Borna disease virus-induced neuronal degeneration dependent on host genetic background and prevented by soluble factors

Yuan-Ju Wu[a,b,c], Herbert Schulz[d], Chia-Ching Lin[a,b,c], Kathrin Saar[d], Giannino Patone[d], Heike Fischer[e], Norbert Hübner[d], Bernd Heimrich[e,1], and Martin Schwemmle[a,1]

[a]Department of Virology, [b]Spemann Graduate School of Biology and Medicine, [c]Faculty of Biology, and [e]Institute of Anatomy and Cell Biology, University of Freiburg, 79104 Freiburg, Germany; and [d]Medical Genomics and Genetics, Max-Delbrück-Centrum, 13092 Berlin, Germany

Edited by Peter Palese, Mount Sinai School of Medicine, New York, NY, and approved December 12, 2012 (received for review August 28, 2012)

Infection of newborn rats with Borne disease virus (BDV) results in selective degeneration of granule cell neurons of the dentate gyrus (DG). To study cellular countermechanisms that might prevent this pathology, we screened for rat strains resistant to this BDV-induced neuronal degeneration. To this end, we infected hippocampal slice cultures of different rat strains with BDV and analyzed for the preservation of the DG. Whereas infected cultures of five rat strains, including Lewis (LEW) rats, exhibited a disrupted DG cytoarchitecture, slices of three other rat strains, including Sprague–Dawley (SD), were unaffected. However, efficiency of viral replication was comparable in susceptible and resistant cultures. Moreover, these rat strain–dependent differences in vulnerability were replicated in vivo in neonatally infected LEW and SD rats. Intriguingly, conditioned media from uninfected cultures of both LEW and SD rats could prevent BDV-induced DG damage in infected LEW hippocampal cultures, whereas infection with BDV suppressed the availability of these factors from LEW but not in SD hippocampal cultures. To gain further insights into the genetic basis for this rat strain–dependent susceptibility, we analyzed DG granule cell survival in BDV-infected cultures of hippocampal neurons derived from the F1 and F2 offspring of the crossing of SD and LEW rats. Genome-wide association analysis revealed one resistance locus on chromosome (chr) 6q16 in SD rats and, surprisingly, a locus on chr3q21-23 that was associated with susceptibility. Thus, BDV-induced neuronal degeneration is dependent on the host genetic background and is prevented by soluble protective factors in the disease-resistant SD rat strain.

hippocampus | neurotropic viruses | postnatal development

Borna disease virus (BDV) is a nonsegmented, negative-sense RNA virus that persistently infects the CNS, resulting in behavioral syndromes and disease in a wide range of mammalian and avian species (1–3). Experimental infection of adult rats provokes an immune-mediated disease with associated meningoencephalomyelitis and progressive movement disorders (1). In contrast, infection of newborn rats causes no overt cellular immune response, but the hippocampus of BDV-infected newborn Lewis (LEW) or Wistar rats' dentate granule cells (DGCs) nonetheless progressively degenerate (1, 4). This BDV-induced neuronal degeneration can also be observed in infected organotypic hippocampal slice cultures from newborn LEW rats (5). Using this ex vivo tissue culture system, we recently showed that DGC degeneration in the hippocampus is a postmaturation event after the dentate gyrus (DG) is completely formed. Consistently, progressive loss of DGCs is observed 14 d post infection (PI) of hippocampal slice cultures, accompanied by the retraction of mossy fiber axons (5). The molecular mechanisms contributing to this pathology are unknown; however, aberrant zinc homeostasis (6) and activation of poly (ADP ribose) polymerase 1 and caspase 3 (7) might contribute to DGC degeneration. Interference with neuronal signaling pathways might also contribute to this pathology, especially because the phosphoprotein of BDV can act as a decoy substrate for cellular kinases including PKC (8).

Viral dissemination and BDV-induced DGC loss in the hippocampus were similar in newborn-infected LEW and histocompatible Fisher rats (9) and after infection of hippocampal slice cultures of these animals (5), suggesting that the BDV-associated susceptibility of certain rat strains to neuronal degeneration may be mirrored in slice culture preparations. However, mice and hippocampal slice cultures of mice do not show any sign of neuronal degeneration in the hippocampus and the remaining area of the CNS despite efficient infection with BDV (10). This could reflect a general species-specific difference in vulnerability. Alternatively, because only a limited number of rat strains had previously been investigated, we hypothesized that there might be rat strains that exhibit similar resistance to BDV-induced pathology as mice. Most intriguingly, such susceptible and resistant rat strains might serve as model systems to identify cellular counter mechanisms that prevent neuronal degeneration in the CNS following infection.

To identify rat strains that are resistant to BDV-induced neuropathology in the hippocampus, we infected hippocampal slice cultures of eight rat strains and determined the extent of neuronal degeneration. Hippocampi of three of eight rat strains, including Sprague-Dawley (SD), were resistant to DGC degeneration despite efficient replication of BDV in these cultures. These results were confirmed in vivo because SD maintained their DGCs after neonatal infection but LEW rats did not. Intriguingly, coculture of BDV-infected hippocampi of LEW and SD rats revealed the presence of neuroprotective factor(s) in the culture media. Treatment of BDV-infected LEW cultures with conditioned media revealed the presence of these protective factors in the supernatant of uninfected SD and LEW cultures, respectively. However, BDV infection suppressed the availability of these factors from LEW but not in SD hippocampal cultures. Finally, based on genome-wide association studies, we provided evidence that there is inheritance of one resistance locus on chromosome (chr) 6q16 and a susceptibility locus on chr3q21-23 in SD rats.

Results

Neuronal Integrity of the Hippocampus Is Dependent on the Rat Strain Background. To assess the vulnerability of granule cells to BDV infection, hippocampal slice cultures were prepared from

Author contributions: Y.-J.W., N.H., B.H., and M.S. designed research; Y.-J.W., H.S., C.-C.L., K.S., G.P., H.F., and B.H. performed research; Y.-J.W., H.S., K.S., N.H., B.H., and M.S. analyzed data; and B.H. and M.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence may be addressed. E-mail: martin.schwemmle@uniklinik-freiburg.de or bernd.heimrich@zfn.uni-freiburg.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1214939110/-/DCSupplemental.

the brains of various rat strains (Table 1), including susceptible LEW rats, and infected with 1,000 focus forming units (FFU) of BDV strain He/80. At 28 d PI, slice cultures were paraformaldehyde-fixed, resliced, and immunostained against BDV nucleoprotein (BDV-N) and calbindin. The majority of rat strains, including LEW rats, showed a severe reduction in calbindin-immunopositive DGCs and their mossy fiber projections (Fig. 1*A* and Fig. S1*A*), which are typical for the cytoarchitectural changes observed after BDV infection (5). In contrast, hippocampi of three rat strains, including SD, showed typical C-shaped calbindin-positive granule cell layers (Fig.1 *A* and *B*). BDV replication efficiencies were comparable in susceptible and in resistant slice cultures as judged by the accumulation of BDV-N and phosphoprotein (BDV-P) 1–4 wk PI by Western blot analysis (Fig. 1*C* and Fig. S1 *B–D*). Moreover, we observed no difference in the distribution of microglia cells between the BDV-infected LEW and SD cultures (Fig. S2). To provide in vivo evidence for the rat strain–dependent vulnerability of hippocampal granule cells observed in slice cultures, newborn LEW and SD rats were infected with BDV (5,000 FFU) intracranially. BDV-N was detectable in neurons, including pyramidal cells and DGCs from both LEW and SD rats at 4 wk PI, and the DG did not exhibit any sign of cell loss (Fig. 2). In sharp contrast, at 8 wk PI, calbindin-positive granule cells had almost disappeared in the DG of the LEW rats, whereas a densely packed granule cell layer and a mossy fiber projection could still be observed in the SD rats. These data suggest that BDV-induced degeneration is dependent on the rat strain genetic background.

Soluble Protective Factor(s) in Conditioned Media Prevent BDV-Induced Granule Cell Disturbances in Susceptible LEW Rats. Slice cultures harboring the DG are not only viable ex vivo, but also survive and are able to project mossy fibers to pyramidal cells when cocultured with intact hippocampal slices (11). To determine whether the DG of LEW rats shows an intrinsic susceptibility to BDV-induced degeneration, we infected cocultures of isolated LEW DG and intact hippocampal slice cultures of SD rats. The granule cell layers of both the LEW and SD rats remained calbindin-positive and showed mossy fiber projections at 4 wk despite being infected with BDV (Fig. S3*A*). As expected, axonal projections were missing in cocultures of LEW DG and intact LEW hippocampal slice cultures (Fig. S3*B*). Similar maintenance of the DG cytoarchitecture was observed in cocultures of intact LEW and SD hippocampi (Fig. 3*A*). These results suggest that close contact to the SD hippocampal slices protects LEW cultures from DG degeneration.

Interestingly, BDV-infected LEW cultures placed 1 cm from infected SD cultures also showed a normal formation of a C-shaped DG and a mossy fiber projection (Fig. 3*B*), suggesting that soluble factor(s) from SD cultures protect cultured LEW cells from neuronal degeneration. To confirm the presence of such factor(s), we supplemented the media of infected LEW hippocampal cultures with media of either infected or uninfected SD cultures at a ratio of 2:1 (SD medium:fresh medium) every 2 d for 28 d. In both cases, this treatment prevented the BDV-induced loss of calbindin-positive granule cells and their mossy fiber projections in >90% of the LEW cultures (Fig. 3*C*). However, supplementation at a ratio of 1:1 or 1:2 did not preserve the DG of infected LEW cultures (Fig. S4 *A* and *B*). Filtration of the supernatant revealed that the bioactive native size of this putative protective factor(s) was between 30 and 50 kDa (Fig. S4*C*). Intriguingly, supplementation with media of uninfected LEW hippocampal cultures at a ratio of 2:1 also prevented BDV-induced pathology in 11 of 14 infected LEW cultures (79%), whereas media from BDV-infected LEW cultures led to a preservation of the DG in only 7 of 16 LEW cultures (44%) (Fig. 3*C*), suggesting that the amount and/or activity of the protective factor(s) released from LEW cultures is suppressed after BDV infection.

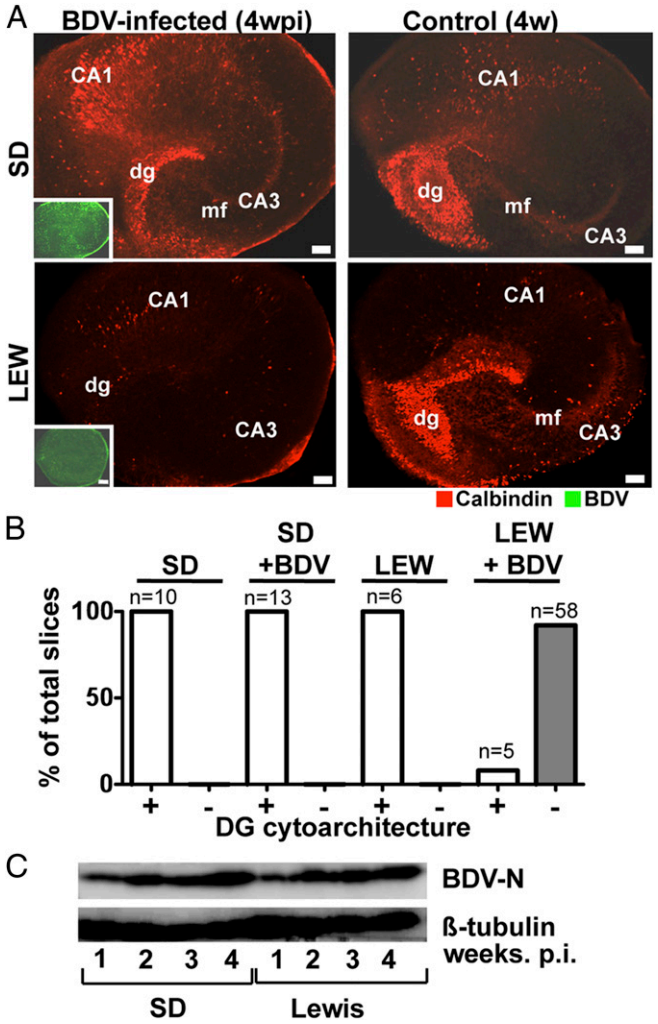

Fig. 1. Disturbances of the granule cell layers in hippocampal slice cultures from different rat strains after BDV infection. (*A*) Hippocampal slice cultures from SD and LEW rat strains prepared after birth (P0-P2) were either infected with *ca.* 1000 focus forming units of BDV immediately or left uninfected (control) and stained for calbindin (red). (*Inset*) Micrographs illustrating BDV-N (green) immunofluorescence. CA1, CA3, hippocampal subfields; dg, dentate gyrus; mf, mossy fiber projection. (Scale bar, 100 μm.) (*B*) Percentage of total hippocampal slice cultures of LEW and SD rats, either infected or uninfected with BDV, were analyzed as described in (*A*). (+), intact DG; (–), loss of calbindin-immunopositive granule cells and mossy fiber projections. Numbers of slice cultures are indicated. (*C*) BDV-N levels in infected hippocampal slices cultures from SD and LEW rats at the indicated time points PI as judged by Western blot analysis. Levels of β-tubulin served as loading control.

Table 1. Hippocampal pathology in BDV infection slice cultures

Rat strain	DG degeneration
SD	No
Brown Norway	No
Spontaneously hypertensive rat	No
Fisher	Yes
LEW	Yes
Piebald-Viral-Glaxo	Yes
Wistar Kyoto	Yes
Wistar	Yes

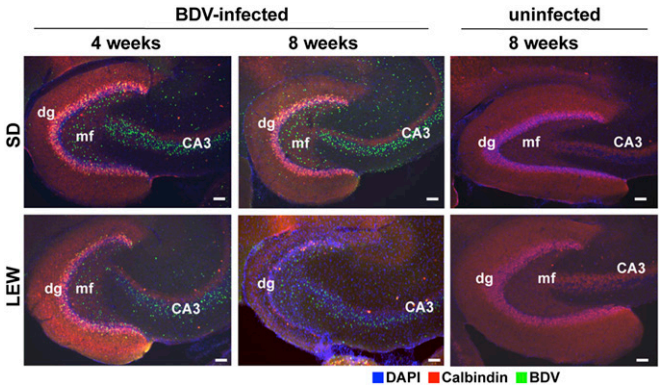

Fig. 2. Differences in granule cell vulnerability between SD and LEW rats after BDV infection. Immunofluorescent micrographs of hippocampi from neonatally BDV-infected LEW and SD rats at either 4 or 8 wk PI and uninfected animals (8 wk after birth) stained for BDV-N (green) and calbindin (red). DAPI nuclear staining was used to illustrate the cytoarchitectural organization. CA3, hippocampal subfields; dg, dentate gyrus; mf, mossy fiber projection. (Scale bar, 100 μm.)

Based on the molecular weight of 28 kDa of the mature brain-derived neurotrophic factor (BDNF) dimer and the known interference of BDV with BDNF-mediated signaling events (12), we speculated that this protein might be involved in the protection of LEW DGC from virus-induced neuronal degeneration. Indeed, exogenous administration of high concentrations of soluble BDNF in BDV-infected LEW slice cultures resulted in partial protection (54% of total slices) (Fig. S5). Consistent with this result, depleting BDNF from the culture media of BDV-infected SD cultures by introduction of neutralizing anti-BDNF antibody increased the pathologic changes in hippocampus (47% of total slices) (Fig. S5). However, attempts to quantify BDNF in the culture media of infected and uninfected LEW and SD cultures by ELISA failed, indicating that only trace amounts of this protein are secreted.

Supply of the Protective Factor Is Required During Postnatal Maturation of the DG. Earlier studies indicated that axonal retraction and neuronal degeneration of the granule cells in BDV-infected cultures occurs after 2 wk when postnatal maturation of the DG is completed (5). Damage of neurons might occur before maturation of the DG, however; thus, the presence of protective factors is likely to be required early during the infection. To test this, we supplemented the media of BDV-infected LEW cultures with media of uninfected SD cultures at different time points PI. The presence of conditioned SD media from 7 to 28 d PI did not protect LEW cultures from DG degeneration (Fig. 3*D*). Similarly, the presence of conditioned media from 14 to 28 d PI resulted in protection of only 13% of cultures (Fig. 3*D*). Significant increase in protection was observed only when conditioned media was present from 2–14 (30%) or from 2–21 d PI. (68%). These results indicate that neuronal protection is required throughout the maturation period of the DG. Consistently, infection of LEW hippocampal slice cultures at 2 wk (after formation of the DG is completed) resulted in an efficient infection of the culture, but no reduction in calbindin-immunopositive DGCs and their mossy fiber projection (Fig. S6), indicating that the BDV-mediated neuronal loss is initiated during postnatal development.

Mapping of Resistance and Susceptibility Loci in SD Rats. To gain insights into the genetic constellation involved in the BDV-mediated hippocampal pathology, we crossed LEW and SD rats and infected the corresponding hippocampal cultures of 31 F1 and 198 F2 offspring with BDV. Whereas only 10% of the cultures from F1 offspring of male SD and female LEW exhibited a normal DG cytoarchitecture, a significantly higher proportion (66%) of the F1 cultures from male LEW and female SD rats were observed with intact DG (Fig. S7*A*). However, independent on the combination

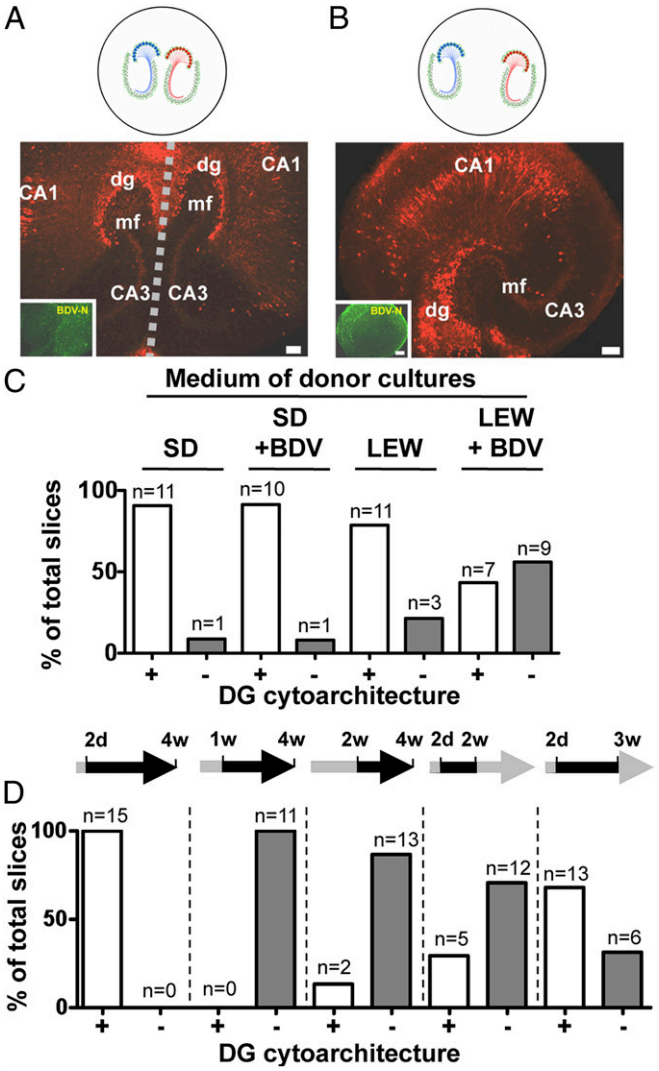

Fig. 3. Granule cell damage in BDV-infected hippocampal cultures of LEW rats can be prevented by addition of conditioned media. (*A* and *B*) Hippocampi from newborn LEW and SD rats were either cultured adjacent or *ca*. 1 cm apart [see schematic drawing: LEW (red) and SD (blue)] and immediately infected with BDV [1,000 focus forming units (FFU)]. After 4 wk incubation, calbindin-immunostaining (red) revealed an intact cytoarchitecture and mossy fiber projections in both LEW cultures. (*Insets*) Staining against BDV-N (green). CA1, CA3, hippocampal subfields; dg, dentate gyrus; mf, mossy fiber projection. (Scale bar, 100 μm.) (*C*) To test for the presence of protective factors released in the nutrient medium, hippocampi from LEW and SD rats were prepared at the same time and either infected with BDV (1,000 FFU) or remained noninfected. The nutrient medium in the hippocampal cultures was changed 3 times per week over 4 wk of the incubation period. At each medium exchange, medium from the indicated donor cultures was mixed with fresh medium at a ratio of 2 (donor medium):1 (fresh medium) and supplied to BDV-infected LEW cultures. At 4 wk PI, the cultures were examined as described in (*A*). Numbers indicate the amount of slices used in this experiment. (*D*) Nutrient media of BDV-infected LEW cultures were substituted with filtered supernatant of uninfected SD hippocampal cultures as described in (*C*) during the indicated periods (black part of the arrows). At 4 wk PI, the cultures were judged as described in (*A*). Numbers indicate the amount of independent slice cultures used. (+), intact DG; (–), loss of calbindin-immunoppositive granule cell layer and axonal mossy fiber projection.

MICROBIOLOGY

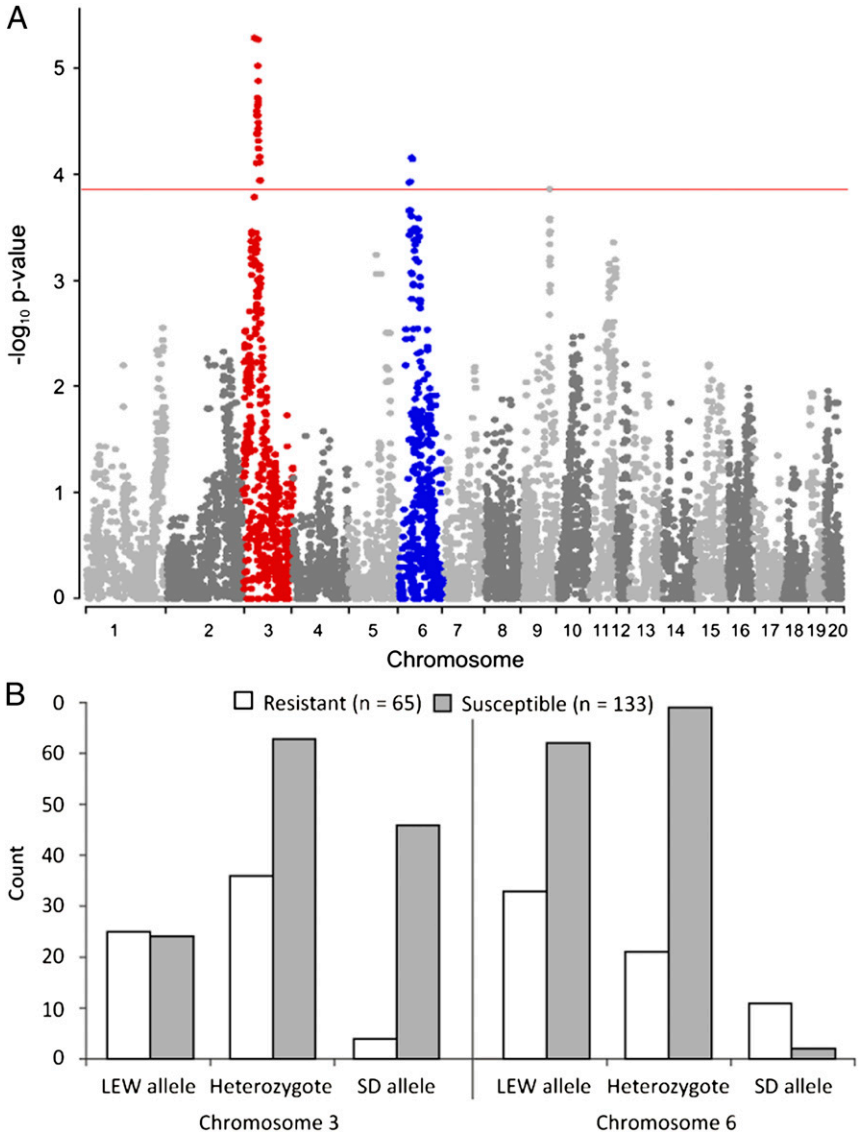

Fig. 4. Identification of genetic loci related to the BDV-induced alteration in the hippocampus. (*A*) Manhattan plot of the association analysis results on 198 (n = 115 SD×LEW and n = 83 LEW×SD) F2 rats. Physical positions of the SDPs and -$\log_{10}$ Fisher test P values are given on X and Y respectively. The red line represents the Benjamini-Hochberg 5% FDR cutoff. Chromosomes 3 and 6, having significant association results, are marked red and blue, respectively. (*B*) Count of SD genotypes, LEW genotypes, and heterozygotes in resistant (white) and susceptible (gray) F2 offspring. The SNP pattern representing the most significant associated SNPs on (*Left*) chr3:39,803,691–39,804,005 (2 SNPs; P value = 5.17×10^{-6}) and on (*Right*) chr6:41,379,239–41,562,454 (13 SNPs; P value = 6.98×10^{-5}), respectively.

of the F0 generation, a comparable degree of DG resistance was observed in cultures of the F2 offspring (~33%, Fig. S7*A*). To identify disease-related loci, SNPs were determined from all animals and used for genome-wide association analysis. This revealed significant loci with a 5% Benjamini-Hochberg false discovery rate (FDR) on chr3q21-23 and chr6q16 spanning 19.3 Mbps and 11.4 Mbps, respectively. Within these loci on chr 3 and 6, 23 and 4, significant genetic patterns comprising 351 and 51 SNPs were present, respectively (Fig. 4*A*, Table S1). Intriguingly, the 3q21-23 loci significantly correlated (P value of 5.17×10^{-6}, 2.5% FDR) in SD allele carriers that exhibited pronounced DG degeneration after BDV infection (Fig. 4*B*, *Left*). In contrast, the 6q16 locus significantly (minimum P value = 6.98×10^{-5}, 3.3% FDR) increased the protective effect in the SD allele carriers (Fig. 4*B*, *Right*). Thus, 11 of 13 homozygote SD allele carriers at 6q16 locus preserved an intact DG cytoarchitecture upon BDV infection. Interestingly, all of the homozygote SD allele carriers at this position were offspring of the female SD founder. Indeed, the allele status analysis revealed that female SD founder had a heterozygote allele, whereas the male SD founder had either a homozygote LEW allele or a heterozygote allele (Fig. S7*B*).

Discussion

In contrast to mice, infection of newborn rats with BDV is associated with a selective degeneration of the hippocampal granule cells. We demonstrate here that this virus-induced pathologic change is dependent on the host genetic background. We further show that cellular factor(s) released into the culture medium of hippocampal slice cultures from resistant SD rats protect BDV-infected cultures of LEW rats from neuronal degeneration. Moreover, the presence of this protective factor(s) is required before and throughout the process of completing the development of connectivity within the hippocampus. Finally, we provide strong genetic evidence that resistance to BDV-induced neuronal degeneration is inherited

and identify both a resistance and a susceptibility locus on chr6 and 3, respectively, of SD rats.

Several lines of evidence indicate that the differences in the BDV-induced neuronal loss in the rat hippocampus depend on the genetic background. In hippocampal slice culture, BDV-associated degeneration is absent in three of eight distantly related rat strains (13), supporting the concept that resistance does not represent a unique feature of one rat strain only. Both the viral replication efficiency and the subcellular tropism were comparable in susceptible and resistant cultures, indicating that resistance to BDV-mediated neuronal losses is not simply mediated by interfering with viral growth in certain rat strains. Furthermore, this pattern of strain-dependent susceptibility is also observed in vivo. Finally, consistent with a genetic contribution, we observed an inheritance of the resistant phenotype during in vivo studies and found a single resistance locus on chr 6 that was associated with SD alleles in genome-wide association studies. Intriguingly, we also identified a susceptible locus on chr 3 that correlated with the degree of DG degeneration in hippocampal slice cultures from SD × LEW F1 and F2 offspring. Thus, it seems likely that the resistant phenotype in SD is the result of a successful genetic buffering that does not occur in susceptible rat strains. In vivo infections of F1/F2 generation animals may have been a useful corroboration of our findings. However, we have previously demonstrated a high level of correlation between results obtained in vivo and those obtained with hippocampal slice cultures (5) and, moreover, the numbers of animals required made it infeasible to pursue such studies within the scope of the current work.

We hypothesize that BDV infection reduces a critical level of a neuroprotective factor(s) in LEW hippocampal cultures that is required for protecting the DG from degeneration. This was based on the observation that media from uninfected but not infected LEW hippocampal cultures provide protection from BDV-mediated neuronal degeneration. We identified BDNF as a potential protective cellular factor because the addition of recombinant BDNF to infected LEW cultures protected from DG degeneration and the depletion of BDNF from infected SD cultures resulted in DG degeneration. However, protective and disease-inducing effects were only partial, suggesting that there are other factors yet to be identified that might also prevent DG degeneration. In this respect, the genes located around the mapped loci appear to be particularly interesting (Table S2).

One interesting gene on chr 3 is *CHN1*, chimerin 1 (chr3q23), a locus that encodes a neuronal GTPase-activating protein that exhibits an essential role in axon guidance and the establishment of neuronal circuits (14, 15). Another candidate gene on chr 3 is *RBM45*, coding for the RNA binding motif protein 45 (chr3q23). This protein has been proposed to have significant function in neurogenesis (16). Moreover, on chr6, one potential candidate is the gene for *KIDINS220*, which codes for the kinase D–interacting substrate of 220 kDa (chr6q16). Kidins220 is an integral membrane protein selectively expressed in the brain and neuroendocrine cells (17). Growing evidence suggests that Kidins220 has a multifunctional role involving neuronal polarity, development, differentiation, survival, and as a neurotransmitter in signaling pathways (18–20). Other interesting gene candidates encode proteins belonging to a neuronal Ca^{2+}-sensor family: *VSNL1* (visinin-like protein 1; at chr6q14) and *HPCAL1* (hippocalcin-like protein-1; at chr6q16). Both proteins are highly expressed in many regions of the CNS, including the hippocampus and the cerebellum (21), and have been implicated in several neuronal disorders, such as Alzheimer's disease (22). Vsnl1 is suggested to play a role in neuronal loss mediated by altered Ca^{2+}-homeostasis, whereas Hpcal1 may function together with cytochrome b5 in an antioxidant system that is impaired in patients with Alzheimer's disease (22).

A sixth and perhaps the most likely candidate gene is *MYCN*, coding for n-myc proto-oncogene protein (chr6q14). Mycn has been shown to colocalize with a transcription repressor, the methyl CpG binding protein 2 (Mecp2), at varies target sites, including the gene coding for BDNF, thereby regulating transcriptional activities (23). Interestingly, comparison of BDV-infected and uninfected primary rat neuronal cultures revealed that the protein level of Mecp2 is down-regulated in infected cells (24). Therefore, it appears possible that Mycn may help protect the DG from damage in SD allele carriers. However, we currently lack a simple cell culture system to evaluate the contribution of gene candidates of the resistant loci, because dissociated primary neuronal cultures from susceptible and resistant rats show no detectable signs of cell damage after infection with BDV (12).

Intriguingly, BDV-induced degeneration was only observed after granule cell layers in slice cultures had completed their maturation, which typically occurs after 2 wk in culture (5). Nonetheless, to prevent the DG from degeneration, supplement of BDV-infected LEW hippocampal cultures with media from SD cultures is required as early as 3 d PI. Together with the observation that granule cells remained intact after being infected at 2 wk in vitro, this raises the possibility that the maturation process per se represents the sensitive phase during which noxious events can have a deleterious impact on specific cell populations (25). The ability of BDV to infect immature transcription factor prospero homeobox protein (Prox 1)-positive DGCs (26) in LEW cultures (5) may relate to the capacity of BDV to trigger cell apoptosis after DG maturation (27). Unfortunately, we currently lack a direct and simple tool to monitor the development of a single BDV-infected cell within slice cultures. It is therefore difficult to illustrate the mechanisms by which BDV infection influences the fate of the DGCs.

Comparison of rat strains that are susceptible or resistant to BDV-induced brain degeneration represents a unique means of studying both neuronal survival and degeneration processes during the maturation of the hippocampus. This model system might be additionally used to identify cellular factors and networks that efficiently prevent virus-induced cell damage in the brain.

Materials and Methods

Preparation and Infection of Organotypic Slice Cultures. Hippocampi were dissected from rat pups (P0 to P2) and processed and infected with 1 μL of BDV virus stock, corresponding to *ca.* 1,000 FFU as described (5). Control cultures received normal medium but without BDV infection.

In Vivo Infection of Newborn Rats. Newborn LEW and SD rats were infected intracranially with 20 μL (250 FFU/μL) of rat brain–derived BDV within 24 h after birth (28). All animal experiments were performed in accordance with the German animal protection law (Tierschutzgesetz). The animal welfare committees of the University Freiburg (Regierungspräsidium Freiburg) approved all animal experiments.

Immunohistofluorescence and Western Blot Analysis. Cultures selected for morphological analysis were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 2 h, resliced, and stained for BDV-N, BDV-P, or calbindin as described (5). Western blot analyses were carried out as described previously (29) using mouse-anti-β tubulin antibody (Sigma) and polyclonal rabbit-anti-BDV N or anti-BDV-P antibodies (29).

Preparation of Conditioned Medium. Medium from donor cultures was collected at every medium change and either directly mixed with fresh nutrient medium at the indicated ratio (donor medium/fresh medium) and applied to slice cultures or first filtered through membranes with different cutoff sizes [300 kDa, 100 kDa (Sartorius Stedim), 50 kDa, 30 kDa, and 10 kDa (Millipore)] according to the manufacturer's instructions.

Treatment of BDNF or BDNF Neutralizing Antibody. The hippocampal slice cultures were prepared from newborn LEW rats (P0 to P2), immediately

infected with BDV (~1,000 FFU) or left uninfected, and supplemented with either 100 ng/mL of recombinant human BDNF (Millipore) or BDNF neutralizing antibody [5 μg/mL of BDNF neutralizing antibody (Millipore)]. At each medium exchange, fresh recombinant BDNF or BDNF neutralizing antibody was added into each well.

Genome-wide Association Analyses. For large-scale SNP genotyping of the LEW and SD founder and their offspring, we used a previously designed custom high-density SNP genotyping array (RATDIV array) containing 803,485 SNPs. For further information, see *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Mady Hornig and Geoffrey Chase for critical reading of the manuscript and Heidi Banse and Simone Zenker for excellent technical assistance. The study was supported by European large-scale functional genomics in the rat for translational research (EURATRANS) Grant HEALTH-F4-2010-241504 (to N.H.) and Deutsche Forschungsgemeinschaft Grants SCHW 632 (to M.S.) and He 1520 (to B.H.).

1. Gonzalez-Dunia D, Volmer R, Mayer D, Schwemmle M (2005) Borna disease virus interference with neuronal plasticity. *Virus Res* 111(2):224–234.
2. Kistler AL, et al. (2008) Recovery of divergent avian bornaviruses from cases of proventricular dilatation disease: Identification of a candidate etiologic agent. *Virol J* 5:88.
3. Honkavuori KS, et al. (2008) Novel borna virus in psittacine birds with proventricular dilatation disease. *Emerg Infect Dis* 14(12):1883–1886.
4. Bautista JR, Schwartz GJ, De La Torre JC, Moran TH, Carbone KM (1994) Early and persistent abnormalities in rats with neonatally acquired Borna disease virus infection. *Brain Res Bull* 34(1):31–40.
5. Mayer D, Fischer H, Schneider U, Heimrich B, Schwemmle M (2005) Borna disease virus replication in organotypic hippocampal slice cultures from rats results in selective damage of dentate granule cells. *J Virol* 79(18):11716–11723.
6. Williams BL, et al. (2006) Metallothioneins and zinc dysregulation contribute to neurodevelopmental damage in a model of perinatal viral infection. *Brain Pathol* 16(1):1–14.
7. Williams BL, Hornig M, Yaddanapudi K, Lipkin WI (2008) Hippocampal poly(ADP-Ribose) polymerase 1 and caspase 3 activation in neonatal bornavirus infection. *J Virol* 82(4):1748–1758.
8. Prat CM, et al. (2009) Mutation of the protein kinase C site in borna disease virus phosphoprotein abrogates viral interference with neuronal signaling and restores normal synaptic activity. *PLoS Pathog* 5(5):e1000425.
9. Pletnikov MV, Rubin SA, Vogel MW, Moran TH, Carbone KM (2002) Effects of genetic background on neonatal Borna disease virus infection-induced neurodevelopmental damage. I. Brain pathology and behavioral deficits. *Brain Res* 944(1-2):97–107.
10. Ackermann A, Staeheli P, Schneider U (2007) Adaptation of Borna disease virus to new host species attributed to altered regulation of viral polymerase activity. *J Virol* 81(15):7933–7940.
11. Gaiarsa JL, Heimrich B (1995) Restoration of mossy fiber projection in slice co-cultures of dislocated dentate gyrus and degranulated hippocampus. *Brain Res Dev Brain Res* 86(1-2):250–258.
12. Hans A, et al. (2004) Persistent, non-cytolytic infection of neurons by Borna disease virus interferes with ERK 1/2 signaling and abrogates BDNF-induced synaptogenesis. *The FESEB Journal* 18(7):17.
13. Saar K, et al.; STAR Consortium (2008) SNP and haplotype mapping for genetic analysis in the rat. *Nat Genet* 40(5):560–566.
14. Wegmeyer H, et al. (2007) EphA4-dependent axon guidance is mediated by the RacGAP alpha2-chimaerin. *Neuron* 55(5):756–767.
15. Beg AA, Sommer JE, Martin JH, Scheiffele P (2007) alpha2-Chimaerin is an essential EphA4 effector in the assembly of neuronal locomotor circuits. *Neuron* 55(5):768–778.
16. Tamada H, et al. (2002) cDNA cloning and characterization of Drb1, a new member of RRM-type neural RNA-binding protein. *Biochem Biophys Res Commun* 297(1):96–104.
17. Iglesias T, et al. (2000) Identification and cloning of Kidins220, a novel neuronal substrate of protein kinase D. *J Biol Chem* 275(51):40048–40056.
18. Higuero AM, et al. (2010) Kidins220/ARMS modulates the activity of microtubule-regulating proteins and controls neuronal polarity and development. *J Biol Chem* 285(2):1343–1357.
19. López-Menéndez C, et al. (2009) Kidins220/ARMS downregulation by excitotoxic activation of NMDARs reveals its involvement in neuronal survival and death pathways. *J Cell Sci* 122(Pt 19):3554–3565.
20. Neubrand VE, Cesca F, Benfenati F, Schiavo G (2012) Kidins220/ARMS as a functional mediator of multiple receptor signalling pathways. *J Cell Sci* 125(Pt 8):1845–1854.
21. Paterlini M, Revilla V, Grant AL, Wisden W (2000) Expression of the neuronal calcium sensor protein family in the rat brain. *Neuroscience* 99(2):205–216.
22. Braunewell KH (2012) The visinin-like proteins VILIP-1 and VILIP-3 in Alzheimer's disease-old wine in new bottles. *Front Mol Neurosci* 5:20.
23. Murphy DM, et al. (2011) Co-localization of the oncogenic transcription factor MYCN and the DNA methyl binding protein MeCP2 at genomic sites in neuroblastoma. *PLoS ONE* 6(6):e21436.
24. Suberbielle E, et al. (2008) Proteomic analysis reveals selective impediment of neuronal remodeling upon Borna disease virus infection. *J Virol* 82(24):12265–12279.
25. Lazarov O, Mattson MP, Peterson DA, Pimplikar SW, van Praag H (2010) When neurogenesis encounters aging and disease. *Trends Neurosci* 33(12):569–579.
26. Kronenberg G, et al. (2003) Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol* 467(4):455–463.
27. Hornig M, Weissenböck H, Horscroft N, Lipkin WI (1999) An infection-based model of neurodevelopmental damage. *Proc Natl Acad Sci USA* 96(21):12102–12107.
28. Carbone KM, Park SW, Rubin SA, Waltrip RW, 2nd, Vogelsang GB (1991) Borna disease: association with a maturation defect in the cellular immune response. *J Virol* 65(11):6154–6164.
29. Geib T, et al. (2003) Selective virus resistance conferred by expression of Borna disease virus nucleocapsid components. *J Virol* 77(7):4283–4290.