Borna Disease Virus Infection Impairs Synaptic Plasticity

Romain Volmer,1,2† Christine M. A. Prat,1,2 Gwendal Le Masson,3 André Garenne,3‡ and Daniel Gonzalez-Dunia1,2*,‡

INSERM, U563, Centre de Physiopathologie de Toulouse Purpan, Toulouse, France; INSERM, U862, and Université Paul-Sabatier, Toulouse, France; INSERM, U563, CPTP bat B, CHU Purpan, BP 3028, 31024 Toulouse Cedex 3, France. Phone: 33 5 6274 4558. E-mail: Daniel.Dunia@toulouse.inserm.fr.

Received 22 March 2007/Accepted 24 May 2007

The mechanisms whereby Borna disease virus (BDV) can impair neuronal function and lead to neurobehavioral disease are not well understood. To analyze the electrophysiological properties of neurons infected with BDV, we used cultures of neurons grown on multielectrode arrays, allowing a real-time monitoring of the electrical activity across the network shaped by synaptic transmission. Although infection did not affect spontaneous neuronal activity, it selectively blocked activity-dependent enhancement of neuronal network activity, one form of synaptic plasticity thought to be important for learning and memory. These findings highlight the original mechanism of the neuronal dysfunction caused by noncytolytic infection with BDV.

Borna disease virus (BDV) is an enveloped virus with a nonsegmented, negative-strand RNA genome belonging to the Bornaviridae family within the Mononegavirales order (6, 22). This neurotropic virus infects a wide variety of mammals (16), and serological evidence suggests that BDV, or a BDV-like virus, also infects humans (4, 13). Infected hosts develop a wide spectrum of neurological disorders, ranging from immune-mediated diseases to behavioral alterations without inflammation (16, 21), reminiscent of symptoms observed in human psychiatric diseases such as schizophrenia, mood disorders, and autism (12). BDV has a noncytolytic strategy of replication and primarily infects neurons of the limbic system, notably the cortex and hippocampus (8). To date, the mechanisms responsible for the cognitive impairment of BDV-infected animals are still poorly understood. One hypothesis is that neuronal infection by BDV impairs signaling pathways that are important for proper neuronal functioning and neuronal communication (9–11, 15).

As a first step to test this hypothesis, we recently showed that BDV specifically interferes with the activity-dependent enhancement of synaptic vesicle recycling, one component of neuronal communication (25). To gain further insight into the consequences of BDV infection on synaptic transmission, electrophysiological recordings of BDV-infected neurons were needed. Since synaptic plasticity is not a static event and is subjected to activity-dependent modulation (18), a real-time analysis of the firing pattern of live BDV-infected neurons would be very informative. However, these studies require intense handling of the neurons and are especially difficult to set up with infected neurons due to biosafety issues. To overcome this difficulty, we took advantage of the recently developed multielectrode arrays (MEA) from Multi Channel Systems (Reutlingen, Germany) (3). MEA consist of a grid of 60 planar electrodes embedded in a culture dish and concentrated on a 1-mm² square (Fig. 1A and B). As neurons grow directly in the recording chamber, the electrical activity pattern of neurons can be easily stimulated and recorded for a prolonged time. In addition, MEA can be sealed under sterile conditions to avoid any direct contact between the experimenter and the cultures infected with BDV.

We prepared primary cortical neurons from embryonic Sprague-Dawley rats at gestational day 18 in accordance with a previously described protocol (3). Double-staining experiments with neuronal and glial markers revealed that >80% of the cultures consisted of neurons, the rest being composed of astrocytes (data not shown). Neuronal cultures were seeded at a density of 10⁵ cells per MEA, and half of the MEA dishes were infected with BDV (strain He/80) on day 1. All experiments were performed on day 15. At this time point, neurons have established mature synapses and BDV has spread to all of them (11, 25). Consistent with the noncytolytic nature of BDV replication and in agreement with previous reports (11, 25), there was no obvious difference in the levels of neuronal survival or in the densities of neuronal networks between control and BDV-infected neurons.

Signals corresponding to the electrical activity from the 60 electrodes of the MEA were recorded using MC Rack software (Multi Channel Systems, Reutlingen, Germany) for online visualization and raw data storage (Fig. 1C). Typically, the signal corresponding to the firing of a single action potential by a neuron in the vicinity of an electrode is revealed by a single spike (Fig. 1D). We also detected series of high-frequency spikes, known as bursts, which represent an important parameter of the analysis of neuronal network activity (20). Spikes and bursts were detected by software developed in-house which computes the signal obtained from the electrodes, calculates a threshold, and detects a spike every time the signal crosses this threshold with a negative slope (Fig. 1D). The threshold was set to 4 standard deviations of the average noise amplitude computed for the whole recording and applied using the average value of the signal as a baseline (14). Bursts were defined as sequences of at least three spikes occurring in less than 100 ms (Fig. 1D).
In order to test for the consequences of BDV infection on neuronal communication, we first studied the spontaneous (i.e., nonstimulated) electrical activity, which is a good indicator of the state of the neuronal network (24). Under these conditions, the activity of the network was characterized by a majority of spikes randomly distributed on the electrodes and by very few bursts (Fig. 2). There was no noticeable difference between control and BDV-infected neurons regarding spike and burst frequencies. Next, we stimulated the neurons for 15 min with 50 μM bicuculline (bicuculline methiodide; Tocris Bioscience, Bristol, United Kingdom), a gamma-aminobutyric acid A receptor antagonist. Treatment with this antagonist leads to the removal of the tonic inhibition imposed by gamma-aminobutyric acid-stimulating interneurons on the network. It
has been shown that this removal results in an increase in synaptic efficacy at excitatory synapses (2). This increase is associated with changes in network activity, from uncoordinated firing of neurons to a highly organized, periodic, and synchronous burst pattern over the whole neuronal network. Indeed, we observed that the treatment with bicuculline induced an increase in spike frequency and the appearance of a synchronous burst activity, with an interburst interval of between 2 and 5 s (Fig. 2). Here again, there were no differences between the results with control and BDV-infected neurons. Quantitative analysis of spike (Fig. 3A) and burst (Fig. 3B) frequencies from at least four independent experiments showed that under spontaneous conditions and during bicuculline stimulation, control and BDV-infected neurons behaved the same way. Together, these results show that although BDV replicates actively in neurons, it has no impact on spontaneous neuronal activity or on the ability of the neurons to modulate synaptic transmission during stimulation.

The synchronous burst firing triggered by bicuculline exposure induces an important influx of Ca\(^{2+}\) through postsynaptic N-methyl-D-aspartic acid receptors, which, in turn, produces an increase in the strength of the synaptic connections (2, 18). This increase in synaptic strength lasts for several hours following stimulation and is thought to represent the cellular basis of learning and memory (17). We therefore compared the network activities of control and BDV-infected neurons after bicuculline removal. We observed that control neurons maintained a high level of activity 1 and 2 hours following bicuculline washout (Fig. 3). This high level of activity was characterized by a regular and synchronous bursting activity distributed over the whole neuronal network (Fig. 2 and 3). In sharp contrast, neuronal activity had returned to basal levels as early as 1 hour poststimulus in BDV-infected cultures (Fig. 2 and data not shown). Altogether, these results indicate that BDV specifically targets activity-induced changes in neuronal network activity while leaving basal properties of neuronal

![Fig. 2. Representative examples of MEA recordings from noninfected (NI) and BDV-infected neurons before, during, and 1 h after bicuculline (bic.) treatment. For each experimental condition, a 15-s-long portion of the recording is shown (total recording, 180 s). For both the NI and BDV-infected neuron results, a signal from a representative electrode is shown on the left. On the right, a raster plot depicts the signal obtained from the 60 electrodes: each line corresponds to an electrode, and each dot represents a spike. Note that bursts of action potential are induced by bicuculline exposure and occur synchronously. This high level of synchronous activity is maintained only in NI neurons following bicuculline washout.](http://jvi.asm.org/.../ Downloaded from)
communication unaffected. This result is consistent with our recent description of the BDV interference with activity-dependent enhancement of presynaptic activity in hippocampal neurons (25).

Signaling by extracellular signal-regulated kinase (ERK)1/2 has been shown to be essential for the development of a long-lasting increase in neuronal network activity, in particular following exposure to bicuculline (2). We therefore tested whether impaired ERK1/2 signaling could play a role in the blockade of the induction of the recurrent synchronous burst activity observed in BDV-infected neurons. Cortical neurons cultured in 35-mm plastic dishes were stimulated for 15 min with bicuculline by using the same protocol used for MEA recordings. Neurons were then rapidly lysed in cold lysis buffer and processed for Western blot analysis according to previously described procedures (25). We observed the same phosphorylation levels of ERK1/2 in control and BDV-infected neurons following bicuculline exposure (Fig. 4A). This suggests that a signaling pathway other than ERK1/2 is likely involved in the BDV-induced blockade of activity-induced enhancement of neuronal network activity. Protein kinase C (PKC) has been reported to be important for the induction of multiple forms of synaptic plasticity (1), and we previously showed that BDV interferes with PKC signaling (25). We therefore analyzed PKC-dependent signaling following exposure to bicuculline by measuring the phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS), a major PKC substrate in neurons (19). In agreement with our previous results (24), we observed lower levels of bicuculline-induced MARCKS phosphorylation in BDV-infected neurons than in control neurons (Fig. 4B). In addition, a strong inhibition of network activity was observed in control neurons following treatment with a cell-permeable specific PKC peptidic inhibitor (PKC inhibitor 20-28; Calbiochem, VWR, Fontenay-sous-Bois, France) used at 4 μM (data not shown), suggesting that the inhibition of PKC signaling indeed leads to altered neuronal network properties. Altogether, these results suggest that BDV interference with PKC signaling may be involved in the blockade of activity-dependent enhancement of neuronal network activity.

In summary, our study describes the use of a novel, noninvasive system suitable for assessing the impact of BDV persistence on synaptic plasticity. Interestingly, this system could, in principle, also be applied to the study of other neurotropic viruses. Moreover, we provide evidence that the effects of BDV on neuronal-network functioning are due, at least in part, to an interference with synaptic plasticity. A remarkable feature of the pathogenesis of neuronal infection by BDV is the...
absence of any detectable effect on spontaneous activity or on the immediate response to stimulation. Given the paramount importance of synaptic plasticity in the experience-dependent modification of brain function (for example, during learning), the selective interference of BDV with these processes may be especially relevant when the cognitive disorders linked to BDV persistence are considered. Synaptic plasticity also contributes to prenatal and postnatal neurodevelopment, through the continuous refinement of synaptic connections (5). Any interference with these mechanisms can impair neuronal survival and thus could represent the initial insult underlying BDV-associated neurodevelopmental disorders (21). We cannot exclude the possibility that BDV interference with neuronal network activity may result, at least in part, from interference with glia (astrocyte)-neuron interaction, which plays an important role in the regulation of intersynaptic communication. We consider this hypothesis unlikely, though, as only a minority of astrocytes were infected at the time that the experiments were performed (15 days postinfection). In any event, it is clear that further work will be necessary to delineate more precisely the underlying mechanisms. In particular, it will be of interest to further document the interference with PKC signaling that is thought to trigger the impairment of synaptic plasticity in BDV-infected neurons. Recently, we suggested that BDV phosphoprotein, which serves as a PKC substrate, could compete with the phosphorylation of endogenous PKC cellular substrates (25). Now that a reverse-genetic system for BDV is available (23), it will be of great interest to generate a virus and to analyze the consequences for the virus life cycle (7).

This work was supported by grants from the Inserm Avenir program, the ANR (ANR-06-MIME-005-01) program, and the CNRS (to D.G.-J.C). R.V. and C.M.A.P. are recipients of doctoral fellowships from the ANR (ANR-06-MIME-005-01) program, and the CNRS (to D.G.-J.C). We thank A. Saoudi, R. Liblau, E. Suberbielle, and C. Duval for critical reading of the manuscript and insightful comments.

REFERENCES