

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**

Open access books available

**122,000**

International authors and editors

**135M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Gene Therapy for Chronic Pain Management

---

Isaura Tavares and Isabel Martins

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55050>

---

## 1. Introduction

This chapter provides an overview of the main current applications of gene therapy for chronic pain in what concerns animal studies and putative clinical applications. The value of gene therapy in unravelling neuronal brain circuits involved in pain modulation is also analysed. After alerting to the huge socioeconomic impact of chronic pain in modern societies and justifying the need to develop new avenues in pain management, we review the most common animal studies using gene therapy, which consisted on deliveries of replication-defective viral vectors at the periphery with the aim to block nociceptive transmission at the spinal cord. Departing from the data of these animal studies, we present the latest results of clinical trials using gene therapy for pain management in cancer patients. The animal studies dealing with gene delivery in pain control centres of the brain are analysed in what concerns their complexity and interest in unravelling the neurobiological mechanisms of descending pain modulation. The chapter will finish by analysing possible futures of gene therapy for chronic pain management based on the development of vectors which are safer and more specific for the different types of chronic pain.

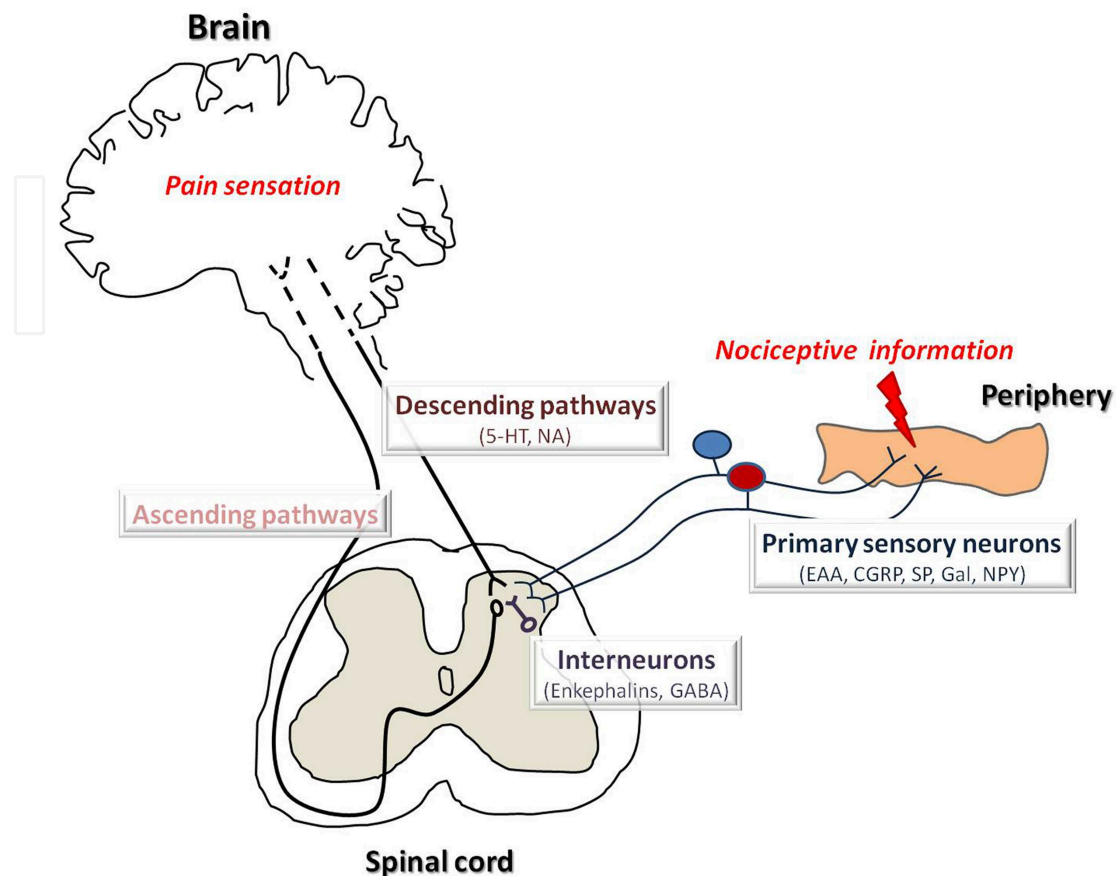
## 2. Chronic pain: A burden for modern societies

Pain is not easy to define since it is a highly subjective experience. The more consensual definition of pain was provided by the International Association for the Study of Pain (IASP) and states that “*Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*” [1]. Acute pain is important as an alert signal to potentially threaten situations (internal or external to the organism) and it is important for survival. Acute pain may progress to chronic pain which, according to IASP, is the pain that lasts more than 3 months and persists beyond the normal tissue healing time [2].

Chronic pain may be divided into "nociceptive" and "neuropathic" [3]. Nociceptive pain is caused by activation of nociceptors, the thin nerve fibers which convey nociceptive input from the periphery to the spinal cord. Neuropathic pain is caused by malfunction or damage of the nervous system. Neuropathic pain is frequently difficult to treat being associated to spontaneous pain, exaggerated responses to nociceptive stimuli (*hyperalgesia*) and nociceptive responses to stimuli which are usually non-nociceptive (*allodynia*).

The number of people affected by chronic pain is increasing due to multifactorial causes such as increasing aging of the population. In Europe, about 20% of people suffer from moderate to severe chronic pain [4]. In the United States the prevalence of chronic pain ranges from 2% to 40%, with a median of 15% [5], which cost the country 560 to 635 million dollars [6]. People suffering from chronic pain are less able to walk, sleep normally, perform social activities, exercise or have sexual relations. Chronic pain strongly affects the productivity. About 60% of chronic pain patients are unable or less able to work, 19% lost their jobs and 13% change jobs due to their pain [6]. Chronic pain is associated to several co-morbidities, namely depression and anxiety [6]. Besides all of these indirect costs, chronic pain is a burden due to direct costs of pain management. Despite major investments in basic and clinical pain research, the available analgesics remain considerably unchanged during the last decades. Opioids are useful to manage several pain types but they have a modest efficacy in several pain conditions (e.g. neuropathic pain). Furthermore, long term treatments with opioids frequently induce severe off-target effects, like nausea, constipation and addiction [7]. Intractable pain remains a clinical problem and a drama for the patients and their families [8]. During the last decade, pain clinicians and pain researchers were challenged to search for alternatives to conventional pain treatment, which should be more specific and sustained than conventional analgesics. Gene therapy outstands as a powerful technique to overcome some current problems of chronic pain treatments.

Neurobiological research in the pain field provided solid information regarding the transmission and modulation of nociceptive information from the periphery to the brain, where a pain sensation is produced (Fig. 1). Nociceptive signals are conveyed by primary afferent fibers from peripheral organs, like the bladder or muscles, to the spinal cord. This is the first relay station involved in the modulation of nociceptive information namely by local inhibitory interneurons that use opioid peptides or aminoacids ( $\gamma$ -aminobutyric acid-GABA- and glycine). Nociceptive information is then transmitted supraspinally, namely to the thalamus, and to several brainstem areas, where additional modulation of the nociceptive signal occurs. The thalamo-cortical pathway ensures that the nociceptive information reaches the somatosensory and prefrontal cortices, where the nociceptive signal is finally perceived as a pain sensation [9, 10]. Some brain areas which directly or indirectly receive nociceptive information from the spinal cord are also involved in descending pain modulation. Both inhibition and facilitation may occur and chronic pain may derive from a reduction of the former and enhancement of the latter [9, 11]. This neurobiological knowledge has been used to design gene therapy studies for chronic pain, namely to choose the somatosensory system areas and neurotransmitters/receptors to be targeted in order to block nociceptive transmission.



**Figure 1.** Schematic diagram of pain pathways involved in pain transmission and modulation. **Nociceptive information** is transmitted from the periphery to the spinal dorsal horn by primary sensory neurons. At the spinal level, these neurons transmit nociceptive information to second order neurons ("Ascending pathways") through the release of neurotransmitters like the excitatory amino acids (EAA) glutamate and aspartate, calcitonin gene-related peptide (CGRP), substance P (SP) galanin (Gal) and neuropeptide Y (NPY). In the brain, the nociceptive information is then perceived as a **pain sensation**. The transmission of nociceptive information at the spinal level is modulated by interneurons (mainly inhibitory) through the release of opioid peptides and GABA and also by supraspinal descending neurons ("Descending pathways") through the release of serotonin (5-HT) and noradrenaline (NA). Descending pathways may inhibit or enhance nociceptive transmission from the spinal cord.

Gene therapy is an especially versatile tool for chronic pain management since it is based in a triad of controllable parameters: the vector, the transgene and the promoter. By knowing the neurobiological features of each chronic pain type, namely the neurotransmitters and receptors affected, it is possible to design gene therapy strategies based on the best combination of vectors, transgenes and promoters. As to **vectors**, gene therapy for pain uses mainly "vehicles" which have a "certified" experience in infecting neurons, namely replication-defective forms of viruses. Non-viral vectors have seldom been used in gene therapy studies for pain but their transduction efficiency and specificity are much lower than those of viral vectors. Some of these vectors have the ability to migrate retrogradely (i.e., contrary to the direction of nerve impulse) which is very useful to target neurons that are located in structures of difficult surgical

access. A good example is the application of replication-defective forms of Herpes Simplex Virus type 1 (HSV-1) at the periphery (e.g. the skin) to transduce neurons at the spinal ganglia (dorsal root ganglia-DRGs), which are difficult to access due to their bone protection. Regarding the **transgenes** to include in the vectors for gene therapy of pain, it is possible to increase the expression of neurotransmitters and receptors involved in nociceptive inhibition (e.g. opioids), neurotrophic factors or substances with anti-inflammatory properties. Finally, and in what concerns the **promoters**, it is possible to choose those that restrict transgene expression to a cell type, such as a neuron or a glial cell, or even target selective neurochemical neuronal populations. Examples of neuron-specific promoters are synapsin I, calcium/calmodulin-dependent protein kinase II, tubulin alpha I and neuron-specific enolase [12]. Some possibilities of controlling the vectors, transgenes and promoters will be discussed in the next two sections using gene therapy in animal models.

### 3. Gene therapy targeting the spinal cord in animal pain models

One of the main advantages of experimental gene therapy studies is that they can be performed using several pain models. This is important since each pain type may induce specific changes in neuronal circuits devoted to the transmission and modulation of nociceptive transmission [13]. Studies of gene therapy for pain have used clinically relevant models of inflammatory [14-22] and neuropathic pain [23-34]. In a much lower incidence, models of acute [35-38], post-operative pain [39] and cancer [40] pain have been used in experimental gene therapy studies. The large majority of studies were performed in pain models affecting the limbs or the trunk, in the latter case being of visceral origin [22, 37]. Two studies used gene therapy to block nociceptive transmission coming from the head/face in pain models that reproduces some types of craniofacial pain, like trigeminal neuralgia [41] or temporomandibular joint disorders [42].

Gene therapy studies for pain in animal models may be divided in studies targeting the spinal cord (Table 1) and studies directed to pain control centres located in the brain (Table 2). Studies directed to the spinal cord mainly aim to manipulate the expression of transgenes in order to block the transmission of nociceptive input at the spinal dorsal horn (Table 1). Most of the spinal cord studies using gene therapy for pain elected HSV-1 as the most suitable vector, due to its natural affinity to the neuron and its ability for retrograde transport [43]. HSV-1 has the additional advantage over other vectors of carrying multiple transgenes or large transgenes and not integrating in the host genome, which reduces the possibility of mutagenic events [44, 45]. After application of replication-defective forms of HSV-1 at the periphery in order to transduce DRG neurons (or trigeminal ganglion neurons), delivery of the transgene product by the spinal branch of transduced neurons at the spinal dorsal horn induced analgesia in several rodent models of pain (Table 1). Gene therapy in animal models of craniofacial pain [41, 42] aimed to release the transgene products at the level of the spinal trigeminal nucleus and this structure is homolog of the spinal cord, which prompted to include these studies in the section devoted to spinal cord studies.

Pain models	Gene product	Inoculation	References
<b>Herpes Simplex type 1</b>			
Acute pain	Pre-proenkephalin	Subcutaneous	[35]
Inflammatory pain	Pre-proenkephalin A	Subcutaneous	[14]
Neuropathic pain	Pre-proenkephalin A	Subcutaneous	[41]
Cutaneous hyperalgesia	Pre-preproenkephalin	Subcutaneous	[36]
Bladder hyperactivity	Pre-preproenkephalin	Bladder wall	[37]
Inflammatory pain	Endomorphin-2	Subcutaneous	[15]
Neuropathic pain	Endomorphin-2	Subcutaneous	[23]
Neuropathic pain	IL-4	Subcutaneous	[24]
Neuropathic pain	sTNFRs	Subcutaneous	[25]
Neuropathic pain	GAD	Subcutaneous	[26, 47]
Chronic pancreatitis	Pre-proenkephalin	Pancreas surface	[22]
Inflammatory pain	Na <sub>v</sub> 1.7 antisense	Subcutaneous	[16]
Incision pain	Pre-proenkephalin	Subcutaneous	[39]
Cancer pain	Pre-proenkephalin	Subcutaneous	[40]
<b>Adenovirus</b>			
Inflammatory pain	GAD	Trigeminal ganglion	[42]
Neuropathic pain	IL-10	Intrathecal	[27]
Inflammatory pain	$\beta$ -endorphin	Intrathecal	[17]
Neuropathic pain	IL-2	Intrathecal	[28]
<b>Adeno-associated vectors</b>			
Neuropathic pain	IL-10	Intrathecal	[29]
Neuropathic pain	shGCH1	Intrathecal	[30]
Neuropathic pain	Prepro- $\beta$ -endorphin	Intrathecal	[31]
Inflammatory pain	$\mu$ -opioid receptors	DRG	[18]
<b>Lentivirus</b>			
Neuropathic pain	GDNF	Intraspinal	[32]
Neuropathic pain	NF $\kappa$ B Repressor	Intraspinal	[33]

**Table 1.** Summary of experimental studies using viral vectors for gene transfer to the spinal cord.

As to the transgenes included in the HSV-1 vectors, opioid peptides or their precursors largely prevail due to their well-known ability to block nociceptive transmission at the spinal cord. HSV-1-based delivery of opioids has additional advantages over classic opioids, namely by

being deprived of major side-effects and preventing tolerance after repeated administrations of the vector [46]. Furthermore, opioid-based gene therapy can be very powerful in inducing analgesia if combined with administration of very low doses of classical opioids [46]. Besides opioid peptides, other transgenes were included in the HSV-1 vectors constructs. A transgene that increases the levels of the inhibitory neurotransmitter GABA, namely by overexpressing its synthesizing enzyme glutamate decarboxylase (GAD), induced analgesia in neuropathic pain models [26, 47]. HSV-1 based vectors have also been used to deliver transgenes that overexpress anti-inflammatory interleukins [24, 48] or the soluble receptor for tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which act as an antagonist of TNF- $\alpha$  in order to block its role as a pro-inflammatory mediator [25, 49]. A decrease in the levels of the  $\alpha$  subunit of the voltage-gated sodium channel 1.7 (Nav 1.7) was also achieved using HSV-1 constructs but with the transgene inserted in antisense orientation [16].

Other viral vectors, namely adenoviruses, adeno-associated viruses and lentiviruses have been used to target the spinal cord, but unlike HSV-1 vectors which have been administered at the periphery following its natural route of retrograde transport to DRG neurons, these vectors were either directly injected into DRGs or trigeminal ganglion neurons, intrathecally or intraspinally (Table 1). The transgenes included in adenoviruses, adeno-associated viruses are similar to those used in HSV-1 vectors, namely opioids [17, 31], interleukins [27-29] and GAD [42]. Adeno-associated vectors have also carried transgenes that overexpress  $\mu$ -opioid receptors [18] or block the expression of GTP cyclohydroxylase (GCH1) using small hairpin RNAs [30]. GCH1 is the rate-limiting enzyme of an essential co-factor for nitric oxide synthase (NOS), which modulates nociceptive transmission. Finally, lentiviral vectors have also been used in gene transfer studies directed to the spinal cord. Based on its ability to restrict transduction to the injection site, lentiviral vectors have been administered intraspinally in the dorsal horn to increase the levels of a neurotrophic factor (glial-derived neurotrophic factor, GDNF) [32] or decrease the expression of Nuclear Factor  $\kappa$ B (NF $\kappa$ B), which regulates cellular inflammation responses [33]. In the latter study, microdelivery of an HIV pseudotyped lentiviral vector into the spinal dorsal horn led to a preferential transgene expression in glial cells. This shows that, besides the promoter, pseudotyping the vector is a way of directing transgene expression and glia is an important target in pain, inasmuch that chronic pain is associated to the activation of glial cells which produce algogenic mediators that exacerbate pain, namely NF $\kappa$ B. All of these approaches showed considerable analgesic efficacy and reduced side effects.

#### **4. Gene therapy targeting for pain: The challenge of targeting pain control circuits in the brain**

Abnormal descending pain modulation from the brain is a common feature of several chronic pain conditions, namely those characterized by widespread pain, like fibromyalgia, which derive from impairments in descending pain inhibition [50]. Studies with gene delivery into the brain (Table 2) are much scarcer than spinal cord deliveries. This is due both to the higher difficulty of surgical approaches to deliver the vectors into the brain and challenges to

manipulate the complex brain neuronal circuits involved in pain modulation. The spinal cord constitutes a less invasive delivery route when the aim is to manipulate descending modulatory pathways (Fig. 1). This delivery route was recently explored by injecting intraspinally an adenovirus vector targeting the expression of a potassium channel into noradrenergic pontospinal neurons, which decreased the activity of those pontospinal neurons and induced hyperalgesia [51]. These experiments confirm the pain inhibiting role of the noradrenergic projections to the spinal cord [52].

Pain models	Gene product	Delivery	References
Acute pain	Proenkephalin	Medullary dorsal reticular nucleus (DRt)	[55]
Neuropathic	Tyrosine Hydroxylase antisense	Medullary dorsal reticular nucleus (DRt)	[34]
Inflammatory	Proenkephalin	Medullary ventral reticular nucleus (VLM)	[19]
Inflammatory	Proenkephalin	Medullary dorsal reticular nucleus (DRt)	[20]
Acute pain	GAD	Insular Cortex	[38]
Inflammatory pain	Preproenkephalin	Amygdala	[21]
Acute and Inflammatory pain	NMDA antisense	Medullary nucleus of the solitary tract	[56]
Inflammatory and neuropathic pain model	Potassium channel (hKir <sub>2.1</sub> )	Pontospinal noradrenergic neurons	[51]

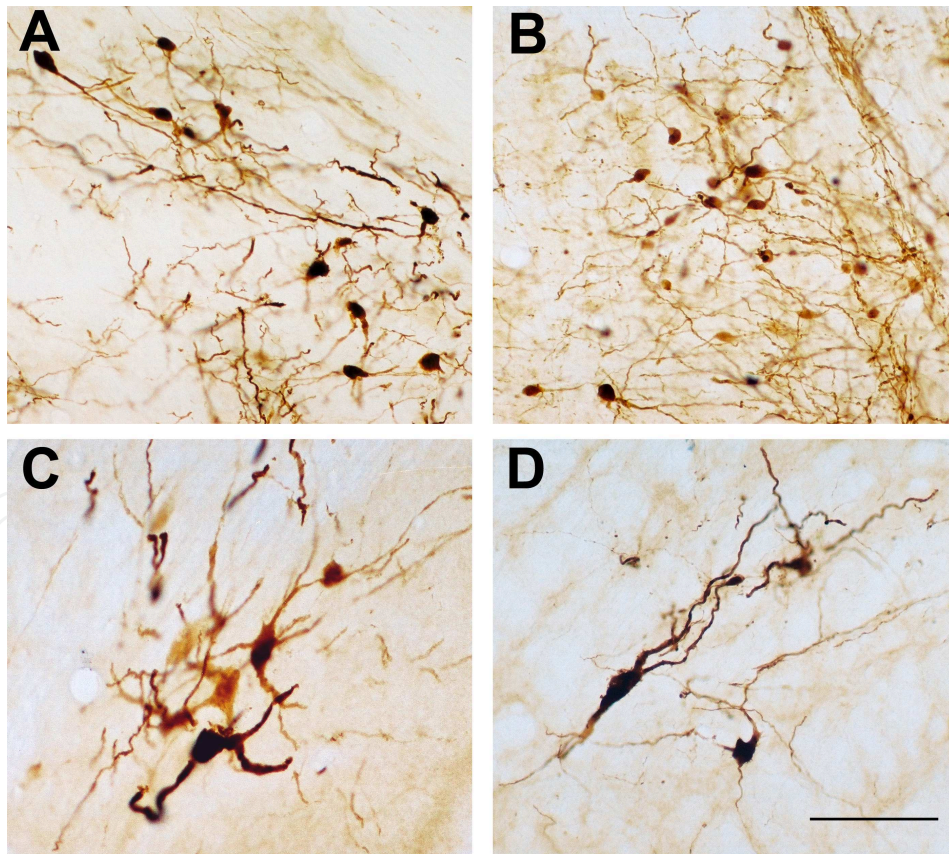
**Table 2.** Summary of animal studies using viral vectors for gene transfer to pain control centers in the brain.

Gene transfer in the brain used almost exclusively HSV-1 vectors to overexpress opioid peptides [19-21, 38] and, in a much more limited extent, GAD [38]. Our research group has a large experience in gene transfer to pain control centres at the medulla oblongata, namely the dorsal reticular nucleus (DRt), the caudal ventrolateral medulla (VLM) and the nucleus of the solitary tract (NTS). These areas were elected based on the extensive neurobiological knowledge of their role in pain modulation [53, 54]. Overexpression of opioid precursors in the DRt and VLM induced analgesia in acute pain tests and models of sustained or chronic inflammatory pain [19, 20, 55]. Brain areas involved in pain control and which are of easier neurosurgical access are the amygdala and the rostral agranular insular cortex. Overexpression of opioid precursors in the central amygdalar nucleus [21] or GAD in the rostral agranular insular cortex induced analgesia in acute pain models [38]. Lentiviral vectors were delivered to the NTS, an area which is crucial in pain and cardiovascular integration, to decrease local expression of N-methyl-D-aspartate (NMDA) receptor, a key receptor for the action of glutamate, and this approach was shown to decrease acute and inflammatory pain [56]. Since glutamate, is the most ubiquitous mediator of excitatory synaptic transmission in the central nervous system



and NMDA receptors are also expressed by glial cells, the effects of gene therapy were restricted to NTS neurons by using the rat synapsin promoter.

There is a puzzling difference between gene therapy studies using HSV-1 vectors at the periphery or the brain. Whereas the ability for HSV-1 to migrate retrogradely is the main feature of studies at the periphery, the migration of HSV-1 in the brain is seldom evaluated. This can confound the effects of gene therapy on pain responses since the effect may derive from transduction of neurons that project to the injected area, and not at the targeted area itself. Our research group has pioneer work in studying the dynamics of HSV-1 migration in the brain after injections of the vector in pain control centres of the medulla oblongata, namely the caudal medulla oblongata (VLM) and the dorsal reticular nucleus (DRt). After injections of a HSV-1 vector expressing the *lacZ* reporter gene, under control of the human cytomegalovirus promoter (hCMV), in pain control centres of the medulla oblongata, migration in VLM and DRt afferents was detected [19, 55] (Fig. 2). However, not all the brain afferents of the VLM and DRt exhibited  $\beta$ -galactosidase ( $\beta$ -gal), the product of *lacZ* expression. For example, the amygdala and the cortex, which are important VLM and DRt afferents [57, 58] did not show neurons expressing  $\beta$ -gal.



**Figure 2.** Dynamics of HSV-1 migration in the brain after injection into the DRt. Photomicrographs of  $\beta$ -gal positive neurons in the cerebellum (A), the parabrachial complex (B), the locus *coeruleus* (C) and the VLM (D) at 7 days post-injection Scale bar D: 100  $\mu$ m (photomicrographs A-C are at the same magnification).

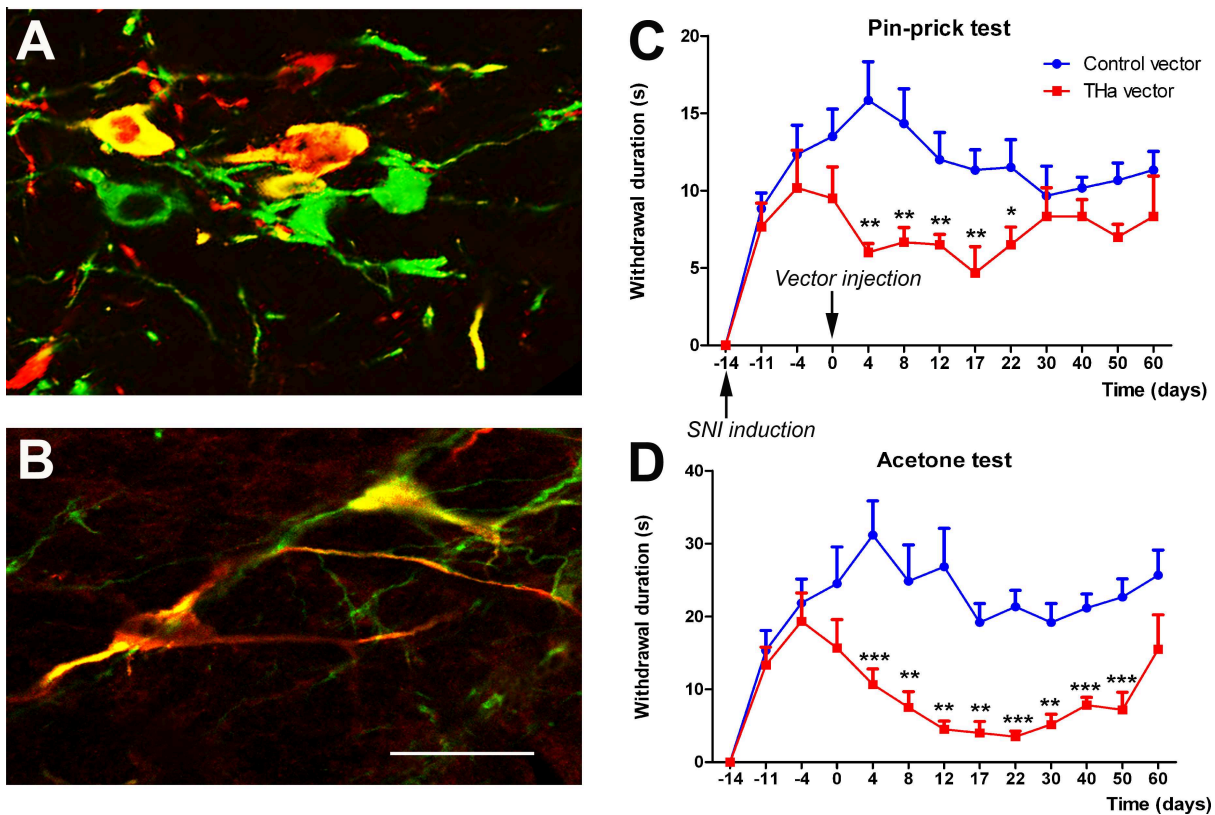
Although it could be argued that this is due to lack of activity of the hCMV promoter in amygdalar and cortical neurons, other studies showed that hCMV is active in those neurons [21, 59]. These results rather point to a selective uptake of HSV-1 vectors injected in the brain parenchyma, probably due to interactions between neuronal receptors and glycoproteins of the HSV-1 envelope. By carefully mapping the brain areas exhibiting retrograde transport after HSV-1 injections in the brain using immunohistochemical detection of the gene reporter and in situ hybridization against the DNA of HSV-1, the problems of affecting brain afferents of the injected area can be circumvented.

The selective migration of HSV-1 in the brain can be a useful feature of the vector. After establishing the dynamics of the migration of HSV-1 in the brain after injection into the DRt (a facilitatory pain control centre of the brain), we used a tissue specific promoter (tyrosine hydroxylase-TH) to direct the expression of the vector to the noradrenergic afferents of the DRt (Fig. 3). Based on the analgesic effects of the administration of  $\alpha$ 1-adrenoreceptor antagonists into the DRt, the TH transgene was inserted in antisense orientation into the vector in order to decrease the levels of noradrenaline in the DRt [34]. A sustained analgesic effect was achieved in a model of neuropathic pain, which reproduces clinically relevant features of neuropathic pain. The fact that the analgesic effects were so long, lasting for 2 months with a single vector injection, and reversed several pain modalities, indicates that targeting pain control centres of the brain needs to be considered both in animal and pre-clinical studies.

## 5. Gene therapy for chronic pain at the bedside: Human studies

The translational perspectives of the studies summarized in section 2, namely those using replication-defective HSV-1 vectors, favoured the approval of clinical trials for gene therapy for chronic pain. An important reinforcement of the proof-of-concept for the potential utility of HSV-based vector in rodent pain models was provided by equivalent studies performed in primates [36]. These studies were important since the translational perspectives of the rodent results were questioned for several reasons, such as the larger size of dermatomes of humans. The first clinical trial of gene therapy for pain was a safety and dose-escalation Phase I study in ten patients with mild to severe intractable pain due to terminal cancer [60]. The protocol consisted in the administration directly in the pain-reporting area of an HSV-1 replication-defective vector containing the transgene of the precursor of enkephalin [61]. A dose-dependent analgesic effect was demonstrated with a reduction of pain scores lasting for at least 2 weeks and with no adverse effects. These encouraging results prompt to implement a Phase II trial in a larger cancer population and the study includes placebo controls, evaluation of the effects of reinoculation of the vector and assessment of the maximal dose [45, 62].

The progress of the clinical trials for cancer pain opened avenues to test gene therapy to block nociceptive transmission in the spinal cord in other pain conditions, such as painful diabetic neuropathy. This pain type, which is increasing to the pandemic occurrence of diabetes, is difficult to treat with conventional analgesics and only about one third of the patients achieve a 50% pain reduction beyond the placebo effect [63]. A clinical trial has recently been approved



**Figure 3.** HSV-1 injected at the DRt transduces noradrenergic afferents of the nucleus (A, B). Photomicrographs representing double-labeled neurons for  $\beta$ -gal and TH (yellowish) in the locus *coeruleus* (A) and the  $A_5$  noradrenergic cell group (B).  $\beta$ -gal positive neurons are shown in red and TH positive neurons are shown in green (A). Scale bar in B: 40  $\mu$ m (A is at the same magnification). The insertion of TH in antisense orientation into HSV-1 (THa vector) induced analgesia in the spared nerve injury (SNI) model of neuropathic pain (C, D). THa induced a sustained attenuation of mechanical hyperalgesia evaluated by the pin-prick test (C) and cold allodynia evaluated by the acetone test (D). THa and the control vector were injected at time 0, i.e., 2 weeks after SNI induction. Data are presented as mean  $\pm$  SEM ( $n=6$  for each group); \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  THa- vs. control-vector.

to use an HSV-1 vector that overexpress GAD to relief painful diabetic neuropathy [45]. Other therapeutic transgenes are being considered for future clinical trials of gene therapy, namely the overexpression of interleukins [45]. The future of gene therapy for chronic pain in humans will depend on the results of the clinical trials that are currently being performed but the promising results obtained so far indicate that gene therapy will add to the armamentarium of available pain treatments.

The application of gene therapy to block nociceptive transmission at supraspinal levels has been proposed by several pain specialists [64]. However, most experimental studies dealing with gene delivery at the brain were directed to pain control areas of the medulla oblongata, which are of difficult neurosurgical approach since they are in close vicinity to areas involved in the control of vital functions, such as cardiovascular and respiratory controls. Moving the focus of the gene delivery studies to areas that are more easy to approach may be useful namely in the context of widespread chronic pain, such as fibromyalgia and complex regional pain syndrome [65]. This can only be considered after a thorough characterization of the pain control

circuits in the brain namely in what concerns the functional changes induced by the chronic pain condition in order to select the best brain areas to target to maximise the balance between efficacy and risk.

## 6. Future challenges

The advances of gene therapy in other diseases of the nervous system rather than pain will be crucial to define the future of gene therapy for chronic pain, namely by the improvements in the delivery systems. Studies which improved the efficacy of non-viral vectors already inspired the construction of a non-viral, non plasmid immunologically defined gene expression (MIDGE) vector that overexpress  $\beta$ -endorphin and induced analgesia after injection into inflamed paws by increasing the concentration of  $\beta$ -endorphin in leukocytes [66]. Since chronic pain requires long-term transgene expression, the duration of the activity of promoters needs to be increased. It could be useful to design constructs that are activated only when pain lasts for longer periods and rises over a certain threshold. This would allow treating chronic pain but still preserve acute pain as an alert signal. An interesting possibility could be to control the activity of the promoter using inducible promoters, which have been used in gene therapy studies other than pain. The activity of these promoters can be induced exogenously, for example, by antibiotics. An ingenious idea was recently applied by using a ligand (glycine) which normally is not expressed in DRG neurons but can be administered to activate HSV-1 vectors to express glycine receptors in animal models of somatic and visceral pain [67]. Besides the vectors and the promoters, an election of effective transgenes for chronic pain will be important to define the future of gene therapy for chronic pain. Transgenes for opioid peptides have been overused in gene therapy studies in animal models but long-term treatments with classic opioids may induce pain, a phenomena known as opioid-induced hyperalgesia [68]. By achieving more sustained and strong transgene expression, it is possible that opioid-induced hyperalgesia could also be induced by gene therapy. New transgenes should be considered in future studies of gene therapy for chronic pain. Based on the role of the vanilloid receptor TRPV-1 (Transient Receptor Potential channel Vanilloid 1) as a pro-nociceptive cationic channel involved in pain signalling, and the clinical relevance of desensitization of TRPV1 receptors [69], this may be an important target molecule in the future. By decreasing the expression of protein kinase C-epsilon (PKC), which phosphorylates TRPV1 receptors, it was possible to induce analgesia in animal models [70]. Even more challenging is the possibility of targeting pain control centres in the brain using gene therapy. These studies will continue for large years to focus on animal pain models in order to determine neurobiological effects of chronic pain installation in pain control centres, using gene therapy as a method to prevent those changes. Finally, the emergent new field in pain research of genetics of pain has recently provided data which may explain the higher susceptibility of some persons to develop chronic pain [71]. Due to its versatility and the possibility of direct gene targeting, gene therapy can be the perfect tool to verify if the holy grail of a personalized pain treatment can be implemented.

## Financial support

This work was supported by FCT and COMPTE project PTDC/SAU-NSC/110954/2009.

## List of abbreviations

$\beta$ -gal-  $\beta$ -galactosidase

DRG- Dorsal root ganglion

DRT- Dorsal reticular nucleus

GABA-  $\gamma$ -amminobutiric acid

GAD- glutamate decarboxylase

GDNF- Glial-derived neurotrofic factor

hCMV- Human cytomegalovirus

HSV-1- Herpes Simplex Virus type 1

IASP- International Association for the Study of Pain

IL<sub>2</sub>- Interleukin 2

IL<sub>4</sub>- Interleukin 4

IL<sub>10</sub>- Interleukin 10

MIDGE- Non plasmid immunologically defined gene expression

Nav 1.7- Voltage-gated sodium channel 1.7

NF $\kappa$ B- Nuclear Factor  $\kappa$ B

NMDA- N-methyl-D-aspartate

NTS- Nucleus of the solitary tract

PKC- Protein kinase C-epsilon

shGCH1- small hairpin RNAs for GTP cyclohydroxilase

sTNFRs- tumor necrosis factor- $\alpha$

TH- Tyrosine hydroxylase

TRPV-1- Transient Receptor Potential channel Vanilloid 1

VLM- Caudal ventrolateral medulla

## Author details

Isaura Tavares<sup>1,2</sup> and Isabel Martins<sup>1,2</sup>

\*Address all correspondence to: isatav@med.up.pt

1 Department of Experimental Biology, Faculty of Medicine of Porto, University of Porto, Portugal

2 IBMC - Instituto de Biologia Molecular e Celular, University of Porto, Portugal

## References

- [1] Merskey, H., et al., Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on Taxonomy. *Pain*, 1979. 6(3): p. 249.
- [2] Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. *Pain Suppl*, 1986. 3: p. S1-226.
- [3] Thienhaus, O. and B.E. Cole, Classification of pain. Weiner RS, 6th ed. 2002: American Academy of Pain Management.
- [4] Breivik, H., et al., Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain*, 2006. 10(4): p. 287-333.
- [5] Verhaak, P.F., et al., Prevalence of chronic benign pain disorder among adults: a review of the literature. *Pain*, 1998. 77(3): p. 231-9.
- [6] Gaskin, D.J. and P. Richard, The economic costs of pain in the United States. *J Pain*, 2012. 13(8): p. 715-24.
- [7] Benyamin, R., et al., Opioid complications and side effects. *Pain Physician*, 2008. 11(2 Suppl): p. S105-20.
- [8] Mao, J., M.S. Gold, and M.M. Backonja, Combination drug therapy for chronic pain: a call for more clinical studies. *J Pain*, 2011. 12(2): p. 157-66.
- [9] Tracey, I. and P.W. Mantyh, The cerebral signature for pain perception and its modulation. *Neuron*, 2007. 55(3): p. 377-91.
- [10] Basbaum, A.I., et al., Cellular and molecular mechanisms of pain. *Cell*, 2009. 139(2): p. 267-84.
- [11] Heinricher, M.M., et al., Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res Rev*, 2009. 60(1): p. 214-25.

- [12] Hioki, H., et al., Efficient gene transduction of neurons by lentivirus with enhanced neuron-specific promoters. *Gene Ther*, 2007. 14(11): p. 872-82.
- [13] Porreca, F., M.H. Ossipov, and G.F. Gebhart, Chronic pain and medullary descending facilitation. *Trends Neurosci*, 2002. 25(6): p. 319-25.
- [14] Braz, J., et al., Therapeutic efficacy in experimental polyarthritis of viral-driven enkephalin overproduction in sensory neurons. *J Neurosci*, 2001. 21(20): p. 7881-8.
- [15] Hao, S., et al., Effects of transgene-mediated endomorphin-2 in inflammatory pain. *Eur J Pain*, 2009. 13(4): p. 380-6.
- [16] Yeomans, D.C., et al., Decrease in inflammatory hyperalgesia by herpes vector-mediated knockdown of Nav1.7 sodium channels in primary afferents. *Hum Gene Ther*, 2005. 16(2): p. 271-7.
- [17] Finegold, A.A., A.J. Mannes, and M.J. Iadarola, A paracrine paradigm for in vivo gene therapy in the central nervous system: treatment of chronic pain. *Hum Gene Ther*, 1999. 10(7): p. 1251-7.
- [18] Xu, Y., et al., Adeno-associated viral transfer of opioid receptor gene to primary sensory neurons: a strategy to increase opioid antinociception. *Proc Natl Acad Sci U S A*, 2003. 100(10): p. 6204-9.
- [19] Martins, I., et al., Reversal of inflammatory pain by HSV-1-mediated overexpression of enkephalin in the caudal ventrolateral medulla. *Eur J Pain*, 2011. 15(10): p. 1008-14.
- [20] Pinto, M., et al., Opioids modulate pain facilitation from the dorsal reticular nucleus. *Mol Cell Neurosci*, 2008. 39(4): p. 508-18.
- [21] Kang, W., et al., Herpes virus-mediated preproenkephalin gene transfer to the amygdala is antinociceptive. *Brain Res*, 1998. 792(1): p. 133-5.
- [22] Lu, Y., et al., Treatment of inflamed pancreas with enkephalin encoding HSV-1 recombinant vector reduces inflammatory damage and behavioral sequelae. *Mol Ther*, 2007. 15(10): p. 1812-9.
- [23] Wolfe, D., et al., Engineering an endomorphin-2 gene for use in neuropathic pain therapy. *Pain*, 2007. 133(1-3): p. 29-38.
- [24] Hao, S., et al., HSV-mediated expression of interleukin-4 in dorsal root ganglion neurons reduces neuropathic pain. *Mol Pain*, 2006. 2: p. 6.
- [25] Peng, X.M., et al., Tumor necrosis factor-alpha contributes to below-level neuropathic pain after spinal cord injury. *Ann Neurol*, 2006. 59(5): p. 843-51.
- [26] Hao, S., et al., Gene transfer of glutamic acid decarboxylase reduces neuropathic pain. *Ann Neurol*, 2005. 57(6): p. 914-8.

- [27] Milligan, E.D., et al., Controlling pathological pain by adenovirally driven spinal production of the anti-inflammatory cytokine, interleukin-10. *Eur J Neurosci*, 2005. 21(8): p. 2136-48.
- [28] Yao, M.Z., et al., Adenovirus-mediated interleukin-2 gene therapy of nociception. *Gene Ther*, 2003. 10(16): p. 1392-9.
- [29] Milligan, E.D., et al., Controlling neuropathic pain by adeno-associated virus driven production of the anti-inflammatory cytokine, interleukin-10. *Mol Pain*, 2005. 1: p. 9.
- [30] Kim, S.J., et al., Effective relief of neuropathic pain by adeno-associated virus-mediated expression of a small hairpin RNA against GTP cyclohydrolase 1. *Mol Pain*, 2009. 5: p. 67.
- [31] Storek, B., et al., Sensory neuron targeting by self-complementary AAV8 via lumbar puncture for chronic pain. *Proc Natl Acad Sci U S A*, 2008. 105(3): p. 1055-60.
- [32] Pezet, S., et al., Reversal of neurochemical changes and pain-related behavior in a model of neuropathic pain using modified lentiviral vectors expressing GDNF. *Mol Ther*, 2006. 13(6): p. 1101-9.
- [33] Meunier, A., et al., Lentiviral-mediated targeted NF-kappaB blockade in dorsal spinal cord glia attenuates sciatic nerve injury-induced neuropathic pain in the rat. *Mol Ther*, 2007. 15(4): p. 687-97.
- [34] Martins, I., et al., Reversal of neuropathic pain by HSV-1-mediated decrease of norepinephrine in a pain facilitatory area of the brain. *Pain*, 2010. 151(1): p. 137-45.
- [35] Wilson, S.P., et al., Antihyperalgesic effects of infection with a preproenkephalin-encoding herpes virus. *Proc Natl Acad Sci U S A*, 1999. 96(6): p. 3211-6.
- [36] Yeomans, D.C., et al., Recombinant herpes vector-mediated analgesia in a primate model of hyperalgesia. *Mol Ther*, 2006. 13(3): p. 589-97.
- [37] Yokoyama, H., et al., Gene therapy for bladder overactivity and nociception with herpes simplex virus vectors expressing preproenkephalin. *Hum Gene Ther*, 2009. 20(1): p. 63-71.
- [38] Jasmin, L., et al., Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. *Nature*, 2003. 424(6946): p. 316-20.
- [39] Cabanero, D., et al., The pro-nociceptive effects of remifentanyl or surgical injury in mice are associated with a decrease in delta-opioid receptor mRNA levels: Prevention of the nociceptive response by on-site delivery of enkephalins. *Pain*, 2009. 141(1-2): p. 88-96.
- [40] Goss, J.R., et al., Herpes vector-mediated expression of proenkephalin reduces bone cancer pain. *Ann Neurol*, 2002. 52(5): p. 662-5.



- [41] Meunier, A., et al., Attenuation of pain-related behavior in a rat model of trigeminal neuropathic pain by viral-driven enkephalin overproduction in trigeminal ganglion neurons. *Mol Ther*, 2005. 11(4): p. 608-16.
- [42] Vit, J.P., et al., Adenovector GAD65 gene delivery into the rat trigeminal ganglion produces orofacial analgesia. *Mol Pain*, 2009. 5: p. 42.
- [43] Glorioso, J.C. and D.J. Fink, Herpes vector-mediated gene transfer in the treatment of chronic pain. *Mol Ther*, 2009. 17(1): p. 13-8.
- [44] Goss, J.R., M.S. Gold, and J.C. Glorioso, HSV vector-mediated modification of primary nociceptor afferents: an approach to inhibit chronic pain. *Gene Ther*, 2009. 16(4): p. 493-501.
- [45] Goins, W.F., J.B. Cohen, and J.C. Glorioso, Gene therapy for the treatment of chronic peripheral nervous system pain. *Neurobiol Dis*, 2012. 48(2): p. 255-70.
- [46] Hao, S., et al., Transgene-mediated enkephalin release enhances the effect of morphine and evades tolerance to produce a sustained antiallodynic effect in neuropathic pain. *Pain*, 2003. 102(1-2): p. 135-42.
- [47] Liu, J., et al., Peripherally delivered glutamic acid decarboxylase gene therapy for spinal cord injury pain. *Mol Ther*, 2004. 10(1): p. 57-66.
- [48] Zhou, Z., et al., HSV-mediated transfer of interleukin-10 reduces inflammatory pain through modulation of membrane tumor necrosis factor alpha in spinal cord microglia. *Gene Ther*, 2008. 15(3): p. 183-90.
- [49] Hao, S., et al., Gene transfer to interfere with TNFalpha signaling in neuropathic pain. *Gene Ther*, 2007. 14(13): p. 1010-6.
- [50] Staud, R., Evidence for shared pain mechanisms in osteoarthritis, low back pain, and fibromyalgia. *Curr Rheumatol Rep*, 2011. 13(6): p. 513-20.
- [51] Howarth, P.W., et al., Retrograde viral vector-mediated inhibition of pontospinal noradrenergic neurons causes hyperalgesia in rats. *J Neurosci*, 2009. 29(41): p. 12855-64.
- [52] Pertovaara, A., Noradrenergic pain modulation. *Prog Neurobiol*, 2006. 80(2): p. 53-83.
- [53] Tavares, I. and D. Lima, The caudal ventrolateral medulla as an important inhibitory modulator of pain transmission in the spinal cord. *J Pain*, 2002. 3(5): p. 337-46.
- [54] Lima, D. and A. Almeida, The medullary dorsal reticular nucleus as a pronociceptive centre of the pain control system. *Prog Neurobiol*, 2002. 66(2): p. 81-108.
- [55] Martins, I., et al., Dynamic of migration of HSV-1 from a medullary pronociceptive centre: antinociception by overexpression of the preproenkephalin transgene. *Eur J Neurosci*, 2008. 28(10): p. 2075-83.

- [56] Marques-Lopes, J., et al., Decrease in the expression of N-methyl-D-aspartate receptors in the nucleus tractus solitarius induces antinociception and increases blood pressure. *J Neurosci Res*, 2012. 90(2): p. 356-66.
- [57] Almeida, A., et al., Brain afferents to the medullary dorsal reticular nucleus: a retrograde and anterograde tracing study in the rat. *Eur J Neurosci*, 2002. 16(1): p. 81-95.
- [58] Cobos, A., et al., Brain afferents to the lateral caudal ventrolateral medulla: a retrograde and anterograde tracing study in the rat. *Neuroscience*, 2003. 120(2): p. 485-98.
- [59] Chiocca, E.A., et al., Transfer and expression of the lacZ gene in rat brain neurons mediated by herpes simplex virus mutants. *New Biol*, 1990. 2(8): p. 739-46.
- [60] Fink, D.J., et al., Gene therapy for pain: results of a phase I clinical trial. *Ann Neurol*, 2011. 70(2): p. 207-12.
- [61] Akil, H., et al., Endogenous opioids: biology and function. *Annu Rev Neurosci*, 1984. 7: p. 223-55.
- [62] Fink, D.J. and D. Wolfe, Gene Therapy for Pain: A Perspective. *Pain Manag*, 2011. 1(5): p. 379-381.
- [63] Turk, D.C., Clinical effectiveness and cost-effectiveness of treatments for patients with chronic pain. *Clin J Pain*, 2002. 18(6): p. 355-65.
- [64] Wu, C.L., et al., Gene therapy for the management of pain: Part I: Methods and strategies. *Anesthesiology*, 2001. 94(6): p. 1119-32.
- [65] Weiss, K. and N.M. Boulis, Herpes Simplex Virus-Based Gene Therapies for Chronic Pain. *J Pain Palliat Care Pharmacother*, 2012. 26(3): p. 291-3.
- [66] Machelska, H., et al., Peripheral non-viral MIDGE vector-driven delivery of beta-endorphin in inflammatory pain. *Mol Pain*, 2009. 5: p. 72.
- [67] Goss, J.R., et al., HSV delivery of a ligand-regulated endogenous ion channel gene to sensory neurons results in pain control following channel activation. *Mol Ther*, 2011. 19(3): p. 500-6.
- [68] Lee, M., et al., A comprehensive review of opioid-induced hyperalgesia. *Pain Physician*, 2011. 14(2): p. 145-61.
- [69] Kissin, I. and A. Szallasi, Therapeutic targeting of TRPV1 by resiniferatoxin, from preclinical studies to clinical trials. *Curr Top Med Chem*, 2011. 11(17): p. 2159-70.
- [70] Srinivasan, R., et al., Protein kinase C epsilon contributes to basal and sensitizing responses of TRPV1 to capsaicin in rat dorsal root ganglion neurons. *Eur J Neurosci*, 2008. 28(7): p. 1241-54.
- [71] Doehring, A., G. Geisslinger, and J. Lotsch, Epigenetics in pain and analgesia: an imminent research field. *Eur J Pain*, 2011. 15(1): p. 11-6.

