Hippocampal and Cerebellar Transcription Profile of the Rat eight Weeks after neonatal Borna Disease Virus Infection

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Abstract

Neonatal Borna disease virus (BDV) infection in the rat, leads to hippocampal and cerebellar degeneration, long term consequences of which are only partially understood. To obtain a wider overview on BDV dependent gene expression changes, we performed a microarray analysis of hippocampal and cerebellar mRNAs from BDV-infected and control treated rats, eight weeks post infection. At a cutoff level of 2.0 we could demonstrate a total of 152/216 hippocampal/cerebellar genes to be up- and 9/39 genes to be downregulated, 97 of which were regulated in both brain regions. Affected transcripts could be assigned to different functional groups including defense related proteins, apoptotic factors, growth factors and their receptors, cytoplasmic signalling molecules, transcription factors, cytoskeletal proteins, cell adhesion and extracellular matrix proteins, enzymes of lipid and steroid metabolism, enzymes of basic energy metabolism, detoxifying and cell protection molecules, factors for protein synthesis, processing, and degradation, and finally a group of neuron and glia related transcripts. For a subset of genes like Hemeoxygenase1, Vimentin, Stat1, Dlhg3, and GFAP, data were confirmed by standard and/or realtime RT-PCR, and by Western blot. In conclusion our study confirms some of the previous results on BDV dependent changes but also adds new candidates to the list of BDV dependent genes, providing new starting points to the study of longterm viral brain effects.

1. Introduction

Borna Disease Virus (BDV) is a non-segmented, non-lytic, negative stranded RNA virus causing infections of the nervous system in most warm-blooded organisms including humans (For a review see Hornig et al., 2003). BDV is suspected as a possible cause for neuropsychiatric diseases like schizophrenia (Waltrip et al., 1995), bipolar disorder (Rott

et al., 1985; Bode et al., 2001) and autism (Pletnikov et al., 2002). However, contradictory results in the past, challenged the role of BDV for etiology of psychiatric illness (Lieb and Staeheli, 2001; Taieb et al., 2001). Nevertheless, BDV-infection in the newborn rat is an established model system to study viral effects on behaviour and neuronal functioning in the mammalian brain (Hornig et al., 2001). Whereas BDV infections of adult rats lead to acute encephalitis, the immature immune system of neonate animals tolerates BDV infection (Hornig et al., 2003), leading to persistent infection resulting in structural and functional changes and neurological and behavioural deficits (Rubin et al., 1999).

Thus, cerebellar and hippocampal degeneration can be found (Carbone et al., 1991; Hornig et al., 1999, Rubin et al., 1999), being accompanied by reduced weightgain (Bautista et al., 1994, Carbone et al., 1991), increased locomotor activity (Bautista et al., 1994, Hornig et al., 1999) altered salt intake and disturbed circadian rhythms (Bautista et al., 1994). In addition, neonatally BDV infected rats show abnormal anxiety and playing behaviour (Dittrich et al., 1989; Hornig et al., 1999; Pletnikov et al., 1999), and deficits in spatial memory and learning (Dittrich et al., 1989; Rubin et al., 1999). Finally, like schizophrenic patients, neonatally BDV infected rats reveal impaired prepulse inhibition of the acoustic startle response (Pletnikov et al., 2002).

Several previous reports about BDV dependent changes in gene expression in the neonatally infected rat brain already exist (Hornig et al., 1999; Sauder and de la Torre, 1999; Sauder et al., 1999; Rauer et al., 2002; Weissenböck et al., 2000; Zocher et al., 2000; Gonzalez-Dunia et al., 2000; Jehle et al., 2003), demonstrating changes of immune related proteins like Inflammatory cytokines (Hornig et al.,1999; Sauder and de laTorre, 1999), chemokines (Sauder et al., 1999) and their receptors (Rauer et al., 2002). This is also acccompanied by microglial proliferation and activation of immune related markers like

MHCI and II, ICAM1, CD4 and CD8 (Weissenböck et al., 2000). In those brain regions with neurodegenerative changes, persistent BDV infection causes also increased rates of apoptosis (Hornig et al., 1999). For the hippocampus this is associated with a reduction of neurotrophins, wellknown inhibitors of apoptotic cell death (Hornig et al., 1999; Zocher et al., 2000). Neonatal BDV infection leads also to synaptic pathology with reduced expression of presynaptic markers like synaptophysin and GAP-43 (Gonzalez-Dunia et al., 2000). An earlier microarray study for 1200 genes (Jehle et al., 2003) revealed mainly immune related genes to be regulated by BDV.

Despite these previous reports, up to now a wide overview on BDV- dependent changes especially during later phases of persistent infection is still missing. We therefore performed a microarray based expression analysis in hippocampus and cerebellum of neonatally BDV infected rats as compared to control treated, and untreated animals. For this we used the Affymetrix Rat230 microarray covering 28.757 genes, representing almost the entire rat transcriptome. The obtained results demonstrate BDV dependent up- or down-regulation of a wide variety of hippocampal and cerebellar genes, that could be confirmed for a small subset by standard and/or real time RT-PCR and Western blot analysis.

2. Materials and Methods

Virus infection and sample dissection

Virus stocks used here were the fifth brain passage of the Giessen strain He/80 of BDV in newborn Lewis rats. Pregnant Lewis rats were purchased from Charles River (Sulzfeld, Germany). All animal research has been done with permission of the government of Lower Saxony (District Government Braunschweig, Code: 509.42502/01-40.03). Animals were kept in standard cages with food and water ad libidum at a 12/12h dark/light cycle at

20°C. Within 12h after birth rat pups were inoculated intracranially with 30 µl of either a brain homogenate from BDV infected, or from non-infected adult rats. The virus homogenate was manually administered 3mm into the right hemisphere using a Hamilton syringe with a stopper. 8 weeks after infection animals were killed by nitrogen intoxication, with the brains being removed immediately and dissected as follows. For RNA isolation from hippocampal samples a 5 mm transverse brain slice (Bregma -1,5 to Bregma -6.5 according to Kruger et al. (1995) through the right hemisphere was cut out and shock-frozen in liquid nitrogen. Likewise the right cerebellar hemisphere was dissected and shock-frozen in liquid nitrogen. For protein isolation similar samples from the left brain were dissected and shock frozen.

RNA isolation and detection of BDV infection

BDV infection of the samples used for this study was verified by either nested RT-PCR or by immunocytochemical detection. For RT-PCR total RNA was isolated by Trizol (Sigma, Taufkirchen, Germany) and further purified using an RNA extraction kit (Qiagen, Hilden, Germany). RNA concentrations were determined spectrophotometrically at 260/280 nm. First strand cDNA synthesis was carried out with Oligo $(dT)_{12-20}$-primers and SuperScript II reverse transcriptase (Invitrogen, Karlsruhe, Germany) using 1 µg of total RNA. PCR amplification was performed in a Mastercycler gradient (Eppendorf, Hamburg, Germany) using a commercial PCR Master Mix (Fermentas, St.Leon-Rot, Germany), with the primary reaction containing 1 µl of cDNA in a total volume of 25 µl. The following primers were used: BDV-P40: (sense 5'-ACG CCC AGC CTT GTG TTT CT-3, antisense 5'-AAT TCT TTA CCT GGG GAC TCA A-3), (GAPDH was used as a positive control). Cycling conditions involved a 2 min denaturation step at 95°C, followed by 40 cycles of a 30'' denaturation step at 94°C, a 45'' annealing step at 58°C, and a 1' extension step at 72°C. Reactions were terminated 10' at 72°C. Reaction products obtained were analyzed by agarose gel electro-

phoresis. To enhance specificity an additional round of nested PCR reactions was performed using 1 µl of the primary PCR template, 11 µl PCR Master Mix, 0.2 µM of each primer in a total volume of 25 µl. Primers were as follows: BDV-P40 nested: (sense 5'-CTC GTG AAT CTT ACC TGT CGA CG-3, antisense 5'-TCC TGC TTT AAT CTT AGA TGA CG-3). Cycling conditions were as described for the primary amplification, but with only 30 amplification cycles. Under the same conditions expression of GFAP was detected using the following primer pairs: sense: 5'-GAA GCA GGG CAA GAT GGA GC-3', antisense: 5'-GCT GTT CCA GGA AGC GGA CC-3'. For the detection of BDV infection by immunocytochemistry a mouse monoclonal antibody specific for BDV p40 was used.

Microarray hybridization and analysis

Quality of the RNA samples used for probe synthesis was controlled by an Agilent 2100 bioanalyzer (Agilent Technologies, Waldbronn, Germany). To minimize the influence of individual variability on the experimental outcome RNAs from **6 individual animals were pooled for probe synthesis**. Each of these RNA pools were then reversely transcribed and retranscribed in order to obtain labeled double stranded cRNA probes. For this, first strand cDNA synthesis was performed with a T7-$(dT)_{24}$ primer and Super-Script II reverse transcriptase (Invitrogen, Karlsruhe, Germany) using 30 µg of pooled total RNA. Then second strand synthesis was performed with the Super-Script Choice System (Invitrogen, Karlsruhe, Germany) including *Escherichia (E.) coli* DNA polymerase I, *E.coli* Ligase and *E.coli* RNase H. Fragment end-polishing was performed using *E.coli* T4-polymerase. In a third step, an *in vitro* transcription reaction was carried out using the BioArray HighYield RNA Transcript Labeling Kit (Enzo, Santa Clara, CA) which produces a biotin-11-CTP labelled cRNA probe. After fragmenting the cRNA by heat at 94°C in a fragmentation buffer (Tris-acetate 40 mM, pH 8.1, Potassium acetate 100 mmol/L, Magnesium acetate 30 mmol/L), each probe was then hybridized over night (45°C) **to a single chip** of the Affy-

metrix rat microarray expression set 230A and B (Affymetrix, Santa Clara, CA) representing 28.757 rat genes. Following hybridization chips were washed in the Genechip Fluidics Station (Affymetrix, Santa Clara, CA), and biotin labeled probes were detected by incubation with R-Phycoerythrin-Streptavidin (Molecular Probes, Göttingen, Germany) and a biotinylated anti-Streptavidin antibody (Vector Laboratories, Burlingame, CA). Fluorescence intensities corresponding to the amount of probe hybridization were estimated by laser scanning. Data were scaled based on total intensity, and the gene expression levels were calculated with the Affymetrix Genechip software. Data are given either as intensity ratio between chips hybridized with the probes from untreated as compared to control treated animals or as intensity ratio between chips from control treated animals as compared to those from BDV infected rats. Members in each cluster were classified according to their biological functions as described in the NetAffix database (Affymetrix, Santa Clara, CA).

Quantification of mRNA expression by realtime RT-PCR

DNA-free total RNA was isolated and extracted using the SV total RNA isolation System (Promega, Mannheim, Germany). First strand cDNA synthesis was carried out with the reverse transcriptase superScript III and Oligo $(dT)_{20}$ primer (Invitrogen, Karlsruhe, Germany). realtime PCR was performed with a Light-Cycler (Roche, Mannheim, Germany) in glass capillaries containing 20 µl of a reaction mixture containing 2 µL cDNA, 10 pmol of each primer, 1U Taq DNA polymerase (Invitrogen, Karlsruhe, Germany), 2 µL 10 x PCR buffer (Invitrogen, Karlsruhe, Germany), 0.25 mM of each dNTP (BioLine, Luckenwalde, Germany), 500 mg/L BSA (New England Bio-Labs, Frankfurt am Main, Germany), 5% DMSO (Sigma, Taufkirchen, Germany), 1µL of 1/1000 SYBRgreen stock (Roche, Mannheim, Germany) and 4mM MgCl2. After an initial 30' denaturation step at 95°C samples were submitted to 45 cycles of 94°C for 0.01' (immediate re-cooling after peak), 60°C for 5', and 72 °C for 10'. For quantification fluorescence emission at 520 nm was measured

every cycle at 83°C for Hemeoxygenase-1, at 80°C for Stat1, at 81°C for GAPDH, and at 85°C for Vimentin. The following primer pairs were used: Hemeoxygenase-1 (sense: 5'-TGC TGA CAG AGG AAC ACA AA-3', an-tisense: 5'-ACA GAG TTC ACA GCC TCT GG-3'), Stat1 (sense: 5'-TTG GTG GAG TAC AGA CTG AAG A-3', antisense: 5'-ATC AGA GTG GGA AGG AAA CAG T-3'), Vimentin (sense: 5'-AGA AGC TGC ACG ATG AAG AG-3', antisense: 5'-GAG AGG TCA GCA AAC TTG GA-3'), Dlgh3 (sense: 5'-CGG TGA ACA ACA CCA ATC TG-3', anti-sense: 5'-CAA GAT AAC CCA GGC TGG AA-3'), GFAP (sense: 5'-GAA GCA GGG CAA GAT GGA GC-3', antisense: 5'-GCT GTT CCA GGA AGC GGA CC-3') and GAPDH (sense: 5'-TCC ACT CAC GGC AAA TTC AAC-3', antisense: 5'-ACT CCA CGA CAT ACT CAG CAC-3'). cDNAs with known concentrations were used to produce quantification standard curves.

Detection of protein expression by Western blot analysis

Frozen samples from hippocampus and cerebellum were thawed in 500 µl of ice chilled homogenization buffer (20 mM Tris pH 7.5, 1 mM EDTA , 1% SDS (w/v), 0.5 mM DTT and 200 µM Pefabloc, Serva, Heideberg, Germany). After mechanical homogenization and so-nification at 4°C, protein concentrations were determined according to Bradford (1976). Samples of 40 µg were denatured in electrophoresis buffer (0.5 mM Tris/HCl pH 6.8, 2% SDS (w/v), 10% glycerol (v/v), 5% β-mercaptoethanol (v/v) and 0.001% bromophenol blue (w/v)) at 95° C and subjected to SDS-PAGE (Laemmli, 1970). Expression was analyzed on 0,75 mm thick SDS-acrylamide gels at concentrations ranging from 5 to 12%. Initially electric tension was set to 80 V and increased to 100 V when bromophenol blue reached the separating gel. Proteins were blotted onto Nitrocellulose membranes (Towbin et al., 1979) and were then blocked over night at 4°C in 5% (w/v) nonfat dry milk in Tris-buffered saline. Blots were probed with antibodies against Hemeoxygenase1 (polyclonal, Stress-gen Bioreagents Ltd., Victoria, CND) Stat1α (monoclonal, Zymed Laboratories Inc., San

Francisco, USA), Vimentin (monoclonal, MBL, Watertown, USA), GFAP (monoclonal, Sigma-Aldrich, Deisenhofen, Germany) and GAPDH (monoclonal, Abcam, Cambridge, UK), diluted in blocking solution. Primary antibodies were detected by peroxidase conjugated secondary antibodies and visualized by chemiluminescenct detection (ECL-Kit, Amersham Biosciences, Freiburg Germany) and film audioradiography. Autoradiographs were scanned and density of the signals evaluated by the metamorph image analysis software (Molecular Devices, Sunnyvale, California). Statistical evaluation was performed using the opensource software OpenOffice.org calc (see http://www.openoffice.org/).

3. Results

Verification of BDV infections

Expression of the BDV p40 nuclear protein was investigated on the mRNA level by a nested RT-PCR protocol and on the protein level by immunocytochemical detection using a mouse monoclonal anti-p40 antibody. As demonstrated in Fig. 1A, BDV p40 could be detected in RNA samples from BDV infected rats by simple and nested RT-PCR, whereas in non-infected control animals no signals were visible. Likewise, immunofluorescent detection of BDV p40 in rat hippocampal sections (Fig. 1B, and C) revealed strong p40 specific immunoreactivity in all cells of the dentate gyrus of BDV infected rats (Fig. 1C), whereas in the hippocampus of non-infected control animals no immunoreactivity was detectable at all (Fig. 1B).

Unspecific changes of hippocampal RNA expression in mock-treated rats

Since the injection procedure for application of the brain homogenate during infection of the animals causes some brain damage that might result in scar formation and thus could cause gene expression changes on its own, we performed a control experiment compa-

ring gene expression in the hippocampus of untreated and mock treated rats. As it is shown in table 1, at a cutoff-level of 2.0, only four genes were altered in mock treated animals as compared to untreated controls, including the four-membrane-spanning-domains protein A2 (Ms4A2), osteomodulin (OMD), phenol preferring sulfotransferase 1A (SULT-1A1), and tumor necrosis factor ligand superfamily, member 4 (TNFSF4). All four transcripts were downregulated at a very moderate range reaching from -2,13 for TNFSF4 up to -3,89 for Ms4A2.

BDV-specfic changes in hippocampal and cerebellar RNA expression in BDV infected rats as compared to mock treated animals

For investigation of the gene expression changes between BDV infected and mock treated rats for each value the fluorescent signal from a chip hybridized to a probe derived from either 6 BDV infected hippocampal or 6 BDV infected cerebellar samples was compared to the signal of a chip hybridized to a probe derived from six corresponding samples from mock treated control animals. The results of this experiments revealed at a cutoff level of 2.0, in the hippocampus transcripts for 143 genes to be up-, and for 9 genes to be downregulated. Likewise in the cerebellum transcripts for 177 genes were up- and for 39 genes were downregulated at a factor ≥ 2.0. Transcripts for 97 of the BDV dependent genes were identical in both brain regions (see table 2 for details). Although many genes serve different functions in different tissues and functional assessment will always be a matter of discussion, we have tried to classify them into 25 groups of functionally related genes, including defense related proteins, apoptotic factors, growth factors and their receptors, cytoplasmic signalling molecules, transcription factors, cytoskeletal proteins, cell adhesion and extracellular matrix proteins, enzymes for lipid and steroid metabolism, enzymes for the basic energy metabolism, molecules for detoxification and cell protection, factors for protein synthesis, processing, and degradation, and finally a group of neuron and glia re-

lated transcripts (see also Table 2). Within the groups genes were arranged in that order that genes upregulated in both brain regions were depicted first, followed by genes upregulated in the hippocampus and cerebellum respectively, followed by the downregulated genes in a similar order.

Validation of gene expression changes by Western blot analysis and realtime PCR

Although the results obtained by microarray analysis are highly reliable with p-values below p = 0.05, validation by alternative methods is necessary. For this purpose we have selected a subset of transcripts including the Transcription factor Stat1, the reactive oxygen species detoxifier Hemeoxygenase1, and the intermediary filament proteins Vimentin, and GFAP (glial fibrillary acidic protein) as genes upregulated in both hippocampus und cerebellum and Dlhg3 (Discs large homolog 3) as a non regulated gene. Expression of these genes was analysed by realtime and/or standard RT-PCR and Western blot experiments, results of which are summarized in table3.

As shown in Figure 2, the microarray results for the upregulation of the transcription factor Stat1, could be confirmed by realtime RT-PCR and Western blot analysis (Fig. 2). In a similar manner the results for hemeoxygenase-1, and Vimentin, as well as the negative result for Dlhg3, could be confirmed by realtime RT-PCR, and Western blot analysis (data not shown, see table3).

Astrogliosis and thus upregulation of GFAP have previously been reported as a characteristic feature of persistent BDV infection (Freude et al., 2002), and therefore the subthreshold levels of GFAP upregulation demonstrated in our microarray analysis needed further validation. As shown in Fig. 4A, standard RT-PCR revealed only a very slight increase in the intensity of GFAP-specific PCR-fragments generated from samples of BDV infected

rat hippocampus, as compared to non-infected controls. In contrast to this, realtime RT-PCR revealed a suprathreshold upregulation of GFAP mRNA by a factor of 2.1 (Fig. 4B). Even stronger changes for the expression of GFAP could be revealed on the protein level by Western blot analysis, where a 3.0 fold upregulation was detected in samples from BDV infected rats as compared to non-infected control animals (Fig. 4C, and D).

4. Discussion

The present study is giving a wide overview on the complex pattern of changes in RNA expression of the rat hippocampus and cerebellum during late phases of persistent infection with the Borna Disease Virus (BDV). It reveals virus dependent regulation of genes from many different functional groups, such as defense related proteins, apoptotic factors, growth factors and their receptors, cytoplasmic signalling molecules, transcription factors, cytoskeletal proteins, cell adhesion and extracellular matrix proteins, enzymes of lipid and steroid metabolism, enzymes of basic energy metabolism, detoxifying and cell protection molecules, factors for protein synthesis, processing, and degradation, and finally some neuron- and glia-specific genes such as neurotransmitter receptors and other synaptic proteins, as well as intermediary filament proteins and transcription factors. Amongst the genes with BDV dependent changes are also some transcripts that have been already demonstrated by earlier studies from other groups to be regulated during BDV infection. However a great deal of genes revealed to be up- or downregulated by BDV in this study are new in that context and have not been previously demonstrated to be affected in the course of neonatal BDV infections.

According to this our results largely confirm the findings of an earlier cDNA microarray study on BDV-dependent expression changes (Jehle et al., 2003), where it has been demon-

strated that at day 75 p.i., β2-microglobulin is upregulated in cerebellum (8.8 fold), and frontal cortex (4.2 fold), as compared to our results of 4.4 fold upregulation in cerebellum and 4.2 fold in the hippocampus. Likewise the Ca^{2+}-transporter ATP-ase3 has been shown by Jehle et al. (2003), to be reduced at a factor of -2.7 as compared to -3.1 in our study. However, other genes like the ribosomal protein L12 and the interferon inducible protein IP-10, have only been shown to be upregulated by the study of Jehle et al. (2003), but not in our approach. These differences might be explained by the different time points and brain regions investigated in both studies.

Although activation of the immune system by postnatal BDV infection has previously been described to be comparably mild (Hornig et al., 1999; Sauder and de la Torre, 1999), the results of the present study reveal a large group of immune related genes to be upregulated. Thus, several markers for the activation of macrophages, B-cells and T-cells are upregulated in hippocampal and cerebellar samples. In accordance to this increased mRNA levels for several immunoglobulin components, genes related to the major histocompatibility complex, and components of the innate immune system are altered. This is in accordance to earlier studies where elevated expression of proinflammatory cytokines (Hornig et al., 1999; Sauder and de la Torre, 1999), chemokines (Sauder et al., 2000) and chemokine receptors have been reported (Rauer et al., 2002). Also upregulation of class I and class II MHC genes has been reported earlier (Weißenböck et al., 2000).

An important new feature with regard to BDV dependent immune modulation revealed by our study is the more than 1000 fold upregulation of an mRNA coding for the VJC region of the kappa chain of the rat anti-ACh receptor antibody. This upregulation could provide a link to the decline in cortical cholinergic innervation reported in earlier studies for BDV infections in adult rats (Gies et al., 1998; 2001). Upregulation of autoantibodies for acetyl-

choline receptors, but of the muscarinic subtype, has also been reported in an argentinian cohort of schizophrenic patients, where circulating antibodies reacting with frontal cortex-activating muscarinic acetylcholine receptors (mAChR) have been detected (Borda et al., 2002). Thus our findings could provide new cues for a link between BDV infection and the etiology of neuropsychiatric disorders i.e. schizophrenia, by an autoimmune mediated process.

Interferons are important extracellular protein messengers regulating cellular responses against viral infections by inhibiting viral uptake and replication, as well as viral shedding (Hengel et al., 2005). However, although interferons are highly effective in preventing the spread of BDV after infection in vitro (Hallensleben and Staeheli, 1999; Staeheli et al., 2001), they are not upregulated in the brains of BDV infected animals in vivo (von Sprockhoff et al., 1975). This finding is supported by our findings, were none of the known interferon genes was induced by BDV infection. The more it is surprising that in our study many interferon dependent genes are upregulated by BDV. This paradoxical behaviour might partially be explained by the observed upregulation of different types of interferon receptors like the γ interferon receptor in the cerebellum.

Degeneration of dentate gyrus granule cells and of Purkinje neurons in BDV infected rats (Carbone et al., 1991; Eisenman et al., 1999; Rubin et al., 1999) have been shown to be a result of programmed cell death, accompanied by induction of caspase1 (Hornig et al., 1999). According to this, the results of our study confirm the upregulation of caspase1, but also extend the list of upregulated caspases by demonstrating for the first time caspase11 and caspase12 to be upregulated in both hippocampal and cerebellar samples of BDV infected rats, and of caspase7 and caspase8 in hippocampus and cerebellar cortex respectively. In addition, our results demonstrate other apoptosis related molecules like the

apoptosis-associated speck-like protein, and a Bcl-2 related protein to be upregulated during late phases of BDV infection. Upregulation of Bcl-2 like proteins might be a cellular response against BDV dependent apoptosis, as it has been shown in mice overexpressing Bcl-2 in their neurons are resistant to experimental autoimmune encephalomyelitis (Offen et al., 2000).

As it has been described in previous studies (Gonzalez-Dunia et al., 2000; Hans et al., 2004) our results demonstrate several neuron specific transcripts to be reduced in the BDV infected brain. Thus, the neuron specific transcription factors homer1, homer3 and noggin, as well as markers for Purkinje neurons like PCP-2, Carbonic anhydrase VIII, and the transcription factor HES-3 are reduced in hipppocampal and/or cerebellar samples. Some synapse related proteins like the synaptic vesicle protein neurogranin are upregulated, suggesting a compensatory remodelling process to occur. This is in accordance to some earlier findings where BDV infection has been shown to interfere with synaptic plasticity (Hans et al., 2004). Likewise several pain related receptors like the opioid receptor and the benzodiazepine receptor are upreguated both in hippocampus and cerebellar cortex of BDV infected rats, providing a possible explanation for earlier findings on impaired pain avoidance in BDV infected rats (Dittrich et al.1989).

An interesting and also new finding of our study are the virus dependent changes in the expression of factors stimulating neuronal differentiation like the β-subunit of CCAAT/enhancer binding protein and the neuronal differentiation factor FoxO1 wich are upregulated in hippocampus and cerebellum of BDV infected rats. Also Deltex3, a strong modulator of Notch signalling and thus of neuronal differentiation is upregulated in both brain regions. This argues towards the induction of a neuronal repair process to be induced probably to compensate for BDV dependent neuron loss. Such a pattern has been demonstrated ear-

lier for neuron loss after ischemia (Yamashita et al., 2006; Tonchev and Yamashima, 2006).

As shown by previous reports neonatal BDV infection is accompanied by an astrogliotic response (Carbone et al., 1991; Bautista et al., 1994; Gonzalez-Dunia et al., 1996). However, in our study GFAP revealed only a subthreshold upregulation, whereas vimentin was upregulated only slightly above threshold levels. We therefore analysed these findings by alternative methods for RNA detection, by which we could largely confirm the microarray data. In contrast to this, distinctly higher levels of upregulation were observed for protein expression as revealed by Western blot analysis. We conclude from this that the weak gliotic response observed here may be a consequence of the late time point investigated.

An important question concerns the comparison of patterns of BDV dependent gene expression to those found in other neurotropic viruses. Thus, if one compares our findings to what has been found in a similar expressional analysis for the pseudorabies virus (PRV; Paulus et al., 2006), it turns out that genes from almost the same functional groups are altered in the PRV infected brain. Thus, as for BDV infections a large group of defense related genes is upregulated in the PRV infected brain including genes for antigen presentation, the complement system, the interferon system as well as several cytokines and chemokines (Paulus et al.,2006). Also with regard to individual genes a certain overlap exists between PRV and BDV dependent expressional changes, since in both infection models transcripts coding for the complement components 1, 2, and 3 are upregulated. In a similar manner both virus models reveal the interferon dependent transcripts Best5, Myxovirus resistance 1, and -2, and the transcription factors Stat1 and Stat3 to be upregulated. An important difference however, between both virus models is the production of autoantibodies, since the high upregulation of the transcripts for the anti acetylcholine receptor anti-

body was only observed in the BDV infected rat brain but not in brain samples from PRV infected animals. This suggests autoimmune mediated processes to play only a role during BDV dependent pathogenesis.

As there exist several reports on long term changes in gene expression after brain injury (Carbonell and Mandell, 2003; Stylli et al., 2000), we wanted to clarify whether brain damage during postnatal application of BDV exerted similar effects. For this we made a control experiment were we compared gene expression in hippocampal samples from our mock treated rats to those from completely unaffected animals. By this we found the four-membrane-spanning-domains protein A2 (Ms4A2; involved in allergic reactions), osteomodulin (OMD; serves as an Integrin receptor), the phenol preferring sulfotransferase 1A (SULT 1A1; Catalyzes the sulfate conjugation of catecholamines, phenolic drugs and neurotransmitters), and the tumor necrosis factor ligand superfamily, member 4 (TNFSF4; involved in T-cell activation), as well as 12 other transcripts with yet unknown functions to be up- or downregulated more than 2-fold in mock-treated rat brain. Irrespective of their putative significance for long term consequences of juvenile brain damage we think that these changes are not of major importance to our study. Towards this direction point also some earlier reports that in the neonatal brain the longterm gliotic reactions to a stab wound are only weak (Balasingam et al., 1994) and depend greatly on the affected brain region (Ajtai et al., 1997).

All microarray based methods share some weak points, since they can detect existence and direction of changes only for relatively abundant transcripts, and magnitude of expression changes cannot be assessed reliably (Draghici et al., 2005). Therefore, all results obtained need confirmation by alternative techniques like quantitative realtime RT-PCR, and Western blot analysis. We applied both methods for a subset of five BDV dependent and

independent genes and were largely able to confirm the individual results for these genes with GFAP being an exception probably due to the threshold levels chosen. For other molecules this may not to be the case and therefore all results presented here need further confirmation before the start of ongoing experimental work. However, despite these weak points microarray studies provide a much broader overview on gene expression changes during pathological processes, and allow a much more differentiated analysis of the underlying molecular pathogenetic processes, as it previously has been possible.

Thererfore, in conclusion our results provide for the first time a wide overview on molecular changes during late phases of postnatal BDV infections, on one hand confirming already known facts, and on the other adding new candidates to the list of BDV dependent genes, opening thereby new aspects to our understanding of longterm viral brain effects.

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Figure Legends

Figure 1: Verification of BDV infection by detecting BDV P40 by standard and nested RT-PCR. The UV-photograph of an ethidium bromide stained agarose gel demonstrates standard and nested RT-PCR products for BDV p40 in RNA samples from the brains of

BDV infected but not of control treated rats.

Figure 2: Expression of mRNA and protein of the transcription factor stat1 in hippocampus and cerebellum of control treated and BDV infected rats as revealed by realtime RT-PCR and Western blot analysis. (A, and B) Diagrams demonstrating the statistical evaluation of the results from the detection of stat1 by realtime RT-PCR in RNA samples from hippocampus (A) and cerebellum (B) of control treated and BDV infected rat brains. (C, and D) Photograph of a Western blot analysis for the immunoreactivity of a Stat1-specific antibody with hippocampal (C) and cerebellar (D) samples from control treated and BDV infected rats. For loading control, blotting membranes were stripped and immunoreactivity for the housekeeping gene GAPDH was detected. (E, and F) Diagrams showing the statistics of the densitometric evaluation of the blots as shown in (C, and D). Both Western blot and realtime RT-PCR confirmed the results obtained by microarray analysis for the upregulation of Stat1 in both hippocampal, and cerebellar samples. (** = $p \leq 0{,}01$).

Figure 3: Expression of the mRNA of the transcription factor Dlhg3 in hippocampus and cerebellum of control treated and BDV infected rats as revealed by realtime RT-PCR. (A, and B) Diagrams demonstrating the statistical evaluation of the results from the detection of Dlhg3 by realtime RT-PCR in RNA samples from hippocampus (A) and cerebellum (B) of control treated and BDV infected rat brains. As already revealed by microarray analysis no statistically significant differences in the expression of Dlhg3 mRNA could be observed between control treated and BDV infected rats by realtime RT-PCR.

Figure 4: Expression of mRNA and protein of the glial intermediary filament protein GFAP in the hippocampus of control treated and BDV infected rats as revealed by RT-PCR, realtime RT-PCR and Western blot analysis. (A) Expression of GFAP mRNA in hippocamal

samples from hippocampus of control treated and BDV infected rat brains as demonstrated by RT-PCR. (B) Diagram showing the statistical evaluation of the results from the detection of GFAP by realtime RT-PCR in RNA samples from the hippocampus of control treated and BDV infected rat brains. (C) Diagram of the statistical evaluation of the densitometry of the Western blot analysis as shown in (D). (D) Photograph of a Western blot analysis for the immunoreactivity of a GFAP-specific antibody with hippocampal samples from control treated and BDV infected rats. For loading control, the housekeeping gene GAPDH was detected on the same membrane. (** = $p \leq 0{,}01$).

Probe Set ID	Transcripts ↑	Factor

Table 1: Genes up- or downregulated at a factor of ≥ 2 in the hippocampus of control treated rats 8 weeks post infection as compared to untreated animals. P values for all genes shown to be regulated were p ≤ 0,05.

Probe Set ID	Transcripts	Factor
1369481_at	tumor necrosis factor (ligand) superfamily, member 4	-2,13
1370019_at	sulfotransferase family 1A, phenol-preferring, 1	-2,16
1387197_at	osteomodulin	-2,33
1369399_at	membrane-spanning 4-domains, subfamily A, 2	-3,89

Probe set ID	Transcripts Factor:	Hippo	Cereb

Probe set ID	Transcripts Factor:	Hippo	Cereb

Table 2: Genes up- or downregulated at a factor of ≥ 2 in hippocampus and/or cerebellum of BDV infected rats 8 weeks post infection as compared to mock treated controls. P values for all genes shown to be regulated were p ≤ 0,05.

Probe set ID	Transcripts Factor:	Hippo	Cereb
Section 01: *Neuron and Glia related molecules*			
1374718_at	Deltex3	13,3	10,7
1390738_at	DAMP-1 Amphiphysin	3,6	5,7
1370249_at	benzodiazepin receptor	2,1	2,2
1369330_at	Munc13-1	5,0	
1387661_a_at	opioid receptor-like	2,3	
1369138_a_at	parkin	2,3	
1369819_at	bassoon	2,1	
1368895_at	neuroligin 2	2,1	
1370211_at	neurogranin		3,0
1370570_at	neuropilin		2,9
1387032_at	cholecystokinin		2,7
1367574_at	vimentin		2,6
1369554_at	synaptogyrin 2		2,5
1388571_at	synaptogyrin 2		2,3
1368028_at	peripherin 1		2,2
1370997_at	homer1	-3,3	
1369777_a_at	proline rich synapse associated protein 1		-2,1
1387482_at	glutamate receptor, ionotropic, delta 2		-2,1
1369432_at	ACh receptor, nicotinic, beta polypeptide 2		-2,3
1371052_at	noggin		-2,4
1370212_at	homer3		-2,5
1369185_a_at	synaptotagmin 7		-2,5
1371013_at	glutamate receptor, ionotropic, AMPA1 (alpha 1)		-2,6
1387712_at	HES-3		-3,3
1387409_x_at	neuroligin 3		-5,6
Section 02: Apoptosis related genes			
1387605_at	caspase 12	2,4	3,8
1389873_at	apopt.-assoc. speck-like protein CARD containing	2,5	2,8
1369186_at	caspase 1	2,3	2,6
1387818_at	caspase 11	3,2	2,0
1369262_at	caspase-8	2,5	
1370512_at	androgen receptor-related apoptosis-associated protein CBL27	2,0	
1368482_at	BCL2-related protein A1		2,3
1369557_at	caspase-7		2,1
Section 03: Calcium related proteins			
1367975_at	Annexin III	4,7	13,5
1368558_s_at	allograft inflammatory factor 1	4,2	11,6
1371173_a_at	calpastatin (Inhibitor of calpain)	2,5	
1369068_at	vasopressin-activated calcium-mobilizing receptor protein	2,3	
1386890_at	S-100 related protein, clone 42C		2,5
1368908_at	Annexin IV		2,0
1367675_at	calcium- and integrin-binding protein		2,0
1388079_at	voltage-dependent calcium channel gamma-8 subunit	-2,3	
1368753_at	CaM-kinase kinase beta	-2,4	
1386939_a_at	calcium channel, voltage-dependent, alpha 1A subunit		-2,0
1387285_at	ATPase, Ca++ transporting, plasma membrane 2		-2,1
1370508_a_at	calcium channel, voltage-dependent, T type, alpha 1G subunit		-2,3
1379464_at	calbindin 1		-2,9
1371165_a_at	ATPase, Ca++ transporting, ubiquitous		-3,1
Section 04: Growth factors and their receptors			
1386893_at	granulin	3,2	3,7
1367715_at	TNF-receptor superfamily 1a	2,0	2,9
1370082_at	transforming growth factor, beta 1	4,7	
1387498_a_at	Fibroblast growth factor receptor 1	2,2	
1390398_at	BMP receptor 1A	2,1	
1395925_s_at	BMP/retinoic acid-inducible neural-specific protein	2,0	
1386552_at	antisense bFGF		2,8
1371194_at	TNF-induced protein 6		2,4
1367648_at	IGF binding protein 2		2,3
1372466_at	TGF-beta receptor II		2,2
1373490_at	glia maturation factor, gamma		2,0
1396152_s_at	IGF-binding protein 5		-2,3
1388096_at	Stem Cell Factor (Kit ligand)		-6,4
Section 05: Membrane proteins			
1388149_at	ATP-binding cassette transp. 1B	62,2	47,5
1376150_at	Edg3	3,8	12,9

Probe set ID	Transcripts Factor:	Hippo	Cereb
1386879_at	lectin galactose binding soluble 3	3,9	9,9
1368187_at	glycoprotein nmb	2,8	7,0
1369716_s_at	lectin galactose binding soluble 5	3,0	6,9
1382692_at	Lectin	4,7	6,6
1387027_a_at	lectin galactose binding soluble 9	3,6	6,4
1386913_at	glycoprotein 38	2,0	2,8
1370516_at	peptide/histidine transporter PHT2	2,2	3,7
1367925_at	major vault protein	2,4	2,0
1375444_at	Adapter-rel. prot. compl. 3 delta 1	2,6	
1371167_at	membrane protein palmitoylated 3	2,5	
1368981_at	aquaporin 4	2,2	
1376835_at	solute carrier family 35, B2	2,1	
1368729_a_at	adenylate cyclase activating polypeptide 1 receptor 1	2,0	
1368930_at	intermediate conductance calci-um-activated potassium channel		25,6
1368582_at	solute carrier family 7, member 3		2,6
1368754_at	pyrimidinergic receptor P2Y, G-protein coupled, 6		2,5
1394490_at	ATP-binding cassette transporter 1, sub-family A		2,3
1370135_at	caveolin 2		2,3
1368052_at	transmembrane 4 superfamily member 3		2,2
1368460_at	solute carrier family 2, member 5		2,0
1372929_at	potassium large conductance calcium-activated channel Ma1		-2,1
1367852_s_at	glycoprotein Ib, beta polypeptide		-2,8
1387161_at	solute carrier family 1, member 6		-3,1
1369165_at	transient receptor protein 3		-3,3
Section 06: Cytoplasmic signalling molecules			
1387413_at	neutrophil cytosolic factor 1	2,7	18,9
1373932_at	endothelial type gp91-phox gene	15,2	4,7
1370090_at	lymphocyte cytosolic protein 2	54,9	3,6
1377835_at	Cytokinesis8	2,8	3,0
1377594_at	Src homology 2 domain-contai-ning transforming protein 1	2,2	2,6
1368679_a_at	lyn protein non-receptor kinase	2,8	2,3
1369129_at	RAS guanyl releasing protein 1	2,0	
1388249_at	C3G protein	2,0	
1387153_at	reversion induced LIM gene	2,1	
1396464_at	SH3 domain protein 2A	2,1	
1369278_at	G-protein, alpha 12	2,1	
1368897_at	MAD homolog 7	2,2	

Probe set ID	Transcripts Factor:	Hippo	Cereb
1369387_at	vav 1 oncogene		9,7
1369484_at	WNT1 induc. signaling protein 2		3,2
1384889_at	thymosin beta-4		3,0
1371131_a_at	upregulated by 1,25-dihydroxyvitamin D-3		2,3
1367698_a_at	septin-like protein		2,2
1387024_at	dual specificity phosphatase 6		2,1
1387198_at	inositol polyphosphate-5-phosphatase D		2,1
1367731_at	G-protein, beta 1	-2,1	
1371183_a_at	discs, large homolog 4	-2,4	
1368462_at	inositol 1,4,5-triphosphate 3-kinase		-2,1
1387403_at	regulator of G-protein signaling 8		-2,3
1387908_at	DEXRAS1		-2,3
1378101_at	G substrate		-2,6
1388072_at	SH3-binding kinase		-3,7
Section 08: Protein phosphorylation			
1370603_a_at	protein tyrosine phosphatase, receptor type, C	16,6	23,1
1368010_at	protein tyrosine phosphatase, non-receptor type 6	2,4	2,4
1370488_a_at	protein tyrosine phosphatase, receptor type, D	2,3	
1369204_at	hemopoietic cell kinase	2,1	
1369297_at	protein phosphatase 2, regulatory subunit B, γ -isoform	2,1	
1368358_a_at	protein tyrosine phosphatase, receptor type, R		-2,2
1373164_at	serine/threonine kinase 17b		-2,8
1369089_at	protein kinase C, gamma		-3,1
Section 10: Transcription factors			
1387134_at	schlafen 4	17,1	12,8
1387087_at	CCAAT/enh. binding protein β	2,3	7,4
1387354_at	Stat 1	9,1	6,1
1367826_at	NF-E2-related factor 2	2,2	2,1
1371070_at	tumor stroma and activated macrophage protein DLM-1	4,1	2,3
1375469_at	SWI/SNF related chromatin regulator A4	9,9	
1380079_at	Myc protooncogene	4,7	
1369382_at	c-mer proto-oncogene tyrosine kinase	3,0	
1393249_at	friend leukemia integration 1 transcription factor	2,8	
1371072_at	nuclear receptor coactivator 6	2,6	
1388088_a_at	transcription factor USF2	2,3	
1372601_at	activating transcription factor 5	2,3	

Probe set ID	Transcripts Factor:	Hippo	Cereb
1370224_at	Stat 3	2,3	
1387391_at	cyclin-dep. kinase inhibitor 1A		5,4
1368632_at	forkhead box O1		2,5
1369959_at	zinc finger protein 36		2,5
1387769_a_at	Inhibitor of DNA binding 3		2,4
1367676_at	high mobility group box 2		2,2
1387276_at	neurotransmitter-induced early gene protein 4		2,1
1367816_at	global ischemia induced protein GIIG15B		2,1
1372031_at	disabled homolog 2		2,0
1369067_at	nuclear receptor subfamily 4, group A, member 3		-2
1375043_at	c-fos oncogene		-2,1
1387410_at	nuclear receptor subfamily 4,A2		-4,7
1387442_at	early growth response 4		-8,0
Section 11: Cytoskeletal proteins			
1386925_at	actin related protein 2/3 complex, subunit 1B	2,5	4,1
1371239_s_at	tropomyosin isoform 6	2,3	2,6
1368948_at	moesin	3,1	2,1
1370875_at	villin 2		2,5
1387402_at	myosin, heavy polypeptide 9	2,1	
1389105_at	dystroglycan 1	2,0	
1387810_at	Kelch-like ECH-associated prot. 1	2,0	
1370854_at	nexilin		-2,1
Section 12: Cell adhesion and cell matrix proteins			
1388046_at	integrin alpha M	16,6	10,0
1370056_at	Ly6-C antigen gene	2,5	
1379103_at	Protocadherin7	2,7	
1368474_at	vascular cell adhesion molecule 1		3,5
1367700_at	fibromodulin		2,1
1398280_at	interphotoreceptor matrix proteoglycan 1		-2,8
1388045_a_at	cadherin22		-2,8
Section 13: Lipid and steroid metabolism enzymes			
1370892_at	palmitoyl-protein thioesterase 2	12,0	9,0
1398892_at	epididymal secretory protein 1	2,4	3,3
1374976_a_at	acyl-coenzyme A, cholesterol acyltransferase	2,5	2,6
1391754_at	Lysophospholipase	4,2	2,2
1369672_at	arachidonate 5-lipoxygenase activating protein		2,6
1370024_at	fatty acid binding protein 7		2,4
1370161_at	steroid sensitive gene 1		2,4

Probe set ID	Transcripts Factor:	Hippo	Cereb
1370391_at	cellular retinoic acid binding protein 2		2,3
1388426_at	sterol regulatory element binding factor 1		2,1
1367939_at	retinol binding protein 1		2,0
1386953_at	hydroxysteroid 11-beta dehydrogenase 1	-2,1	
1388184_at	isoprenylcysteine carboxyl methyltransferase		-2
Section 14: Basic energy metabolism			
1370377_at	Cytochrome P450	4,7	
1369794_a_at	6-phosphofructo-2-kinase	2,8	
1383519_at	hexokinase 2	2,5	
1387914_at	cytochrome P450, family 27, A1		3,4
1368669_at	uncoupling protein 2		2,1
1370219_at	cytochrome b558 alpha-subunit		2,1
1376667_at	cytochrome P450, family 26, B1		-2,7
Section 15: Detoxifying and protecting proteins			
1370080_at	heme oxygenase 1	4,4	12,6
1371237_a_at	Metallothionein	4,3	6,3
1374367_at	Glutaredoxin	3,4	3,4
1368419_at	ceruloplasmin	3,9	3,3
1387759_s_at	UDP glycosyltransfer. 1 family, A6	2,0	2,1
1368973_at	adenosine deaminase, RNA-spec.	2,3	
1387964_a_at	ERO1-like protein	2,1	
1367774_at	glutathione S-transferase, alpha 1		4,3
1387339_at	selenoprotein P, plasma, 1		3,4
1367612_at	microsomal glutath. S-transf. 1		2,3
1387280_a_at	tumor-associated protein 1		2,0
1387052_at	glutamic-pyruvate transaminase	-2,5	
Section 16: Chaperones			
1367577_at	heat shock 27kDa protein 1	2,9	3,8
1368270_at	apolipoprotein B editing compl. 1		6,0
1387282_at	crystallin, alpha C		2,4
1394200_at	heat shock protein-related hst70		2,4
1369590_a_at	DNA-damage inducible transcr. 3		2,4
1370956_at	decorin		2,3
Section 17: Protein degrading enzymes			
1370186_at	proteosome subunit, beta type 9	5,5	7,3
1370885_at	cathepsin Y	4,7	7,1
1367786_at	proteosome subunit, beta type 8	4,6	6,6
1372254_at	serine proteinase inhibitor, clade G, member 1	4,4	6,2

Probe set ID	Transcripts Factor:	Hippo	Cereb
1367663_at	protease 28 subunit, alpha	2,9	3,5
1386899_at	cathepsin H	2,4	3,0
1399161_a_at	leucyl-specific aminopeptid. PILS	2,0	2,1
1367712_at	TIMP1		5,7
1374778_at	cathepsin C		4,5
1387005_at	cathepsin S		3,3
1386940_at	TIMP2		3,3
1367710_at	protease 28 subunit, beta		2,5
1368430_at	protease, cysteine, 1		2,2
1386884_at	protease, serine, 11	-2,3	
Section 18: CD antigens of immune cells			
1369483_at	CD4 antigen	3,6	4,6
1368518_at	CD53 antigen	2,2	3,9
1368555_at	CD37 antigen	2,4	3,7
1370891_at	CD48 antigen	2,0	3,4
1390659_at	CD44 antigen	2,1	3,1
1377943_at	CD86 antigen	2,0	2,8
1368490_at	CD14 antigen		2,3
Section 19: MHC genes			
1371209_at	RT1 class Ib gene(Aw2)	308,7	855,1
1370883_at	MHC class II RT1.u-D-alpha chain	14,8	26,5
1367679_at	CD74 antigen	18,1	23,8
1370822_at	butyrophilin-like 2	14,3	17,4
1388212_a_at	MHC class Ib RT1.S3	8,5	16,3
1370383_s_at	MHC RT1 class II E-beta chain	9,3	15,2
1371033_at	MHC class II antigen RT1.B-1 β	12,4	14,2
1379496_at	MHCI	8,5	12,4
1371440_at	beta-2 microglobulin	4,0	4,4
1370882_at	MHCII, DM beta		3,7
1370904_at	MHCII, DM alpha		2,6
Section 20: Blood clotting factors			
1369852_at	coagulation factor 10	19,8	40,8
1392894_at	fibrinogen-like 2	2,1	4,4
1389470_at	B-factor, properdin	4,2	3,8
1395617_at	Plasminogen activator	-2,6	
Section 21: Complement system			
1368000_at	complement component 3	31,6	31,3
1393219_at	complement component 2	38,9	19,2
1370215_at	complement component 1, qβ	6,5	10,4
1387893_at	complement component 1, s	2,5	3,8

Probe set ID	Transcripts Factor:	Hippo	Cereb
1387029_at	complement component factor h		2,7
Section 22: Immunoglobulins			
1387902_a_at	Anti-ACh receptor antibody, kappa-chain, VJC region	1168,1	1128,4
1370967_at	Ig active lambda2-like chain	11,6	46,5
1370394_at	Anti-ACh receptor antibody IgG-2a chain, VDJC region	17,6	29,7
1371079_at	low affinity IgG FC region receptor II precursor	2,7	29,2
1371262_at	Ig heavy chain	9,5	21,3
1398246_s_at	Fc receptor, IgG, low affinity III	2,0	4,2
1388166_at	Ig delta heavy chain constant region and 3' ut		3,0
Section 23: Cytokines and their receptors			
1387969_at	CXC motif chemokine ligand 10	6,2	18,0
1379365_at	CXC motif chemokine ligand 11	3,1	2,6
1377671_at	IL-3 receptor	3,2	2,6
1370728_at	interleukin 13 receptor, alpha 1	3,6	
1367973_at	small inducible cytokine A2		24,4
1369665_a_at	interleukin 18		2,7
1371774_at	spermidine/spermine N1-acetyl transferase		2,1
Section 24: Interferon related and antiviral defense			
1368332_at	guanylate bp2, IFN-inducible	15,1	21,9
1370913_at	Best5 protein	21,6	20,3
1371015_at	myxovirus resistance 1	20,0	16,9
1384180_at	IFN induced protein	9,6	16,1
1387770_at	IFNalpha-inducible protein 27-like	5,4	7,9
1387995_a_at	IFN induced transmembrane protein 3-like	4,9	6,9
1387242_at	IFN-inducible protein kinase dsRNA dependent	5,1	2,9
1368073_at	IFN regulatory factor 1	2,0	2,5
1387283_at	myxovirus resistance 2		6,9
1369956_at	IFNgamma receptor		2,2
Section 25: Others			
1387946_at	peptidylprolyl isomerase C-associated protein	8,9	12,5
1371152_a_at	25 oligoadenylate synthetase	8,2	8,9
1383658_at	lysosomal-associated transmembr. prot. 5	2,2	6,4
1369427_at	macrophage expressed gene 1	4,3	4,2
1368840_at	TORID	2,1	4,1
1367581_a_at	secreted phosphoprotein 1	3,1	3,2
1389851_at	parathyroid hormone regulated sequence	4,1	2,3
1379691_at	Sarcoma antigen	4,9	

Probe set ID	Transcripts Factor:	Hippo	Cereb
1375215_x_at	pyroglutamyl aminopeptidase-1	2,1	
1370141_at	myeloid cell leukemia sequence 1	2,1	
1368728_at	P2Y12 platelet ADP receptor		2,3
1370483_at	non MHC restricted killing assoc.		2,1
1370154_at	lysozyme		2,1

Probe set ID	Transcripts Factor:	Hippo	Cereb
1392170_at	pituitary tumor X2CR1 protein		2,0
1388337_at	nucleoside phosphorylase		2,0
1370320_at	MAWD binding protein		-2,5
1368459_at	prepro bone inducing protein		-5,0

Table 3: Validation of a subset of microarray data on the mRNA level by realtime RT-PCR and on the protein level by Western blot analysis (Blot). Numbers indicate the factors of upregulation for the specified genes in either hippocampal or cerebellar samples as revealed by microarray analysis (Chip), realtime RT-PCR (RT-PCR) and Western blot analysis (Blot).

	Hippocampus			Cerebellum		
Gene	Chip	RT-PCR	Blot	Chip	RT-PCR	Blot
Stat1	6,4	7,0	13,3	5,7	5,1	11,6
Hemeoxygenase1	4,4	8,5	3,0	12,6	13,9	3,8
Vimentin	1,7	1,7	3,0	2,6	1,8	1,8
Dlhg3	1,3	1,0	--	1,0	0,7	--
GFAP	1,7	2,1	3,0	--	--	--

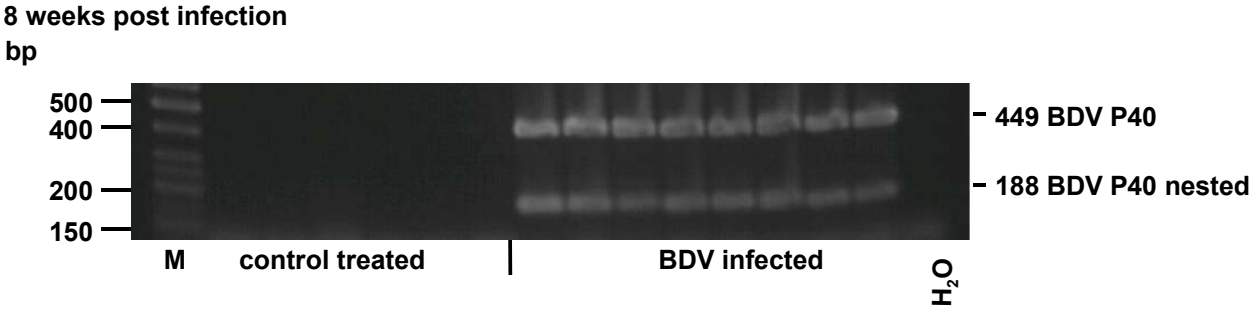


Figure 1 Hosseini et al. BDV Microarray

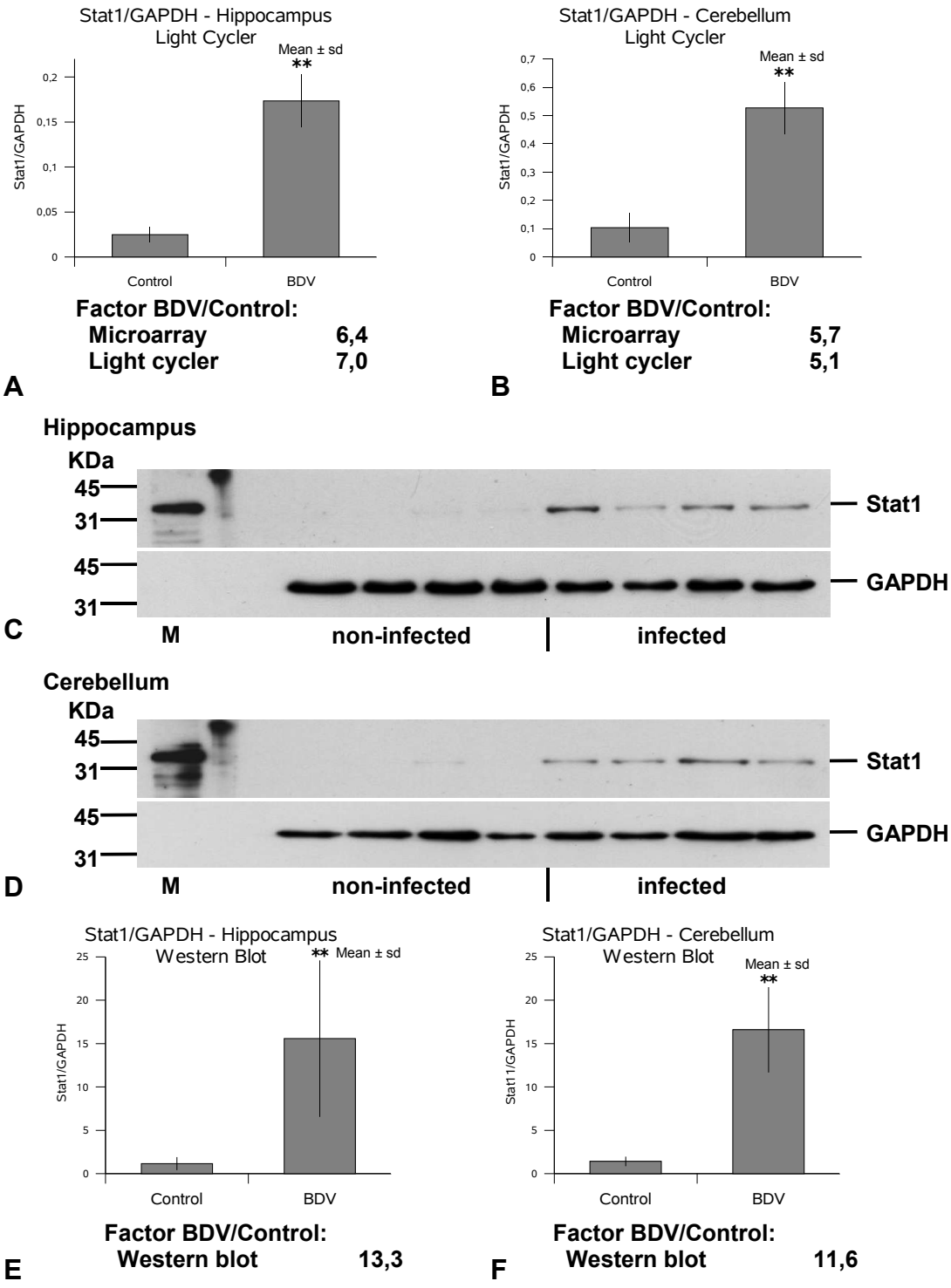
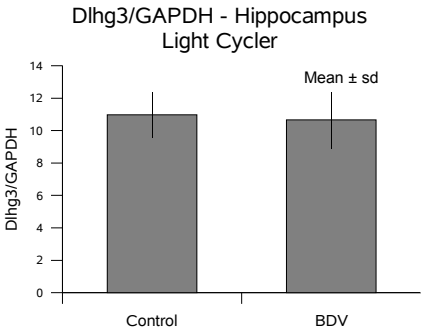


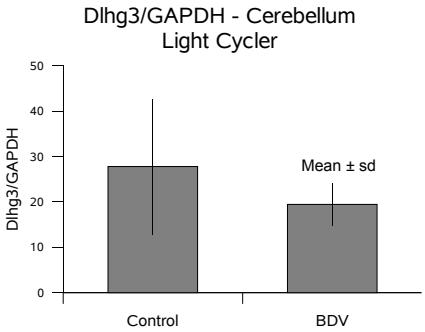
Figure 2 Hosseini et al. BDV Microarray



Factor BDV/Control:

Microarray	**1,3**
Light cycler	**1,0**

A



Factor BDV/Control:

Microarray	**1,0**
Light cycler	**0,7**

B

Figure 3 Hosseini et al. BDV Microarray

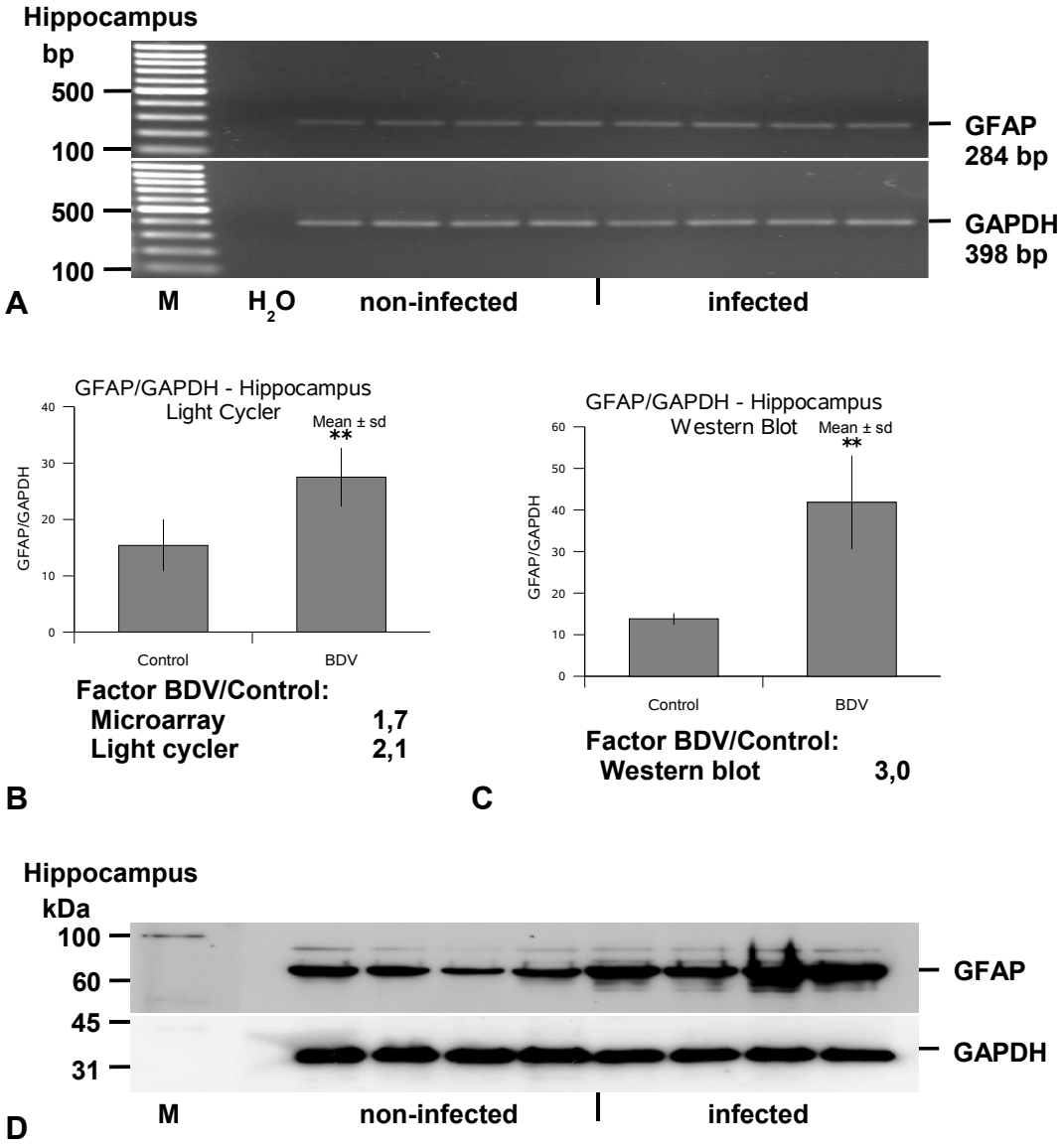


Figure 4 Hosseini et al. BDV Microarray