Transplantation of the rat suprachiasmatic nucleus. Functional studies and ex vivo adenoviral vector-mediated gene transfer
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Adenoviral vector-mediated expression of neurotrophin-3 increases the number of vasopressinergic and vasoactive intestinal polypeptidergic neurons in suprachiasmatic nucleus grafts


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Transplantation of a fetal suprachiasmatic nucleus (SCN) can to some extent restore circadian rhythmicity in several behavioral parameters in SCN-lesioned adult rats or hamsters. In an attempt to improve survival and neuritic outgrowth of transplanted SCN neurons, SCN grafts were transduced with an adenoviral vector encoding for neurotrophin-3 and transplanted in the SCN-lesioned brains of Wistar or Brattleboro rats. Rats that received mock-transduced grafts or grafts transduced with an adenoviral vector with the marker gene LacZ were used as controls. The transplants were evaluated for the presence of vasopressin (VP) and vasoactive intestinal polypeptide (VIP)-positive SCN neurons. The size of the transplants and the numbers of VP- and VIP-positive cells and fibers were semiquantitatively determined. AdNT-3-transduced transplants displayed more VP- and VIP-positive SCN cells in comparison with AdLacZ-transduced transplants. AdNT-3-transduced transplants revealed, moreover, a tendency towards more neuritic outgrowth of VP-positive fibers into the Brattleboro host brain. The number of VP- and VIP-positive fibers that crossed the graft-host border was not affected by the treatment. The results indicate that NT-3 increases the number of the grafted fetal SCN neurons with VP or VIP immunoreactivity, either through stimulation of cell survival or by stimulation of VP and VIP peptide expression, and suggest also a positive effect on the outgrowth of VP-positive SCN fibers. Adenoviral vector-mediated gene transfer appears to be an effective method to direct expression of neurotrophic factors in grafts of fetal CNS tissue at such levels that it significantly increases the number of surviving grafted neurons.
Introduction

The suprachiasmatic nucleus (SCN) of the hypothalamus plays a pivotal role in the generation and regulation of circadian rhythmicity of a variety of physiological functions in mammals. Although the importance of the SCN as a biological clock has been widely recognized, the molecular basis of circadian rhythm generation is only beginning to be understood. The first clock gene was identified in Drosophila melanogaster [28 reviewed in 23], and recently the first mammalian homologues have been identified [7, 27]. The clock genes appear to encode for proteins which resemble transcription factors and thus might regulate transcription of target genes in a circadian fashion. The circadian signal of the SCN is transmitted to other brain regions via extensive fiber projections of SCN neurons, expressing amongst others the neuropeptides vasopressin (VP) and vasoactive intestinal polypeptide (VIP) (see [13]). Transplantation of the SCN has been one of the strategies to study the contribution of these subpopulations of SCN neurons to the clock function. Grafting of the fetal SCN in adult SCN-lesioned (SCNX) rats and hamsters can reinstate circadian rhythmicity of several behavioral parameters to some degree [1, 2, 10, 17, 19, 21, 32, 38]. Re-establishment of proper neural projections is presumed to contribute to the occurrence of restoration of circadian rhythms. Outgrowth of fibers from the grafted SCN towards SCN target areas of the host is indeed observed, but the density of reinnervation is low and the pattern highly variable [2, 10, 21, 30, 32, 44]. Griffioen et al. [21] reported that SCN efferent outgrowth is always present in drinking rhythm-restored SCNX rats, while Sollars and Pickard [44] reported a correlation between outgrowth of VIP fibers in the paraventricular nucleus of the thalamus and restoration of a circadian wheel-running rhythm of SCNX hamsters. However, in hamster, encapsulated SCN transplants were also capable of restoring circadian wheel-running rhythm of SCNX animals, suggesting that at least in this species a rhythmically released humoral factor is sufficient for rhythm restoration of locomotor activity [42].

Restoration of circadian rhythms in SCNX rodents by a transplanted SCN is restricted to overt behavioral rhythms, like wheel-running and drinking rhythms, whereas hormonal rhythms are not restored (see [31]). In rat, recovery of circadian rhythmicity is found in only 40-50% of the transplanted animals [3, 10, 21]. Transplantation results might be improved by application of neurotrophic factors, as shown in other transplantation models. Cell survival and neuritic growth of transplanted fetal dopaminergic and chromaffin cells was increased, and concomitantly functional restoration of behavioral deficits was improved by neurotrophin 4/5, basic fibroblast growth factor and nerve growth factor [16, 24, 46]. Exposure of a SCN transplant to neurotrophic factors may thus counteract the considerable neuronal loss that has been observed in SCN transplants during the first week following transplantation [12, 48, 49] and might promote neuritic outgrowth of transplanted SCN neurons. Expression of TrkB (the receptor for brain-derived neurotrophic factor, BDNF) and TrkC (the receptor for neurotrophin-3, NT-3) has been reported in the perinatal and adult hypothalamus [4, 29, 34, 36, 43, 47], indicating that either BDNF or NT-3 could potentially promote survival and integration of transplanted fetal SCN neurons.
In the present study expression of NT-3 was directed in SCN transplants by means of adenoviral vectors. Previous studies have shown that ex vivo adenoviral vector-mediated gene transfer results in long-term expression of the reporter gene LacZ in neurons and glial cells in SCN transplants [12,48]. The adenoviral vector used in the present study (AdNT-3), had previously been shown to direct expression of biologically active NT-3 [18]. SCN-lesioned Wistar and Brattleboro rats received mock-transduced, AdLacZ-transduced or AdNT-3-transduced fetal SCN transplants at the site of the lesion. The expression of adenoviral vector-derived LacZ- and NT-3 mRNA was monitored by in situ hybridization and β-galactosidase (β-gal) was visualized by immunocytochemistry. The effect of NT-3 on survival and neuritic outgrowth of transplanted SCN neurons was investigated by immunocytochemistry for the C-terminal of the VP precursor molecule (C-terminal propressophysin; CPP) and for VIP. Vasopressin-deficient Brattleboro rats were used as hosts to study the effect of NT-3 on the outgrowth of VPergic fibers of the transplanted SCN into SCN target areas in the host brain.

Material and methods

Animals and experimental groups
Male Wistar rats and VP-deficient (di/di) Brattleboro rats (Harlan, Zeist, The Netherlands) were kept under standard conditions and a 12 h light/12 h dark (LD) regime (lights on at 7.00 a.m., 250 lux), with food and water available ad libitum. Animals were SCN-lesioned when weighing 280-350 g (Wistar rats) or 220-280 g (Brattleboro rats). Fetal SCN tissue was obtained from E17 Wistar donors (E0 being the day after overnight mating). Fetal E17 and E19 and postnatal P1 and P9 Wistar rats were used for in situ hybridization for the TrkC receptor.

Three experiments were performed to investigate the effect of the viral infection procedure and the effect of adenoviral vector-mediated NT-3 expression on the VP and VIP cell populations of the grafted SCN. One series of SCN lesioned (SCNX) Wistar rats received a mock-transduced graft (n=7) or an AdLacZ-transduced graft (n=10) at 4 to 9 weeks post-lesion and were perfusion fixed at 10 or 30 weeks post-grafting. The data of the rats were pooled for statistical analysis of the results, since it has previously been shown that the final staining pattern of the transplanted E17 SCN for CPP and VIP is established 4 to 5 weeks post-grafting [21]. A second series of SCNX Wistar rats received an AdLacZ-transduced graft (n=6) or an AdNT-3-transduced graft (n=9) at 9 to 13 weeks post-lesion. Two rats of each treatment group were decapitated at 10 weeks post-grafting for in situ hybridization of LacZ and NT-3 mRNA. The remaining rats were perfusion fixed at 25 weeks post-grafting. A third series consisted of SCNX Brattleboro rats in order to specifically evaluate the effect of NT-3 on the outgrowth of VPergic fibers of the grafted SCN into the host brain. These rats received an AdLacZ-transduced graft (n=6) or an AdNT-3-transduced graft (n=7) at 4 weeks post-lesion and were decapitated for in situ hybridization (2 rats of each treatment group) or perfusion fixed at 10 weeks post-lesion. The experimental procedures were approved by the Animal Care and Use Committee of the Dutch Royal Academy of Sciences.
Construction of the recombinant adenoviral vector

Construction of the adenoviral plasmids and the recombinant adenoviral vectors containing the LacZ or the NT3 gene under control of the CMV promotor, has been described in detail before [18,25]. In the present study, adenoviral vector stock solutions were used of $1.7 \times 10^{11}$ plaque forming units (pfu)/ml AdLacZ and $9 \times 10^{10}$ pfu/ml AdNT-3.

Surgery

Rats were subjected to bilateral electric SCN lesions and donor SCN tissue for transplantation was dissected from E17 Wistar rats fetuses as described previously [21]. Prior to grafting, the tissue of a single fetus was cut into several pieces and incubated for 18 h (35°C, 5% CO₂) in 50 µl of a chemically defined medium (R16 [37]) containing either no adenoviral vector (mock-transduced), AdLacZ ($5 \times 10^8$ pfu/ml), or AdNT3 ($5 \times 10^8$ pfu/ml) [12,48,49]. Subsequently, the tissue was rinsed with culture medium, and the pieces of two fetuses were pooled and kept in the incubator until transplantation. SCN tissue was aspirated in a metal transplantation cannula (OD 0.9 mm, ID 0.6 mm) and implanted at the lesion site (third ventricle) of the SCNX acceptor rats within 5 h after the viral transduction period. SCN lesion and transplantation surgery was performed under Hypnorm anaesthesia (0.1 ml i.m./ 100 g b.wt.; Janssen Pharmaceutica, Tilburg, The Netherlands), and after surgery the animals received Temgesic (0.01 ml s.c./ 100 g b.wt.) in order to minimize distress.

In situ hybridization

Digoxigenin-labeled riboprobes for trkC, LacZ and NT-3 were generated by in vitro transcription of the respective cDNAs [20]. The trkC riboprobe was transcribed from the first 1.1 kb of the human TrkC receptor comprising the extracellular domain and a small part of the transmembrane region [33]. After decapitation of the fetal, postnatal and grafted adult rats, the heads (fetal rats) or the brains (postnatal and adult rats) were quickly isolated and immediately frozen on dry ice-cooled isobutane and stored at -80°C until use. Coronal cryostat sections (20 µm) were thaw-mounted on 3-aminopropyl-triethoxysilane-coated slides, fixed for 20 min with 4% paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.4 (PF) and washed twice with 0.1 M phosphate buffered saline pH 7.4. Hybridization was performed as described [18] with a hybridization temperature of 60°C for the trkC probe and 55°C for the LacZ and NT-3 probes. Digoxigenin was detected with alkaline phosphatase-conjugated anti-digoxigenin antibody using nitroblue tetrazolium (0.34 mg/ml) and bromochloroindolyl phosphate (0.18 mg/ml) as substrates. Alternate sections were stained with thionin to visualize the anatomy of the developing brain or the location of the transplant. Structures of the developing brains were identified on the basis of the brain atlas of Altman and Bayer [6].

Immunocytochemistry

Animals were transecardially perfused with 100 ml ice-cold saline followed by 300 ml ice-cold PF under deep Nembutal anaesthesia. Brains were removed and post-fixed for 48 h.
A 1 cm thick coronal slice of the hypothalamus containing the transplantation site was gelatin-embedded [22] and fixed for 18 h. Alternate 50-μm vibratome sections were stained for CPP, VIP and β-gal. The procedure entailed overnight incubation at 4°C of free-floating sections with the polyclonal rabbit antibodies against CPP (C3final 1:2000 [50], kindly provided by J.P.H. Burbach, Rudolf Magnus Institute for Neurosciences, University of Utrecht, The Netherlands) or VIP (Viper 1:2000) [14], or with a monoclonal mouse antibody against β-gal (Gal-13 1:2000, Sigma Immunochemicals, St. Louis, MO). Sections for CPP and VIP staining were subsequently incubated for 1 h with goat-anti-rabbit IgG serum (1:100) at room temperature, followed by 1 h of incubation with rabbit peroxidase-antiperoxidase (PAP 1:1000). Sections for β-gal staining were incubated for 1 h with rabbit-anti-mouse IgG coupled to horseradish peroxidase (1:100, DAKO, Glostrup, Denmark). All antibodies were diluted in 0.05 M Tris-buffered saline pH 7.6 containing 0.5% Triton X-100 and 0.25% gelatin. Antigen-antibody binding sites were visualized with the diaminobenzidine (DAB) reaction in the presence of 0.2% ammonium nickel sulphate.

Data evaluation
Microscopical evaluation of the sections stained for CPP, VIP and β-gal was performed without knowledge of the treatment of the transplant. Sections were semiquantitatively analyzed for: i) location and size of the transplants (+ small transplants, up to +++ large transplants filling nearly the third ventricle), ii) the estimated number of β-gal-positive cells in the transplants (- no cells, + scattered cells, ++ high density of cells), iii) the number of SCN cell clusters in the transplants, iv) the estimated number of CPP-positive and VIP-positive cells (+/- a small cluster of cells or some scattered cells, + and ++ for increasing numbers of cells, +++ for several large cell clusters), v) the estimated number of CPP- and VIP-positive fibers in the transplant (+/- for a few fibers, + and ++ for increasing density of fibers, +++ for a dense fiber network throughout large parts of the transplant, and vi) the occurrence of CPP- and VIP-positive fibers crossing the graft/host border (- no fibers, +, ++ and +++ increasing numbers of fibers). The parameters were compared between mock- and AdLacZ-transduced transplants and between AdLacZ- and AdNT-3-transduced transplants in order to respectively investigate the effect of the transduction procedure and of the NT-3 expression on the transplanted SCN. Results are presented as median scores for each treatment group. Differences between the treatment groups were tested by two-tailed Mann-Whitney U test corrected for ties (see [15]). For the AdLacZ- and AdNT-3-transduced SCN transplants in the Brattleboro rats, evaluation of the CPP immunocytochemistry was extended to the localization of the CPP-positive SCN fibers in the host brain. The following areas were screened for the presence of these fibers (according to the atlas of Swanson [45]: anteroventral periventricular nucleus of the hypothalamus (AVPV), suprachiasmatic preoptic nucleus (PSCN), median preoptic nucleus (MEPO), preoptic periventricular nucleus (PVpo), medial preoptic area (MPO), subparaventriculaire zone (sPVz), paraventricular nucleus of the hypothalamus (PVN), retrochiasmatic area (RCH), and the dorsomedial nucleus of the hypothalamus (DMH) (scores from - to ++++, see above).
Results

TrkC mRNA expression in the perinatal hypothalamus

Expression of trkC mRNA was observed throughout the fetal and postnatal hypothalamus including the SCN. At E17, the age at which the premature SCN is dissected for transplantation, the expression levels in the SCN and the rest of the hypothalamus were relatively low except for the anteroventral nucleus. At E19 (Figure 1), P1 and P9 (not shown) expression of trkC was observed in a compact cell cluster corresponding to the developing SCN in the thionin-stained sections. At E19 (Figure 1) and P1, the anteroventral nucleus showed the highest expression level in the hypothalamus. In extra-hypothalamic areas, relatively high expression was found in the hippocampus and the striatum, which corresponds to the fetal expression pattern documented by others [29]. In the thalamus, high expression was found in the anteromedial and anteroventral nuclei of E19, P1 and P9 brains. Sections hybridized with a sense trkC probe displayed no specific staining pattern (not shown).

Adenoviral vector-mediated transgene expression in SCN transplants

Viable transplants were found in all rats. The transplanted tissue pieces had merged into one or two transplants which were in almost all rats located in the third ventricle close to the lesion site. All transplants contained one or more SCN cell clusters as shown by

Figure 1. Distribution of trkC mRNA in E19 rat hypothalamus.

(A) Coronal section of the anterior hypothalamus. TrkC mRNA is present in low levels throughout the hypothalamus, and in higher levels in the SCN and the anteroventral nucleus of the hypothalamus (AVH). (B) Adjacent thionin-stained section confirming the location of the SCN. OC, optic chiasm. Scale bar = 50 μm.
Figure 2. Longterm transgene expression in SCN grafts upon adenoviral mediated gene transfer. (A) Immunocytochemistry for β-gal at 25 weeks after transduction and transplantation reveals the presence of many transduced neurons and glial cells. (B,C) In situ hybridization for respectively LacZ mRNA or NT-3 mRNA at 10 weeks after transduction and transplantation reveals comparable numbers of transduced cells throughout the transplant expressing variable levels of the transgene. 3V, third ventricle. Scale bar = 25 μm.

Effect of adenoviral vector-mediated expression of NT-3 on the transplanted SCN

The transplant size, the number of SCN clusters and the different features of the CPP and VIP staining showed no statistical differences between the mock- and AdLacZ-transduced transplants (series 1; Table 1). The AdNT-3-transduced SCN transplants were larger compared to AdLacZ-transduced transplants (Table 1), but this effect was not significant (Wistar host: p=0.21, Brattleboro host: p=0.076). The AdNT-3-transduced transplants contained more SCN cell clusters in the Brattleboro hosts (p=0.016), but not in the Wistar hosts (p=0.32). In both Wistar and Brattleboro hosts, the number of CPP-positive cells and VIP-positive cells were higher in the AdNT-3-transduced transplants, although the...
increase in VIP neurons in the Wistar hosts just failed to reach significance (CPP: p=0.050 for both series, VIP: p=0.076 and 0.016, respectively). The median scores for CPP- and VIP-positive fibers in the AdNT-3-transduced transplants were higher in the Brattleboro hosts, but this increase was statistically significant for the VIP-positive fibers only (p=0.15 and 0.036, respectively). No effect was observed on the number of CPP- or VIP-positive fibers in the Wistar hosts, nor on the number of CPP- and VIP-positive fibers crossing the graft-host interface in both the Wistar and Brattleboro hosts (Table 1). Figure 3 illustrates several SCN cell clusters and fiber growth in two AdNT-3-transduced SCN transplants.

Although the number of CPP-positive fibers showing graft-to-host passage appeared not to be significantly different between AdNT-3- and AdLacZ-transduced transplants in the Brattleboro rats (Table 1), the median scores of CPP-positive fiber density found in the AVPV, MEPO, PVpo, MPO, sPVz, RCH and DMH, and especially the PSCN, were higher for the AdNT-3 group (Table 2). The variability in the scores was however high in both groups and the difference in scores was statistically not significant for any of the areas (p=0.15, 0.10 and 0.13 for the AVPV, PSCN and RCH, respectively). A striking observation was the predominant frontally directed outgrowth of the CPP-positive fibers: the PSCN and the PVpo were relatively densely innervated in some AdNT-3 rats, whereas more caudal parts like the sPVz and the DMH were usually more sparsely innervated. Examples of transplant-derived CPP fiber growth in the host brain are shown in Figure 4.

**Discussion**

In the present study, fetal SCN transplants were transduced with an adenoviral vector encoding for NT-3 (AdNT-3) to examine the effect of this neurotrophin on survival and

| Table 1. Microscopical evaluation of mock-transduced vs AdLacZ-transduced SCN transplants (series 1) and of AdLacZ-transduced vs AdNT-3-transduced transplants placed in SCNX Wistar or Brattleboro rats (series 2 and 3). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | series 1 Wistar | series 2 Wistar | series 3 Brattleboro |
| number of animals | 7               | 10              | 4               | 7               | 4               | 5               |
| transplant size  | ++              | +/+             | ++              | ++              | ++              | ++              |
| number of SCN clusters | 3               | 3               | 2               | 3               | 2               | 4*              |
| CPP staining density of cells | ++              | ++              | ++              | ++              | ++              | ++              |
| fibers           | ++              | ++              | ++              | ++              | ++              | ++              |
| crossing fibers  | ++              | +               | ++              | +++             | ++              | ++              |
| VIP staining density of cells | ++              | ++              | ++              | ++              | ++              | ++              |
| fiber            | ++              | +               | ++              | ++              | ++              | ++              |
| crossing fibers  | +/+              | +               | +               | +               | n.a.           | +               |

Results are given as median scores. For explanation of the scores see Materials and Methods section. Asterisks indicate a significant difference (two-tailed Mann-Whitney U test corrected for ties; p<0.05). N.a., not available.
Table 2. Survey of the innervation pattern of CPP-positive fibers in the brain of Brattleboro host rats 70 days after grafting of AdLacZ- or AdNT-3-transduced fetal SCN tissue.

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nr., rat number; AVPV, anteroventral periventricular nucleus of the hypothalamus; PSCN, suprachiasmatic preoptic nucleus; MEPO, median preoptic nucleus; PVpo, preoptic periventricular nucleus; MPO, medial preoptic area; sPVz, subparaventriculaire zone; PVN, paraventricular nucleus of the hypothalamus; RCH, retrochiasmatic area; DMH, dorsomedial nucleus of the hypothalamus. For explanation of scores see Materials and Methods.

NT-3 effect on VP and VIP neurons

NT-3 has been shown to promote neuronal survival [8], cellular proliferation and differentiation [26,53] and neuritic outgrowth [35,39,40].

The observed increase in VP (CPP)- and VIP-positive cell numbers in the AdNT-3-transduced transplants might, first of all, be attributed to a cell survival-enhancing effect of NT-3. A considerable cell loss is reported in several transplantation studies. In fetal SCN transplants, cell loss could be inferred from central necrosis in solid SCN transplants one week post-grafting [12,48] and from a limited survival of VP- and VIP-positive neurons in SCN cell suspensions grafts [11,41]. Counteraction of this cell death might explain the observed effect of NT-3 on the number of SCN neurons, and on the consistent, but nonsignificant, increase in size of the solid SCN transplants observed in the present study.

A proliferative effect of NT-3 on the SCN cells is not likely to explain the increase in VP and VIP neurons, since the neurons that are destined to form the SCN have ceased proliferating at E17, the time point of viral transduction and transplantation [5,9]. Stimulation of proliferation of non-SCN cells in the transplant might have occurred however, since the E17 hypothalamic transplant contains parts of the third ventricle germinal zones. Such a proliferative effect might have contributed to the increased size of the AdNT-3-transduced transplants. Differentiating effects of NT-3 have been described...
Figure 3. Immunocytochemistry for CPP and VIP of AdNT-3-transduced SCN transplants. 
(A,B) CPP staining and VIP staining of adjacent sections reveals the presence of a large cluster of SCN neurons in a transplant in a Wistar host. (C) CPP staining of a transplant in a Brattleboro host reveals two large clusters of SCN neurons and extensive fiber growth throughout the transplant. 3V, third ventricle; HNST, hypothalamo-neurohypophysial system tract; OC, optic chiasm. Scale bar = 25 μm (A,B) or 50 μm (C).

for several cell types, including fetal hippocampus neurons and fetal motor neurons [51,52]. In these studies, the numbers of cells expressing respectively calbindin and choline acetyltransferase, and the levels of expression were increased. In the present study, staining intensities for CPP and VIP were equally high in the mock-, AdLacZ- and AdNT-3 transduced transplants, suggesting that expression of these peptides is not greatly enhanced by NT-3. A differentiating effect of NT-3 on the VP- and VIP-positive SCN neurons of the grafts can, however, not be excluded.

A neuritic outgrowth-stimulating effect of NT-3 is suggested by an increase in the number of CPP- and VIP-positive fibers in the transplants. The higher number of fibers is however likely to be related to the higher number of CPP- and VIP-positive cells in the AdNT-3-transduced transplants. The effect of NT-3 on cell numbers was most pronounced for VIP in the transplants in the Brattleboro rats, coinciding with the most pronounced increase in VIP fibers in these hosts. Quantification of neuritic growth in a SCN cell suspension culture could elucidate whether NT-3 has an effect on the neuritic outgrowth of the VPergic and VIPergic SCN neurons.

A possible effect of NT-3 on the outgrowth of CPP- and VIP-positive fibers of the transplanted SCN into the host brain is difficult to evaluate because of the presence of
VPerigic and VIPergic fiber pathways of non-SCN origin and the presence of remnants of the endogenous SCN in some of the host brains. As reported in previous studies [10,21],

Figure 4. Immunocytochemistry for CPP reveals fibers derived from the transplanted SCN neurons in the VP-deficient Brattleboro host brain. (A) A high density of CPP-positive fibers is present in the preoptic periventricular nucleus (PVpo) and the suprachiasmatic preoptic nucleus (PSCN) of rat #507-II (score +++ and ++, respectively; see Table 2). The AdLacZ-transduced transplant of this animal is located at the bottom of the third ventricle caudal from the present section. The CPP-positive fibers of the transplanted SCN neurons ran bilaterally in a fronto-dorsal direction along the third ventricle. (B,C) A frontal section at the level of the anteroventral periventricular nucleus of the hypothalamus (AVPV) and a more caudal section at the level of the transplant (T) and the subparaventricular zone (sPVz) of rat #5000-IV, illustrating outgrowth of CPP-positive fibers of an AdNT-3-transduced transplant. The density of CPP-positive fibers in the AVPV and the sPVz area was given the scores +++ and ++, respectively (see Table 2). Note that no fibers are visible that cross the graft-host interface at this level (C). (D) A SCN cell cluster in the AdNT-3-transduced transplant of rat #503 at the level of the endogenous SCN showing CPP-positive fibers that cross the midline and run into the contralateral side. (E) The AdNT-3-transduced transplant (T) of rat #5000-I, located relatively caudal, revealed prominent outgrowth of CPP-positive fibers into the retrochiasmatic area (RCH) (score +++; see Table 2). 3V, third ventricle. Scale bar = 25 μm.
only a limited number of CPP- and VIP-positive fibers appeared to cross the graft-host interface. This number was not different between the AdLacZ-transduced grafts and AdNT-3-transduced grafts in the Wistar and Brattleboro hosts. However, the number of these fibers appeared not to predict the number of fibers that eventually reach host target areas, since in the Brattleboro rat, which allowed a good evaluation of the outgrowth of CPP-positive fibers, the number of CPP-positive fibers showed a tendency to be higher in the AdNT-3 treatment group. Previous studies have indicated that the density and the direction of neuritic outgrowth of transplanted SCN neurons into the host brain depends on disruption of the ventricular wall and on the location of the transplanted SCN, respectively [2,21,30,44]. These factors result in a high variability in the pattern of neuritic outgrowth, also in the present study.

In conclusion, ex vivo transduction of fetal SCN tissue with an adenoviral vector encoding for NT-3 resulted in a higher number of VP- and VIP-positive SCN neurons in the grafts. Moreover, a positive effect on the outgrowth of CPP-positive fibers in the Brattleboro host is suggested. The present results illustrate the potential of ex vivo gene transfer to transplants of CNS origin as a way to improve survival of the transplanted neurons. Adenoviral vectors can transduce most if not all CNS cell types, so that the neurotrophins can be produced in the transplant itself. The currently used transduction method could not only be applied to other transplantation models, as well, but offers the exciting possibility to study the effect of expression of for instance the newly discovered clock genes on circadian rhythmicity.

References


Chapter 9