

## Use of a Recombinant Pseudorabies Virus to Analyze Motor Cortical Reorganization after Unilateral Facial Denervation

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**A unilateral facial nerve injury (n7x) was found to influence the transcallosal spread of the attenuated strain of pseudorabies virus (PRV Bartha) from the affected (left) primary motor cortex (MI) to the contralateral MI of rats. We used Ba-DupLac, a recombinant PRV strain, for the tracing experiments since this virus was demonstrated to exhibit much more restricted transportation kinetics than that of PRV Bartha, and is therefore more suitable for studies of neuronal plasticity. Ba-DupLac injection primarily infected several neurons around the penetration channel, but hardly any transcallosally infected neurons were observed in the contralateral MI. In contrast, after right facial nerve injury, Ba-DupLac was transported from the primarily infected neurons in the left MI to the contralateral side, and resulted in the labeling of several neurons due to a transneuronal infection. These results reveal that a peripheral nerve injury induces changes in the Ba-DupLac infection pattern in the related cortical areas. These findings and the literature data suggest that this phenomenon may be related to the changes in the expression or to the redistribution of cell-adhesion molecules, which are known to facilitate the entrance and/or transmission of PRV into neurons.**

**Keywords:** herpes, neuronal plasticity, peripheral nerve injury, primary motor cortex, pseudorabies virus

### Introduction

Since the early 1990s, studies demonstrating plasticity in the cortical somatotopic representation maps of the primary motor cortex (MI) of adult animals have brought about a dramatic change in the concept of the function and role of motor cortical areas as information-processing structures (Sanes and Donoghue, 2000). In a series of electrical stimulation mapping experiments, Sanes and Donoghue demonstrated that transection of the facial motor nerve (n7x), which supplies the rat facial whisker musculature, led to a functional loss of the MI whisker area. Consequently, this area was occupied by representations of the adjacent forelimb or eye/eyelid regions (Donoghue *et al.*, 1990; Sanes *et al.*, 1992). This reorganization emerged within hours of the nerve lesion, and persisted for a period of months (Sanes *et al.*, 1990). The earliest sign of motor cortical plasticity induced by n7x can be observed within minutes after the intervention (Toldi *et al.*, 1996, 1999). An important insight into the possible mechanism was revealed by the observation that the blockade of cortical GABAergic inhibition unmasked existing horizontal connections that are probably normally blocked by feed-forward inhibition (Jacobs and Donoghue, 1991). This kind of local GABAergic release yielded map changes parallel to

those following nerve lesions, demonstrating that the MI has the intrinsic circuitry necessary to support reorganization, in which the intracortical horizontal connections play a decisive role (Sanes and Donoghue, 2000). Our earlier studies revealed that the motor cortices of both hemispheres, interconnected commissurally, are involved in n7x-induced cortical plasticity (Toldi *et al.*, 1999; Farkas *et al.*, 2000). Most of the studies cited above were based on experiments in which electrophysiological methods were used.

However, it is more than likely that the changes in the cortical representational maps are consequences of molecular biological and biochemical changes in the neurons and the glial cells and in their connections. It has been found, for instance, that n7x leads not only to the activation of astrocytes in the corresponding facial nerve nucleus (Rohlmann *et al.*, 1993, 1994), but also, a few minutes after a peripheral nerve injury, to histochemical and immunohistochemical changes throughout the cortical areas (Nagyessy *et al.*, 2000; Hoyk *et al.*, 2002).

Here we demonstrate that the changes induced by n7x in the motor cortical neuronal connections can be studied by neuronal tracing with the pseudorabies virus (PRV).

PRV-Bartha is an attenuated strain of PRV developed as a vaccine (Bartha, 1961). It has been used widely for transneuronal tracing (Enquist *et al.*, 1998; Card, 1999). We used Ba-DupLac, a recombinant PRV strain, for the present tracing experiments, since this virus has been demonstrated to exhibit much more restricted transportation kinetics than that of PRV-Ba (Boldogkői *et al.*, 2002), and is therefore more suitable for studies of neuronal plasticity. Indeed, we found that the method based on the use of this virus is sensitive enough to detect fine plastic changes induced in the central nervous system by estrogen application (Horváth *et al.*, 2002). In fact, utilization of Ba-DupLac allowed us to reduce the problem to an all-or-none labeling paradigm.

The literature cited above leads us to suppose that n7x induces complex changes, e.g. surface molecule [heparan sulfate-proteoglycan (HSPG) and lectins] redistribution or the gene activation of cortical neurons, which lead to alterations in the functions of their connections. The HSPGs are a group of glycoproteins that carry covalently bound large, unbranched polymers composed of ~20–200 repeating heparin/heparan sulfate disaccharide units, which are usually attached to the core proteins through a serine residue and characteristic carbohydrate linkage regions. It appears that the HSPGs can regulate long-term potentiation (LTP) and may be involved in the morphological maturation of dendritic spines through

multiple ligand interactions; this may be critically dependent on the balance between the different heparin-binding molecules available (Bandtlow and Zimmermann, 2000). Heparan sulfate chains on cell surface proteoglycans also provide initial docking sites for the binding of PRV to eukaryotic cells (Campadelli-Fiume *et al.*, 2000). Nectin-1, a member of the immunoglobulin superfamily, is a component of a novel cell-to-cell adhesion system, localized within the cadherin-catenin system at cell-to-cell adherens junctions (Ajs). It has been shown to play an important role in synapse formation (Mandai *et al.*, 1997; Takahashi *et al.*, 1999; Miyahara *et al.*, 2000; Tachibana *et al.*, 2000; Mizoguchi *et al.*, 2002). Nectin-1 serves as an entry and cell-cell spread mediator of PRV (Geraghty *et al.*, 1998; Campadelli-Fiume *et al.*, 2000).

As concerns our model, the crucial question is whether cell surface molecules exist which can change their expression or distribution following n7x, and which therefore influence the entry of virions into the neurons and/or their cell-to-cell spread.

As the first step, in this study we tested the hypothesis that n7x induces changes in the neuronal connections of the MIs in both hemispheres, which influence the transcallosal PRV labeling pattern.

## Materials and Methods

### Cells and Virus

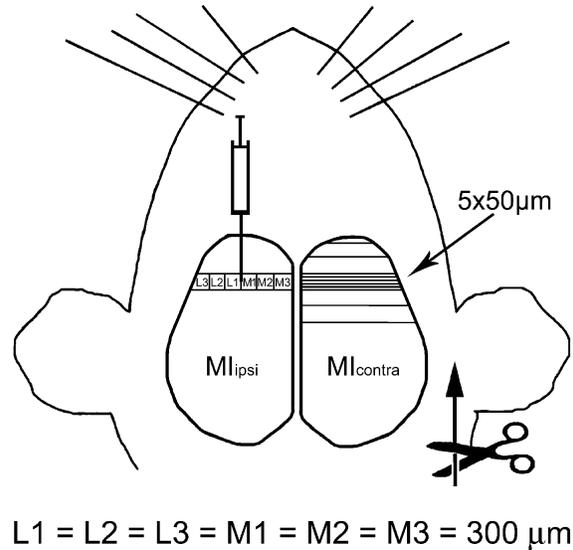
A porcine kidney cell line, PK-15, was used for the propagation and titration of PRV. Cells were grown in Dulbecco's modified minimum essential medium (DMEM) supplemented with 5% fetal calf serum at 37°C in a CO<sub>2</sub> incubator. Aliquots of PRV (1000 µl/vial) were stored at -80°C, and single vials were thawed immediately prior to injection. Ba-DupLac was constructed by the insertion of a pair of lacZ expression cassettes to a putative latency promoter (antisense promoter) of PRV-Ba, located in the inverted repeat of the virus (Boldogkoi *et al.*, 2000, 2002).

### Animals and Surgical Procedures

The experimental procedures used in this study followed the protocol for animal care approved by the Hungarian Health Committee (1998) and the European Communities Council Directives (86/609/EEC). A total of 28 adult Sprague-Dawley rats were raised with access to water and food pellets (Altromin) *ad libitum*. Sixteen animals were used to study the postinjury/preadministration time (see later and Fig. 2). In 7 of the remaining 12 animals, the right facial nerve trunk was transected 1 h before PRV injection; 5 sham-operated animals served as controls. The nerve cut was made near the stylomastoid foramen. All the surgical procedures were carried out under deep ketamine/xylazine anesthesia (ketamine 10.0 mg/100 g and xylazine 0.8 mg/100 g body wt, *i.P.*).

### Injection of the Virus

The head of each rat was fixed in a stereotactic headholder. PRV was injected with special care; the inoculations were made by the same person at the following coordinates: frontal: +2.0 mm to the bregma, lateral 2.0 mm, vertical 800 µm from the cortical surface (Paxinos, 1998). PRV (0.1 µl) was injected over 5 min by pressure (PRV concentration: 10<sup>9</sup> P.f.u./ml, vehicle: DMEM + 5% fetal calf serum). The coordinates of the injection site (and of its homotopic point) were determined by prior physiological mapping; we have been carrying out the mapping of MIs since 1996. It is known from the literature (Sanes and Donoghue, 2000) and from our own studies that the injection site (with the coordinates given above) at which intracortical microstimulation induces contralateral whisker movements is in the MI center of the contralateral whiskers (Toldi *et al.*, 1996). The homotopic point in the contralateral hemisphere was determined by intracortical stimulation at the injection site, which transcallosally evoked responses in the homotopic point (Farkas *et al.*, 2000). After determination of the location, and following the completion of the injection, the pipet was

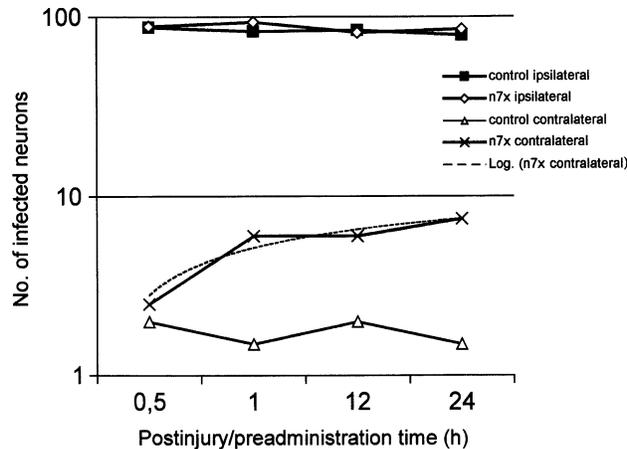


**Figure 1.** A schematic drawing to explain the experimental paradigm. PRV was injected into the whisker representation area in the left motor cortex (MI ipsi). PRV-immunoreactive (PRV-IR) neurons were counted in five consecutive 50 µm wide coronal sections of both hemispheres (as shown in the right hemisphere: MI contra). To facilitate the analysis, the photomicrographs of the five coronal sections (forming the 250 µm wide bands of the cortices) were divided in the mediolateral direction into six 300 µm wide areas (on the coronal surface): L1 = L2 = L3 = M1 = M2 = M3 = 300 µm. The numbers of PRV-IR cells obtained in five sham-operated and five n7x animals were quantified according to this cortical division. In one animal each in both the sham-operated and the n7X group, serial sections (50 µm wide) of the rostral part of both hemispheres were made (also as shown in the right hemisphere: MI contra). The PRV-IR neurons were counted in every sixth section in these two animals. The three-dimensional distributions of the PRV-IR neurons in Figure 5 were constructed on the basis of these studies. Scissors plus arrow denotes the right side facial nerve cut. The divisions (L3-M3) in the left hemisphere, the labeling of the 50 µm wide cortical slides and the mark of every sixth section in the rostral half of the right hemisphere) serve only for demonstration and, of course, are not proportionate in this schematic drawing.

left in the tissue for an additional 5 min in order to prevent any backflow of the PRV and/or its spread into the surrounding areas. After the PRV injection, the incision on the head was closed and each animal was housed individually in a plastic isolation cage. The presence and location of PRV-immunoreactive (IR) neurons were checked in all animals. The positions of the tip of the Hamilton syringe and the lesion induced by the PRV injection were verified histologically in cresyl violet-stained sections.

### Perfusion and Immunocytochemistry

After survival for 72 h, the animals were deeply anesthetized as described above and perfused transcardially with ~200 ml of phosphate-buffered saline (PBS, 0.1 mol/l, pH 7.3), followed by ~200 ml of Zamboni's fixative (2.0% aqueous paraformaldehyde solution — from a 16% stock solution containing 15% picric acid — in 0.1 M sodium phosphate buffer stock, pH 7.3) (Stefanini *et al.*, 1967). Brains were postfixed in fresh Zamboni's solution overnight. Coronal sections (50 µm thick) of the brain were cut using a Vibratome (Campden Instruments) and were processed for PRV immunocytochemistry. Only those animals were evaluated ( $n = 10$ ) in which the infection was successful, *i.e.* infected neurons (PRV-IR) were seen in the left motor cortex and the whole length of the penetration channel was situated within the cortex. The sections were blocked in 5% normal goat serum (diluted in PBS) for 1 h, and incubated with a rabbit polyclonal antibody (Rb133; 1:10 000, courtesy of Professor L.W. Enquist, Department of Molecular Biology, Princeton University, Princeton, NJ, USA) overnight at 4°C. The sections were then treated with biotinylated anti-rabbit IgG (1:200, Vector Laboratories) for 2 h at room temperature. The immunohistochemical reaction was visualized with the ABC-DAB technique



**Figure 2.** The number of infected neurons increased logarithmically with the postinjury/preadministration time only in the right hemisphere of the n7x animals (n7x contralateral; its log fit is depicted by a broken line). The other three curves are linear and parallel to the x-axis. The postinjury/preadministration time on the abscissa means that PRV was injected into the animals 0.5 h ( $n = 2$ ), 1 h ( $n = 2$ ), 12 h ( $n = 2$ ) or 24 h ( $n = 2$ ) after n7x. The study was also made on eight sham-operated animals. The PRV infection was followed in each case by a 72 h survival time.

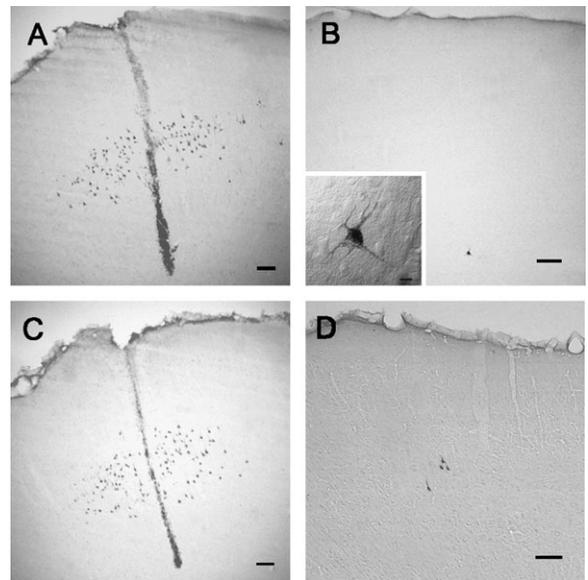
(ABC-Elite Kit, Vector Laboratories); sections were mounted on gelatinized slides, dehydrated and coverslipped with Entellan® (Merck).

#### Statistical Analysis

To prevent experimental bias, the facial nerve status was decoded for statistical analysis after cell counts had been collected. In our experiments, five consecutive (50  $\mu\text{m}$  thick) coronal sections from both hemispheres of the animals were processed for PRV immunocytochemistry. Accordingly, in both hemispheres, all of the PRV-IR neurons within these 250  $\mu\text{m}$  wide bands of the MIs were encountered. In the left hemisphere, this 250  $\mu\text{m}$  wide cortical band contained the penetration channel too (Fig. 1). To check whether the infection pattern exceeded the 250  $\mu\text{m}$  wide band, one animal randomly selected from the n7x group and one from the sham-operated group were treated and processed as described previously, but serial sections of the rostral part of the hemispheres were made. In these cases, we selected every sixth section for data sampling (Figs 1 and 5). We used the nonparametric Mann-Whitney *U*-test to analyze the difference between the total number of infected cells on each cortical side in the control and n7x groups. Repeated-measures ANOVA was applied to test the mean effects on the number of infected cells and the interactions between the facial nerve status (between-subject), the cortical side (within-subject) and the cortical area (within-subject). The slides were processed digitally (Olympus BX51, DP11, Camedia Master 2.0). The coronal sections of the motor cortices were then divided into six 300  $\mu\text{m}$  wide areas on the cortical surface (Figs 1 and 4), making the infection pattern easier to analyze. Statistical analysis was performed with the aid of the SPSS 11.0 for Windows program. The results are expressed as means  $\pm$  SD;  $P < 0.05$  was regarded as significant.

#### Data Presentation in Figures

The PRV-IR neurons in Figure 3 are shown in microphotographs. In Figure 4, diagrams of coronal sections were constructed to demonstrate in two dimensions the distribution and localization pattern of PRV-IR cells observed within 250  $\mu\text{m}$  wide bands of both cortices of five controls and five n7x animals. Since the differences in the numbers of labeled neurons within the detailed studied 250  $\mu\text{m}$  wide bands in the five slides were very small, the average number of labeled neurons in a slide could be calculated and given (see the small SDs in Fig. 4). In these drawings, the motor cortical slices were divided into 300  $\mu\text{m}$  wide areas. The black areas denote the medial and lateral areas closely adjacent to the injection channel. The gray areas are homotopic to them. In Figure 5, the schematic surface diagrams depict in three dimensions the distribution of PRV-IR neurons in the frontal part of both hemi-



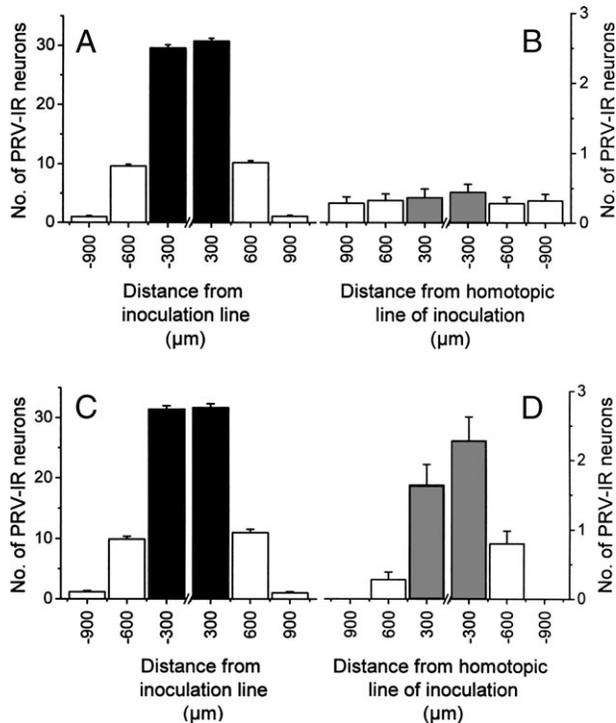
**Figure 3.** Labeled neurons in the left (A) and right (B) primary motor cortices (MIs) of a control animal, and in the left (C) and right (D) primary motor cortices of a rat in which the right facial nerve was transected 1 h before the PRV infection. Several PRV-infected neurons are localized around the injection channels (A, C). We could not usually observe labeling in the contralateral MI of the controls (B), with some exceptions, where a few labeled cells were detected. Inset in B: higher magnification of the one labeled pyramidal neuron found in this control animal. (A and B are corresponding slides.) (D) PRV-IR neurons in the homotopic area of the right hemisphere of an n7x animal, after facial denervation. Calibration: 100  $\mu\text{m}$  in A–D. In the inset in B, the bar is 10  $\mu\text{m}$ .

spheres of a sham-operated and of an n7x animal. L1, L2 and L3 denote the three 300  $\mu\text{m}$  wide bands on the cortical surface lateral to the injection site. M1, M2 and M3 denote the three 300  $\mu\text{m}$  wide bands on the cortical surface medial to the injection site. O1, O2 and O3 denote the three 300  $\mu\text{m}$  wide bands on the cortical surface in the oral direction from the injection site. C1, C2 and C3 denote the three 300  $\mu\text{m}$  wide bands on the cortical surface caudal to the injection site (see also Fig. 1).

## Results

### n7x Influences the Transcallosal Spread of PRV in a Time-dependent Manner

To determine whether the peripheral injury of the nervous system has a virus immunohistochemically detectable effect on the synaptic connections, the right facial nerve of the animals was cut or the animals were sham-operated before administration of the PRV suspension. Synaptic reorganization can reveal viral glycoprotein receptors or can induce other protein–protein interactions, which can modulate the entry or transmission of viral particles. We were interested in determining the time course of the possible reorganization, and we therefore applied different postinjury/preadministration times. The results obtained with PRV are shown in Figure 2. The postinjury/preadministration duration did not have a significant effect on the inoculation side in either group. On the contralateral side of the injured animals (n7x contralateral in Fig 2), the number of infected neurons increased in a time-dependent manner. The number reached a plateau at  $\sim 1$  h postinjury/preadministration. In the sham-operated animals, there was no significant effect of the resting time (control contralateral and control ipsilateral in Fig 2). It is likely that the changes in the motor cortex affect the neuronal transmission of PRV in a short period, i.e. within 1 h.



**Figure 4.** Schematic diagrams of the distribution of labeled cells in two slides of controls (A, B) and in two slides of n7x animals (C, D). The average number of labeled cells per slide is given in these diagrams. The averages of the cell numbers were calculated from the corresponding data on five controls and five n7x animals, i.e. from 5 × 5 slides. The motor cortices are divided into 300 μm wide areas (see also Fig. 1). The black areas denote the 300 μm wide cortical areas in the close medial and lateral environment of the injection channel in the left hemispheres (A, C). The gray areas are homotopic to them in the right hemispheres (B, D). Values are means and SDs for PRV-IR neurons (n = 25) (see also Fig. 1).

#### **PRV Immunoreactivity Was not Different on the Inoculation Side in the Controls and the n7x Animals**

To examine the effects of n7x on the entry and/or cell-to-cell spread of PRV, we compared the numbers and distributions of infected cells on the inoculation side of the cerebral cortex. The primarily infected neurons were found around the penetration channel in both the controls and the n7x animals (Fig. 3A,C). In all cases, the primarily infected neurons were located discretely in the third lamina of the motor cortex. This is consistent with the selective uptake of the virus by the middle-layer cells. The highest number of labeled neurons was found close to the injection channel, and the number decreased with increasing distance from this. These results seem to be consistent with the diffusion of PRV-containing solution around the cannula.

There was no significant difference between the control and n7x groups in the number of labeled neurons ( $82.3 \pm 3.36$  versus  $86.2 \pm 3.82$ ;  $P = 0.119$ ), or in the infection patterns on the injected side (Figs 3A,C and 4A,C). These results indicate that the entry of PRV into the motor cortical neurons is not dependent on facial denervation.

#### **PRV Immunoreactivity Differs in the Contralateral MI in the Controls and the n7x Animals**

To test whether n7x affects the cell-to-cell spread of PRV, we compared the numbers and distributions of infected cells in the cortex contralateral to the inoculation. The transcallosally infected cells displayed cytoplasmic staining (as shown in

Fig 3B, inset), and by 72 h following inoculation strong PRV immunoreactivity could be seen in these neurons. In the control animals, there were significantly fewer labeled neurons in the contralateral (right side) cerebral cortex than in the n7x animals ( $2.0 \pm 1.86$  versus  $5.0 \pm 1.83$ ;  $P < 0.001$  (see Figs 3B-D and 4B,D). In the n7x animals, these neurons were located close to the homotopic line of the injection channel (Fig. 4D). n7x not only increased the number of transcallosally labeled neurons, but also affected their distribution. ANOVA indicated a significant three-way interaction between the facial nerve status, the cortical side and the cortical area [ $F(5,40) = 15.64$ ,  $P < 0.0001$ ], i.e. the mean of the PRV-IR cell number is dependent on the cortical side, the cortical area and the unilateral n7x.

In the control animals, the distributions of the transcallosally infected neurons were identical in the divided cortical areas (Fig. 4B). There was no significant difference between the divided areas in the number of labeled neurons.

Although we did not perform a detailed study, in the course of a rough survey, PRV-IR neurons were not found in any other cortical area (e.g. in the somatosensory cortex) apart from the motor cortices on both sides.

#### **The Motor Cortex Is Homogenous for Viral Spread in Both Mediolateral and Orocaudal Directions**

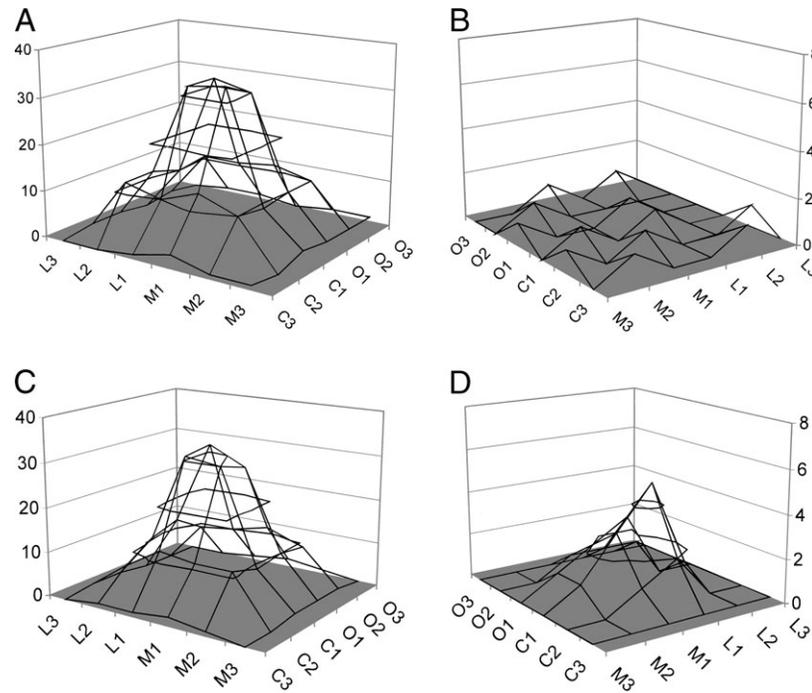
To confirm that this infection pattern does not exist in only two dimensions, one animal randomly selected from each group was treated and processed as described previously, and serial sections of the brains were made. The surface diagrams of the distribution of the PRV-IR neurons revealed a cone-like shape on the ipsilateral side in both groups (Fig. 5A,C) and also on the contralateral side in the n7x animals (Fig. 5D). On the contralateral side of the sham-operated animal, the diagram was nearly planar (Fig. 5B). A possible explanation is the diffusion of the viral suspension on the inoculation side — the farther from the injection channel, the lower the probability of infected neurons. Envelope proteins of PRV and other herpes viruses play an essential role in target cell recognition, attachment and receptor-mediated fusion of virions to permissive profiles. Additionally, some envelope proteins exhibit an affinity for extracellular matrix molecules such as HSPG that are present in the extracellular milieu of the nervous system. These affinities act to limit the diffusion of virions from the injection site and thereby contribute to the ability to carry out localized injections of PRV. Finally, the large size of the PRV particle may further aid in limiting the diffusion of injected tracer (Enquist *et al.*, 1998; Aston-Jones and Card, 2000). In this case, it means that at least this part of the cerebral cortex is homogenous concerning viral infection.

On the contralateral side of the control animal, the distribution of infected neurons was uniform (Fig. 5B), which means that the neurons around the infection channel received afferents from all parts of the contralateral side. After n7x, this afferentation was more focused; the surface diagram exhibited a distribution of PRV-IR neurons similar to that observed on the inoculation side (Fig. 5D).

These results indicate that the transcallosal cell-to-cell spread of PRV within the MIs of both hemispheres is influenced by n7x.

#### **Discussion**

The present study has demonstrated that n7x influences the transcallosal spread of PRV from the MI on the affected side to



**Figure 5.** Surface diagrams for a sham-operated (*A, B*) and n7x animal (*C, D*). The diagrams exhibit a cone-like shape, which demonstrates the distribution of PRV-IR neurons on the inoculation side (*A, C*). In contrast, the diagram of the contralateral cortex is virtually planar (*B*), while in the right hemisphere of the n7x animal there is an impressive peak in the surface diagram at the homotopic point of the injection channel (*D*). It should be mentioned that the distance of sampling was in 300  $\mu\text{m}$  in the mediolateral direction, but 50  $\mu\text{m}$  in the rostrocaudal direction in these animals.

the contralateral MI in rats. The main observations were as follows: in the controls, PRV injection primarily infected several neurons around the penetration channel, but hardly any transcallosally infected neurons were found in the contralateral MI. In coronal sections, these neurons exhibited an almost constant distribution from medial to lateral in the cerebral cortex. In contrast, after right n7x, PRV was transported from the primarily infected neurons in the left MI to the contralateral side, and resulted in the labeling of several neurons via transneuronal infection. These transcallosally labeled neurons were concentrated near the homotopic line of the injection channel. The number of infected neurons reached a plateau 1 h postinjury/preadministration.

In our tracing study, we did not find any other infected brain areas associated with a motor function apart from the MIs. In the MIs, the interhemispheric connections between the homotopic representation fields of the vibrissal muscles undergo rapid disinhibition (minutes after denervation) (Toldi *et al.*, 1999; Farkas *et al.*, 2000). The question arises of whether this disinhibition of interhemispheric connections might play a role in the observed enhanced transcallosal labeling. Our results suggest that a new transcallosal path is unmasked quickly after the peripheral n7x. In adult rats, the MI exhibits a noteworthy capacity to react to peripheral nerve lesions, with changes in the perisynaptic glia and synaptic reorganization, with latencies of from 1 h up to 1 day. The results we have presented here also show that the changes in the motor cortex affect the neuronal transmission of PRV within 1 h. Our results, supported by statistical analysis, suggest that n7x not only facilitates, but also augments the transcallosal spread of PRV from the left MI to the contralateral side. Unilateral n7x did not affect the entry of PRV into the neurons (infected primarily).

To explain this result, it should be taken into account that the entry of alpha herpes viruses into the cells usually requires multiple interactions between the viral envelope and the cell surface proteins. At least two groups (HSPGs and nectins) of these cell surface (glyco)proteins are known to play roles in these processes (Mettenleiter, 2000; Spear *et al.*, 2000). It should also be considered that HSPGs and nectins participate in the development and plasticity in adulthood of tissues of neuroepithelial origin (Carey, 1997; Rauvala and Peng, 1997; Suzuki *et al.*, 2000; Mizoguchi *et al.*, 2002).

Our present results suggest that n7x does not affect the entry of PRV, but increases the efficiency of its cell-to-cell spread. Thus, we may speculate that the n7x-dependent infection pattern appears to be related not to cellular components (HSPGs) involved in the attachment of the virus, but rather to cellular components located in the synaptic region of the membrane of presynaptic neurons.

Many articles (see the review by Sanes and Donoghue, 2000) or our own results (Toldi *et al.*, 1999; Farkas *et al.*, 2000) demonstrate that n7x induces changes in cortical activity in extended areas. On the basis of these results, we suggest that, as a consequence of these changes in cortical activity (or in parallel with them), changes also take place in the expression of the cell surface molecules in the presynaptic terminals of transcallosal axons of motor cortical origin on the right side. Accordingly, we consider that the virus transport in our experiments was mainly transsynaptic and retrograde. There are additional indications in support of retrograde transport. (i) In the course of our experiments, we never observed labeled axon terminals in the right hemisphere. This also holds for the retrograde transport. (ii) The firmest evidence is the recent observation by Enquist and co-workers (Enquist *et al.*, 2002;

Pickard *et al.*, 2002) that PRV-Bartha was transported trans-synaptically only in the retrograde direction (i.e. from post-synaptic to presynaptic neuron).

The suggested relationship between the n7x-induced changes in cortical activity and the changes in the neuronal surface molecules is further supported, and partially explained, by recent results indicating that the expression of the cell surface molecules which we suggest might play roles in the retrograde, trans-synaptic cell-to-cell spread of PRV is controlled dynamically and locally, and modulated by synaptic activity (Tanaka *et al.*, 2000) or via activity-dependent regulatory pathways (Pierre *et al.*, 2001; Murase *et al.*, 2002).

However, in addition to the adhesion molecule (nectin-1 $\alpha$ )-aided cell-to-cell spread of the virus (Sakisaka *et al.*, 2001), its enhanced direct uptake and retrograde axonal transport into the contralateral hemisphere after n7x cannot be completely excluded either. With regard to the enhanced number of PRV-IR neurons in the contralateral primary motor cortex after n7x, due either to trans-synaptic retrograde cell-to-cell spread or to direct uptake and retrograde transport of the virus, the role of the cell adhesion molecules in these processes is hardly disputable.

Nectin-1 and nectin-2, components of a novel cell-to-cell adhesion system, and localized within the cadherin-catenin system at cell-to-cell AJs, have been shown to play an important role in synapse formation (Mandai *et al.*, 1997; Takahashi *et al.*, 1999; Miyahara *et al.*, 2000; Tachibana *et al.*, 2000; Mizoguchi *et al.*, 2002). The synaptic scaffolding molecule (S-SCAM) is localized at the AJs in the CA3 area of the hippocampus in a nectin-dependent manner. This finding indicates that S-SCAM serves as a scaffolding molecule at the AJs after maturation of the synapses and at the synaptic junctions during the maturation. S-SCAM is a neural scaffolding protein which interacts with many proteins, including *N*-methyl-D-aspartic acid (NMDA) receptors (Yamada *et al.*, 2003). The nectin-afadin system may be involved in the structural changes that occur at synapses during the maintenance phase of LTP by modulating the redistribution of synaptic components.

The remodeling of cortical circuits (including new synapse formation) might also play a part in the plasticity of the motor cortex, which contains both the substrate (the horizontal connection system) and the mechanisms (LTP and long-term depression) for reorganization after peripheral nerve injury (Sanes and Donoghue, 2000). The mechanism by which n7x increases the efficiency of cell-to-cell spread or the direct uptake of PRV in the cortical network *in vivo* remains to be elucidated, but the dense and focused PRV-IR suggests changes in the background (in cell surface molecules), which should be of significance in the cortical reorganization after a peripheral nerve injury. We have recently started to study this aspect of cortical plasticity.

Whatever the underlying mechanism is, the peripheral nerve injury-induced changes in the Ba-DupLac infection pattern seem to be a suitable model for the study of injury-induced neuronal plasticity. Such studies reveal another aspect of peripheral nerve injury-induced cortical reorganization.

## Notes

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