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RESEARCH ARTICLE

Distribution of Borna Disease Virus in the Brain of Rats Infected with an Obesity-inducing Virus Strain

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Experimental infection of Lewis rats with Borna disease virus (BDV), a nonsegmented, single-stranded RNA virus, usually causes an immune-mediated biphasic neurobehavioral disorder. Such animals develop a persistent infection of the CNS with viral antigen expression in all brain regions and a disseminated nonpurulent meningoencephalitis. Interestingly, intracerebral infection of Lewis rats with a BDV-variant (*BDV-ob*) causes a rapid increase of body weight with the development of an obesity syndrome without obvious neurological signs. The obese phenotype is correlated with a characteristic distribution of inflammatory lesions and BDV-antigen in the rat brain. Infiltration with mononuclear immune cells and viral antigen expression are restricted to the septum, hippocampus, amygdala and ventromedian tuberal hypothalamus. Therefore, infection with the obesity-inducing *BDV-ob* results most likely in neuroendocrine dysregulations leading to the development of an obesity syndrome. This might be due to the restriction of viral antigen expression and inflammatory lesions to brain areas which are involved in the regulation of body weight and food intake. The BDV-induced obesity syndrome represents a model for the study of immune-mediated neuroendocrine disorders caused by viral infections of the CNS.

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Introduction

Natural and experimental infection with Borna disease virus (BDV) usually causes a severe immune-mediated neurobehavioral disorder. Horses and sheep represent the main natural hosts (17, 25, 58), but recent reports on spontaneous BDV-infections in cattle (13), cats (29), dogs (54) and various zoo animals (hippopotamus, sloth, vari monkey and llama alpacas; 46, 47) indicate a wider spectrum of hosts. In addition, recent seroepidemiological data reveal that humans can also be infected with BDV or a related agent. Mainly patients with psychiatric and neurological disorders have a significantly higher seroprevalence (1, 2, 3, 8, 9, 19, 42, 53).

BDV is a single stranded, nonsegmented RNA virus of negative polarity (16, 18), which has been classified as the prototype of the new family *Bornaviridae* within the order *Mononegavirales*.

In general, adult Lewis rats develop a persistent infection of the CNS with a characteristic biphasic course of the disease when infected experimentally with the isolate BDV-biphasic (*BDV-bi*; 32, 33). Clinical signs of hyperactivity, aggressiveness and weight loss are noted in the first stage of the disease, but later on only apathy can be observed. The onset of clinical signs correlates with the occurrence of mononuclear inflammatory lesions in the brain. The major infiltrating cell type consists of T-cells, macrophages and at later stages also of B-cells (20, 38, 39, 49, 50). Therefore, BD most likely is the result of a virus-induced delayed type hypersensitivity reaction (DTH) in the CNS.

In its biological properties, BDV seems to be highly variable. Thus, after experimental infection of adult Lewis rats with different isolates of BDV, other clinical signs such as paralysis (6) or the development of an obesity syndrome (12, 23, 41) were noted.

In order to obtain more information on the differences in the biological behavior between the isolates BDV-obese (*BDV-ob*) and BDV-biphasic (*BDV-bi*), the clinical course of the disease, the presence of BDV and

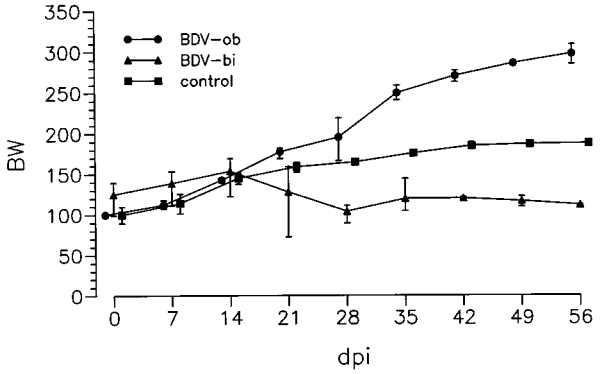

Figure 1. Development of the body weight of Lewis rats in an investigation period of 56 days (n: 17). The body weight of animals infected with *BDV-ob* increased significantly more than the one of the animals infected with *BDV-bi* or of the non-infected control rats ($p < 0.0001$). Abbreviations: BW - body weight (g); dpi - days post infection.

BDV-specific antigen as well as the occurrence of inflammatory lesions in the rat brain infected with *BDV-ob* or *BDV-bi* were compared.

Material and Methods

Experimental BDV-infection. Thirty anaesthesized 4-week-old Lewis rats were inoculated intracerebrally into the left frontal cortex with 10^4 ID_{50}/ml of infectious brain homogenate of the obesity-inducing BDV-isolate (*BDV-ob*). The *BDV-ob* was obtained from a field isolate of a horse (He/80) with spontaneous Borna disease, which was passaged twice in rabbit brain and finally for several times in the brain of newborn Lewis rats. Control rats were infected simultaneously (10^4 ID_{50}/ml) with a BDV-isolate (*BDV-bi*) known to induce the biphasic course of BD (32, 33). This BDV-preparation was also derived from the same equine field isolate (He/80), passaged twice in rabbit brains and for several times in newborn and adult Lewis rats. During an investigation period up to 56 days post infection (dpi) clinical signs and body weight of BDV-infected animals were investigated. The development of the body weight of both BDV-infected cohorts as well as of the noninfected control group was analyzed by the two way ANOVA with repeated measures in the factor "time" using the BMDP Statistical Software (21).

Tissue preparation. BDV-infected animals were killed weekly in deep anaesthesia by heart puncture and decapitation, and brains were removed immediately. For histological and immunohistological analysis brains were fixed overnight in 10% non-buffered formalin and embedded in paraffin at 60°C. Coronal sections of 4μm were cut at approximately *bregma* 6.7mm, 3.7mm, 0.7mm, -3.3mm, -5.8mm, -7.8mm, -10.8mm (36).

For the isolation of infectious virus a fresh and sterile piece of various brain regions (olfactory bulb, motory and sensory cortex, septum, hippocampus, median eminence, amygdala, thalamus, tectum mesencephali, medulla oblongata and cerebellum) were shock frozen and stored at -70°C.

Serum was obtained from coagulated blood samples after centrifugation and stored at -20°C.

Histology and immunohistology. Formalin-fixed and paraffin-embedded brain sections were stained with hematoxylin and eosin for the detection of inflammatory lesions in the brain. The inflammatory reaction of leptomeningeal, perivascular and parenchymal infiltrates and reactive astrocytosis were scored as follows: 0: no inflammatory lesions/reactive astrocytosis, 1: mild inflammatory lesions/reactive astrocytosis, 2: moderate inflammatory lesions/astrocytosis, 3: strong inflammatory lesions/astrocytosis. The distribution of inflammatory lesions in rat brains infected with *BDV-ob* or *BDV-bi* was investigated employing at least 10 different brain regions (hippocampus, septum, hypothalamus, amygdala, thalamus, mesencephalon, basal ganglia, isocortex, medulla oblongata, cerebellum). Data obtained between day 21 and day 49 p.i. were used for statistical analysis (exploratory data analysis) by the two way ANOVA with repeated measures in the factor "localization" using the BMDP Statistical Software (21).

Distribution of BDV-antigen in the rat brain was demonstrated using a mouse monoclonal antibody (Bo18) specific for the putative BDV-nucleoprotein p38 (24). Additionally, a polyclonal rabbit anti-glial fibrillary acidic protein (GFAP) antiserum was used to demonstrate reactive astrocytosis.

For immunohistology, brain sections were deparaffinized in xylene and hydrated through graded alcohols; endogenous peroxidase was quenched with 0.03% H_20_2-diluted in methanol. Brain sections were incubated with the mouse monoclonal anti-BDV-antibody (mAB Bo 18, 1:500 in 0.05M TRIS-buffered saline [TBS], pH 7.6) or the rabbit anti-GFAP antibody (1:500 in TBS, Dako, Hamburg, Germany) overnight at 4°C. After several wash steps, primary antibodies were detected by incubation with a biotinylated horse anti-mouse antibody (1:110 in TBS, Vector, Burlingame, USA) or with a swine anti-rabbit antibody (1:100 in TBS, Dako, Hamburg, Germany) for the rabbit anti-GFAP antisera.

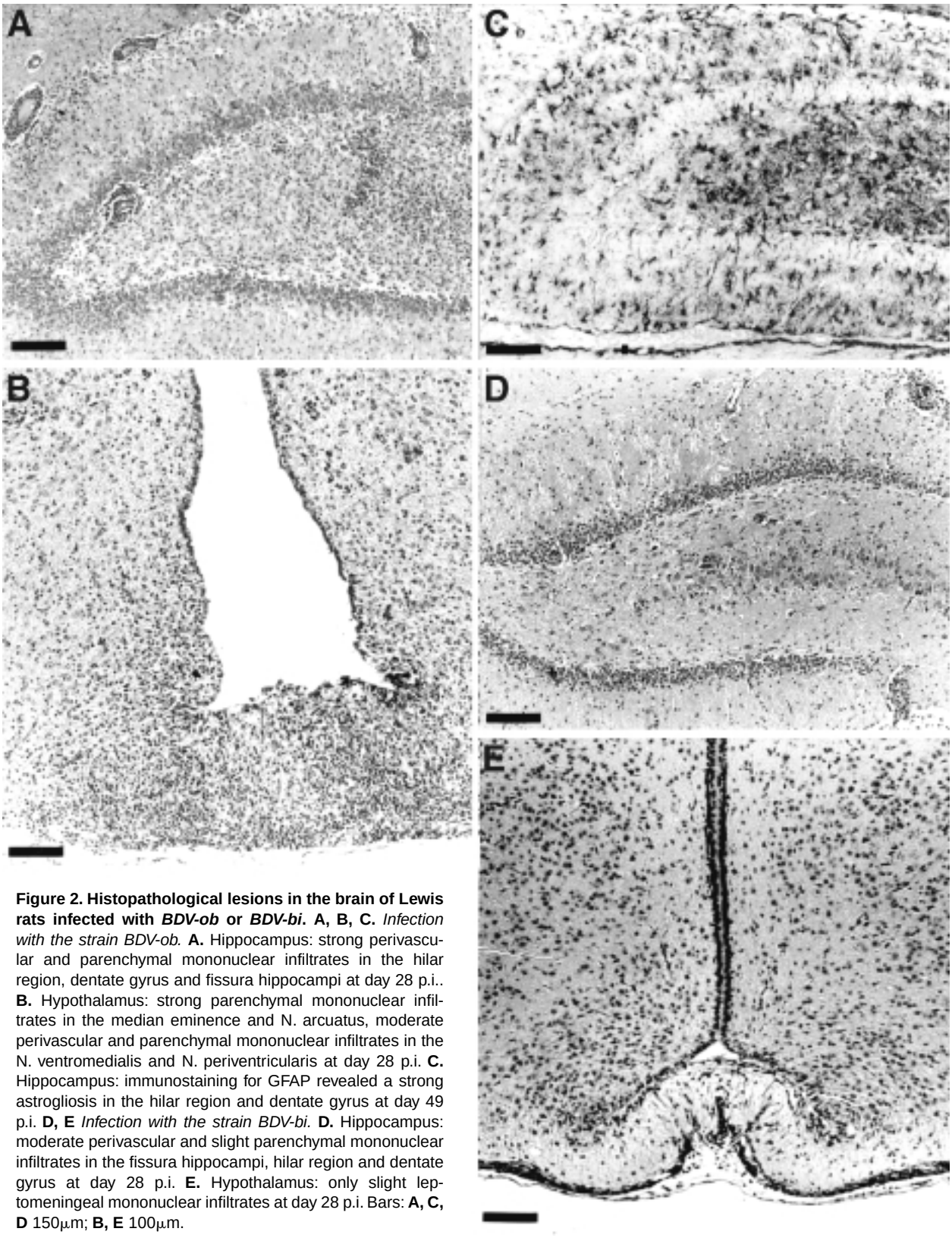

Figure 2. Histopathological lesions in the brain of Lewis rats infected with *BDV-ob* or *BDV-bi*. A, B, C. *Infection with the strain BDV-ob.* **A.** Hippocampus: strong perivascular and parenchymal mononuclear infiltrates in the hilar region, dentate gyrus and fissura hippocampi at day 28 p.i.. **B.** Hypothalamus: strong parenchymal mononuclear infiltrates in the median eminence and N. arcuatus, moderate perivascular and parenchymal mononuclear infiltrates in the N. ventromedialis and N. periventricularis at day 28 p.i. **C.** Hippocampus: immunostaining for GFAP revealed a strong astrogliosis in the hilar region and dentate gyrus at day 49 p.i. **D, E** *Infection with the strain BDV-bi.* **D.** Hippocampus: moderate perivascular and slight parenchymal mononuclear infiltrates in the fissura hippocampi, hilar region and dentate gyrus at day 28 p.i. **E.** Hypothalamus: only slight leptomeningeal mononuclear infiltrates at day 28 p.i. Bars: **A, C, D** 150μm; **B, E** 100μm.

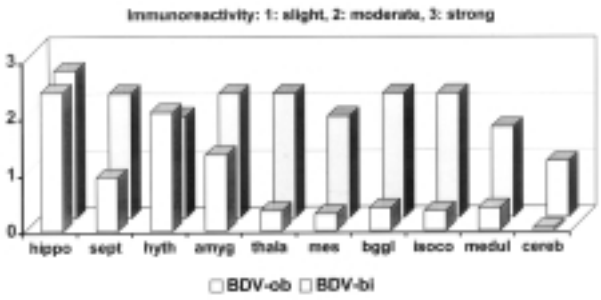

Figure 3. Distribution of BDV-antigen in the brains of Lewis rats infected with *BDV-ob* or *BDV-bi*.

At times of maximal BDV-antigen expression (21-49 dpi) after infection with *BDV-ob*, BDV-specific antigen was mainly restricted to hippocampus (hippo), hypothalamus (hyth), septum (sept) and amygdala (amyg), whereas in thalamus (thala), mesencephalon (mes), basal ganglia (bggl), isocortex (isoco) and medulla oblongata (medul) only single immunoreactive cells could be detected inconstantly. In the cerebellum (cereb) almost no viral antigen could be demonstrated.

At times of maximal BDV-antigen expression (21-56 dpi) after infection with *BDV-bi*, a strong and disseminated expression of BDV-antigen all over the brain was detectable.

Thereafter, brain sections were incubated with the avidin-biotin-peroxidase (ABC) complex (1:110, Vector, Burlingame, USA). When using the antibody specific for GFAP a peroxidase-anti-peroxidase solution (PAP, 1:100 in TBS, Dako, Hamburg, Germany) was applied for 30 minutes at room temperature. To visualize specific antigen-antibody bindings a 3,3′-diaminobenzidine-tetrahydrochloride (DAB-)H_20_2 - reaction was run in 0.1M imidazole, pH 7.1 for 10 minutes. Brain sections were slightly counterstained with Papanicolaou. Between all steps, brain sections were extensively rinsed in TBS. Controls were as follows: incubation of the BDV-infected brains with a monoclonal antibody specific for chicken T-lymphocytes instead of the mAB Bo18 or incubation with preimmune rabbit serum instead of the polyclonal sera. Additionally, a BDV-positive brain section from an animal with verified BDV-infection was used as positive control tissue.

Immunostaining of the brain sections was scored as follows: 0: no antigen, 0.5: traces of antigen, 1: mild antigen expression, 2: moderate antigen expression, 3: strong antigen expression (Figure 3). The distribution of BDV-specific antigen in rat brains infected with *BDV-ob* or *BDV-bi* was investigated employing at least 10 different brain regions (hippocampus, septum, hypothalamus, amygdala, thalamus, mesencephalon, basal ganglia, isocortex, medulla oblongata, cerebellum). Data obtained at times of maximal antigen expression (21-49 dpi) were used for statistical analysis (exploratory data analysis) by the two way ANOVA with repeated measures in the factor "localization" using the BMDP Statistical Software (21).

Isolation of infectious virus. The isolation of infectious virus from different brain regions and determination of the virus titer were performed employing susceptible fetal rabbit brain cells; visualization was done by indirect immunoflourescence techniques as previously described (27).

Serology. BDV-specific serum antibodies were demonstrated as described elsewhere (27). Briefly, sera of BDV-infected animals were titrated on persistently BDV-infected Madin-Darby-Canine-Kidney-Cells (BDV-MDCK) and visualized by indirect immunoflourescence techniques.

Results

Clinical signs. After intracerebral inoculation of the obesity-inducing virus isolate (*BDV-ob*), the body weight of the infected animals increased dramatically from day 14 until the end of the investigation period 56 dpi ($p < 0.01$). The increase of body weight of *BDV-ob* rats was significantly higher than the one of the *BDV-bi* rats and of the control animals ($p < 0.0001$, Figure 1). Neurological signs were minimal or absent. Rats infected with *BDV-bi* developed the typical biphasic form of BD beginning 14 dpi with ataxia, hyperactivity and initial weight loss. In the later stages, apathy and somnolence were noted (32, 33).

Histology. All rats infected either with *BDV-ob* or *BDV-bi* developed a nonpurulent meningonencephalitis with mononuclear perivascular and parenchymal infiltrates (Figure 2). In each BDV-infected cohort, a distinct distribution pattern of inflammatory lesions could be detected which varied significantly from each other ($p < 0.0001$).

Infection with the strain BDV-ob. In rats infected with the strain *BDV-ob*, inflammatory lesions in the brain were restricted mainly to the septum, hippocampus, ventromedian tuberal hypothalamus and amygdala (Figure 2a, b).

Already 7 days after infection with *BDV-ob*, moderate leptomeningeal and periventricular mononuclear infiltrates occurred. On day 14 p.i., the distribution of mononuclear infiltrates increased. They were located predominantly in the perivascular space and could be detected in various areas of the cortex cerebri, hip-

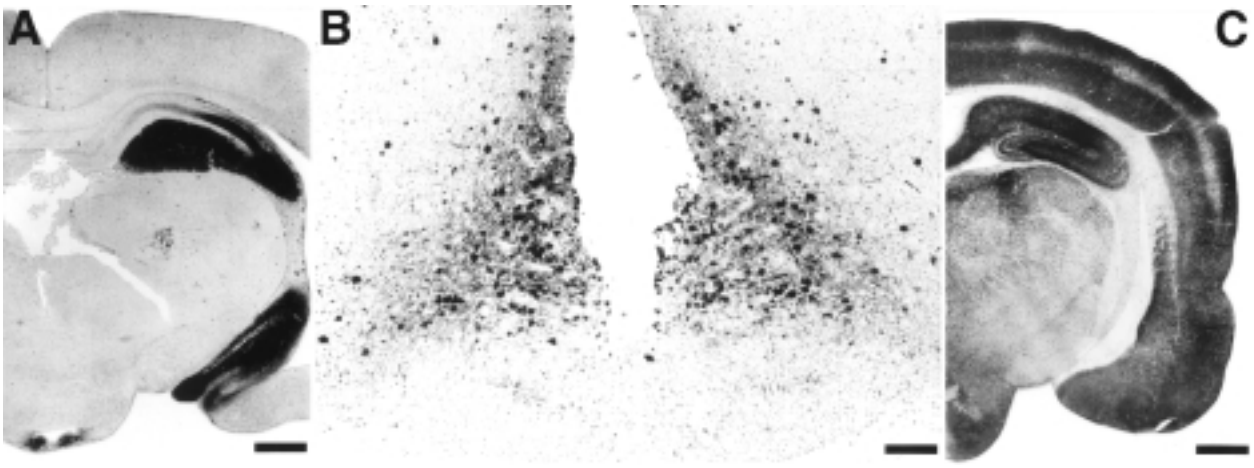

Figure 4. Immunohistological demonstration of BDV-antigen in brains of Lewis rats infected with *BDV-ob* or *BDV-bi*. A, B. *Infection with BDV-ob* **A.** BDV-antigen is predominantly located in the hippocampus and hypothalamus, only single immunoreactive cells in the thalamus, 49 days p.i. **B.** Higher magnification of the ventromedian hypothalamus. BDV-antigen expression is mainly found in the N. arcuatus. **C.** *Infection with BDV-bi.* BDV-antigen expression could be detected in all brain areas, 49 days p.i. Bars: **A, C** 1000μm; **B** 100μm.

pocampus, septum, amygdala, ventromedian hypothalamus, thalamus and globus pallidus. At this time point, the adjacent parenchyma of the hippocampus was already infiltrated with mononuclear immune cells. From day 21 p.i. on, parenchymal infiltrates increased further, mainly in the amygdala, ventromedian hypothalamus (Figure 2b) and septum. The inflammatory reactions were present in these brain areas until the end of the investigation period 56 days p.i.. The parenchymal infiltration in the hypothalamus was severe and mainly restricted to the median eminence and the N. arcuatus (Figure 2b). Only slight mononuclear infiltrates were noted occassionally in other brain regions from day 21 p.i. on.

Neuronal necrosis (data not shown) was observed in the hippocampal pyramidal layer CA1, CA2 and CA3 between day 14 to 28 p.i.. A reduction of pyramidal cells in the CA2- and CA3-area occurred already 21 days p.i.. The mononuclear infiltrates consisted of macrophages and lymphocytes. Interestingly, a significant number of plasma cells could be detected from day 21 p.i. until the end of the investigation period 56 dpi.

Reactive astrocytes occurred already 14 days p.i. in the dentate gyrus and dentate hilus of the hippocampus. In the course of the infection, the astrocytosis increased in the inflamed brains areas, mainly in the hippocampus (Figure 2c).

Infection with the strain BDV-bi. The findings after infection of rats with *BDV-bi* were in accordance with previous descriptions of the *BDV-bi* rat model (32, 33). Shortly, *BDV-bi* infected animals developed inflammatory lesions in many brain areas (cortex cerebri, thalamus, hipppocampus, periventricular regions of the third and fourth ventricle). This is in contrast to the restricted distribution of inflammatory lesions found after infection with *BDV-ob*.

After inoculation with the *BDV-bi*, 7 dpi only slight to moderate leptomeningeal and periventricular mononuclear infiltrates were observed. Single mononuclear cells were already found in perivascular spaces. From day 14 p.i. on, perivascular and parenchymal infiltration increased in the frontal, parietal and occipital cortex, basal ganglia, thalamus and hippocampus (Figure 2d). In the medulla oblongata, strongest inflammation with mononuclear immune cells occured in the periventricular area. In late stages of the infection (56 dpi), the mononuclear infiltration decreased in the affected brain regions.

14 days p.i. single pyramidal cells of the CA3-area of the hippocampus were necrotic (data not shown). 21 days p.i. necrosis in the hippocampal fascia dentata was observed. In late stages of the infection (49-56 days p.i.), a thinning of the fascia dentata of the dentate gyrus was noted, whereas the pyramidal cell layer of the hippocampus was not affected.

The infiltrating immune cells consisted of macrophages and lymphocytes; plasma cells could be found from day 28 p.i. on in increasing numbers. However, this increase was not as dramatic as noted in brains infected with *BDV-ob*.

Some reactive astrocytes were detected already 14 days p.i. in the dentate gyrus and dentate hilus of the

	olfactory bulb	frontal cortex	parietal cortex	septum	piriform cortex/ amygdala	hippocampus	median eminence	thalamus	mesencephalon	medulla oblongata	cerebellum
BDV-ob											
7 dpi	$5x10^1$	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^4$	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^3$	6x102
14 dpi	$5x10^1$	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^4$	$5x10^1$	$6x10^3$	$6x10^3$	-	-
21 dpi	-	-	-	$6x10^2$	-	$6x10^2$	$6x10^2$	$6x10^2$	-	-	-
BDV-bi											
7 dpi	5x101	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^4$	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^3$	$6x10^3$
14 dpi	$5x10^3$	$6x10^4$	$6x10^4$	$6x10^4$	$6x10^4$	$6x10^4$	$6x10^3$	$6x10^3$	$6x10^4$	$6x10^4$	$6x10^4$
21 dpi	$6x10^5$	$6x10^6$	$6x10^6$	$6x10^5$	$6x10^6$	$6x10^6$	$6x10^5$	$6x10^5$	$6x10^5$	$6x10^5$	$6x10^5$

Table 1. Isolation of infectious virus in certain brain areas of Lewis rats infected with *BDV-ob* or *BDV-bi* (ID_{50}/ml). A total amount of 12 animals was used for the isolation of infectious virus. Virus titers were calculated as described elsewhere (27, 37).

hippocampus. The reactive astrocytosis increased in the affected brain regions, mainly in the hippocampus until the end of the investigation period (data not shown). The astrocytosis observed in these animals was not as severe as in brains of rats infected with the virus strain *BDV-ob*.

Immunohistology. At times of maximal expression of viral antigen in rat brains infected either with *BDV-ob* or with *BDV-bi*, significant differences in the localization of viral antigen between both groups could be observed ($p < 0.0001$).

Infection with the strain BDV-ob. After infection with the obesity-inducing virus strain *BDV-ob*, expression of viral antigen was restricted to the same brain areas as described for the inflammatory lesions. BDV-specific antigen could mainly be demonstrated in the hippocampus, septum, ventromedian hypothalamus and amygdala (Figure 3, 4a). In the septum, most immunoreactive neurons were located in the lateral nuclei (LSD, LSV) and the nucleus of the ventral band of Broca (VDB). In the hypothalamus, viral antigen was localized predominantly in the N. arcuatus (Arc) (Figure 4a, b) and N. periventricularis; in the amygdala many immunoreactive neurons were observed in the amygdalohippocampal area (Ahi) and the caudomedial nucleus (PmCo). In the hippocampus, viral antigen was mainly detected in the CA3-pyramidal layer and in the polymorph layer of the dentate gyrus as well as in the fascia dentata. In other brain regions, BDV-specific antigen could be demonstrated only inconstantly and only in single cells. These results indicate a significant difference of viral antigen expression between the investigated brain areas ($p < 0.01$)

It should be noted that at early time points (7 dpi), traces of viral antigen were present in a variety of brain areas (septum, hippocampus, hypothalamus, amygdala, cortex cerebri, thalamus, basal ganglia, raphe nuclei of the mesencephalon) and in ependymal cells. While BDV-specific antigen expression increased in the septum, hippocampus, amygdala and ventromedian hypothalamus during the course of the infection, only single immunoreactive cells or no viral antigen expression could be detected in other initially immunopositive brain areas. Especially in the cortex cerebri, a brain area with strong viral antigen expression after infection with *BDV-bi*, only few immunopositive cells or no BDV-antigen could be detected. On day 56 p.i., a reduced expression of the BDV-specific protein was observed also in the brain areas characteristic for infection with *BDV-ob*. It was surprsing that during the course of *BDV-ob*-infection BDV-antigen positive cells were present in certain brain areas during the entire observation period, whereas in other brain areas the number of BDV-antigen positive cells decreased.

Infection with the strain BDV-bi. After infection with *BDV-bi*, expression of viral antigen was disseminated all over the brain beginning at day 7 p.i. (Figure 3, 4b). In some animals, in the isocortex, medulla oblongata, pons, cerebellum and bulbus olfactorius expression of BDV-antigen was diminshed when compared to the other brain regions. In most animals, the immunoreactivity was strong and nearly the same in all brain areas (Figure 3, 4b). In the hippocampus, viral antigen expression was very intense in the CA3-area and in the fascia dentata.

Already early after infection (7 dpi), BDV-specific antigen could be found in single cells in nearly all brain areas and in ependymal cells; in the mesencephalon and cerebellum, no viral antigen was observed.

Viral antigen expression increased between day 7 and 14 p.i. mainly in the hippocampus, cortex cerebri, septum, amygdala, hypothalamus, thalamus and also mesencephalon. At later time points, viral antigen were found in similar amounts in all brain regions and persisted until the end of the investigation period of 56 days.

Isolation of infectious virus and detection of BDV-specific serum antibodies. At day 7 p.i., infectious virus could be isolated from the different brain regions (olfac-

tory bulb, frontal and parietal cortex, hippocampus, amygdala, thalamus, septum, mesencephalon, cerebellum, medulla oblongata) of both BDV-infected cohorts. Virus titers at this time point ranged between 6×10^3 to 6×10^4 ID_{50}/ml. At day 21 p.i. after infection with *BDV-bi* virus titers increased up to 6×10^6 ID_{50}/ml in frontal and parietal cortex, amygdala and hippocampus (Table 1). In contrast, 21 days p.i.,. brain material of animals inoculated with *BDV-ob* contained only low amounts of infectious virus (6×10^2 ID_{50}/ml) in the median eminence, thalamus, septum and hippocampus. In these animals, no infectious BDV could be demonstrated in other brain regions anymore (Table 1). From day 28 up to the end of the investigation period, only hippocampal material of brains infected either with *BDV-ob* or *BDV-bi* was analyzed for the presence of infectious virus. Hippocampi of rats inoculated with *BDV-bi* contained high virus titers (6×10^6 ID_{50}/ml) until the end of the investigation period of 56 days, whereas only very low titers of infectious virus were found in hippocampi of animals infected with *BDV-ob* (6×10^1 ID_{50}/ml at day 56 p.i.).

BDV-specific serum antibodies were detected already at day 7 p.i. after infection with *BDV-ob*,whereas after inoculation of *BDV-bi* such antibodies were found earliest at day 14 p.i.. In the course of both infections, titers of BDV-specific serum antibodies increased up to day 28 p.i. with maximal titers ranging between 1:10000 and 1:20000 for both virus isolates (Table 2). These high serum titers were present until the end of the observation period of 56 days (Table 2).

	BDV-ob	BDV-bi
7 dpi	1:80	-
14 dpi	1:640	1:40
21 dpi	1:5000	1:10000
28 dpi	1:10000	1:20000
49 dpi	1:10000	1:20000
56 dpi	1:10000	1:20000

Table 2. Detection of BDV-specific serum antibodies. A total amount of 12 animals was used for the detection of BDV-specific serum antibodies.

Discussion

The fact that BDV-infection induces different clinical manifestations such as neurological and behavioral changes, obesity and paralysis, lead us to compare the alterations in the brains of animals experimentally infected with two BDV-strains. Therefore, in the present study Lewis rats were infected intracerebrally with rat-adapted BDV-isolates, either inducing a neurobehavioral illness with similar histopathological, virological and serological results as previously described (32, 33) or causing an obesity sydrome without obvious neurological signs. In contrast to the infection with *BDV-bi*, the development of the BDV-induced obesity syndrome was associated with a characteristic restriction of inflammatory lesions and BDV-specific antigen to certain brain areas (septum, hippocampus, ventromedian hypothalamus, amygdala). In the brain of the animals infected with *BDV-ob*, virus infectivity was low when compared to animals infected with *BDV-bi* (Table 1) and decreased already early after infection (21 days p.i.). Interestingly, detection of positive and negative sense BDV-RNA by *in situ* hybridization at later stages of infection (42-56 days p.i.) was minimal or not possible (data not shown), whereas BDV-antigen was still present. In animals infected with *BDV-ob*, infectivity was low or not detectable anymore at late time points (>350 days p.i.,data not shown). This indicates that infection with *BDV-ob* causes only transient low level virus replication and this only in certain brain areas. This is in contrast to *BDV-bi* infections, where a persistent infection of the CNS is regularly observed for more than 210 days p.i. (32, 33).

The viruses used for this study were not plaque purified and could therefore represent a heterogeneous population. Nonetheless, these viruses were different and were able to maintain their distinct phenotypes throughout several passages. The genetic basis of the different BDV-phenotypes is not known. Recent studies with isolates from different animal species revealed that the BDV-genome seems to be highly conserved (7, 26, 45). However, even single amino acid exchanges within viral proteins can alter the virulence and tropism of the respective viruses (34, 51, 52). This was shown with influenza A virus where a single amino acid exchange within the viral hemagglutinin-glycoprotein altered the antigenicity, the cell tropism and the pathogenetic property of the mutant (35). In the present study, no evidence for antigenic variation between *BDV-ob* and *BDV-bi* was found using the monoclonal BDV-antibody Bo18 specific for the putative nucleoprotein p38. Using this monoclonal antibody, antigenic variation due to a point mutation within the N-terminal region of p38 was previously described (26). Immunohistological demonstration of various other BDV-proteins revealed that p10, p24 and gp94 were expressed in the rat brains infected with *BDV-ob* or *BDV-bi*. However, expression of BDV-gp94 was absent in many brain cells infected with both isolates as shown by the expression of the other BDV-specific proteins (data not shown).

It is notable that in the early phase of infection with *BDV-ob*, viral antigen can be found in more brain areas than at later stages of *BDV-ob* infection. The loss of viral antigen in some brain regions might be the result of strong and early cell-mediated immune response as indicated by the presence of perivascular mononuclear infiltrates in the respective brain areas. In this context, it should be mentioned that an antiviral cellular immune response induced very early after BDV-infection is able to limit viral spread and eventually eliminate BDV from the CNS (38, 44). The fact, that BDV-specific antibodies were found earlier in animals infected with *BDV-ob* might support this hypothesis. The observed loss of viral antigens in certain brain areas of *BDV-ob* infected rats already at early time points p.i. might be due to an early antiviral cellular and humoral immune response in these animals. Elimination of virus and viral antigen from the CNS is not observed after infection with *BDV-bi*.

Inflammatory lesions as a reaction towards viral antigen expression in hippocampus, hypothalamus, septum and amygdala are most likely responsible for the development of the obesity syndrome after infection with *BDV-ob*. These brain areas, especially the hypothalamus are involved in the regulation of body weight and food intake (10, 11). The importance of the hypothalamus for these physiological functions was confirmed when animals with experimental lesions of the ventromedian hypothalamus induced by chemical or surgical methods became obese (10). A similar pattern of histopathological lesions and viral antigen expression was observed after experimental infection of mice with canine distemper virus (CDV; 5, 30). In contrast to the BDV-induced obesity syndrome, the CDV-infected mice developed an obesity syndrome only at late stages of the infection when histopathological lesions and viral antigen were not detectable anymore (5, 31). This indicates that the initial CDV-infection is responsible for the delayed neuroendocrine disorder, even in absence of virus markers (5). Similarly, experimental infection of mice or hamster with the scrapie agent (14, 15, 28) induces an obesity syndrome, most likely due to a dysfunction of the hypothalamo-pituitary-adrenal axis (15, 28, 57). Interestingly, the BDV-induced obesity syndrome seems to be directly related to the occurrence of inflammatory lesions and viral antigen expression in certain brain areas (especially the hypothalamus), since the strong increase of body weight started already early after infection (Figure1).

In general, the expression of many hypothalamic neurotransmitters and -peptides known to be involved in the regulation of appetite and satiety (f.e. neuropeptide Y, galanin, corticotropin releasing factor) is altered in various obesity models, either based on genetic background, on food restriction or on virus infection (4, 30, 40, 43, 55, 56). There is evidence that BDV-infections can alter the expression of neurotransmitters, e.g. leading to disturbances of the dopamine system or the cholinergic innervation of the cortex cerebri (22, 48). Similarly, in CDV-infected mice with an obesity syndrome, dopamine expression was reduced in the hypothalamic N. arcuatus (31). The modulation of neurotransmitters/-peptides in the CNS after BDV-infection seems to be the result of the infiltration with mononuclear immune cells since infection of newborn rats with *BDV-ob* does not cause clinical signs or the development of an obesity syndrome.

The study presented here describes initial investigations on an animal model where infection with a neurotropic virus disturbs the neuroendocrine network resulting in a virus-induced obesity syndrome. Many questions on the biochemical and endocrinological alterations remain to be solved. Infection of rats with *BDV-ob*, however, could serve as a favorable model to study interactions of mononuclear immune cells and its mediators within the neuroendocrine network.

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