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AAV-mediated targeting of gene expression to the peri-infarct region in rat cortical stroke model

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Abstract

Background—For stroke patients the recovery of cognitive and behavioral functions is often incomplete. Functional recovery is thought to be mediated largely by connectivity rearrangements in the peri-infarct region. A method for manipulating gene expression in this region would be useful for identifying new recovery-enhancing treatments.

New Method—We have characterized a way of targeting adeno-associated virus (AAV) vectors to the peri-infarct region of cortical ischemic lesion in rats two days after middle cerebral artery occlusion (MCAo).

Results—We used magnetic resonance imaging (MRI) to show that the altered properties of post-ischemic brain tissue facilitate the spreading of intrastrially injected nanoparticles towards the infarct. We show that subcortical injection of green fluorescent protein-encoding dsAAV7-GFP resulted in transduction of cells in and around the white matter tract underlying the lesion, and in the cortex proximal to the lesion. A similar result was achieved with dsAAV7 vector encoding the cerebral dopamine neurotrophic factor (CDNF), a protein with therapeutic potential.

Comparison with existing methods—Viral-vector mediated intracerebral gene delivery has been used before in rodent models of ischemic injury. However, the method of targeting gene expression to the peri-infarct region, after the initial phase of ischemic cell death, has not been described before.

Conclusions—We demonstrate a straightforward and robust way to target AAV vector-mediated over-expression of genes to the peri-infarct region in a rat stroke model. This method

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will be useful for studying the action of specific proteins in peri-infarct region during the recovery process.

Keywords

Gene transfer; Focal ischemia; Animal models; Functional recovery; Adeno-associated virus; Peri-infarct region

1. Introduction

Despite advances in modern medicine and risk factor control contributing to the decreased mortality rate associated with acute ischemic brain injury in developed countries (Go et al., 2014), stroke remains the leading cause of long-term disability because the functional recovery is often incomplete. The positive effect of physiotherapy and cognitive therapy, or other stimulatory experience, on the recovery process is well known. However, these are currently the only ways to accelerate the recovery process in stroke patients, as there are no drug therapies to promote it. Recovery of cognitive and behavioral functions results largely from the remodeling of neuronal connectivity in the peri-infarct region (Brown et al., 2009, Brown et al., 2007, Li and Murphy, 2008). The peri-infarct region is defined as the region surrounding the necrotic core of the ischemic lesion. It largely overlaps with the penumbra area – the area that undergoes temporary damage induced by partial loss of perfusion and harmful influence of the adjacent infarct core. By understanding the biological mechanisms of recovery in the peri-infarct region, we may be able to identify pharmacological targets for enhancing the recovery process. Towards this end, it is necessary to experimentally manipulate gene expression in the peri-infarct region. One approach is to use viral vector-mediated gene delivery in rodent models of ischemic brain injury, and target the viral vectors to the area surrounding the infarct. A variety of viral vectors have been used for intracerebral delivery, such as lenti-, herpes-, adenoviruses and adeno-associated viruses (AAV-vectors) (Lim et al., 2010). Out of these, AAV-vectors are the most widely used, both in clinical studies and as research tools, as AAVs do not cause any known disease and AAV-vectors do not elicit any cellular immune reaction (Logan and Alexander, 2012). AAV-mediated gene delivery before the ischemic period has been used to assess the neuroprotective effect of vector-encoded proteins in cerebral ischemia (Airavaara et al., 2010, Harvey et al., 2011). Similar attempts to modulate the recovery process have been less frequent. There are some studies where virus particles have been injected after reperfusion (Sun et al., 2011, Watanabe et al., 2004), but only rarely has it been done after the initial phase of massive neuronal death has passed (Sugiura et al., 2005). Comparably, expression of viral vector-delivered genes in the peri-infarct region has been reported only in a few studies (Shen et al., 2011, Sun et al., 2011, Zhu et al., 2009). Unfortunately, in these studies the peri-infarct region targeting is not well documented and the injections have been done during the initial ischemic events. Thus, the aim of our study was to fully characterize the method of AAV vector-mediated gene delivery to the peri-infarct region in the brain of rats in which MCAo has resulted in differently sized focal ischemic lesions. Here we describe the details and robustness of this approach so that it can be reproducibly used in future studies.

2. Materials and Methods

2.1. Animals and surgery

All animal experiments were approved by Finnish National Ethics Board and carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals. The ligation of the right MCA and common carotid arteries (CCAs) bilaterally was performed as described previously (Chen et al., 1986). Briefly, male Sprague Dawley rats (RGD: 737903, average weight 300 g, from Harlan, Netherlands) were anesthetized with intra-peritoneal chloral hydrate injection and the bilateral CCAs were identified and isolated through a ventral midline cervical incision. Rats were placed in stereotaxic apparatus and a craniotomy was made in the right hemisphere. The right MCA was ligated with a 10-0 suture and CCAs were ligated with non-traumatic arterial clamps for 60 minutes. After sixty minutes of ischemia, the suture around the MCA and arterial clips on CCAs were removed. After recovery from anesthesia, the rats were returned to their home cage.

2.2. Triphenyltetrazolium chloride (TTC) staining

The infarction area was measured by TTC staining two days after MCAo, as described previously (Airavaara et al., 2010).

2.3. Nanoparticle injection

Dextran-coated magnetic iron oxide particles with a hydrodynamic diameter of 50nm (fluidMAG-DX from Chemicell, product nr. 4104-1) were injected into two sites at a concentration of 0.2mg/mL (in phosphate-buffered saline solution, PBS, pH 7.4). The injection volume, speed and stereotaxic coordinates were the same as described below for the AAV injections.

2.4. Nanoparticle magnetic resonance imaging (MRI)

MRI studies were performed with a 4.7 T scanner (PharmaScan, BrukerBioSpin, Ettlingen, Germany) using a 90 mm shielded gradient capable of producing a maximum gradient amplitude of 300 mT/m with an 80 μ s rise time. The linear birdcage RF coil used had an inner diameter of 38 mm. The head was fixed in a holder with tooth bar to minimize motion artifacts during imaging. The rats were imaged 10 min and 3h after the injection of dextran-coated iron oxide nanoparticles. T2 weighted images were acquired using a rapid acquisition with relaxation enhancement (RARE) sequence (repetition time=3100 ms, effective echo time=60 ms, matrix size=256 $\times$ 256, field of view=40 $\times$ 40 mm, with slice thickness=1.0 mm).

2.5. AAV production

Viral stocks of AAV7-eGFP, AAV7-hMANF (mesencephalic astrocyte-derived neurotrophic factor, GenBank: NM_006010.5) and AAV7-hCDNF (GenBank: NM_001029954.2) were prepared using the triple-transfection method (Howard et al., 2008, Xiao et al., 1998). Twenty 15 cm dishes containing HEK293 cells at 85-95% confluency were transfected by the CaCl₂ method with pHelper (Stratagene, La Jolla, CA), pdsAAV-GFP, pdsAAV-MANF or pdsAAV-CDNF and a plasmid containing rep/cap genes for serotype7, pAAV7.(Gao et al., 2002) Approximately 48 hours post-transfection, cells were

harvested, lysed by freeze/thaw, and purified by centrifugation on a CsCl gradient. Final samples were dialyzed in PBS containing 12.5 mM MgCl₂, aliquoted and stored at -80 °C until use. All vectors were titrated by quantitative PCR using the cytomegalovirus (CMV) promoter as the target sequence. Viral titers are recorded as viral genome/mL (vg/mL).

2.6. AAV injection

Animals were anesthetized with isoflurane and placed into stereotaxic frame. AAVs were injected intracerebrally into two subcortical sites with the following stereotaxic coordinates: site 1 A/P +1.6; L/M +2.2; D/V -5.0 (from the surface of the skull) and site 2 A/P -0.4; L/M +4.0; D/V -5.0 (from the surface of the skull). Two and a half microliters of AAV7-eGFP (titer 1.1×10^{13} vg/mL), AAV7-hCDNF (3.9×10^{12} vg/mL) or AAV7-hMANF (8.1×10^{13} vg/mL) were injected using a 10 µl Hamilton syringe with a 30 G blunt needle. The injection was started 30 seconds after lowering the needle, and the needle was kept in place for two minutes after the injection. The rate of infusion (1.0 µl/min) was controlled using a microprocessor-controlled injector mounted to a stereotaxic frame (UMP4; World Precision Instruments, Sarasota, FL, USA).

2.7. Perfusion and tissue processing

Twelve days after AAV injections, rats were perfused transcardially with 0.9% NaCl solution followed by 4% paraformaldehyde (PFA) in PBS. The brains were dissected out and post-fixed for 2 days in 4% PFA in PBS. After dehydration and clearing with xylene, the brains were embedded in paraffin wax and sectioned into 5 µm coronal sections.

2.8. Immunohistochemistry

Rabbit anti-GFP (sc-8334, Lot # H101, Santa Cruz Biotechnology, AntibodyRegistry: [AB_641123](#)) and affinity-purified rabbit anti-hCDNF antibody (DDV1, a gift from Dr. Johan Peränen and Prof. Mart Saarma) were both used at 1:500 dilution. Sections were deparaffinized and rehydrated through graded alcohol series. After heat-induced epitope retrieval in microwave oven (10 min 95°C in 5.5 mM citraconic anhydride solution, pH 7.4) the samples were allowed to cool down to room temperature and rinsed 3 times 5 minutes with TBS buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl), followed by H₂O₂ quenching of endogenous peroxidase activity and two washes in TBS-T (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20). Primary antibodies were incubated in TBS-T at +4°C overnight, followed by three washes with TBS-T. Biotinylated goat anti-rabbit IgG antibody (1:200, BA-1000, AntibodyRegistry: [AB_2313606](#)), Vectastain ABC kit (PK4000) and DAB Peroxidase Substrate Kit (SK-4100) were used for the detection of primary antibody (all from Vector Laboratories, Inc.). After dehydration in graded alcohol series and clearing in xylene, the sections were mounted with DePeX mounting medium and imaged with Panoramic 250 Flash II slide scanner (3DHitech Ltd.) using the combined 20X/0.8 NA objective (in Genome Biology Unit, Institute of Biotechnology, Helsinki). The contrast was increased slightly and equally in all images of immunostained sections to make the unstained regions more easily visible.

3. Results

In this study, we used the rat model of focal cerebral ischemic stroke, performed by extravascular ligation of the distal middle cerebral artery together with clip-occlusion of the common carotid arteries for 60 minutes (Chen et al., 1986). We chose this stroke model because it is rather commonly used and results in a cortical lesion. However, as in other MCAo-based models, the size and position of the lesion varies from animal to animal (Figure 1A-D) (Howells et al., 2010), causing the exact position of the cortical peri-infarct region to be different. This makes it unfeasibly difficult to target the peri-infarct region by direct injections into the cortex. On the other hand, the exact position of the subcortical peri-infarct region is far less variable compared to the cortical peri-infarct region (Figure 1 A-D) (Chen et al., 1986). Therefore, we decided to analyze how widely and reproducibly the peri-infarct region can be targeted by injecting AAV into the underlying subcortical structures, such as external capsule/corpus callosum/striatum (Figure 1E).

First we wanted to characterize the spreading of virus particle-sized molecules in the ischemic rat brain. For that, we used magnetic resonance imaging to follow the spreading of magnetic nanoparticles injected into the subcortical areas of ischemic rat brain. The rats had undergone a 60 min temporary MCAo two days before the injection. We chose to do the injections on day 2 after the MCAo, since, when studying potential therapeutic agents, injections before MCAo or closer to the MCAo could have neuroprotective effects that can confound studies of recovery-enhancing treatments (Liu et al., 2009). We focused on the rostral part of the ischemic lesion to minimize the number of injection sites and also to target the cortico-striato-thalamic pathway. Nanoparticle solution was injected into two subcortical sites in the lesioned hemisphere, namely the lateral striatum (Figure 1E) and external capsule. When examined 10-20 minutes after the injections, the particles had spread towards and along the white matter underlying the rostral lesioned cortex (Figure 2A-C). This pattern of distribution remained largely unchanged over 3 hours (data not shown). To compare the spreading and diffusion of virus-sized particles in ischemic and unlesioned brain, we also injected nanoparticles into the brain of a naïve rat using the same stereotaxic injection coordinates. The intrastriatally injected nanoparticles were mostly confined to the striatum in the naïve animal (Figure 2D), and, therefore, had spread differently compared to the lesion brain (Figure 2C). The difference in the distribution of nanoparticles in unlesioned versus lesioned brain can be partly due to the edema which is causing a midline shift (misalignment of the brain midline relative to bregma, most clearly seen on Figure 2B), but there is also a clear difference in the spreading of nanoparticles injected into the striatum (compare site 1 on Figures 2C and D)(see discussion).

Next, we used the same stereotaxic coordinates to inject adeno-associated virus expressing enhanced green fluorescent protein (dsAAV7-eGFP) into the brains of rats with cortical ischemic lesion from the MCAo surgery done 2 days earlier. A double-stranded rather than single-stranded AAV vector was chosen, based on the improved speed of transduction, higher level of gene expression and expression stability of the former.(Wang et al., 2003) After the injection, the animals were left to recover for 12 days, after which they were sacrificed and the pattern of eGFP expression was determined by immunohistochemical staining. Based on the anti-GFP immunoreactivity, virus-transduced cells were detected

mostly around the cortical ischemic lesion and also in a ventrally extending area around the external capsule (Figure 3). More specifically, coronal sections proximal to the injection sites had eGFP-positive cells in the external capsule and in the adjacent lateral striatum (Figure 3M). eGFP expression could also be seen above the external capsule in the peri-infarct cortex, mostly in the deeper cortical layers adjacent to the necrotic tissue of the core region (Figure 3 A, B, D, E, G, H, M). However, on some coronal brain sections eGFP expression was absent in the dorsal part of peri-infarct cortex (Fig. 3D, indicated by *). eGFP-expressing cells could be seen in the proximity of external capsule in coronal sections taken from positions 3–4 mm caudal to the injection site (Figure 3 F, I) and in some cases at positions even farther (approx. 6 mm caudal to the bregma; data not shown). The extent of eGFP expression in the caudal direction was apparently dependent on the size of the lesion, as smaller lesions resulted in more restricted distribution of eGFP-positive cells (compare Fig. 3 C to Fig. 3 F and I). On these more caudal sections eGFP expression could also be detected in the thalamus (Figure 3 C, F and I). In the unlesioned rat brain, dsAAV7-eGFP transduced cells almost exclusively in the striatum (Figure 3 J), just as predicted by the distribution of injected nanoparticles. In summary, when the AAV-vectors were injected into subcortical areas in lesioned rat brain, they transduced cells in both the subcortical and cortical areas of the peri-infarct region.

We next examined whether the peri-infarct targeting was specific for eGFP or could be used for other genes as well. We chose to study whether a potentially therapeutic protein could be similarly targeted to the peri-infarct region. For this we injected an AAV-vector encoding the human cerebral dopamine neurotrophic factor (dsAAV7-hCDNF), a protein that has been shown to be neurorestorative and to reverse the neurological deficits in the rodent models of Parkinson's disease (Airavaara et al., 2012, Lindholm et al., 2007, Ren et al., 2013), but is yet to be tested in models of cerebral ischemia. The observed distribution of hCDNF-expressing cells indicated that the expression of other genes besides eGFP can also be targeted to the peri-infarct region (Figure 4).

By now we have used these injection coordinates in the injection of 34 rats with ischemic lesion, and seen a similar AAV-transduction pattern in all of them. The infarct volumes have ranged from 188 to 426 mm³ (average $\pm$ SEM; 281 $\pm$ 13.5 mm³) as determined by T2-weighted MRI on day 2 after MCAo. In summary, the method of AAV-mediated peri-infarct region targeting is highly reproducible from animal to animal over a wide range of ischemic lesion sizes.

4. Discussion

In this study we have characterized a straightforward and practical way to target different genes to the peri-infarct region of rats without the need to analyze each rat's stroke volume individually. This was achieved by combining a cortical ischemic injury model with subcortical injection of AAV-vector near the infarct on day two after the stroke surgery. This method, described here in detail, can potentially be useful for studying the role of human and rodent proteins in the recovery process after stroke.

Recovery from stroke, on the level of cognitive and behavioral ability, reflects both behavioral compensation (Whishaw, 2000) and true recovery in sensory or motor function e.g. regain of control over a certain limb muscle. Changes that underlie recovery from cortical ischemic lesion can take place in areas distant from the site of ischemic infarct, such as rearrangements in the contralesional hemisphere or brainstem (Reitmeir et al., 2011, Takatsuru et al., 2009). Perhaps even more important to the recovery of function is the remodeling of connections made by neurons that reside in the peri-infarct region (Brown et al., 2009, Winship and Murphy, 2008), which may also include connections to distant sites e.g. thalamus or contralateral cortex. The method of AAV-mediated targeting of genes to the peri-infarct region, which we have characterized here, could be used to study neuronal repair and remodeling of connections in this region directly e.g. by over-expressing or knocking down expression of a neurotrophic factor or a neurite guidance cue molecule. Furthermore, the method may be used to influence neuronal repair and connectivity in a more indirect manner e.g. by influencing local inflammation, angiogenesis or remyelination. Our findings could be especially useful for modulating recovery mechanisms that involve changes in the peri-infarct white matter, as most of the AAV-transduced cells were observed in or adjacent to the external capsule. The corpus callosum, for example, is a source of oligodendrocyte progenitor cells (Roy et al., 1999) and an increased density of these cells and myelinating oligodendrocytes in the peri-infarct striatum has been shown to correlate with recovery of motor function in rat MCAo model (Zhang et al., 2010). As additional examples, functional recovery has been shown to correlate with increased thickness of corpus callosum and white matter remodeling (Liu et al., 2011, Shen et al., 2006).

Regardless of which cellular process is affected, restricting the targeting to the peri-infarct region allows one to attribute the resulting functional changes with greater certainty to alterations to that area. In addition, the restricted targeting helps to minimize possible side-effects of the injected agent. As a limitation of our method, the distribution of AAV-transduced cells was not uniform in the peri-infarct cortex, being especially variable in the cortical area dorsal to the ischemic lesion. We have focused on the rostral part of the lesion and, therefore, do not know whether injecting into more caudal positions would result in a similar distribution of virus-transduced cells. However, even with the injection sites used in this study, we saw viral vector-transduced cells in subcortical peri-infarct region several millimeters caudal to the site of injection. It is left for future studies to reveal whether varying the needle insertion coordinates results in altered distribution of transduced cells. Similarly, we leave it to future studies to determine the importance of the time of injection i.e. the optimal time for injection and whether there is a critical time-window for achieving the peri-infarct distribution. In this study we have only done injections on day 2 after the MCAo surgery to show that it is possible to achieve the expression of AAV vector-delivered genes in the peri-infarct region at the time when most of the ischemic cell death has already taken place (Liu et al., 2009). Thereby one can exclude the possible effects of the treatment on cell survival and get a more correct estimate of its effect on the abovementioned mechanisms of recovery. It is possible that injections done on day 1 or after day 2 can result in different distribution of the vector, since the edema is decreased over time (see below). It should also be emphasized that the onset of expression can vary depending on the AAV type used (Lim et al., 2010).

The appeal of the method presented here lies in our observation that AAV-injections, using the same coordinates for all rats, resulted in comparable transduction patterns in all MCAo-lesioned brains that presented a range of lesion sizes (34 animals have been analyzed so far). Thus, the consistent spreading of AAV particles to the peri-infarct region, despite the variability in the lesion size and position, offers great promise for vector delivery in the MCAo model. This consistency is remarkable because the actual position of the injection needle was dependent on the midline shift, which is caused by the edema and is itself dependent on the size of the lesion. The similarity of AAV-transduction pattern in all lesioned rats can partly be explained by the altered properties of brain tissue on day 2 after the ischemic insult, which facilitate the spreading of intrastrially injected virus-sized particles towards and along the white matter tract. In our experiments this manifested in the observations that the spreading of intrastrially injected virus-sized nanoparticles was different in unlesioned versus lesioned brain. This was well in agreement with the different AAV-eGFP transduction patterns seen in unlesioned versus lesioned brain. These results show that the changed properties of post-ischemic brain make it possible to target the expression of AAV vector-delivered genes to the peri-infarct region by subcortical injections. We want to stress that, despite the robust and consistent delivery by our method, it is unlikely that the specific injection site coordinates we have used will be applicable to targeting the peri-infarct region in other models of focal cerebral ischemia. For example, targeting the peri-infarct region in models of lacunar stroke, requires empirical determination of injection coordinates as the direction and extent of virus particle spreading is likely dependent on the extent of the edema and orientation of nearby white matter tracts. However, given the apparent prominence of spreading along the corpus callosum/external capsule, it is probable that the peri-infarct region can be comparably targeted in other ischemic models in which the lesion occurs in the cortex. Targeting the expression of therapeutic genes to the peri-infarct region could perhaps be a way to enhance functional recovery in focal ischemia patients. However, translation of this approach from animal experiments is difficult because of the challenges that intracranial injection in human patients imposes.

5. Conclusion

We demonstrate a straightforward and robust way to target AAV vector-mediated over-expression of genes to the peri-infarct region in a rat stroke model. This method will be useful for investigating the role of specific proteins in the peri-infarct region during the recovery process after stroke.

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Abbreviations

AAV	adeno-associated virus
A/P	anterior-posterior
CCAs	common carotid arteries
dsAAV7	double stranded adeno-associated virus vector serotype 7
D/V	dorsal-ventral
eGFP	enhanced green fluorescent protein
hCDNF	human cerebral dopamine neurotrophic factor
hMANF	human mesencephalic astrocyte-derived neurotrophic factor
L/M	lateral-medial
MCAo	middle cerebral artery occlusion
MRI	magnetic resonance imaging
PBS	phosphate-buffered saline solution
TTC	Triphenyltetrazolium chloride

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Highlights

We describe the overexpression of genes in peri-infarct region after stroke in rat.

Injected virus-sized nanoparticles spread differently in rat brain after ischemia.

Subcortically injected AAV-vector transduces cells in peri-infarct region.

The described method of peri-infarct region targeting is robust and reproducible.

Figure 1.

Cortical ischemic lesions resulting from the distal middle cerebral artery occlusion model used in this study. Variability of cortical infarction following MCAo is represented by example images from four different animals, showing relatively large (**A** and **C**) and small (**B** and **D**) cortical lesions, as determined by T2-weighted magnetic resonance imaging (**A** and **B**) or TTC staining (**C** and **D**) on day 2 after surgery. Note that the lesion is limited to the cortex. The images are taken from position approx. 1.0 mm anterior to bregma (A/P +1.0). (**E**) Schematic representation of the cortical infarct (black area), peri-infarct region (hatched area) and the approximate position of injection needle inserted to a subcortical injection site 1.6 mm rostral to the bregma.

Figure 2.

Distribution of subcortically injected magnetic nanoparticles along the external capsule underlying the ischemic lesion. (A-C) T2-weighted MRI images of a lesioned rat brain injected with dextran-coated iron oxide nanoparticles into the striatum (A, site 1, A/P +1.6) and, in a more caudal position, into external capsule (B, site 2, A/P -0.4). (C) The spreading of nanoparticles along the white matter is also evident when imaged in the horizontal plane. (D) Distribution of injected magnetic nanoparticles in unlesioned brain. The injection sites are marked with arrows. The MRI scans were taken 10-20 minutes after injection into the second site.

Figure 3.

Targeting AAV vectors to peri-infarct region. Subcortical dsAAV7-eGFP injection transduces cells close to the ischemic infarct area. Rats were injected with dsAAV7-eGFP (A-I) or control AAV (dsAAV7-hMANF, human mesencephalic astrocyte-derived neurotrophic factor) (K) into two sites on day 2 after MCAo surgery and sacrificed 12 days after the injection. Coronal sections of the lesioned cerebral hemisphere were immunostained using anti-GFP antibody. Sections from positions close to site 1 at approx. A/P +1.6 (A, D, G, J), site 2 at approx. A/P -0.4 (B, E, H, K) and more caudal from the

injection site (**C**, **F**, **I**) are presented. The core of the ischemic lesion is outlined. (**J**) anti-GFP immunostaining in unlesioned rat brain injected with dsAAV7-eGFP. Lack of anti-GFP immunoreactivity in dsAAV7-hMANF-injected brain (**K**) (approx. position A/P -0.4) and unlesioned brain (**L**) (approx. position A/P -4.0) shows specificity of the anti-GFP immunostaining. Arrows mark peri-infarct cortex where the deeper layers are dsAAV-eGFP-transduced. Lack of AAV-transduction in peri-infarct cortex dorsal to the lesion is marked with asterisk. Anti-GFP immunoreactive structures in the thalamus are marked with arrowheads. (**M**) Close-up of GFP-expression in the peri-infarct region encompassing cortex (ctx), striatum (str) and external capsule (ec). Scale bar equals 100 μ m.

Figure 4.

Subcortical dsAAV7-hCDNF injection transduces cells close to the ischemic infarct area. Rats were injected with dsAAV7-hCDNF into two sites on day 2 after MCAo surgery and sacrificed 12 days after the injection. Coronal sections of the lesioned cerebral hemisphere were immunostained using anti-hCDNF antibody. Sections from positions close to the injection site 2 (at approx. A/P -0.4) are presented. The core of the ischemic lesion is outlined. Arrows mark peri-infarct cortex where the deeper layers are dsAAV7-hCDNF-transduced. Lack of AAV-transduction in peri-infarct cortex dorsal to the lesion is marked with asterisk.