

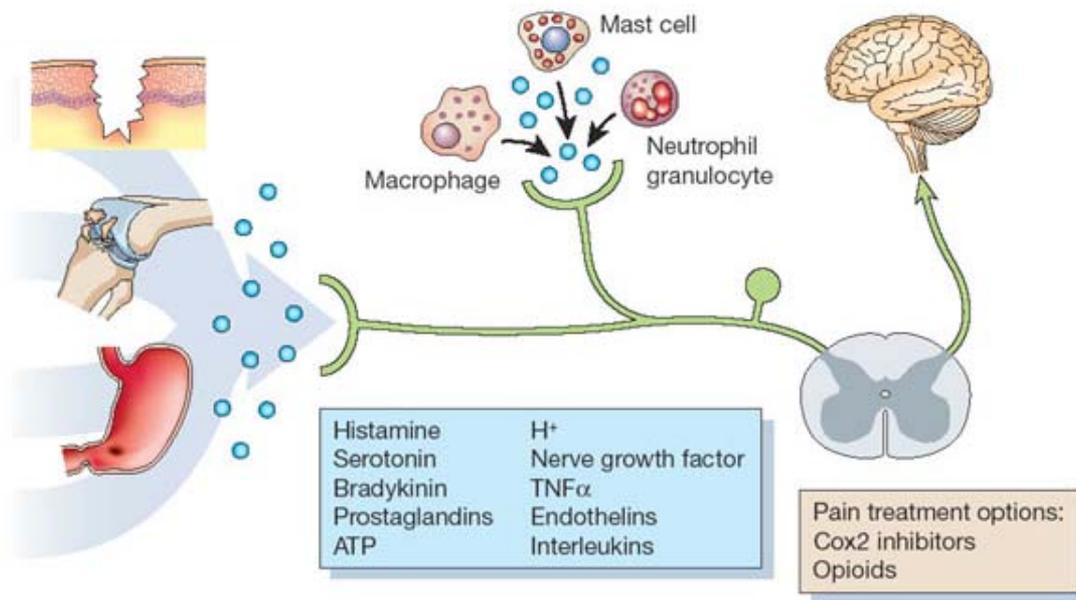
# HSV-mediated transfer of glutamic acid decarboxylase for painful diabetic neuropathy

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University of Michigan

Darren Wolfe, PhD  
Diamyd, Inc

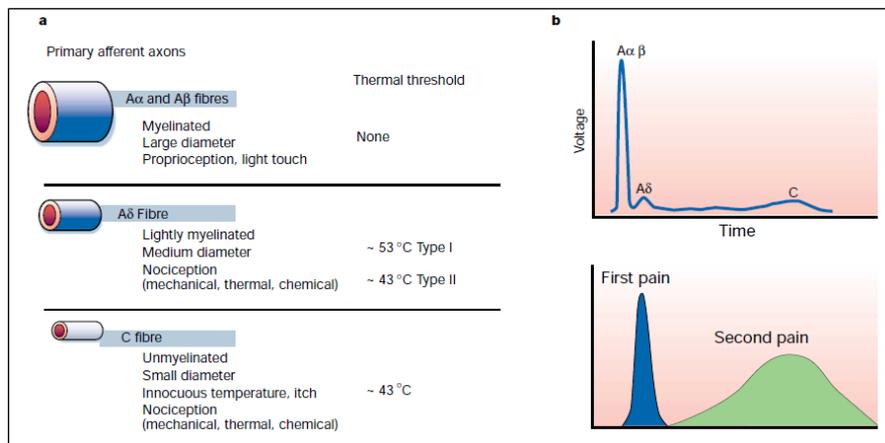


# Inflammatory pain begins with tissue damage

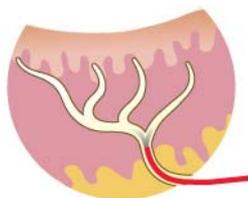
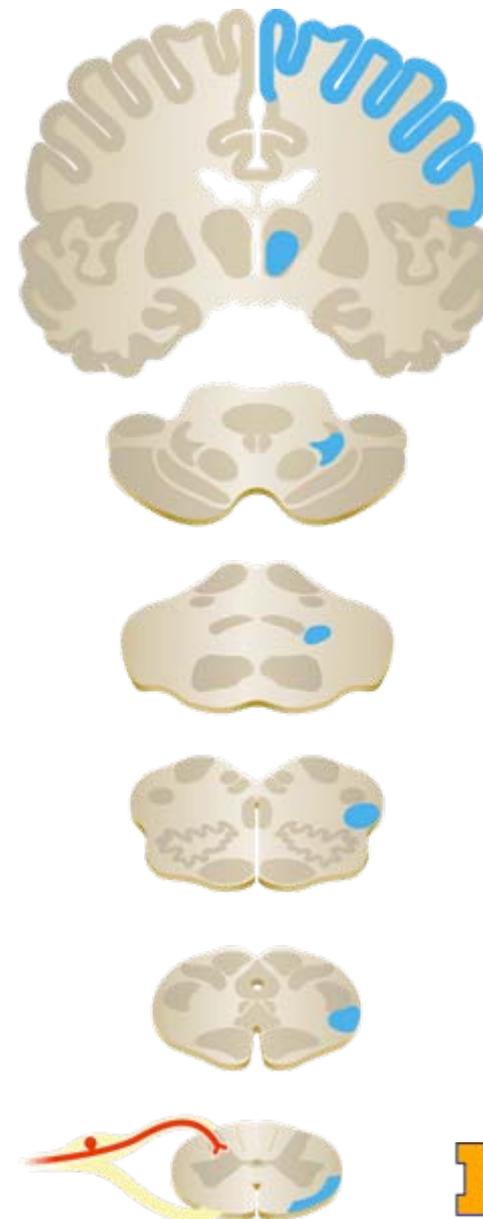


Scholz & Woolf 2002

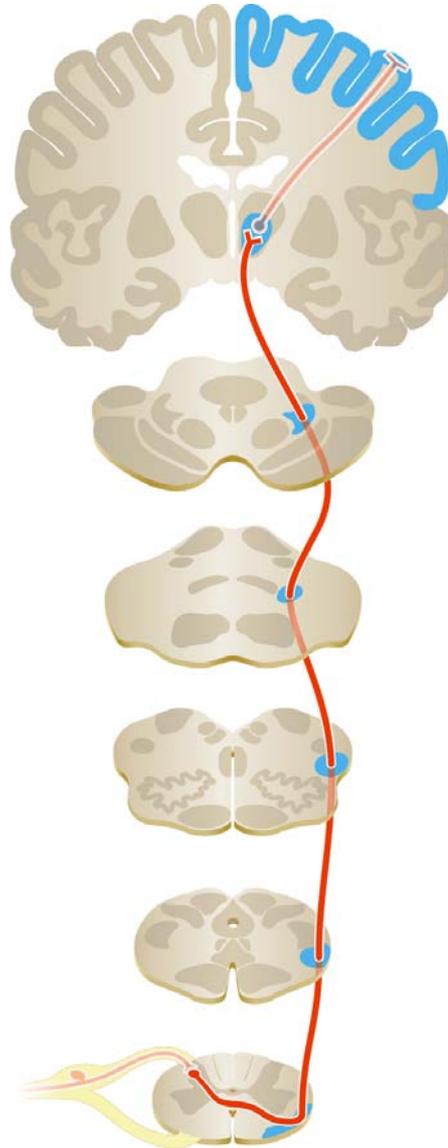
Pain-causing molecules in the periphery activate specialized primary sensory afferents whose cell bodies lie in the dorsal root ganglia

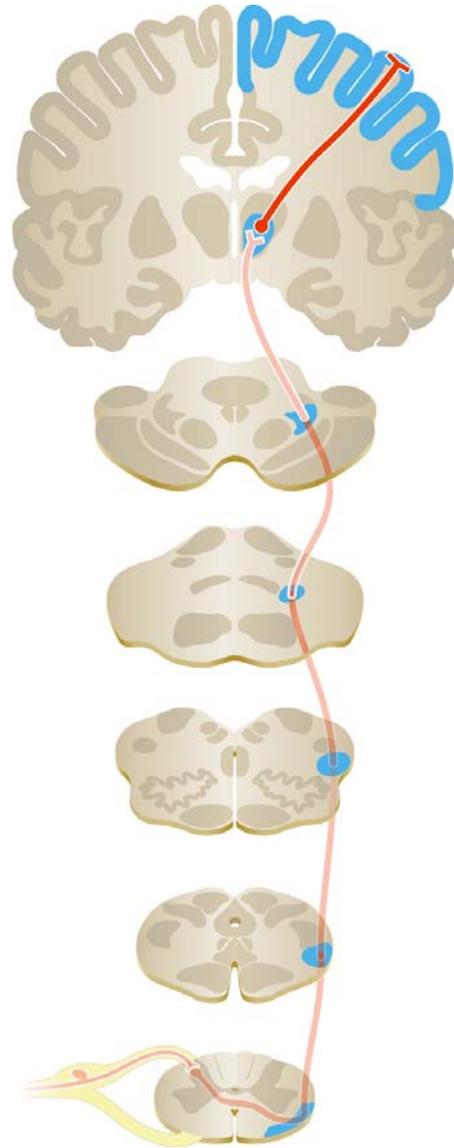


Julius & Basbaum 2001



"Second order" neurons with cell bodies in the dorsal horn of the spinal cord project rostrally to the brain

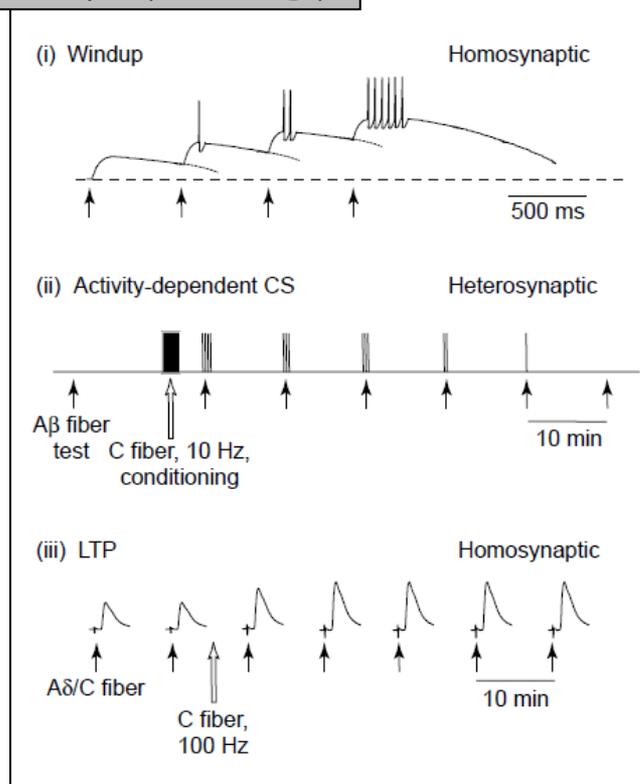




Third order neurons transmit pain-related information to sensory and limbic cortical structures in the cerebral hemispheres

# Continued activation of nociceptors results in alterations in function and changes in gene expression in DRG and spinal cord

## electrophysiology



## gene expression

DRG:  
BDNF, substance P,  
bradykinin receptor...

Dorsal horn neurons  
c-Fos, Cox-2  
prodynorphin, NK1, TrkB...

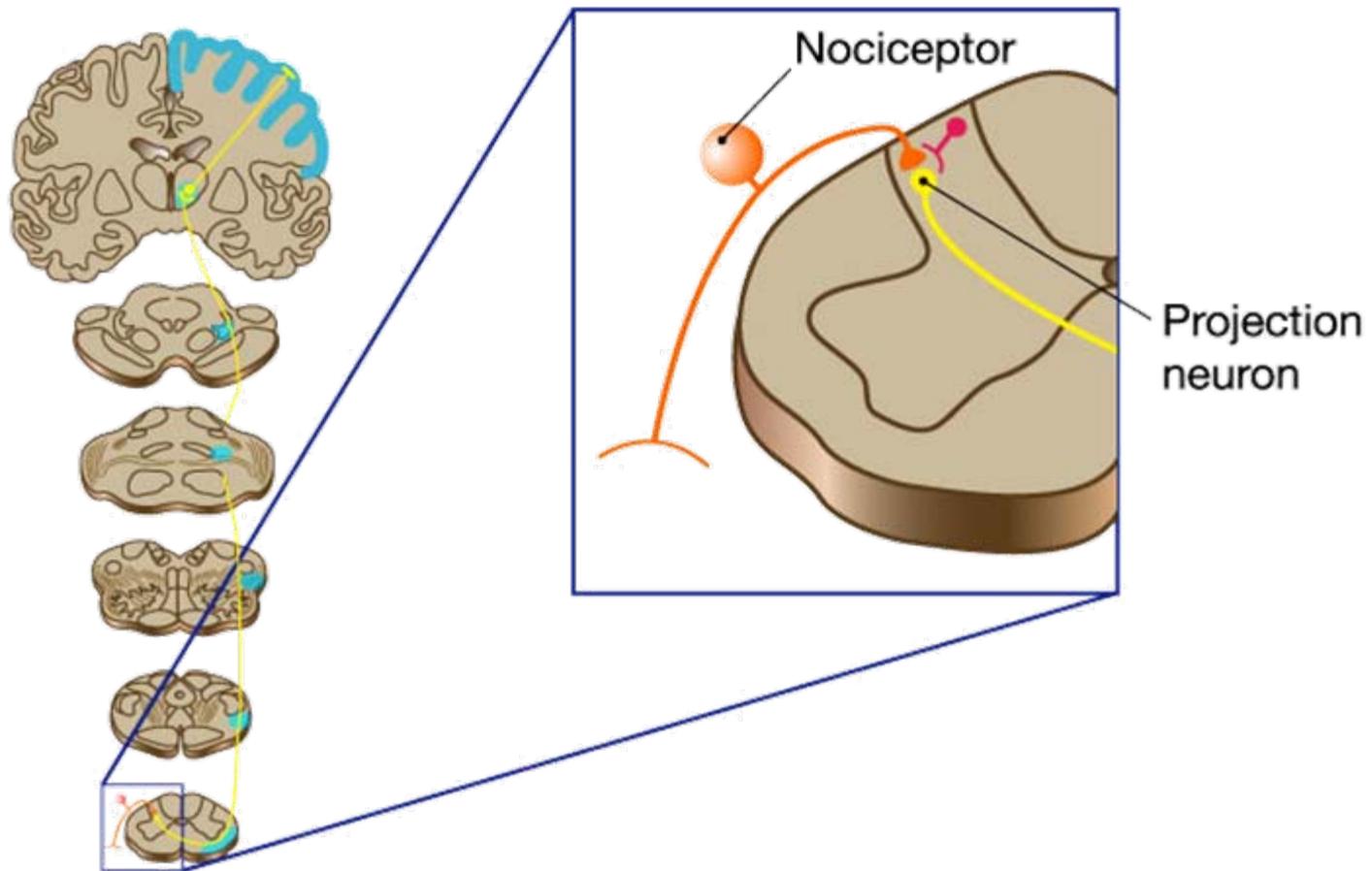
Spinal microglia & astrocytes  
ERK, IL-1 $\beta$ , TNF $\alpha$ ...

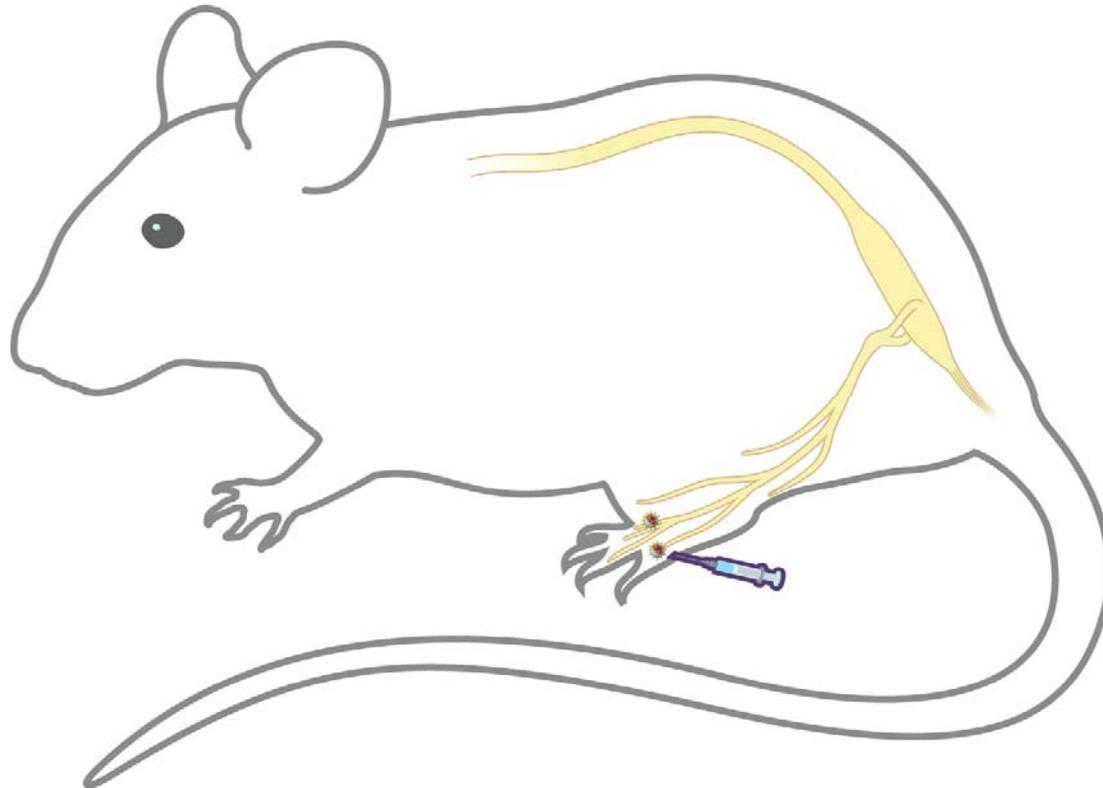
## Premise:

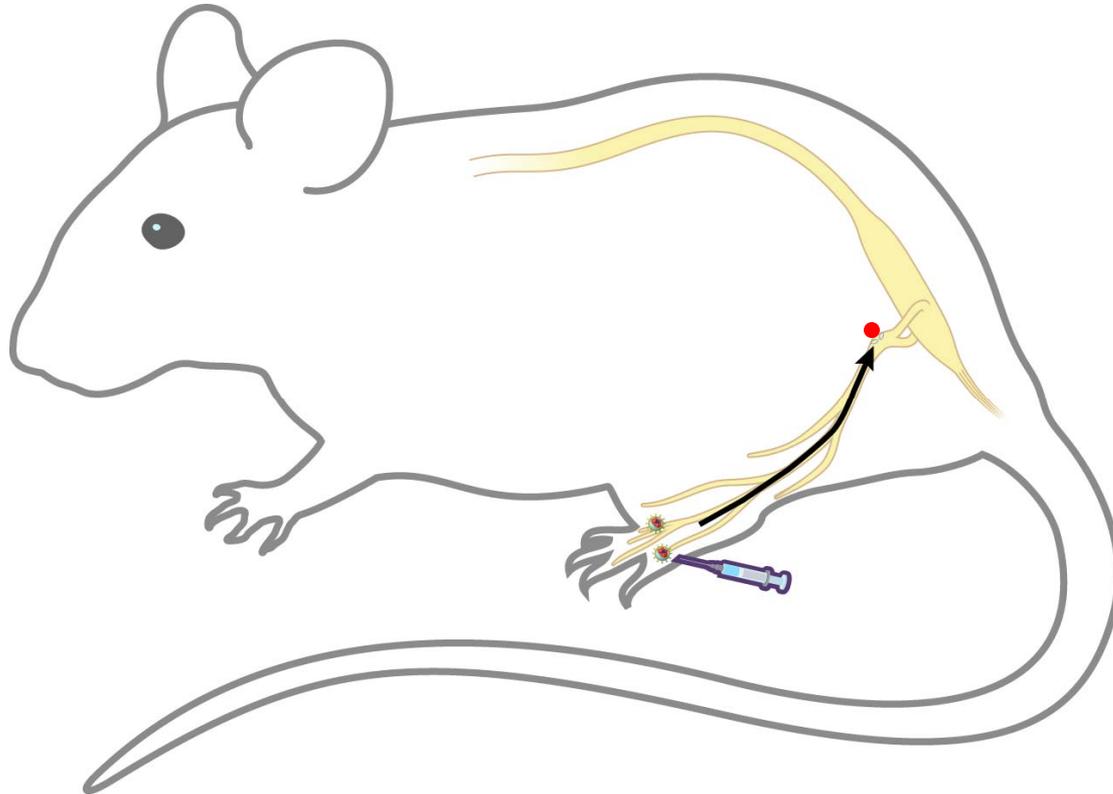
Effective pain control using conventional analgesic agents is limited by the widespread distribution of the drug targets in both pain and non-pain pathways within (and outside) the nervous system.

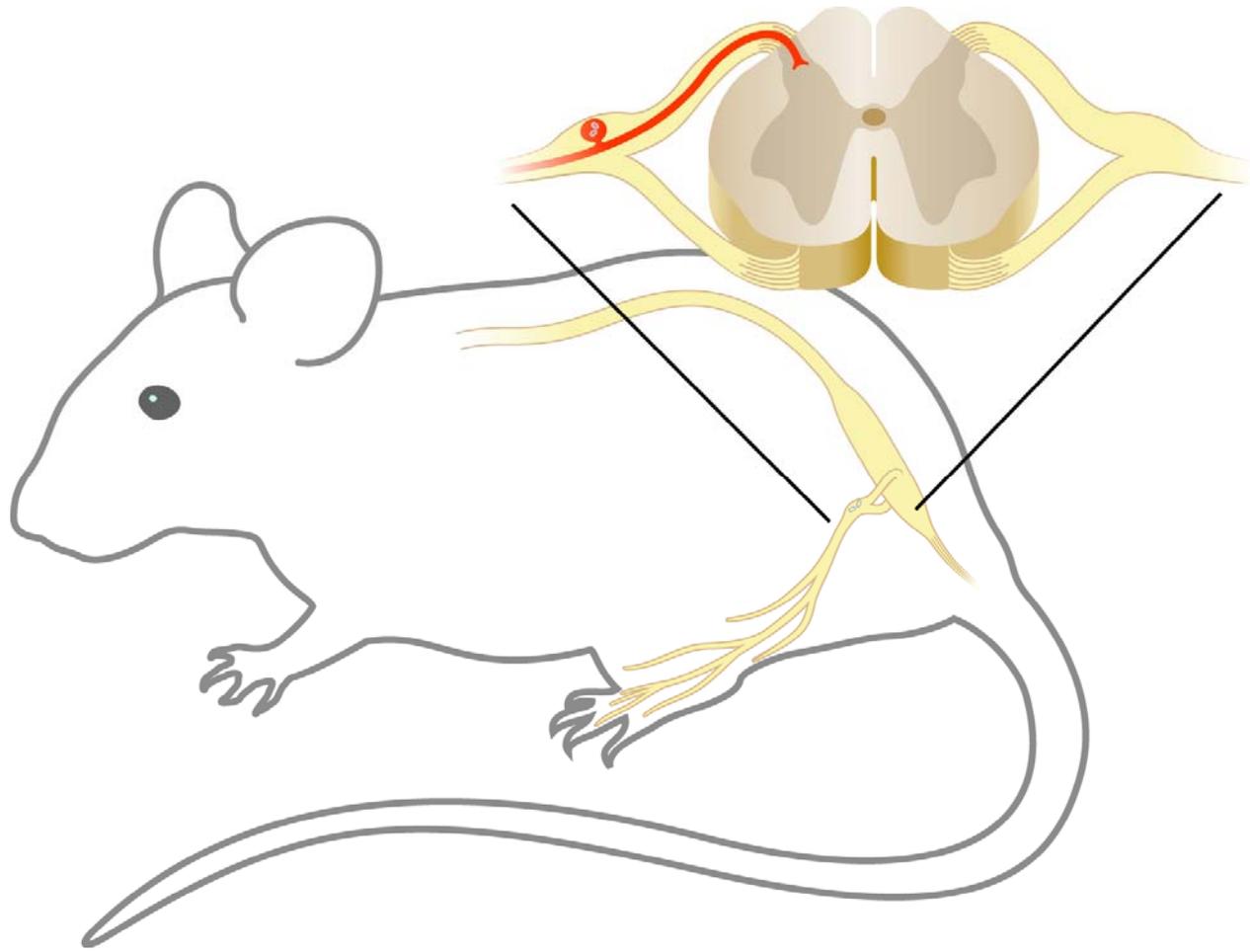
## Rationale:

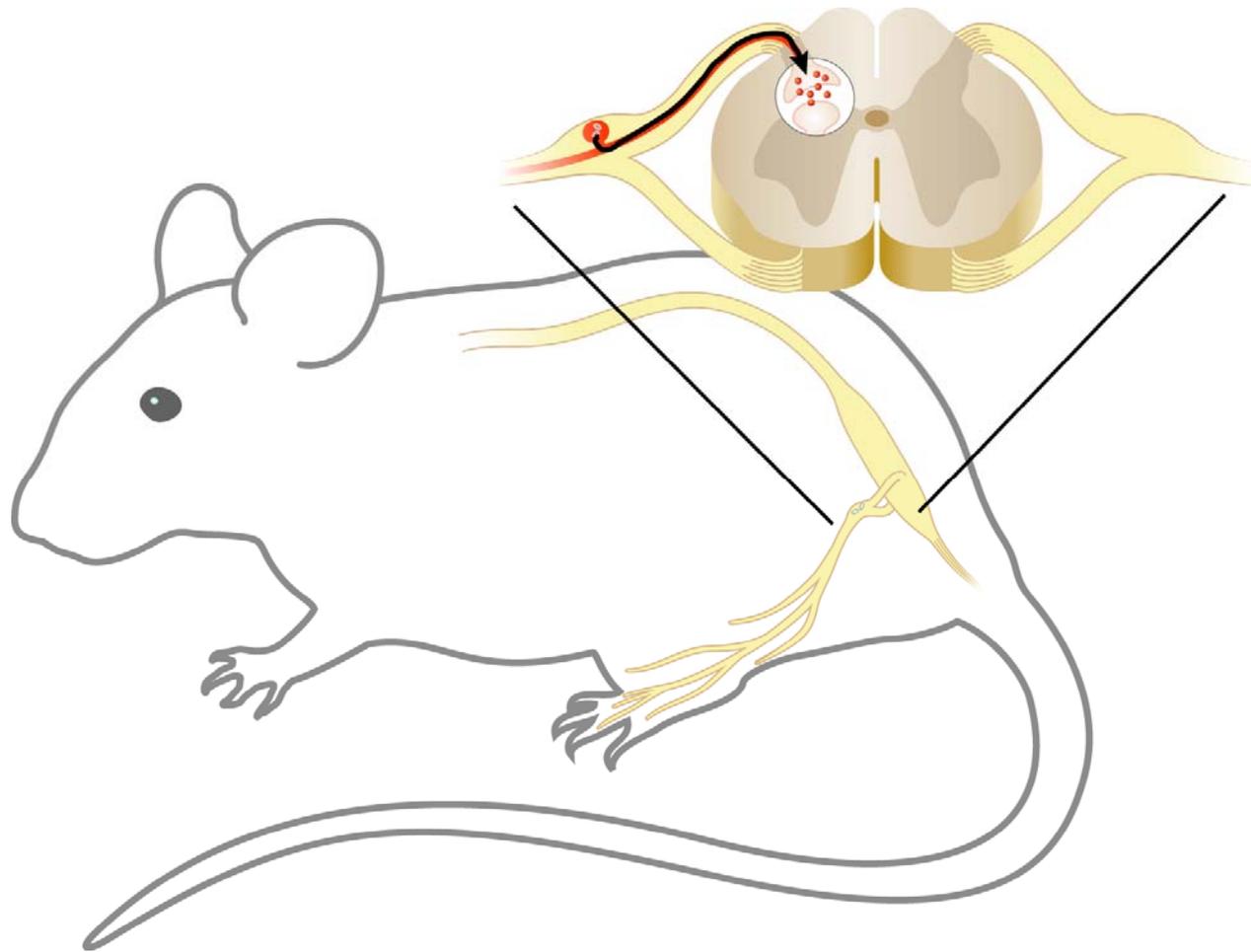
Gene transfer can be used to achieve focal release of an analgesic gene product to selectively interrupt nociceptive neurotransmission.









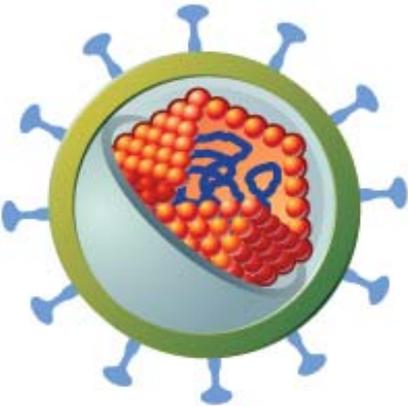


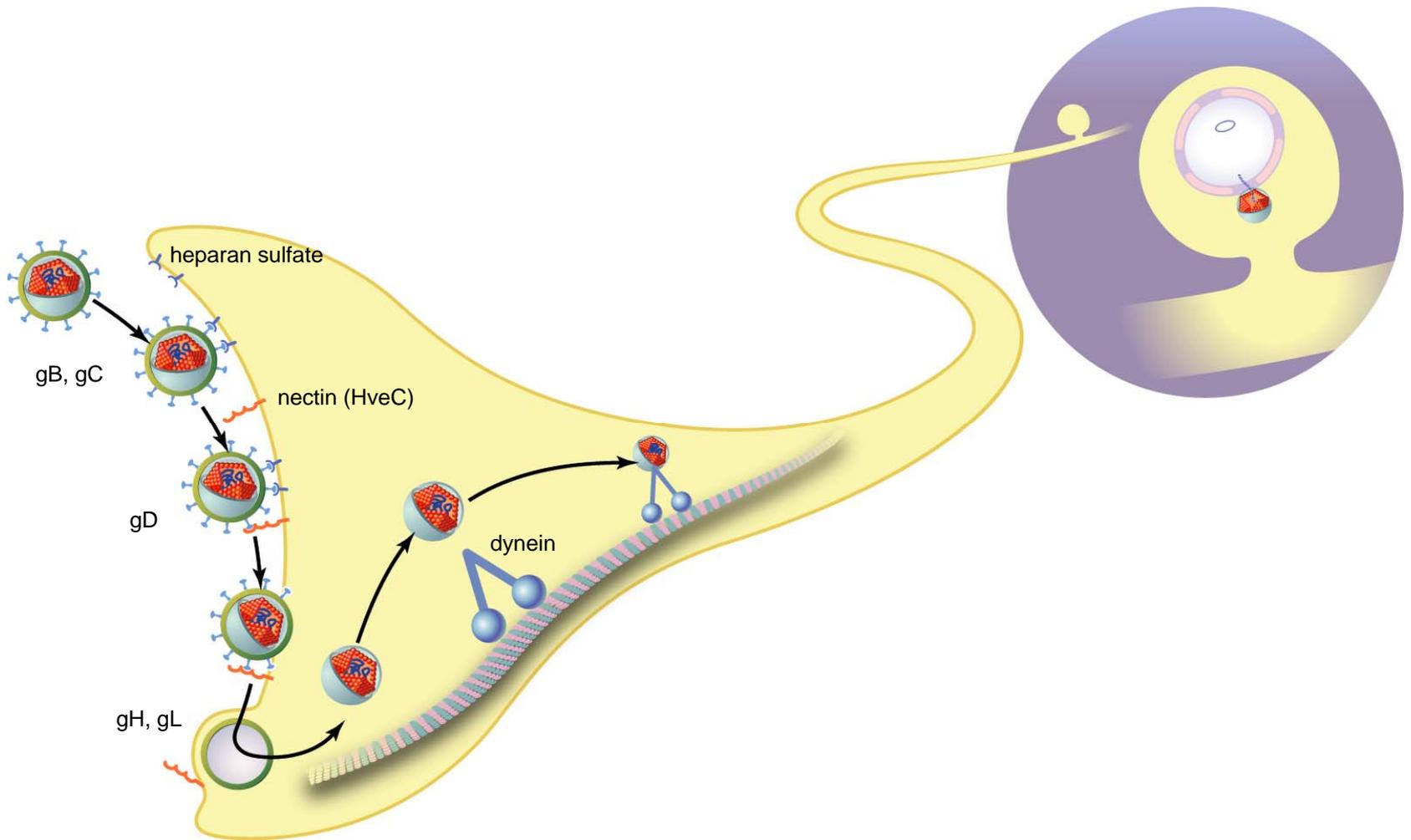
## Gene Transfer Vectors

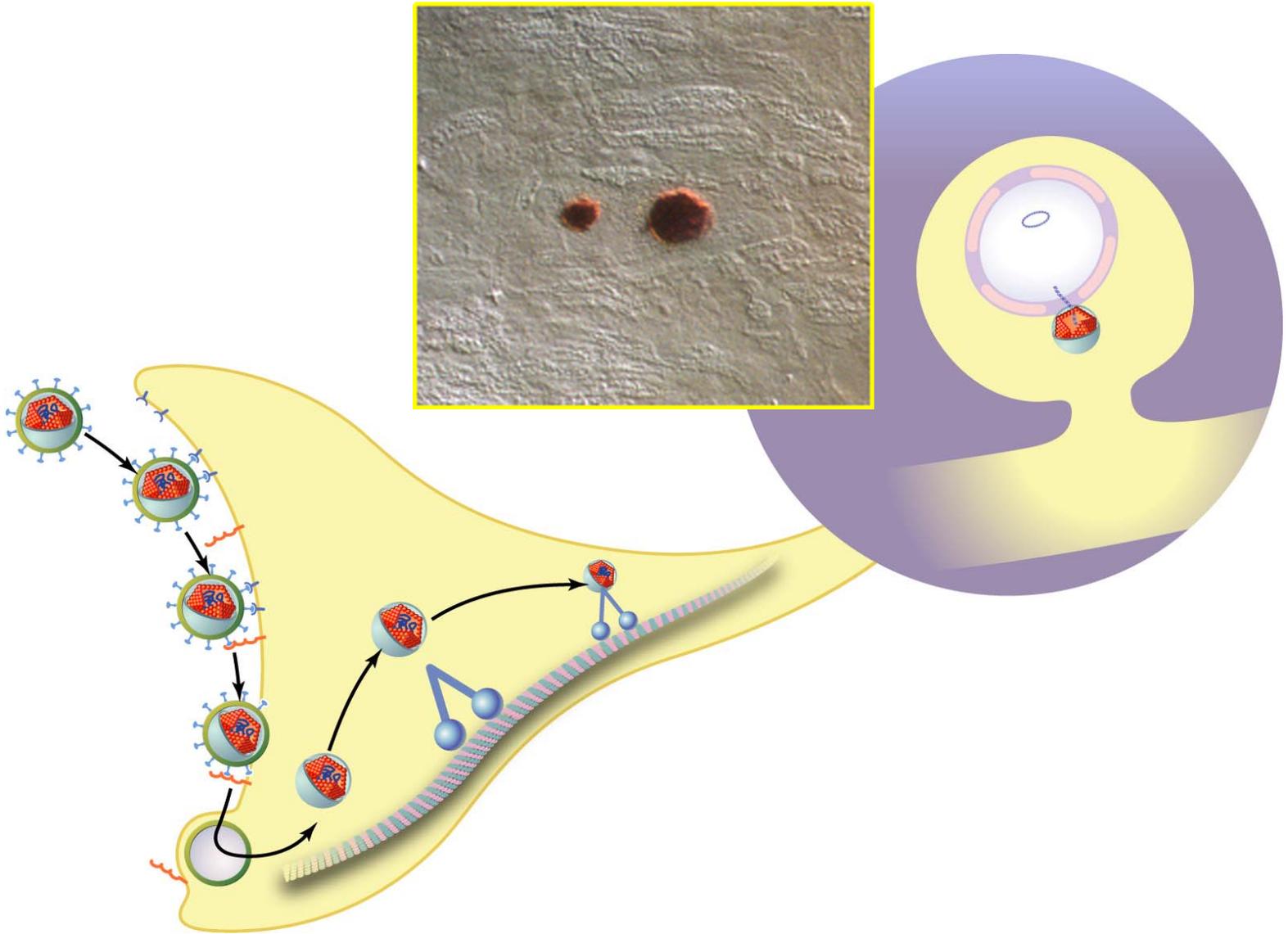
VECTOR	SIZE (nm)	GENOME (kb)	TYPE	INSERT (kb)
liposome			DNA	
retrovirus	100	8	ss-RNA	8
lentivirus	100	10	ss-RNA	8
AAV	20	5	ss-DNA	5
adenovirus	100	36	ds-DNA	8, 30
HSV	200	152	ds-DNA	50

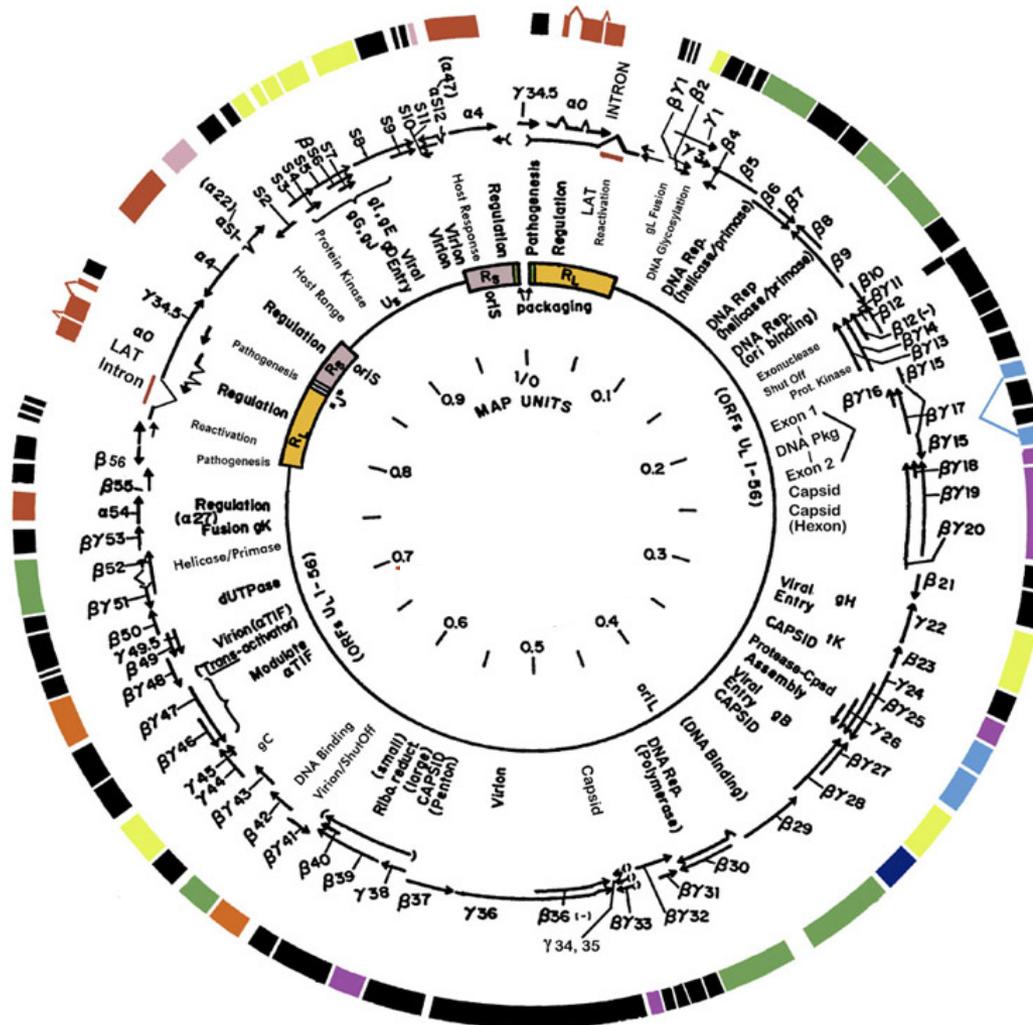


HSV

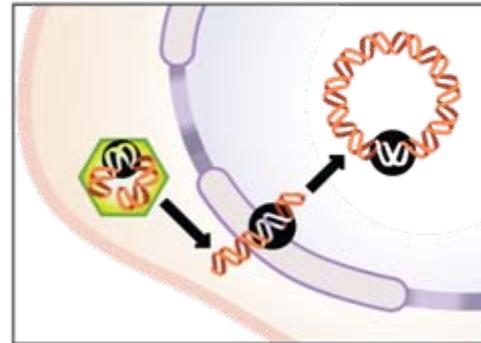
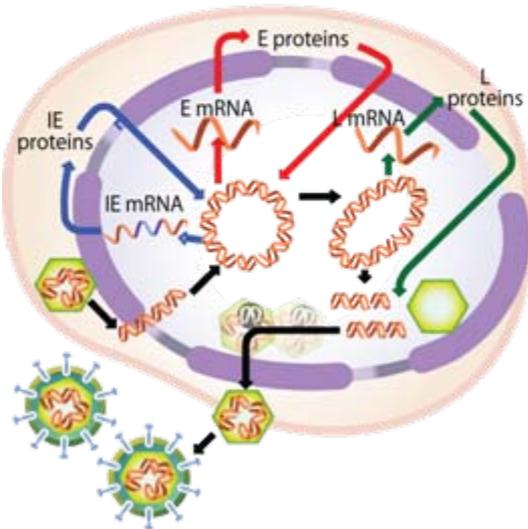




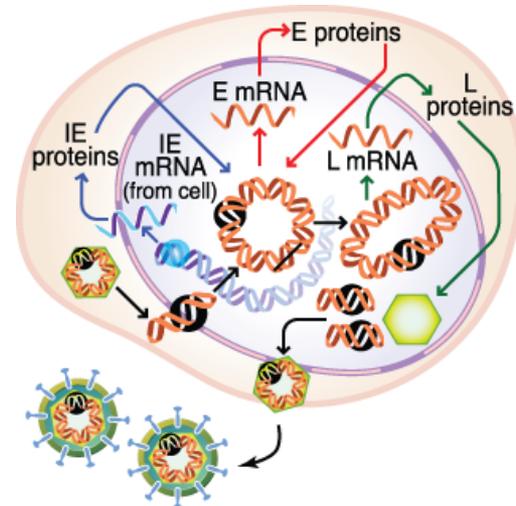




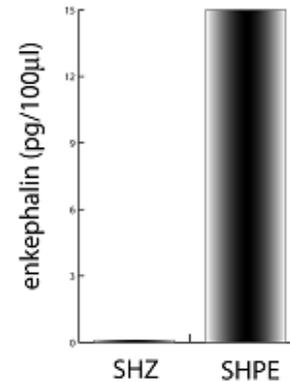
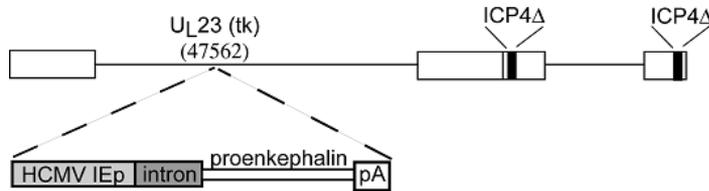
A non-replicating HSV vector is created from the wild type virus by selective deletion of essential Immediate Early (IE) HSV genes



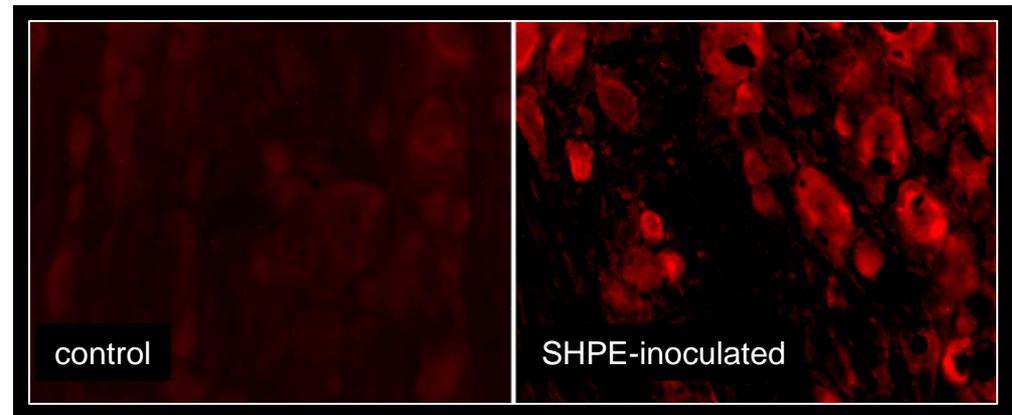
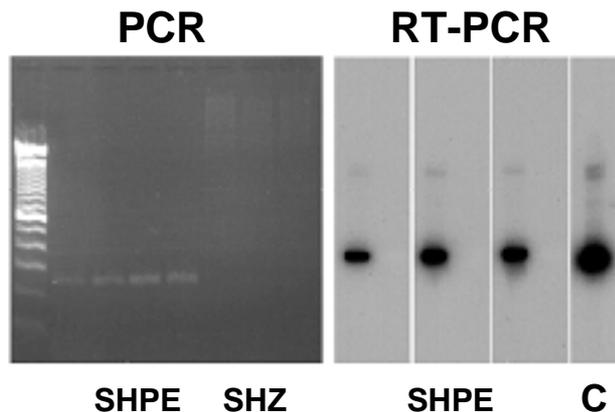
The replication incompetent vector is propagated to high titer on a complementing cell line that provides the missing gene product *in trans*



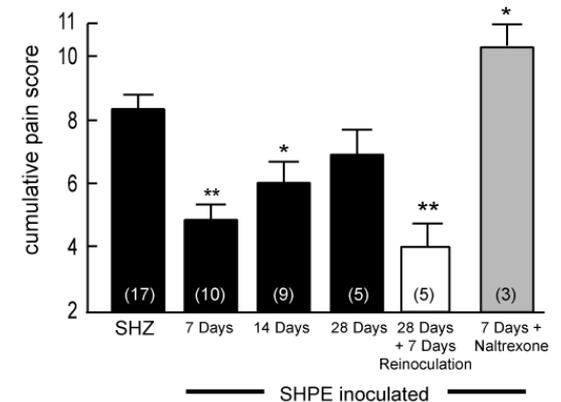
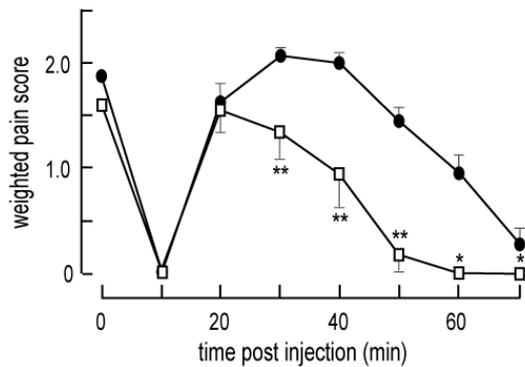
An HSV vector containing the preproenkephalin gene produces enkephalin in DRG neurons *in vitro*...



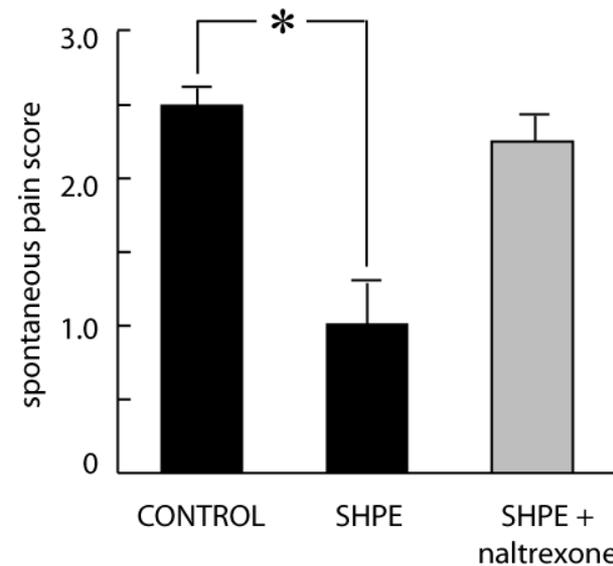
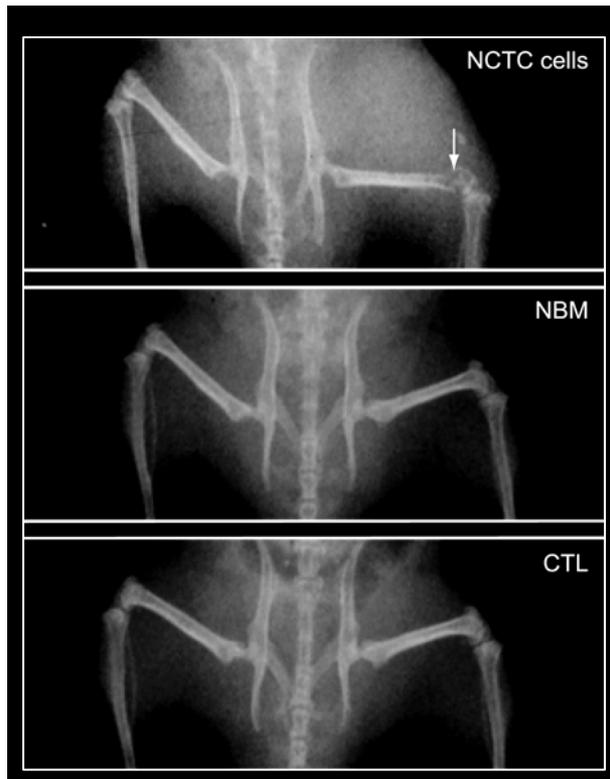
...and in DRG *in vivo* following subcutaneous inoculation in the foot



# HSV-mediated enkekephalin provides an analgesic effect in delayed phase of the formalin model of inflammatory pain



# HSV-mediated enkephalin reduces cancer-related pain in the osteogenic sarcoma model in the mouse



## NP2 protocol

RAC review (June 2002)

Insertion of transgene into human-grade vector backbone

GLP production of toxicology lot and GMP manufacturing optimization

cGMP production and certification of Master Cell Bank

cGMP production and certification of Master Viral Bank

FDA-approved GLP toxicology studies

FDA-approved GLP biodistribution studies

Drafting and submission of IND application to FDA

University of Michigan institutional approvals

Cancer Center Protocol Review Committee (PRC)

Institutional Biosafety Committee (IBC)

General Clinical Research Center (MCRU) Review

Institutional Review Board (IRB)

Certificate of Analysis

First patients enrolled (December 2008)

## Human grade enkephalin-expressing HSV vector (NP2)

Completely deleted for:

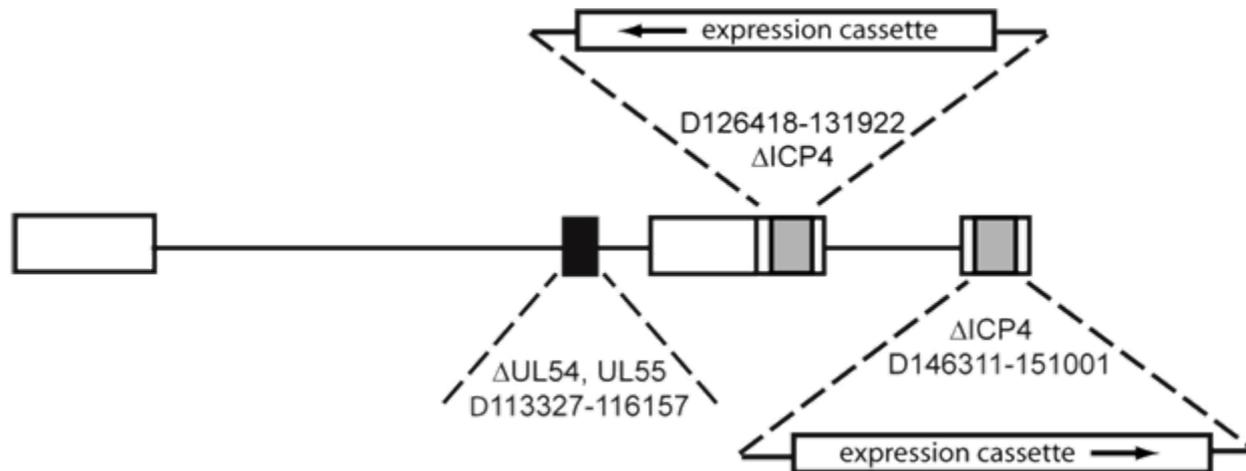
ICP4

ICP27

Defective in promoter function for:

ICP22

ICP47



# HSV PE gene transfer for cancer pain: Phase I trial

## *Inclusion criteria*

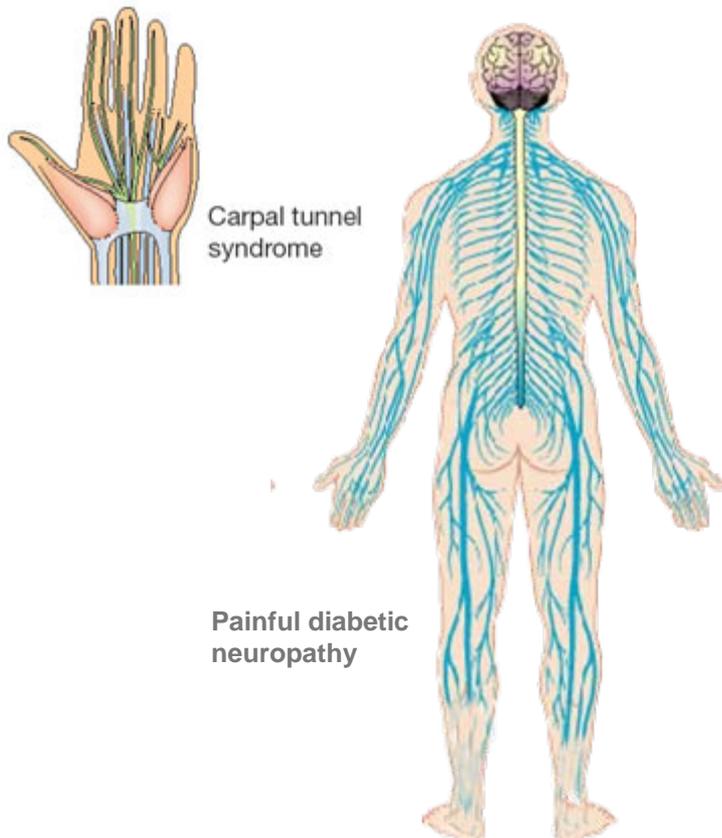
- Patients with terminal malignancy  
Focal pain consistently  $\geq 4$  on a 10 point scale  
despite morphine  $> 200$  mg/d or equivalent
- Dose escalation design

	Period 1	Period 2	Period 3	Period 4
Cohort 1	$1 \times 10^7$ pfu			
Cohort 2		$1 \times 10^8$ pfu		
Cohort 3			$1 \times 10^9$ pfu	
Cohort 4				MTD

## HSV PE gene transfer for cancer pain: Phase I trial *Protocol*

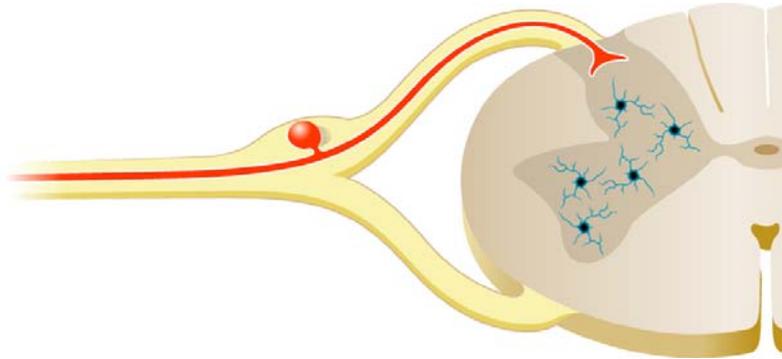
- Clinical grade vector, inoculated in ten 100 microliter intradermal injections in the dermatome(s) corresponding to the radicular distribution of pain
- Physical examination at days 1, 3, 7, 10, 14, 21, 28 at monthly intervals up to 4 months and yearly thereafter
- **Primary Outcome:** Adverse Events
- **Secondary Outcome:** Pain Measures
  - Numeric rating scale
  - McGill Short-Form Pain Questionnaire
  - Diary of concurrent analgesic use

Neuropathic pain is pain that occurs in the setting of injury to elements of the nervous system without peripheral tissue damage

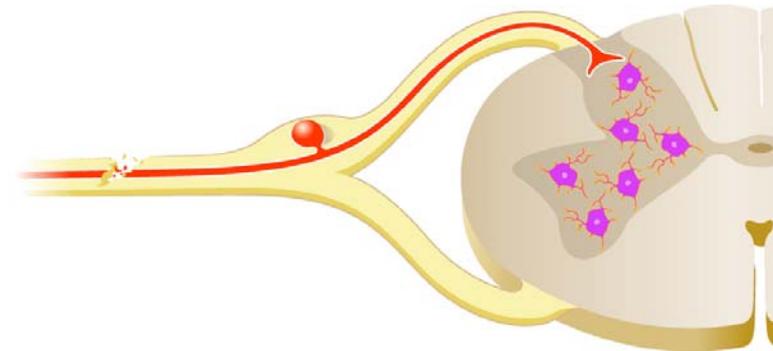


Neuropathic pain treatment options:  
Tricyclic antidepressants  
Anticonvulsants  
Sodium channel blockers  
Opioid drugs

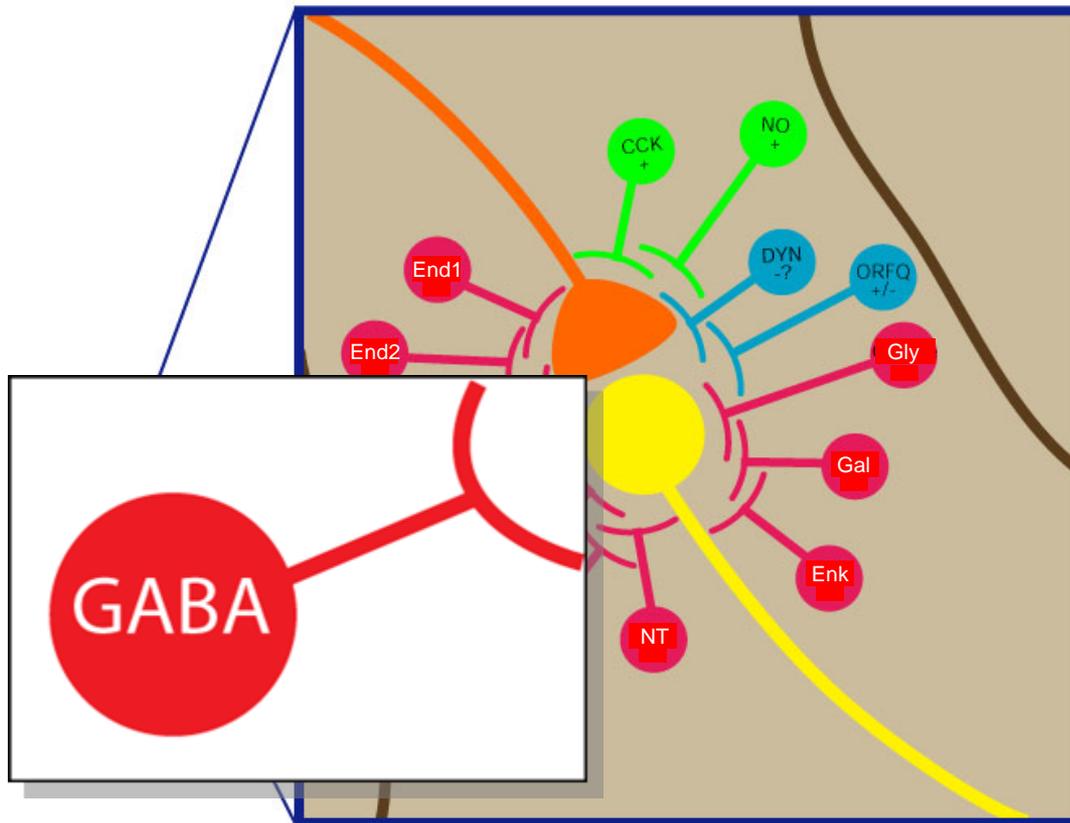
NNT greater than 2 to achieve a 50% reduction in pain intensity



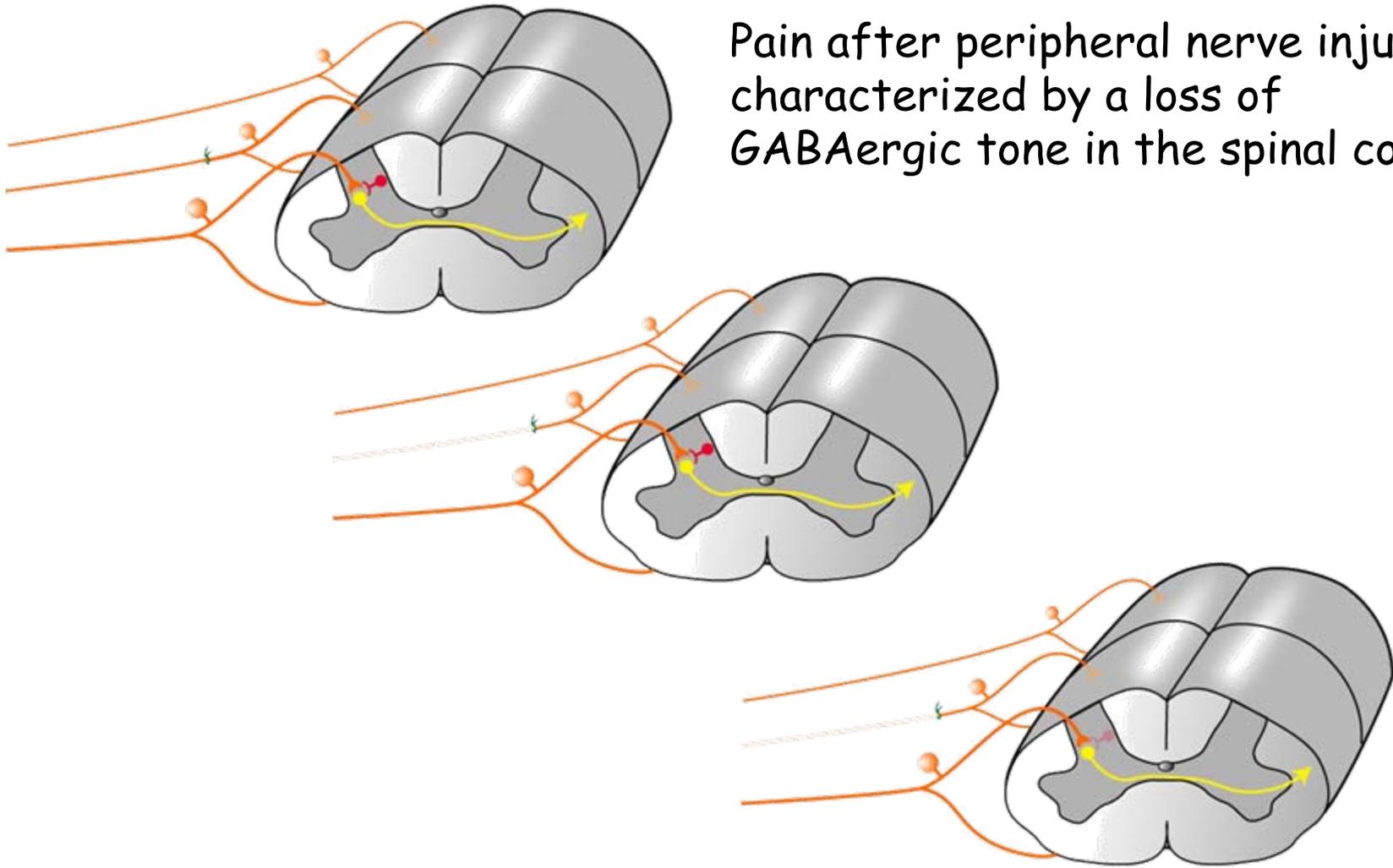
Pain after peripheral nerve injury is characterized by activation of microglia in gray matter of spinal cord

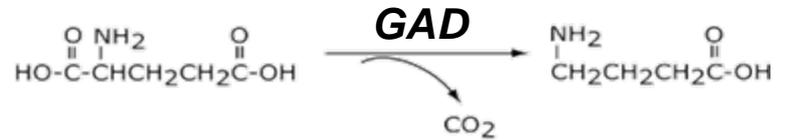


HSV vectors expressing anti-inflammatory peptides IL-4 or the p55 soluble  $\text{TNF}\alpha$  receptor produce analgesic effects that are statistically significant

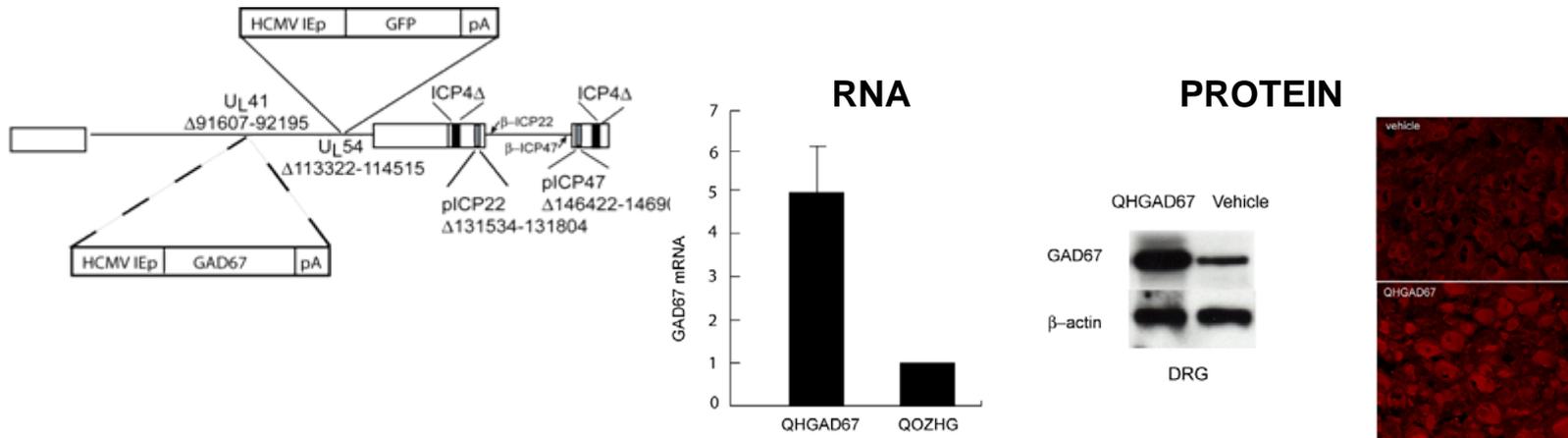


Pain after peripheral nerve injury is characterized by a loss of GABAergic tone in the spinal cord

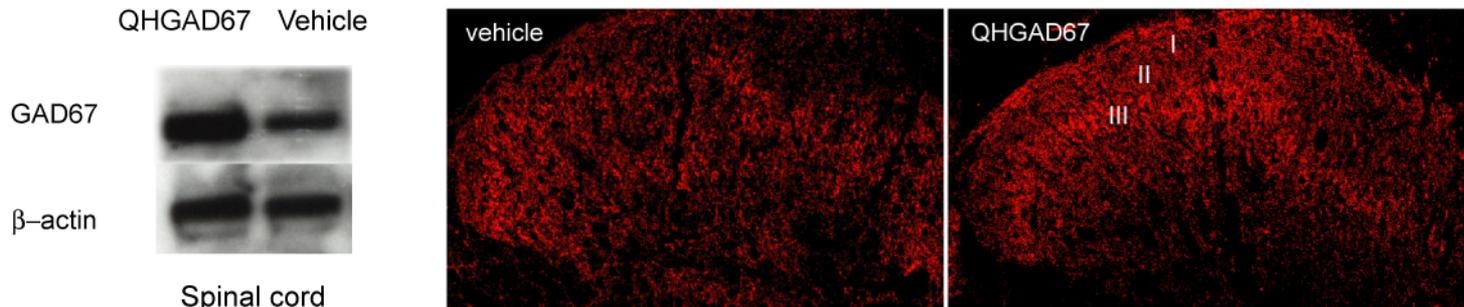




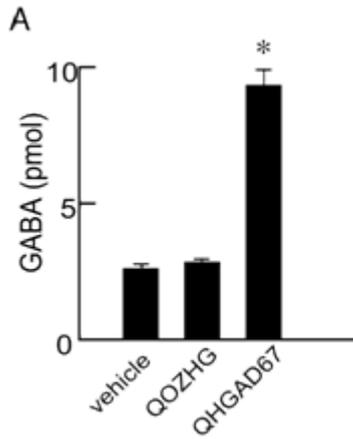
The *GAD*-expressing HSV vector produces *GAD* in DRG *in vivo*



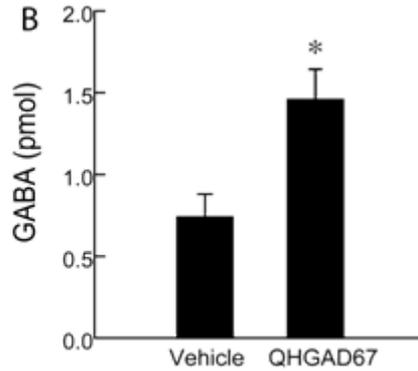
*GAD* protein is transported to afferent terminals in the spinal cord...



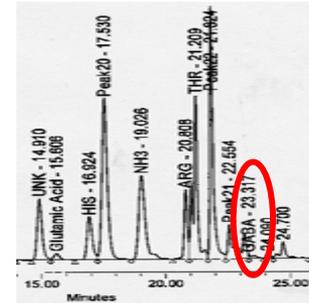
...resulting in the release of GABA...



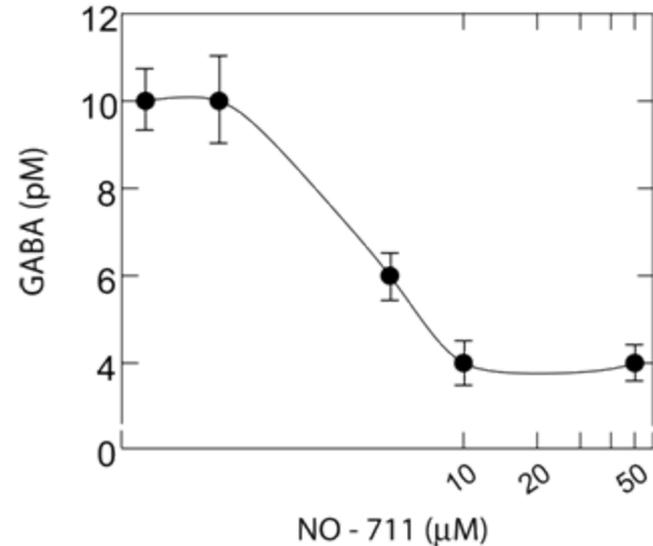
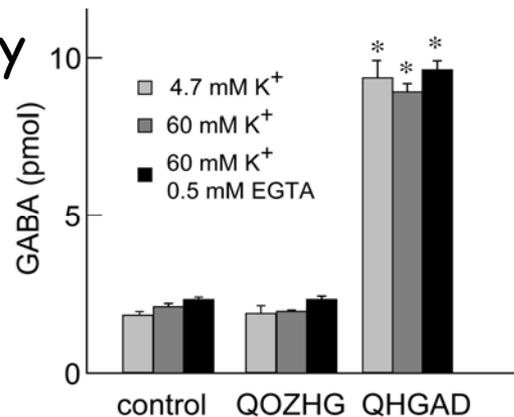
DRG neurons *in vitro*



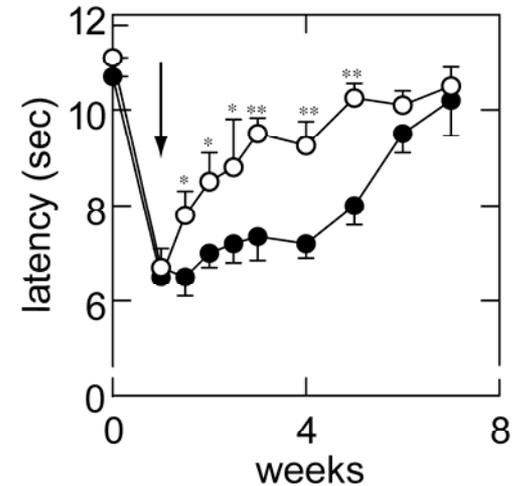
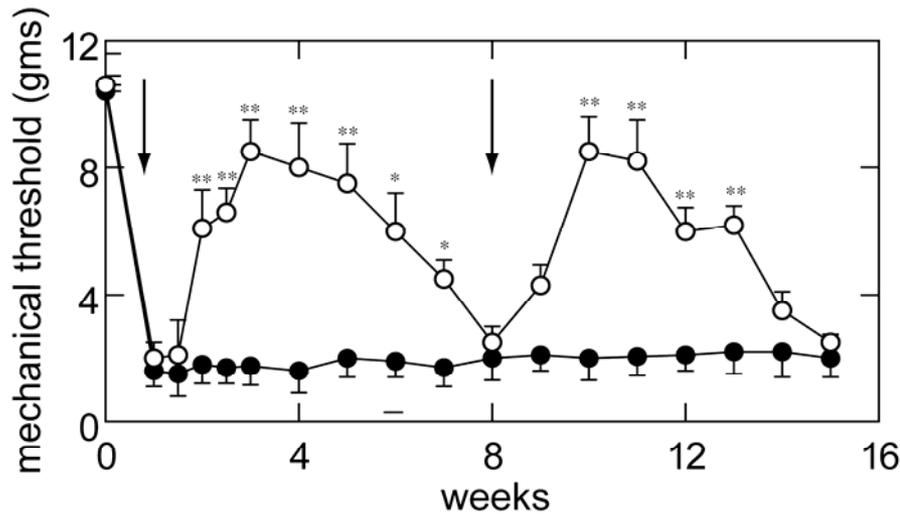
dorsal horn *in vivo*  
(microdialysis)



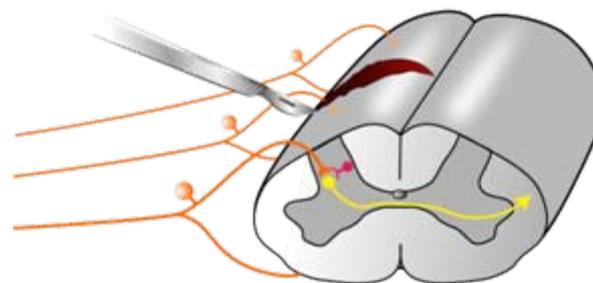
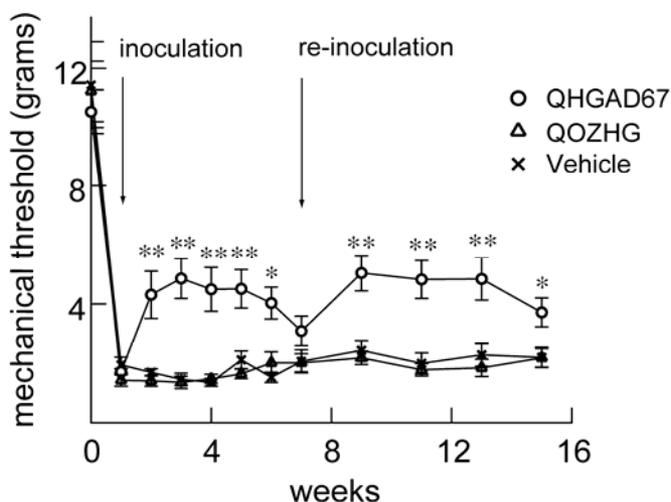
...by a constitutively active mechanism



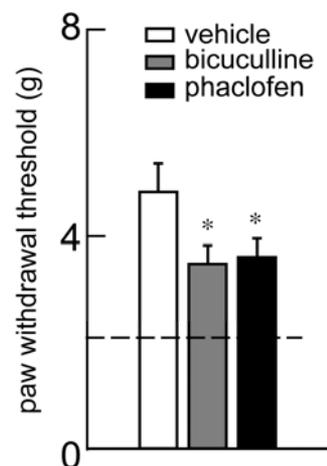
GABA release by the GAD-expressing vector produces a robust antiallodynic effect in the spinal nerve ligation model of neuropathic pain



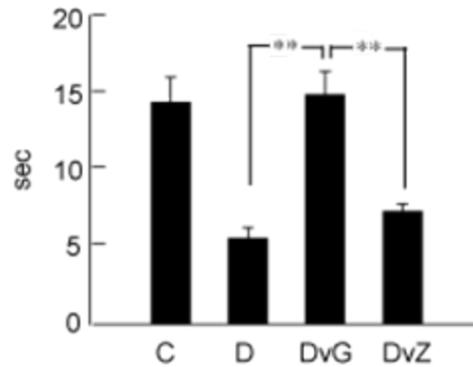
# HSV-mediated GABA reduces below-level central neuropathic pain in the lateral hemisection model spinal cord injury pain



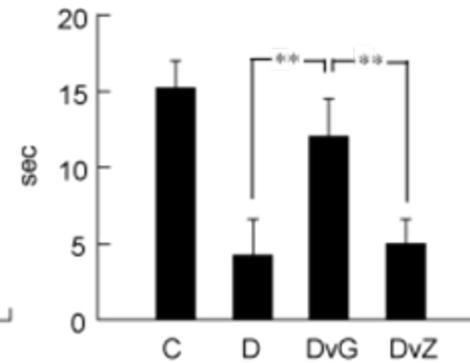
The GABA-mediated effect is blocked in part by both bicuculline and by phaclofen



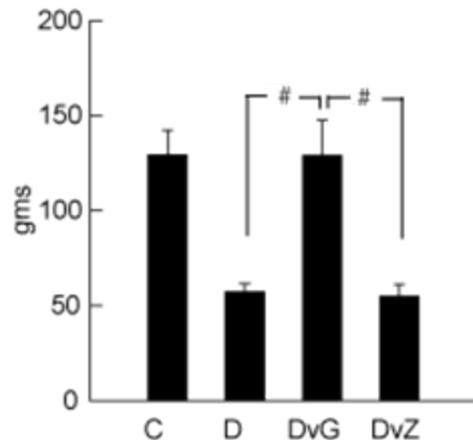
# GABA release by the GAD-expressing vector reverses pain-related behaviors in the STZ model of painful diabetic neuropathy



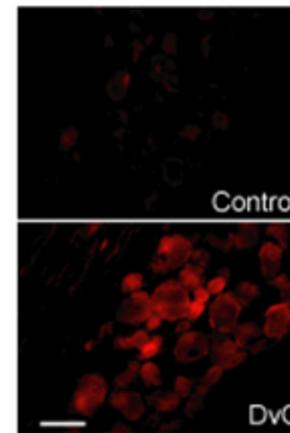
Thermal hyperalgesia



Cold allodynia



Mechanical hyperalgesia



# HSV GAD gene transfer for PDN: Phase I/II trial

## *Inclusion criteria*

- Patients with painful diabetic neuropathy  
Present for at least 6 months  
Pain consistently  $\geq 4$  on a 10 point scale
- Stable glycemic control  
Glycosylated hemoglobin  $\geq 12\%$
- Intraepidermal nerve fibers present  
on punch biopsy of the skin in the distal lower extremity

## HSV GAD gene transfer for PDN: Phase I/II trial *Protocol*

- Clinical grade GAD-expressing vector, inoculated in ten 100 microliter intradermal injections in the L4/L5 dermatomes in the distal leg
- 60 patients randomly assigned to receive vector or placebo in a 2:1 ratio
- **Primary Outcome Phase I: Safety**
- **Primary Outcome Phase II: Efficacy**  
determined by a 30% reduction in pain measured by the 24 hour average pain intensity at 2, 4 and 6 weeks after vector inoculation

Q1. What is the rationale for using the HCMV IEp to drive transgene expression?

A1. Self-limited expression provides safety in case of an unanticipated adverse event. Should the treatment prove effective and prolonged therapy preferable, we will proceed construct a similar vector with the LAP2 promoter driving GAD expression.

Q2. Will pre-existing anti-HSV immunity compromise the therapeutic benefit?

A2. Our data, demonstrating that re-inoculation re-establishes the therapeutic effect in several different models and with several different transgenes, suggests that this will not be a problem.

Post-hoc comparison of the therapeutic effect in patients with and without pre-existing anti-HSV titers will ultimately provide a definitive answer to this question.

Q3. Will the treatment result in the production of anti-GAD antibodies resulting in a disease phenotype?

A3. We don't anticipate a problem, because:

(1) Single injections of the vector do not appear to cause a significant immune response.

(2) While antibodies to GAD65 are found in autoimmune disease, antibodies against GAD67 are not.

(3) Intentional immunization with GAD65:Alhydrogel is currently in Phase III trial as an immune modulating therapy for diabetes mellitus. There has been no evidence of adverse immune consequences in toxicology studies or in the trial to date.

Q4. Is there data regarding spread of vector genomes beyond the injection site?

A4. FDA-mandated biodistribution studies of 320 mice from 1 to 90 days following subcutaneous inoculation of the preproenkephalin-expressing HSV vector showed quantifiable genomes only at the injection site (early) and in DRG.

Q5. In the absence of safety data, please provide additional rationale for the phase 1/2 design.

A5. Safety and dose-finding data for HSV-mediated gene transfer will be provided by the phase 1 study of the preproenkephalin-expressing vector in patients with terminal cancer, that we anticipate will be completed by the end of this calendar year. The results of that study will inform us regarding ultimate design of the GAD PDN study.

## HSV GAD protocol

RAC review (March 2009)

Insertion of transgene into human-grade vector backbone

GLP production of toxicology lot and GMP manufacturing optimization

cGMP production and certification of Master Cell Bank

cGMP production and certification of Master Viral Bank

FDA-approved GLP toxicology studies

FDA-approved GLP biodistribution studies

Drafting and submission of IND application to FDA

University of Michigan institutional approvals

Cancer Center Protocol Review Committee (PRC)

Institutional Biosafety Committee (IBC)

General Clinical Research Center (MCRU) Review

Institutional Review Board (IRB)

Certificate of Analysis

First patients (?)

Q6. HSV delivered subcutaneously has been shown to present antigens and...elicit an immune response. Please comment on the immunological issues.

A6. (1) The data from the re-inoculation experiments suggest this should not be a problem.

(2) Drs. Federoff and Bowers have demonstrated that two subcutaneous inoculations of an HSV amplicon expressing  $A\beta_{1-42}$  does not lead to a detectable immune response.

(3) There is extensive data from clinical trials in which replication competent but compromised "oncolytic" genomic HSV recombinants have been injected into tumors with no reports of systemic immunologic problems.

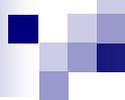
Q7. Provide additional rationale for the anatomical compartments that are planned for HSV gene transfer.

A7. (1) The vector will be injected intradermally to provide access to sensory nerve terminals.

(2) The injections will be targeted to the skin of distal leg dermatomes innervated by L4 and L5 roots, in order to achieve transgene-mediated release of GABA in the dorsal horn of spinal cord at the relevant rostro-caudal level.

Q8. Consent form issues regarding “therapeutic misconception”, research related injuries, randomization and request for autopsy.

A8. These issues will be addressed and corrected when we draft the final consent form in consultation with our Institutional Review Board.



University of Michigan

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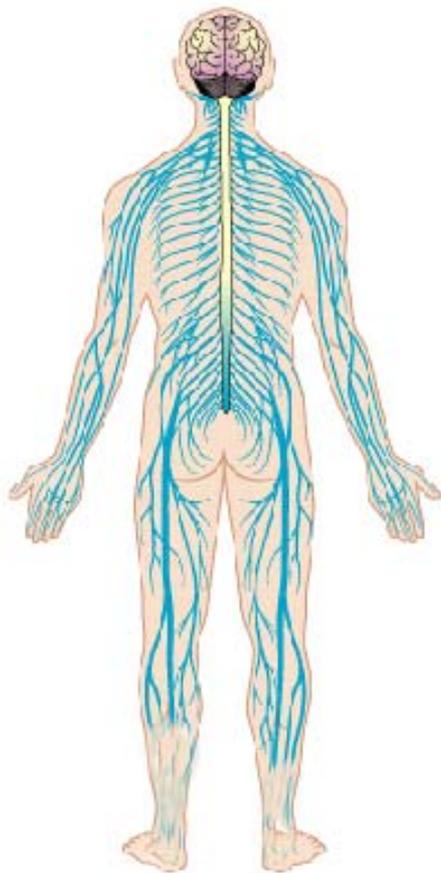
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Susan Urba  
Srinivas Chiravuri  
Frank Worden  
Suzette Walker  
Heidi L'Esperance  
Mary Orr  
Gayle Estep

# Peptide neurotrophic factors can be used to treat polyneuropathy in animal models



## Research Report

### Nerve growth factor administration protects against experimental diabetic sensory neuropathy

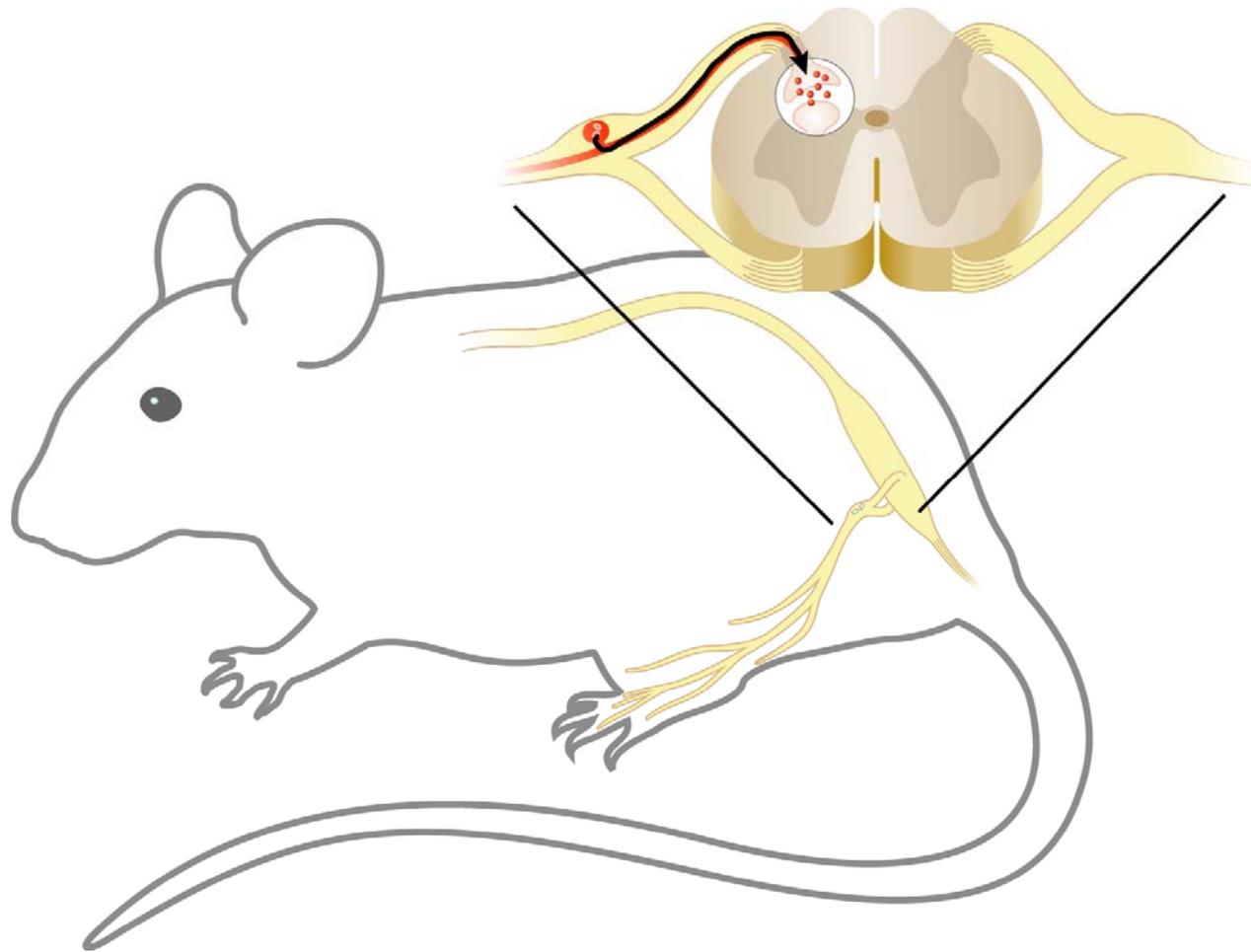
Stuart C. Apfel <sup>a,c,\*</sup>, Joseph C. Arezzo <sup>a,c</sup>, Michael Brownlee <sup>b</sup>, Howard Federoff <sup>b,c</sup>,  
John A. Kessler <sup>a,c</sup>

*Departments of <sup>a</sup> Neurology, <sup>b</sup> Medicine, Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA*

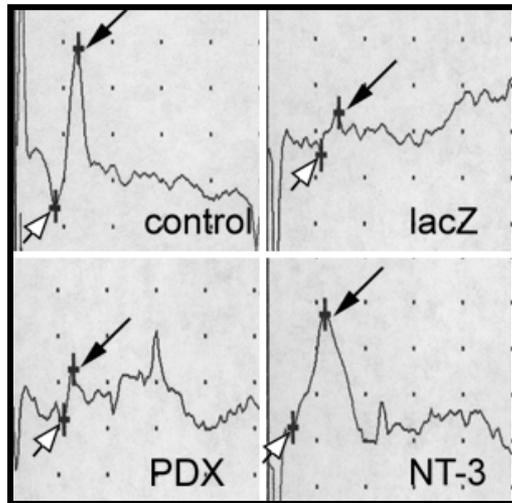
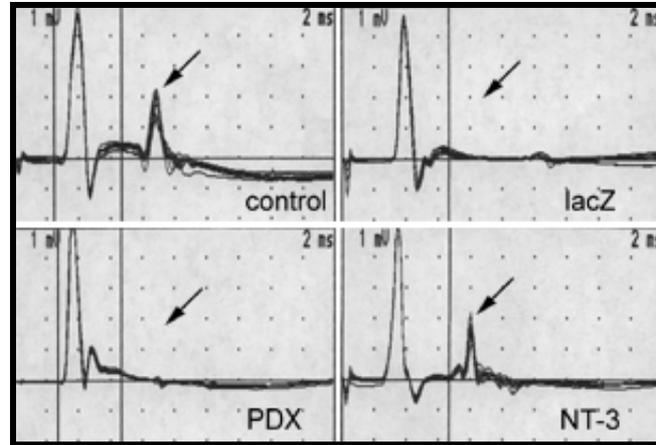
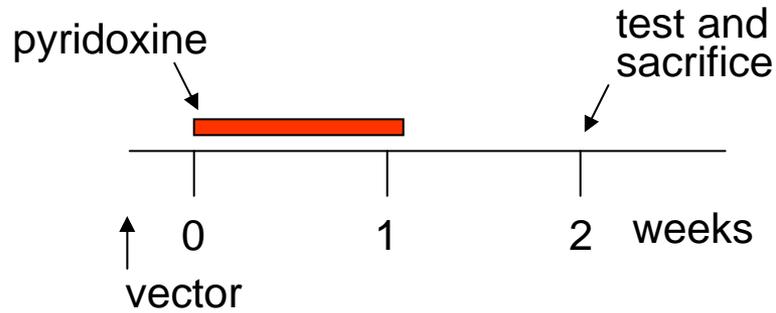
(Accepted 10 August 1993)

## but have proven ineffective in human trials

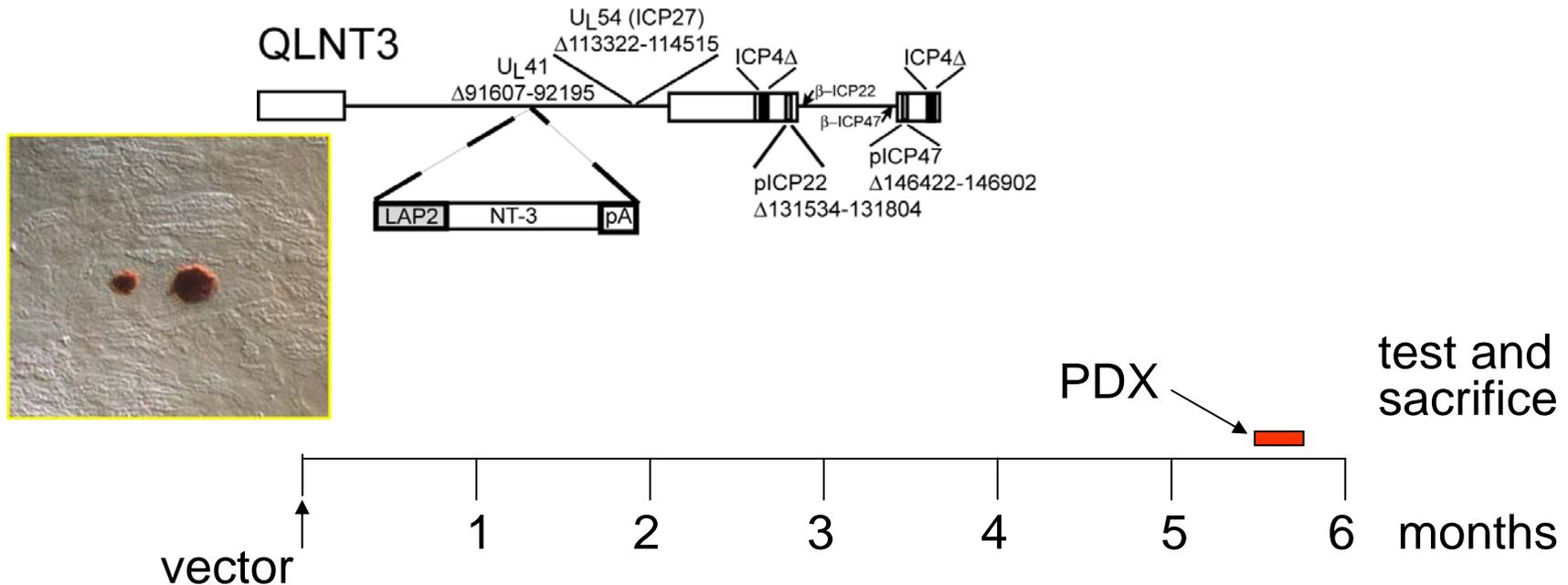
NGF	Diabetic neuropathy	Phase 1–2, placebo controlled, 6 months	250 patients	Subcutaneous, 0.3 µg/kg, 3 times a week
NGF	Diabetic neuropathy	Phase 3, placebo controlled, double blind, 48 weeks	505 patients NGF treated, 515 patients in placebo group	Subcutaneous 0.1 µg/kg, 3 times a week
NGF	HIV neuropathy	Phase 2, placebo controlled, 18 weeks	270 patients	Subcutaneous, 0.1–0.3 µg/kg, twice a week
NGF	HIV neuropathy	Phase 2, open label follow-up study, 48 weeks	200 patients	Subcutaneous, 0.1–0.3 µg/kg, twice a week



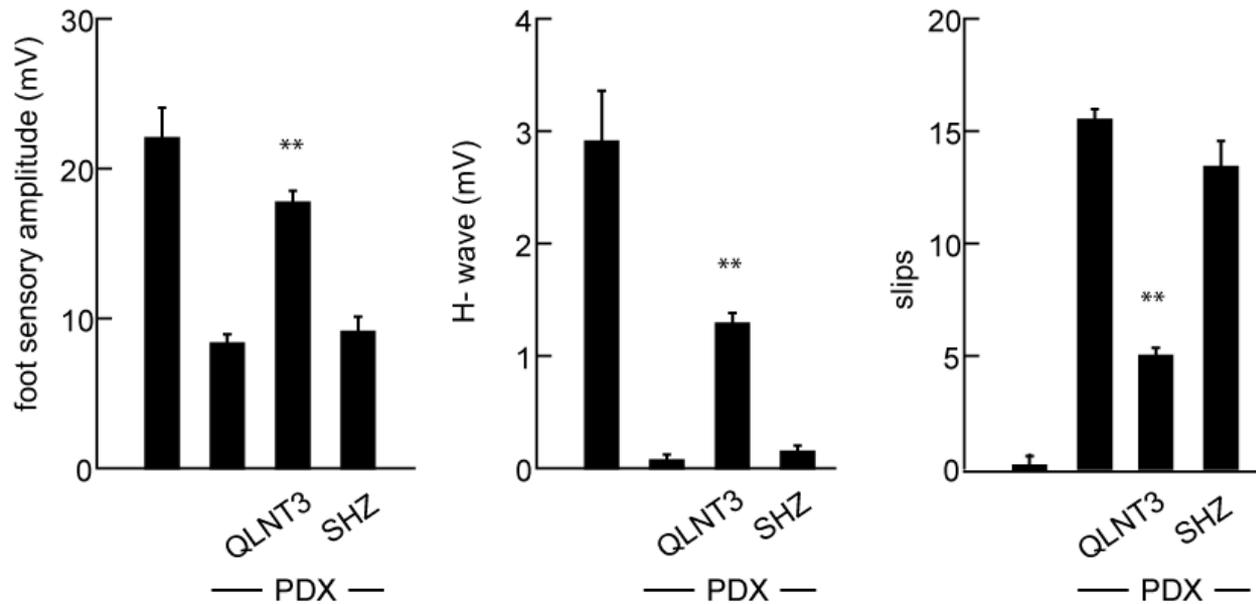
# HSV-mediated expression of neurotrophin-3 prevents neuropathy caused by subacute overdose of pyridoxine (400 mg/kg bid)



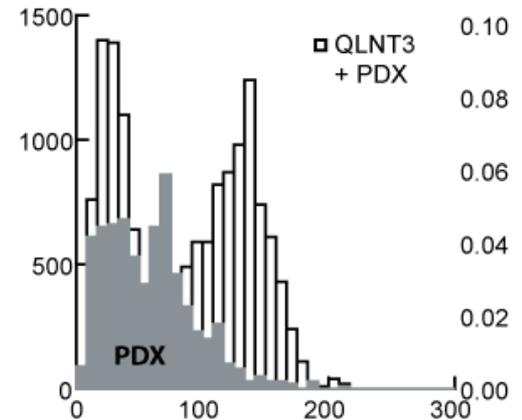
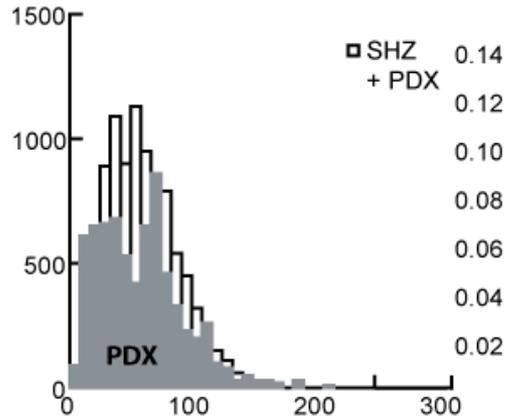
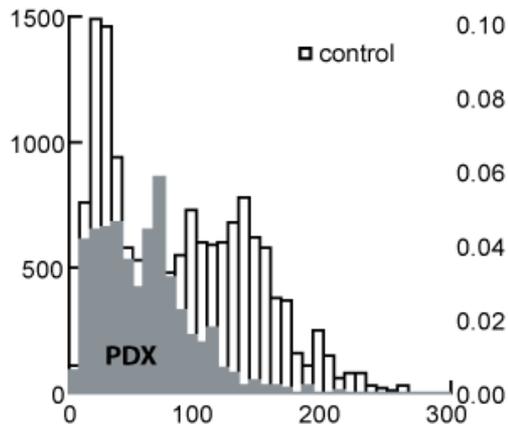
# LAP2-driven expression provides biologically active transgene expression at 6 months after inoculation - PDX neuropathy model



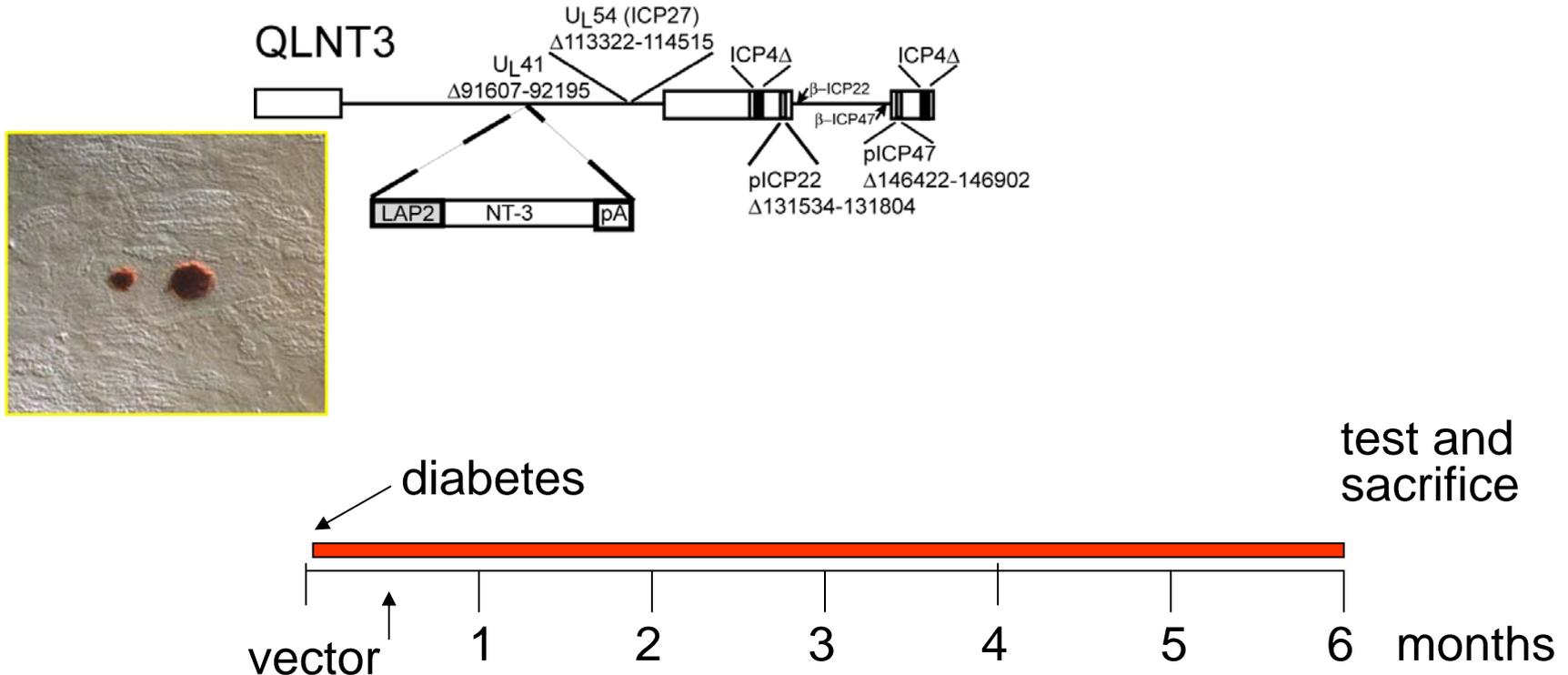
# LAP2-driven expression provides biologically active transgene expression at 6 months after inoculation - PDX neuropathy model



# LAP2-driven expression provides biologically active transgene expression at 6 months after inoculation - PDX neuropathy model



# LAP2-driven expression provides biologically active transgene expression for 6 months after inoculation in diabetic neuropathy



# Subcutaneous inoculation of an NT-3 expressing HSV vector protects against the development of diabetic neuropathy over 6 months

