startle response data appear to be in

agreement with the suggestions about the multidimensional nature of anxiety and support the proposed multiple-testing approach to the study of emotionality in animals (37).

Persistent BDV Infection of the Rat Brain May Cause Chronic Emotional Abnormalities

Regarding different forms of anxiety, Lister (32) has invoked in psychopharmacological research two concepts originated in the clinical field: 1) state anxiety experienced at a particular moment, in the presence of an anxiety-provoking situation; 2) trait anxiety is constant throughout the time as a permanent feature of the individual. Traditional animal models of anxiety state may not correspond to the human anxiety disorders associated with chronic pathological, trait-like anxiety (32,39). It is important to develop animal models of chronic pathological anxiety, for example, genetic models (37,45). In addition, because emotional deficits have also been associated with abnormal brain development (39), animal models utilizing the early harmful influence of some environmental agents (e.g., viral infection) may also be useful. In these models of chronic/trait anxiety, emotional deficits develop on a distinctive neuropathological background and are endogenous and chronic, and, thus, in many respects, similar to human anxiety disorders. In this context, neonatal BDV infection of the rat brain may be a valuable animal model of endogenous, chronic anxiety.

The present work demonstrates that neonatally BDV-infected rats exhibit hyperreactivity to aversive stimulation, suggesting chronically disturbed emotionality. Hyperreactivity of BDV-infected rats may be associated with an elevated escape behavior. An increased escape tendency is believed to be due to an imbalance in functions of the brain regions associated with escape/flight responses, i.e., transdiencephalic circuit connecting the lateral and central amygdaloid nuclei with the central gray and lower autonomic control areas of the brain stem (35). In addition, deficient functioning in the septo-hippocampal and/or the cerebellar inhibition systems, which exert inhibitory control over the above-mentioned flight circuitry, may also facilitate active avoidance behavior (25). The histological findings are in a good agreement with both neurobiological hypotheses, in that neonatal BDV infection of the rat brain results in developmental damage to the cerebellum and the hippocampus (5,13). Additionally, BDV antigenes are seen in the amygdaloid neurons (data not shown), suggesting abnormal functioning of this brain region.

Clinical, epidemiological and virological data indicate that viruses persist in the CNS and sporadically induce progressive neurological disorders, which are associated with alterations in emotional and cognitive spheres (23,51). Studies of the rat model of BDV infection provide new evidence to support the hypothesis that neurotropic viruses can cause specific behavioral abnormalities, even in the absence of encephalitis (4,5). Given recent evidence for isolation of BDV from humans (48), understanding BDV disease pathogenesis may have direct application to human disease. Moreover, factors such as persistence of the BDV infection, the distinctive neuropathological outcome, and abnormal emotional responses of BDV-infected adult rats suggest the utility of BDV neonatal infection of the rat brain as an animal model system for endogenous chronic anxiety.

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