Viral vectors as tools for neuroscience

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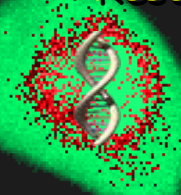

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The Physiological Society

Plan of this workshop

Lecture 1 (SK)

Introduction: WHY using viral vectors?

What are viral vectors and how they work

Why they are good research tools, what are the limitations? Can you use them?

Lecture 2 (AGT)

How to make viral vectors for cell type-specific transgene expression in the brain:

- Viral vector construction
- Targeting gene expression to a specific cell type

Lecture 3 (SK)

Practical aspects of viral vector application

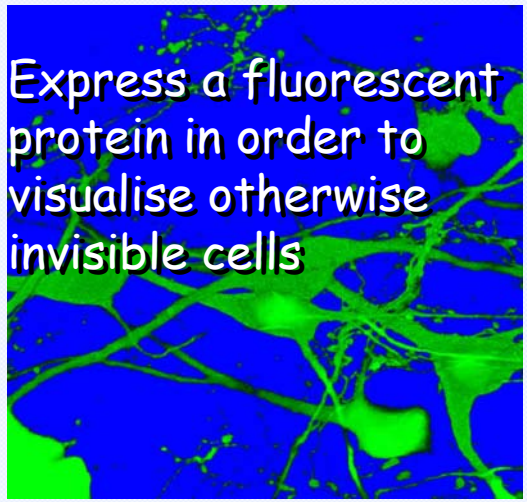
How to modulate cell's function using viral gene transfer

http://www.bris.ac.uk/Depts/Physiology/Staff/Pysk/virallab/index.htm

Introduction: WHY using viral vectors?

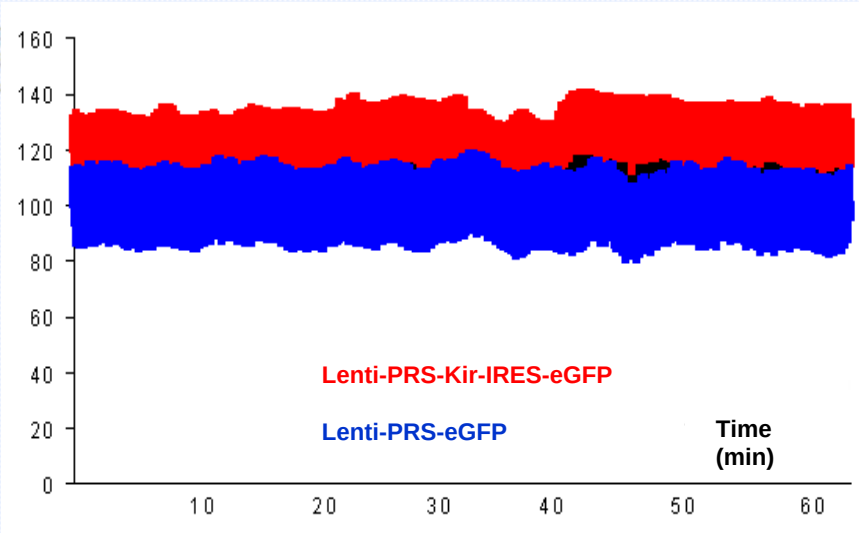
Powerful tools for manipulating functions of mammalian cells:

Increase or decrease levels of endogenous proteins to enhance or inhibit certain functions or to introduce completely novel proteins which confer new properties to a cell



Express a K^+ ion channel in a neurone to decrease its activity

A chronic effect of viral gene expression on blood pressure

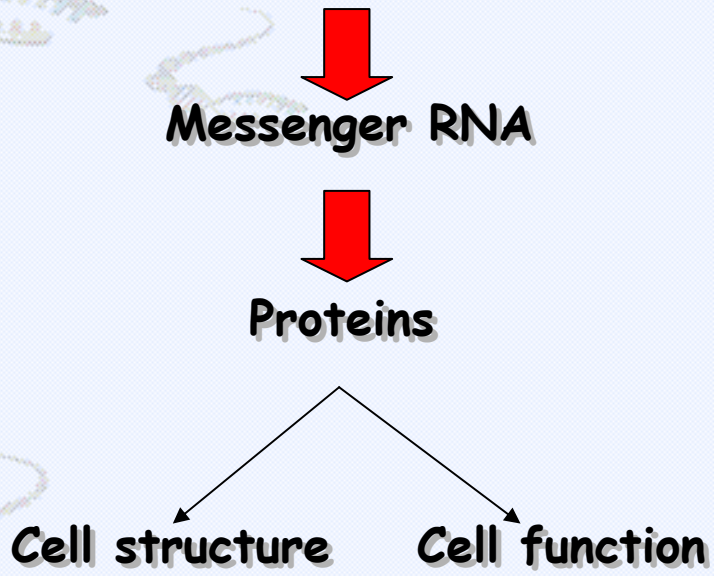


What's great about this approach?

1. The effect *in vivo* lasts weeks to months – thus you can study chronic processes under physiological conditions
2. You can direct it to a particular brain area (inject viral vectors into a specific nucleus) or subset of cells (using cell-specific vectors)
3. You can use rats of any strain or mice or even bigger animals. Your animals are otherwise "normal", not the highly inbred lines as all transgenic mice.
4. It is MUCH cheaper than generating and maintaining transgenic mice
5. It is easily transferable: vectors can be produced in one lab and delivered on dry ice to the other end of the world
6. Viral vectors are actually quite easy and safe to use (provided you know what you are doing!)
7. YOU CAN DO IT!

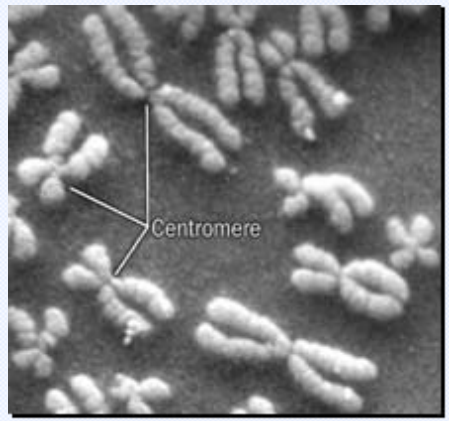
Introduction

The basic flow of genetic information in a mammalian cell



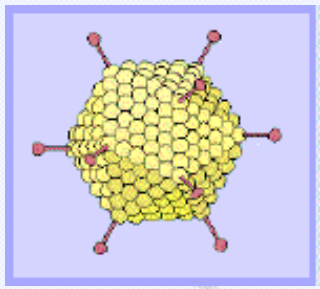
Viral vector allow delivery of foreign genes into the mammalian cells.
In this way we can change the cell's structure and function.

Endogenous cellular genes – in chromosomes



Chromosomes – supercoiled DNA just before cell division

Chromosomes are locked up in the nucleus. Nucleus contains all the transcriptional machinery of the cell and for the control of gene expression.



Wild type viruses introduce their genes into the host cells and make them produce many copies of the virus… ("highjack" cell's machinery for gene expression)
TO PROPAGATE THEMSELVES

Viral vectors are disabled viruses

They:

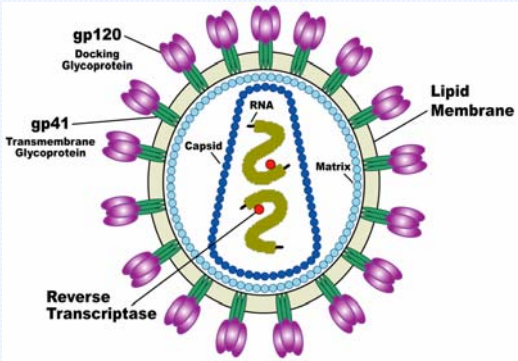
1. **Do NOT replicate in the infected cell**
2. **Do NOT (strongly) interfere with its normal function**
3. **Do NOT kill it**

4 … Just deliver your gene of interest:

There are some vectors which are made "partially" disabled which actually DO multiply in the host cells and eventually kill them. Examples: pseudorabies-based vectors, Semliki forest virus-based vectors and some others. Physiological relevance of observations made using such vectors is questionable.

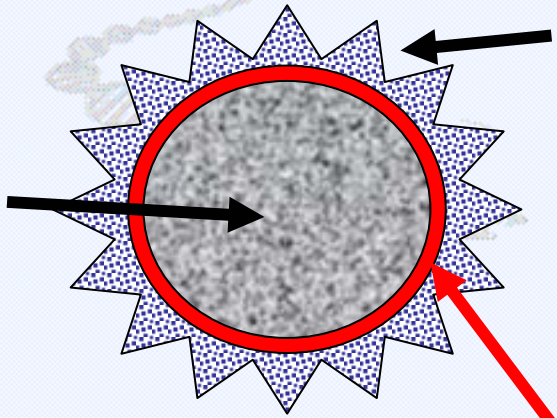
Viraluses are professional parasites. They have evolved to be highly efficient. They are better tools for gene delivery than any currently known chemical reagents, because they provide:

- Higher efficiency of gene delivery *in vivo*
- High levels of gene expression
- Much more stable expression



HIV

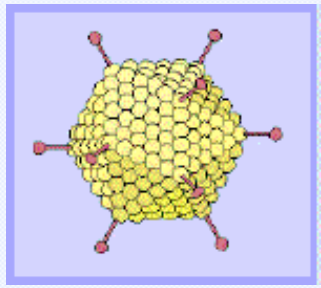
Generic structure of a virus



Densely packaged DNA or RNA

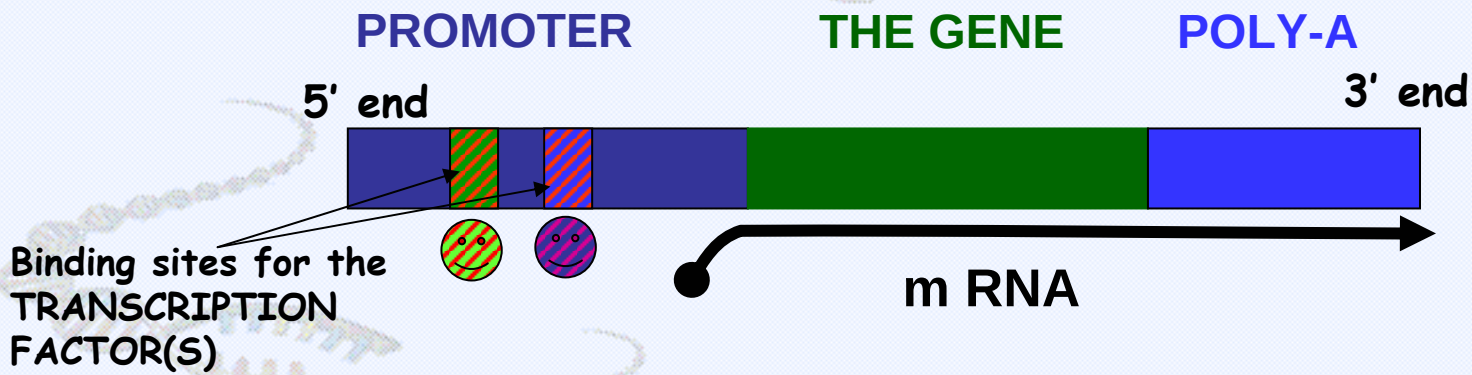
Sometimes - an"envelope" made of glycoproteins & lipids

Capsid - a "box" made of protein(s)



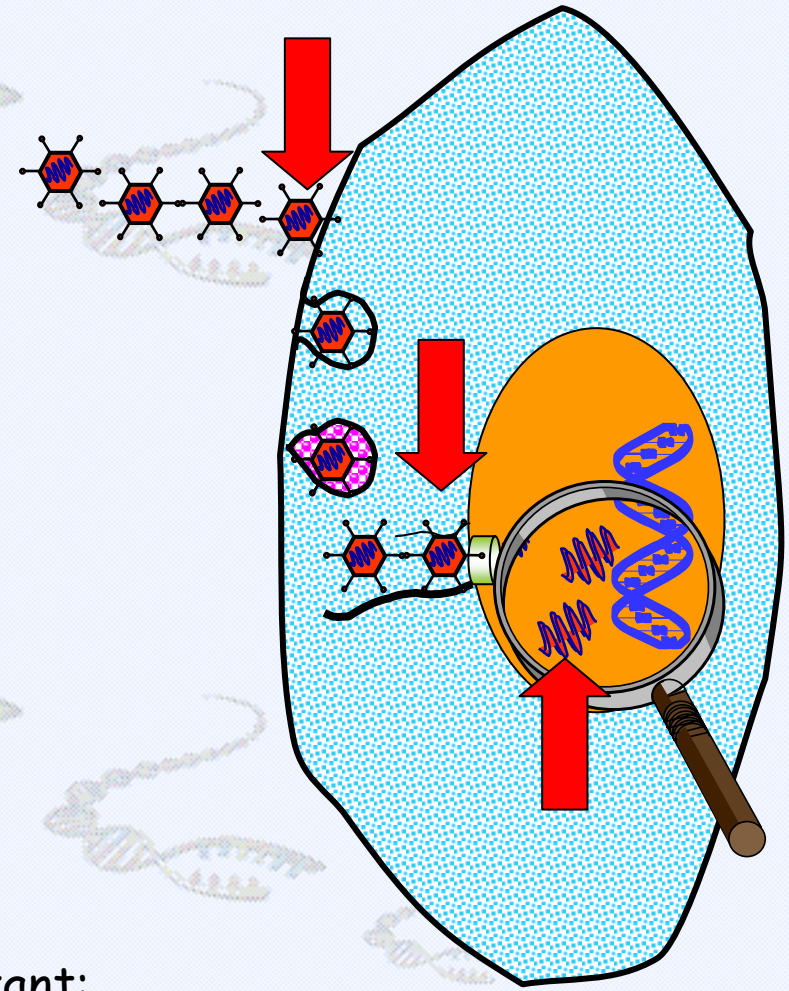
Adenovirus

Expression cassette – a piece of DNA containing elements (promoter, coding part and polyadenylation signal) necessary for expression of a transgene



1. Promoter sequences are located upstream of the gene
2. Specific proteins known as transcription factors can bind to these sequences and facilitate transcription

Delivery of a viral genome into the host cell.



Important:

a) Entry requires binding to the cell's membrane
b) Viruses have "learned" how to overcome the nuclear barrier
c) In most cases more than one viral genome enters a single cell

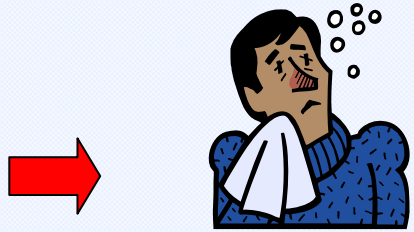
In order to disable a wild type virus into a vector we need to delete part of its genome in order to:

1) Make it unable to replicate and therefore to cause a disease

2) Clear room to accommodate the expression cassette, i.e. that genetic material which will result in expression of our protein of interest

Vectors derived from:

1) Adenovirus
(common cold, serotypes V and II)



2) Lenti/Retrovirus (HIV)

3) Adeno-associated virus
4) Herpes simplex virus

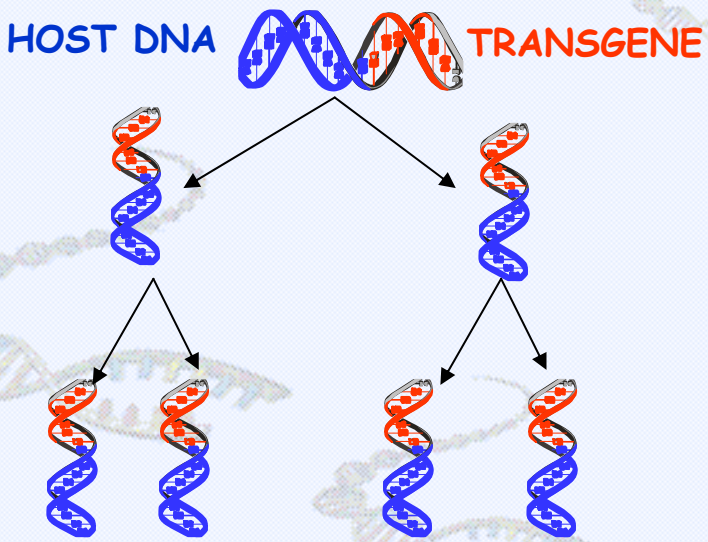
WHAT ARE THE DIFFERENCES BETWEEN DIFFERENT VECTOR TYPES?

1. Which cell types may be transduced?
   (neurones vs glia in the brain, microglia, endothelium – hard to transduce – the gene delivery problem)

2. Does the transgene incorporate into the host cell's genome?

Incorporation of viral genome in the host DNA results in a more stable expression

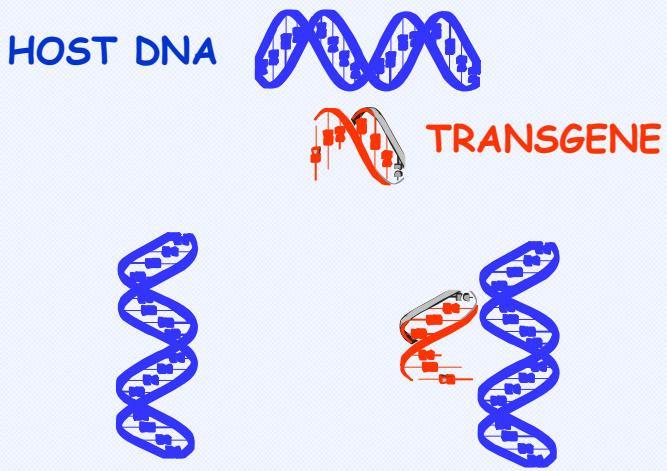
Transgene integrates into the host's DNA (lentivirus)



Long-lasting (~permanent) gene expression. If the cell divides, the transgene will be passed to its progeny.

May be important for gene expression in blood cells

Transgene remains extra-chromosomal (episomal) - adenovirus



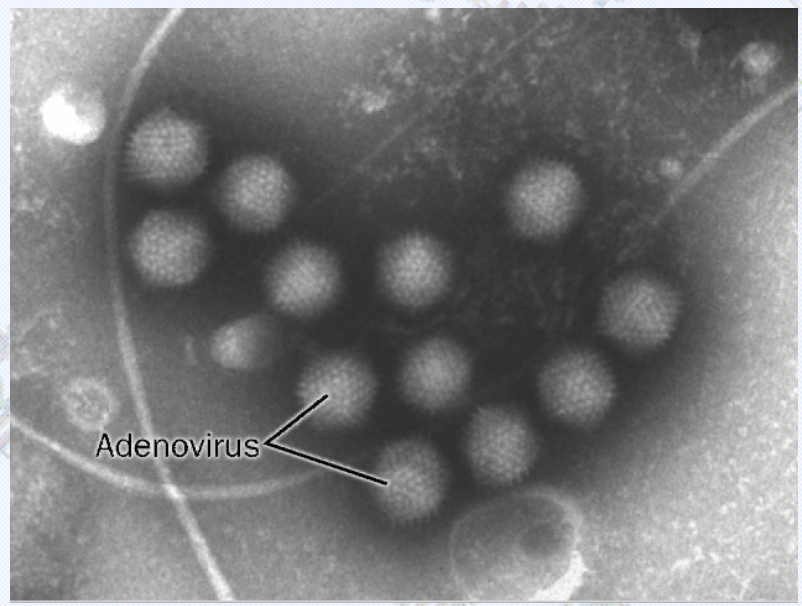
Transgene may be eliminated. Dividing cells lose the transgene.

Fine for CNS – cells do not divide

DIFFERENCES BETWEEN VECTOR TYPES CONTINUED

3. What is the capacity of a vector (how big a transgene it may carry)? (LVV > AVV > AAV)
4. Does the vector cause an immune response? (ADV!!!)
5. Is it safe? (Both AVV and LVV are class I! But watch out for the transgene!)
6. Is it easy to produce and purify to high titres?

Our gene delivery vehicles



Adenovirus

Lenti/Retrovirus (HIV)

More in the next lecture

What one can do using viral gene transfer (examples for experimental neuroscience)

1. To increase concentration of a certain protein and study its function (over-expression studies)

2. To antagonise function of a certain protein (expression of dominant negative proteins and RNAi constructs)

3. To make the cell produce fluorescent indicator proteins (for example, EGFP or Ca^{2+} sensitive proteins). These may be used to monitor various variables within the living cells

4. Control neuronal excitability using light-sensitive ion channels

AND LOTS OF OTHER THINGS!!!

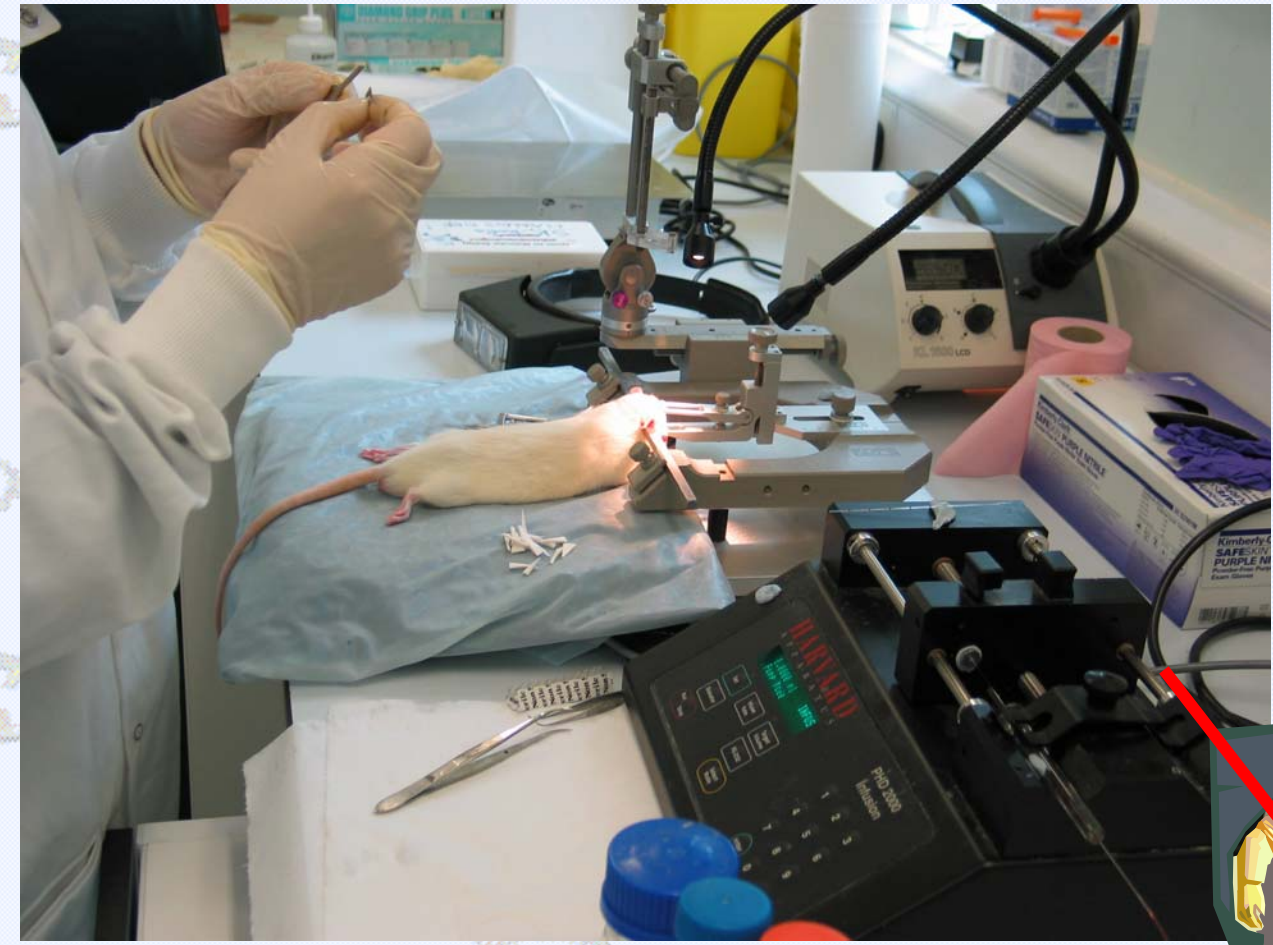
What is not so great about the viral vectors:

1. You have to inject every animal (or put them on any slice culture or other in vitro preparation). You cannot just take a transgenic mouse out of a cage and start using it. Because you have to inject, there will always be a residual risk of an accidental hand prick.

2. You cannot place dangerous constructs into viral vectors. A gene which can cause cancer may become very dangerous if placed into a virus. This risk, however, should not be over-estimated.

3. You use them up, so you need to establish your production line. A properly trained PhD student can do it.

4. You cannot achieve a global effect: in a knock-out mouse all cells of the body at all stages of the development will lack the knocked-out gene. You cannot achieve the same using a viral vector.


HARVARD
PHD 2000

CAN DO IT!

Useful terms:

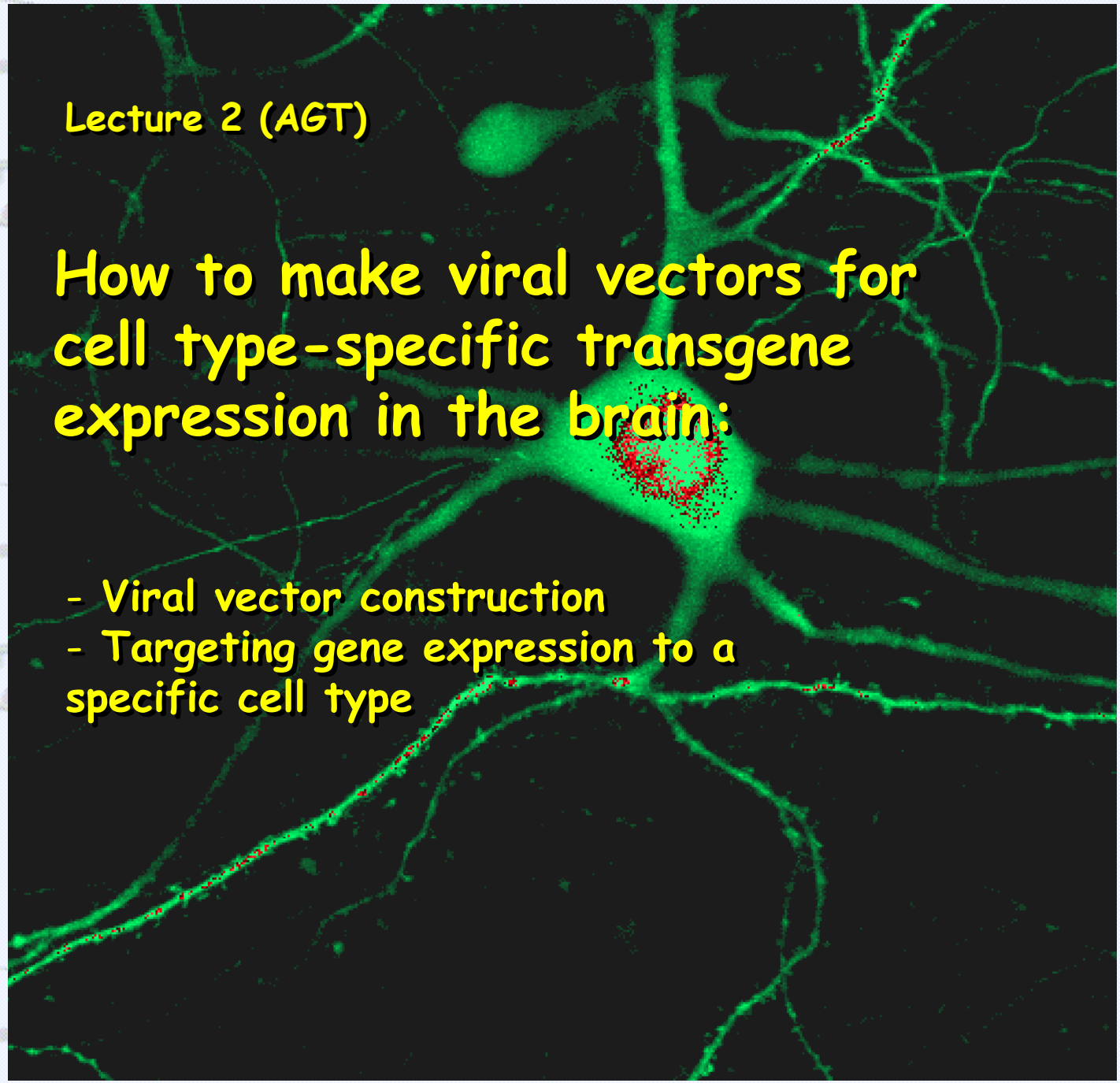
Gene expression – the process of formation of messenger RNA (mRNA) of a DNA template which then is translated into the sequence of aminoacids at the ribosome to make proteins

Transgene – a foreign gene introduced into the cell (for example by a viral vector)

Transduction – process of delivery of a foreign gene into the target cell

In order to make a clear distinction between our constructs and wild type virulent viruses we use the term "vectors"

Expression cassette – a piece of DNA containing elements (promoter, coding part and polyadenylation signal) necessary for expression of a transgene



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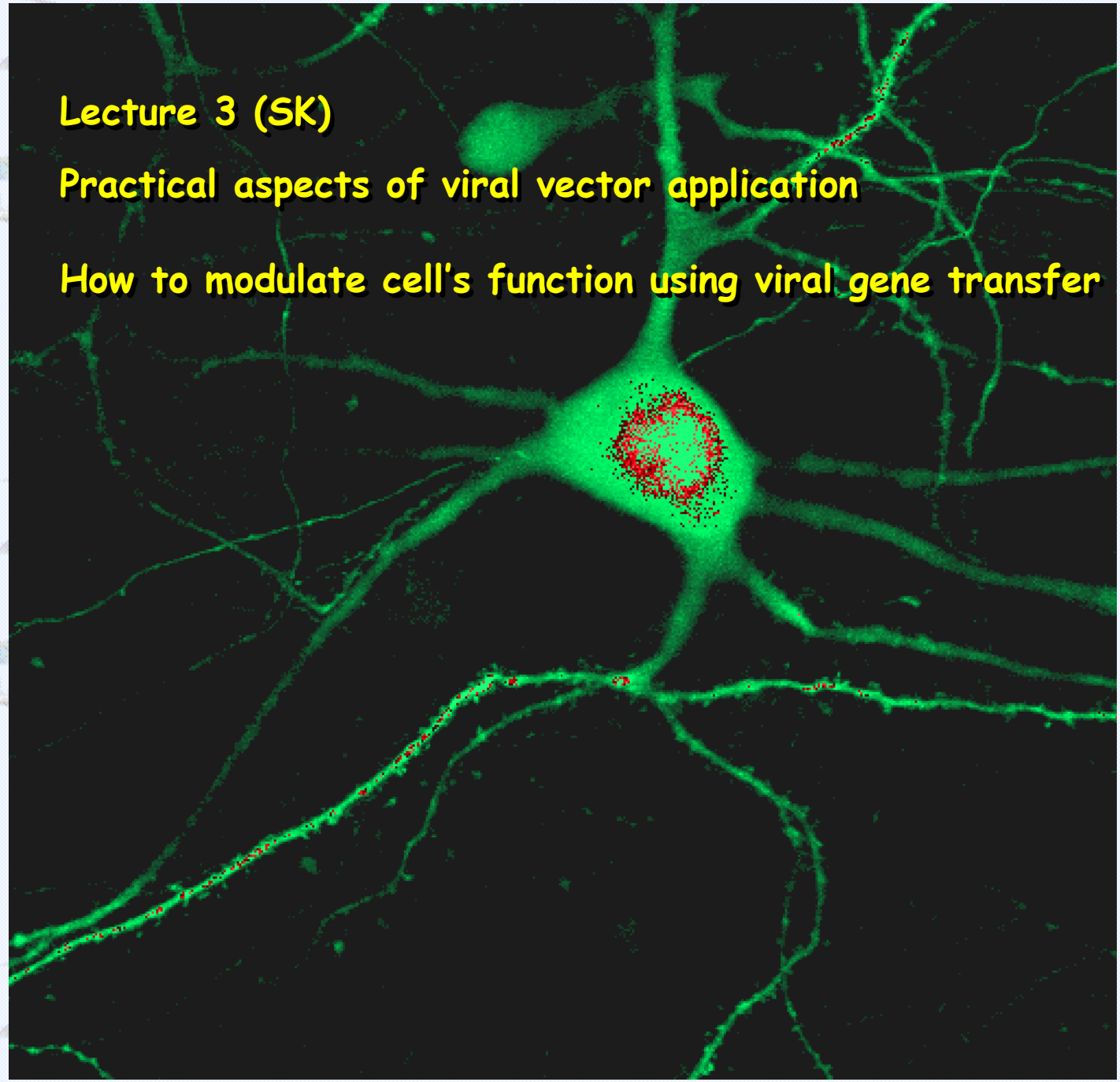
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Practical aspects of viral vector application
How to modulate cell's function using viral gene transfer

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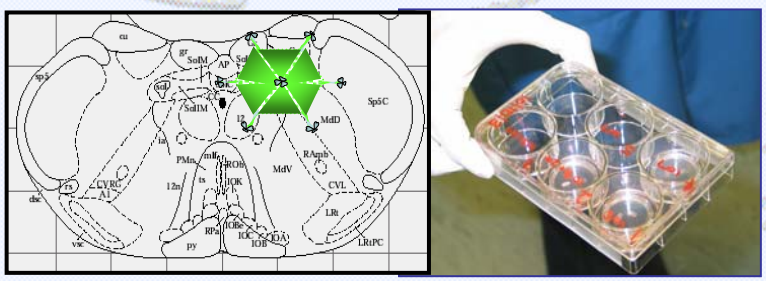
Practical aspects of viral vector application

in vitro:

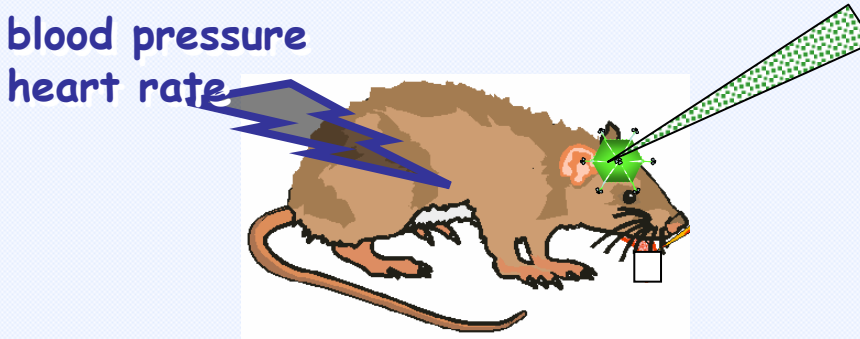
viral transduction in organotypic brainstem slice culture



See:
Wang S et al FASEB J 2006;
Chiti et al 2007

in vivo:

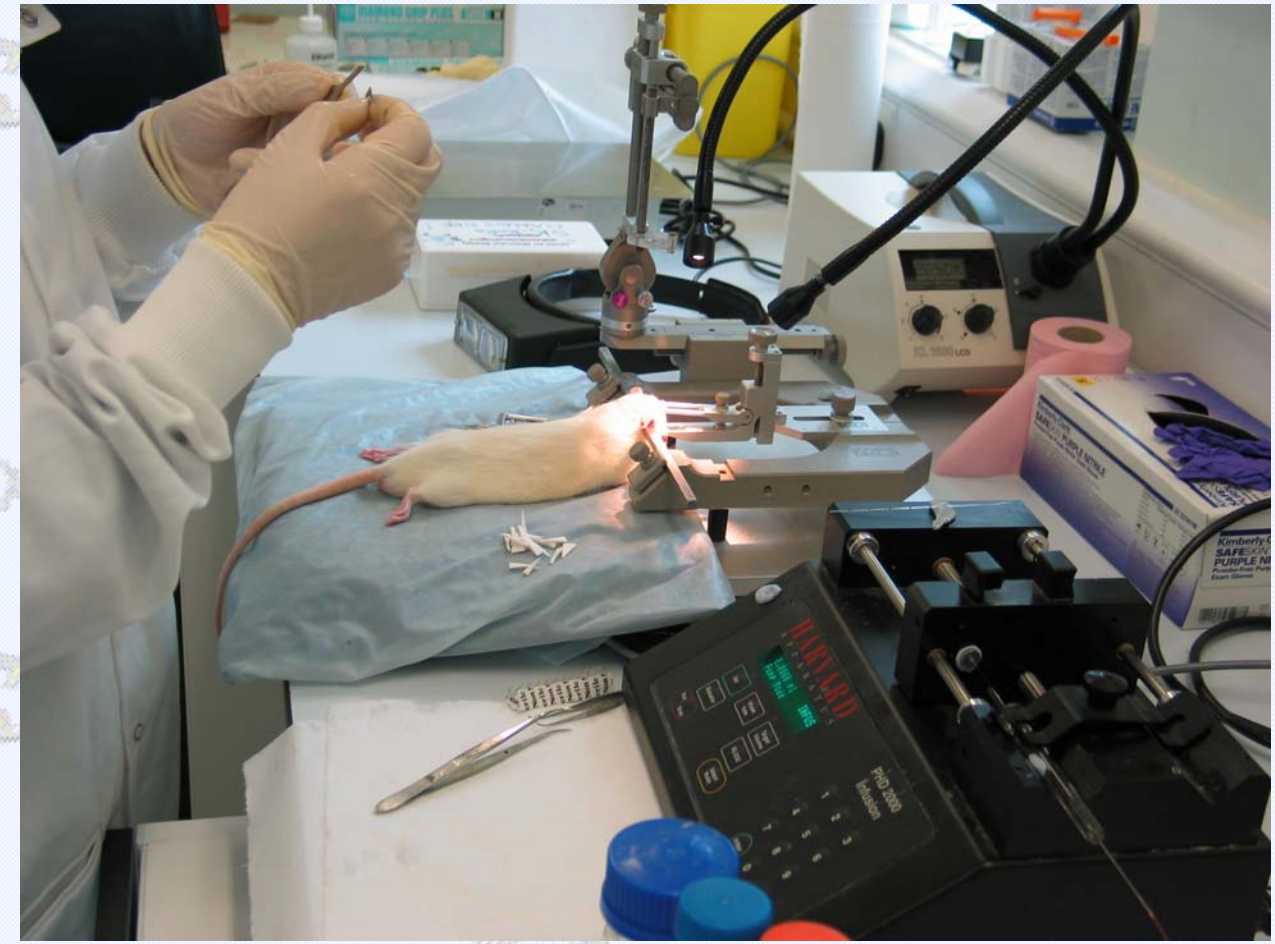
viral microinjections, outcome evaluated in freely moving animals



See: Waki,H. et al. 2003 – 2007 papers,
Duale et al 2007 – Cardiovasc Res in press

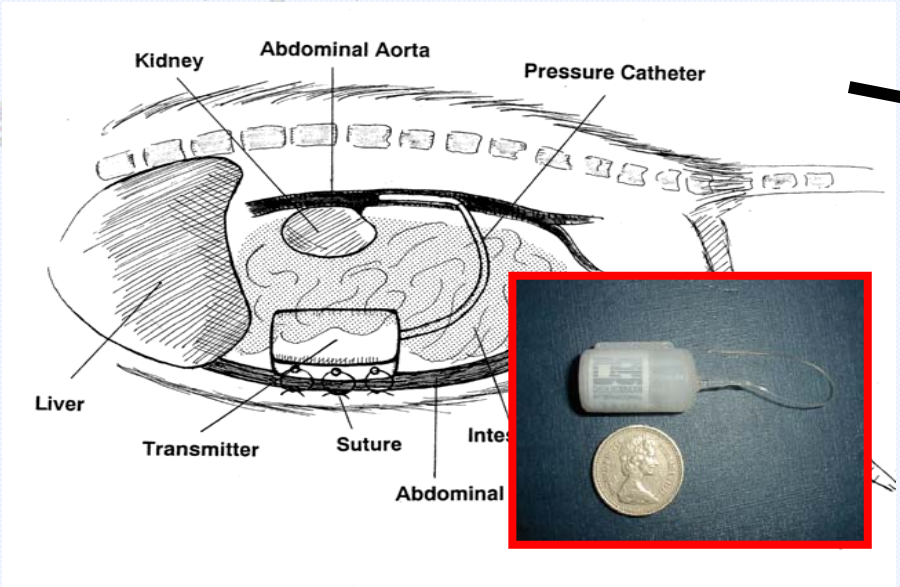
In Vivo:

Injection of viral vectors into a rat brain

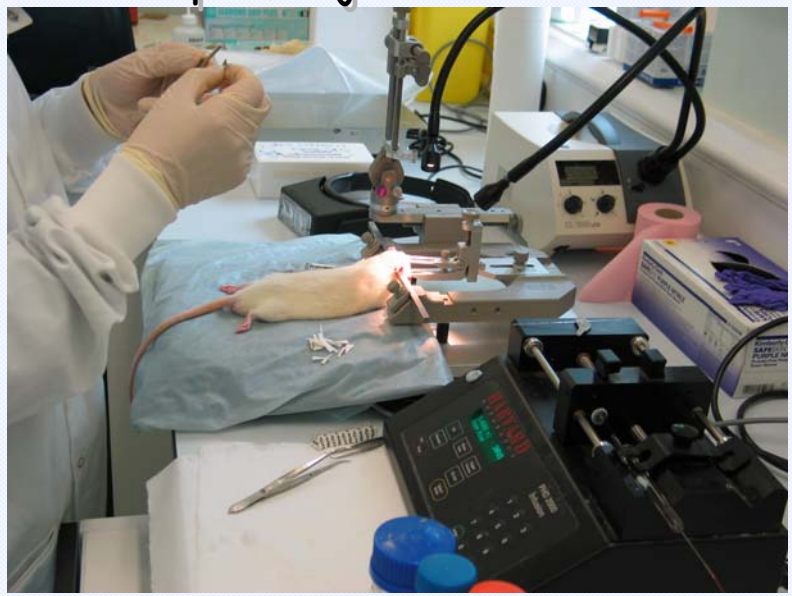


Vectors in most cases need to be injected into the target area directly

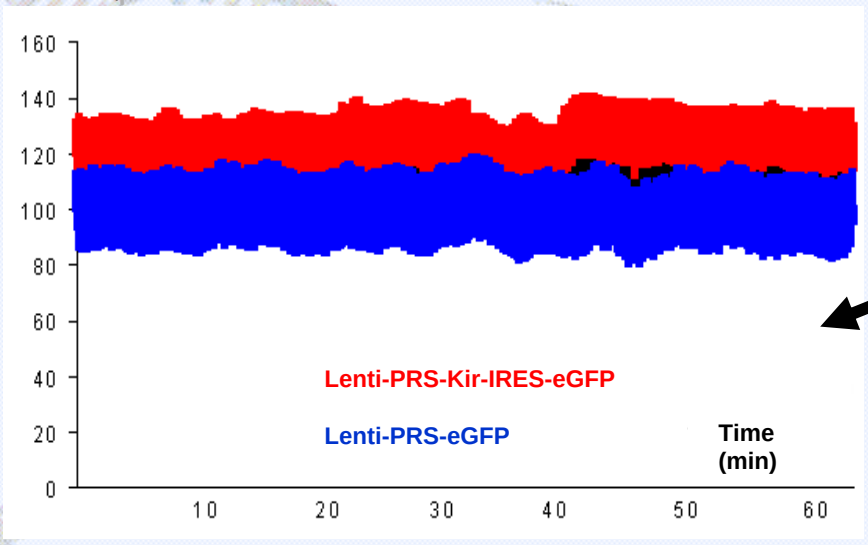
Step 1. Implant BP transmitter



Step 2. Inject virus

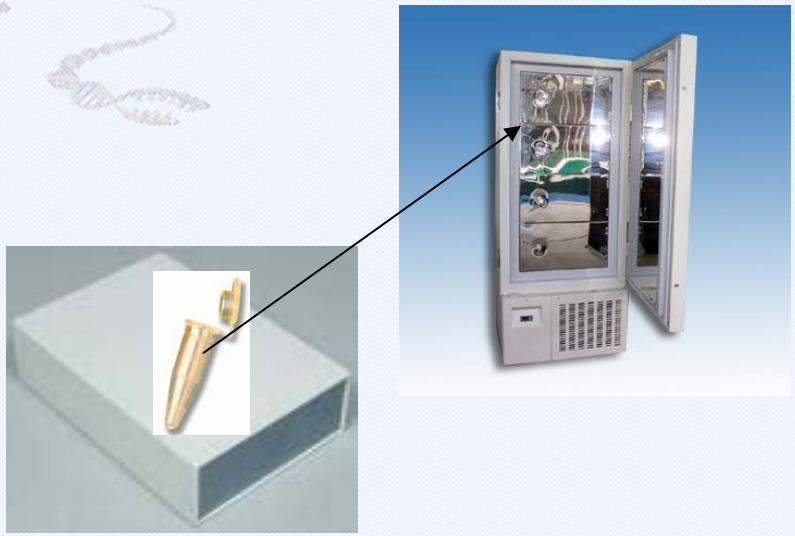


Step 3. Evaluate chronic effect of viral gene expression in noradrenergic neurones of NTS on blood pressure



Procedure for vector injection into the brain

1. Vectors are stored at -80^{o}C until they get injected



2. They are aliquoted into numerous test tubes usually @ 5 mkL for LVV and @10 – 20 mkl for AVV. Once the tube has been thawed, you do not re-freeze it or the titre drops.

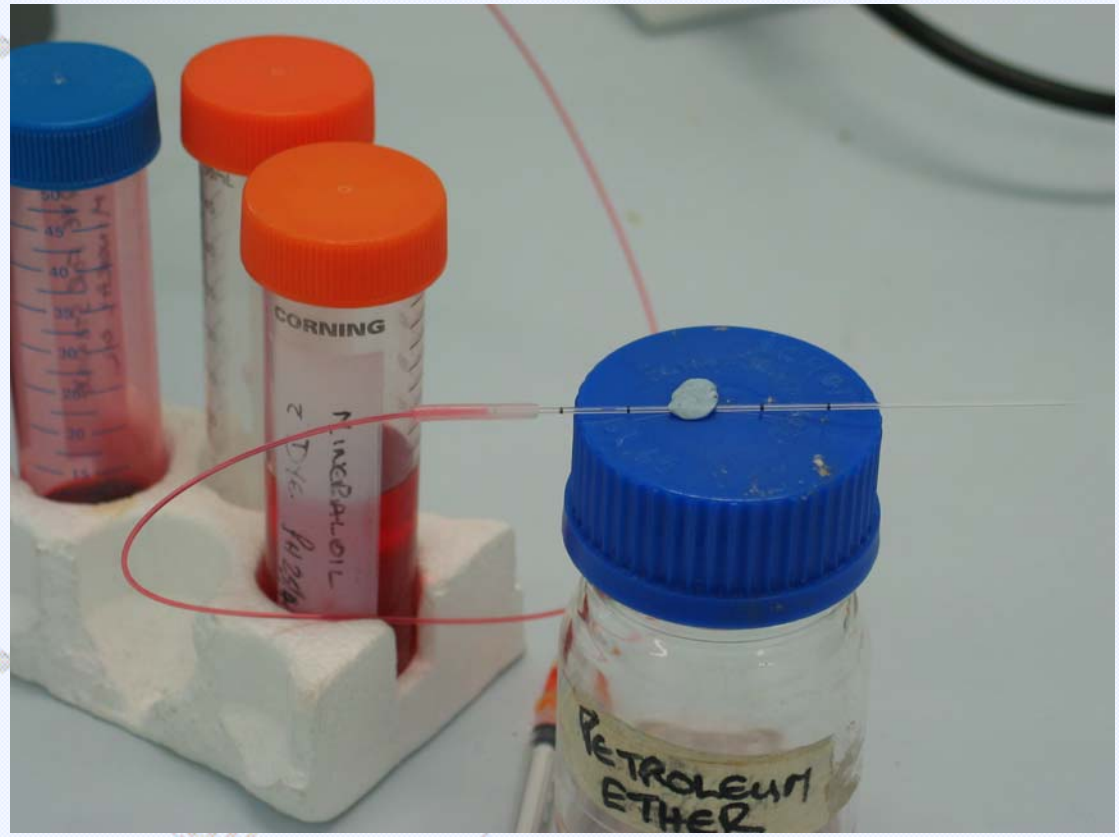
This is almost all what you need for viral vector injection

Relatively large volumes (0.5 -1mkl), slow infusion. Use oil-filled system.

Standard puller is used to pull long pipettes

Capillaries we use for injection

Oil-filled system we use for injection



Safety issues:

As such, both AVV and LVV are harmless… but the transgenes may be dangerous!!!

Never use *in vivo* any vector with a gene which can cause cancer!

Some companies are marketing pre-made viral vectors. Watch out for the nasty genes!!!

Safety issues cont:

1. Avoid formation of aerosol – no high pressure push-pull actions. Some labs require loading of vectors into the pipette in a safety hood.
2. Wear gloves when handling viral stock. However, even on direct contact with skin they will hardly transduce any cells.
3. Avoid hand pricks, handle the injection needles with great care.
4. Dispose the rest of the viral stock into special biohazard containers for incineration or dump in Vircon (strong antiseptic which kills viruses and bacteria).
5. Wipe the surfaces clean with ethanol
6. Animals transfected with viral vectors are treated as normal. No isolation or barriers are required.
7. Viral vectors are very unstable in the organism and those which do not get taken up by the cells, get destroyed within minutes to hours.

Viruses ≠ DRUGS

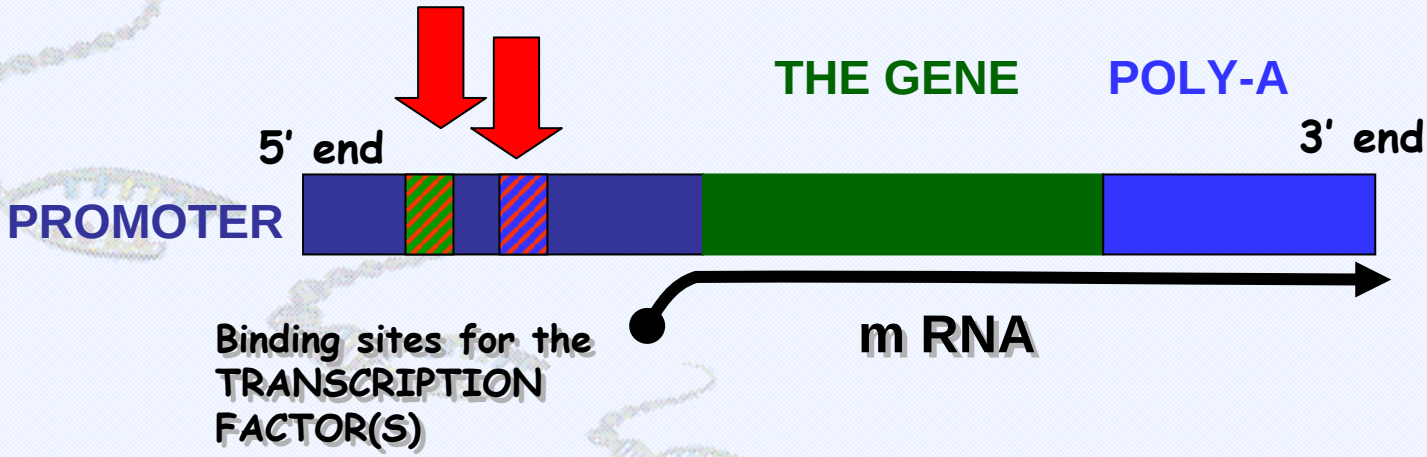
Viral vectors are radically different from drugs in that:

1. In most cases they will affect some cell types more than the others and in some cases they will simply NOT produce enough transgene in certain cells.

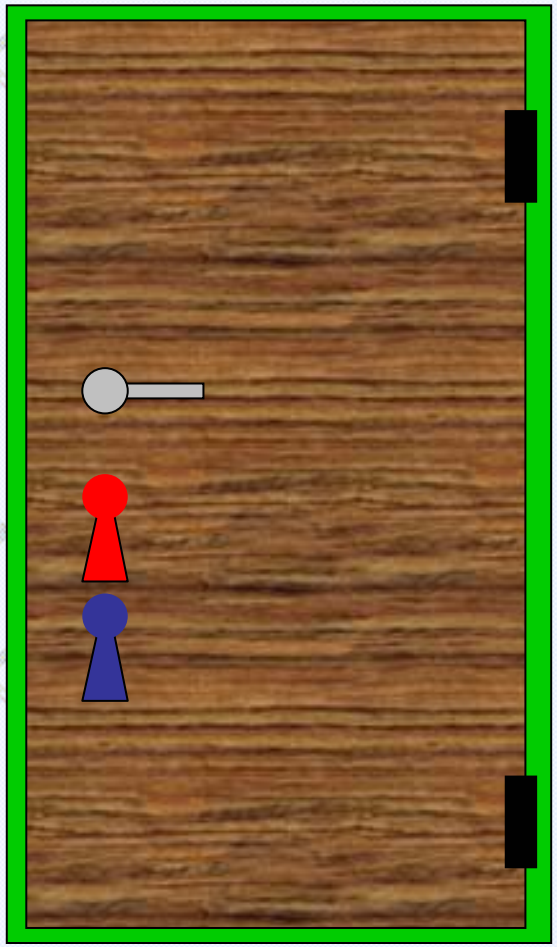
2. In some cases viral vectors may affect cells outside of the area of injection

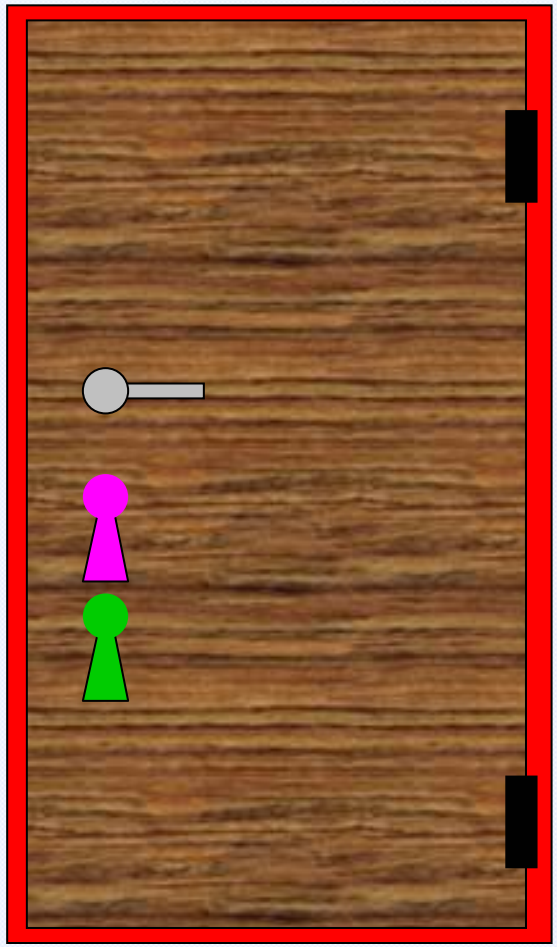
1. In most cases they will affect some cell types more than the others and in some cases they will simply NOT produce enough transgene in certain cells.

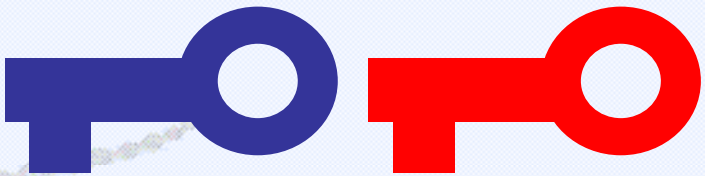
An "expression cassette"

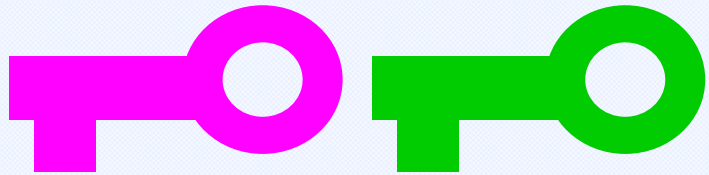


In order for the vector to produce the effect your expression cassette must be transcriptionally active in your target cells.

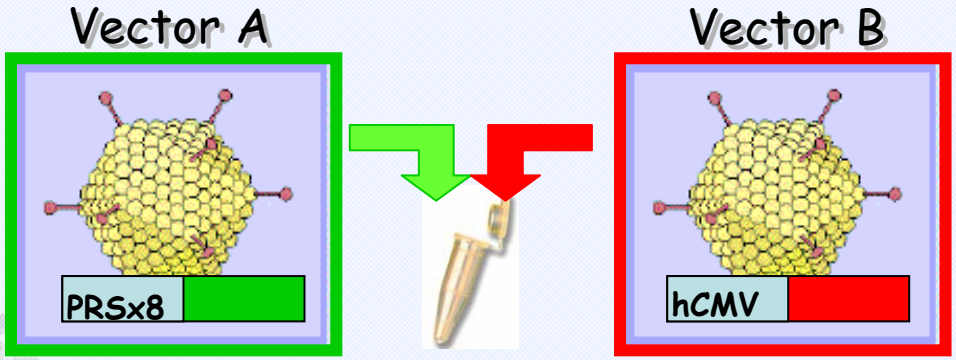


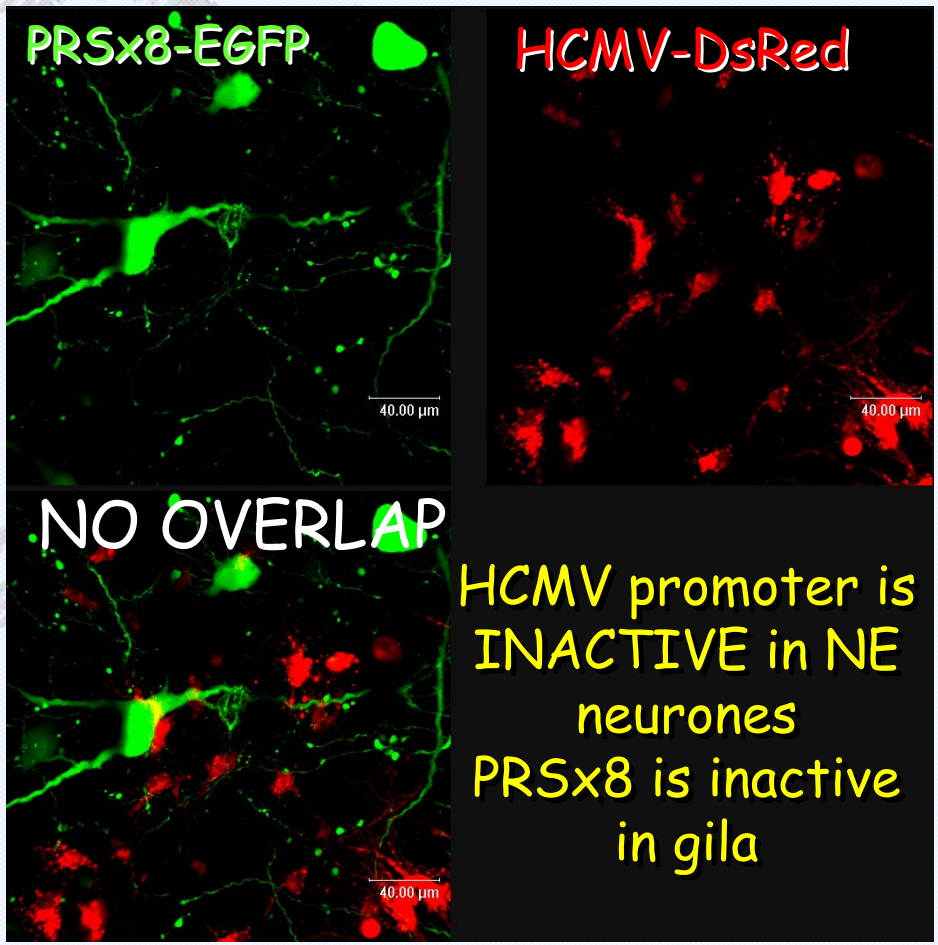




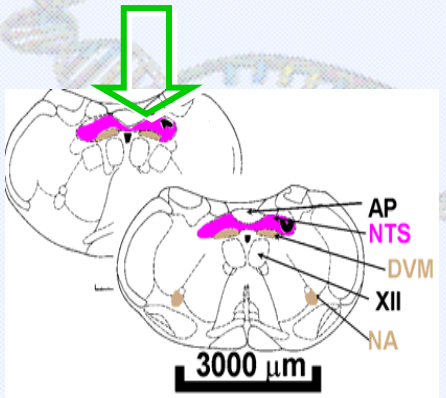


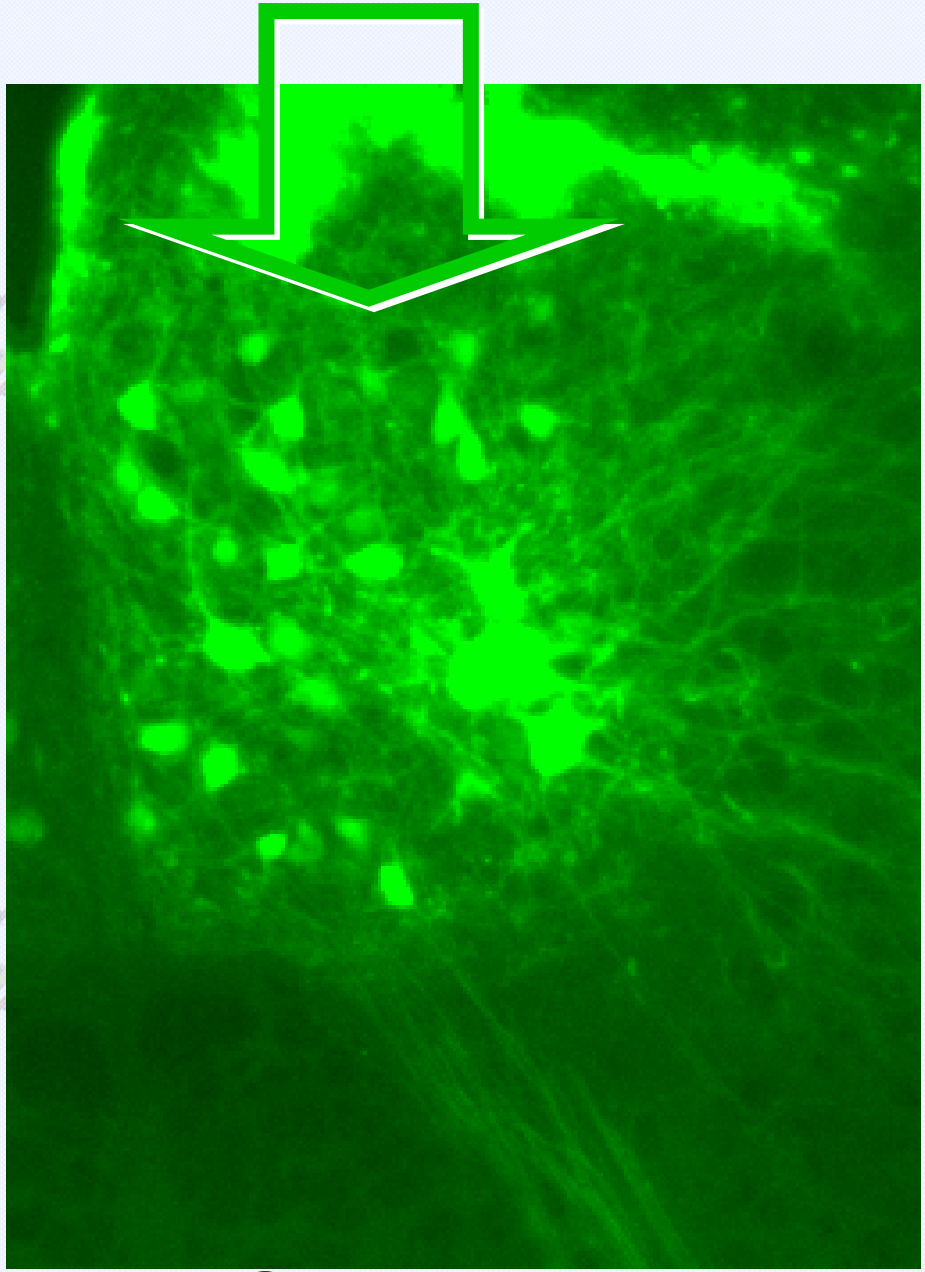
Experiment1

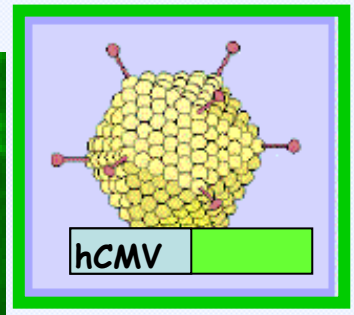




Experiment2







HCMV promoter IS ACTIVE in motoneurones in DVM & hypoglossal nucleus

IMPORTANT:

You need to establish that the vector which you are using IS actually active in the cells which you want to affect!

DO NOT TRUST ASSUMPTIONS, especially based on cell line experiments!!!

This is why it is good to have a marker, like EGFP co-expressed with your gene of interest!

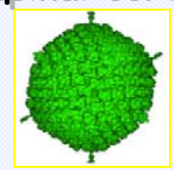
A rule of thumb:

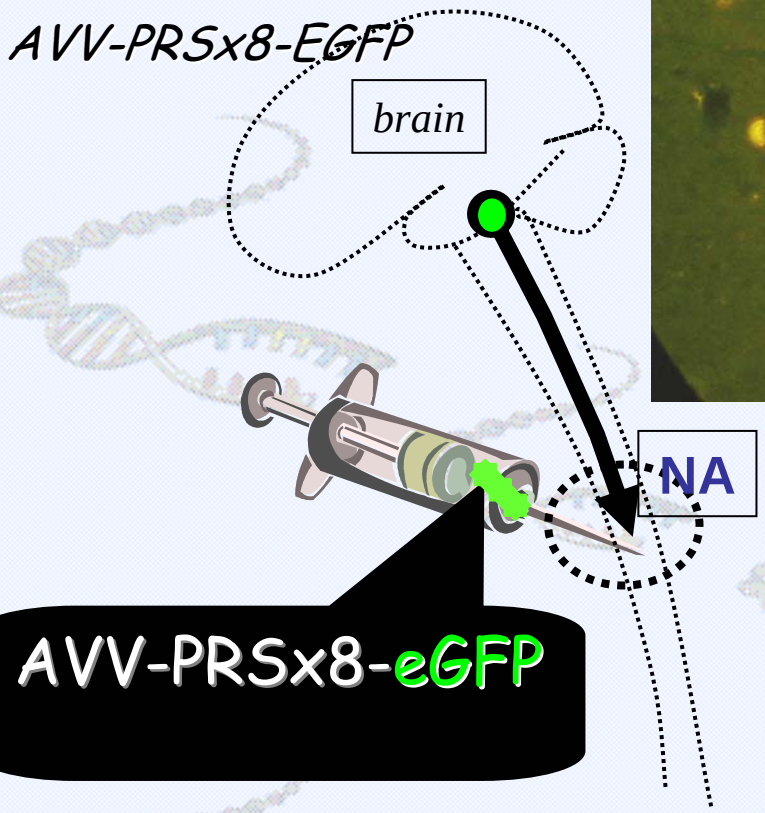
If you can see un-stained EGFP in your target cells, they probably express medium nM to micromolar concentration of EGFP.

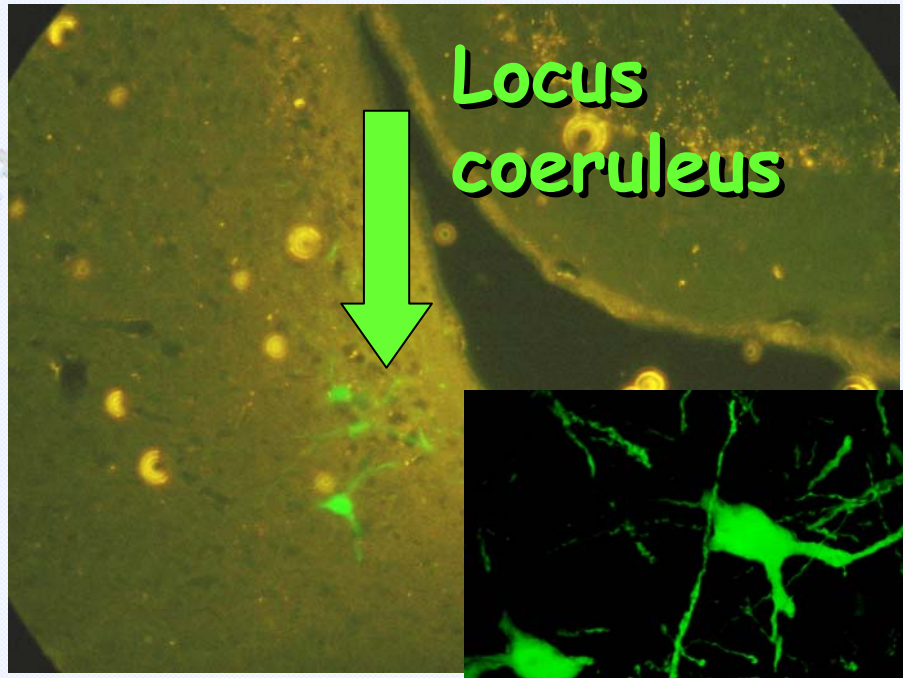
If your transgene is not degraded very fast, this should be enough to get a physiological outcome (most cellular proteins are expressed at low nM levels).

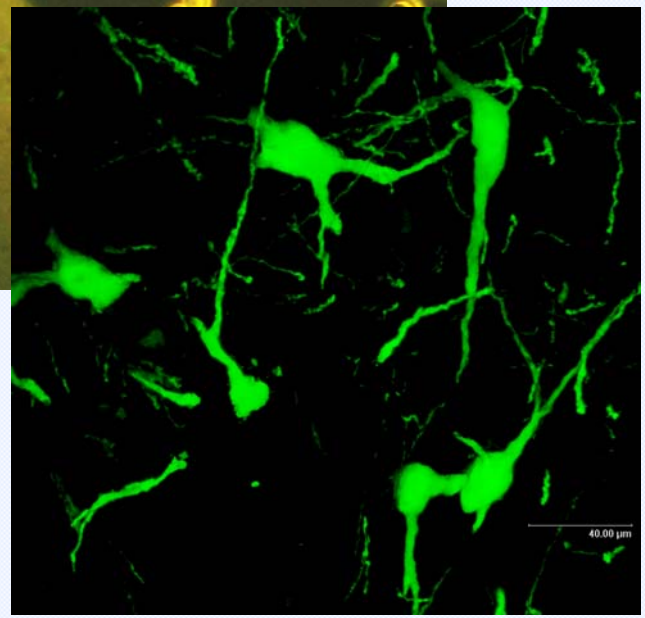
2. In some cases viral vectors may affect cells outside of the area of injection

Example 1: retrograde transduction of LC from the spinal cord

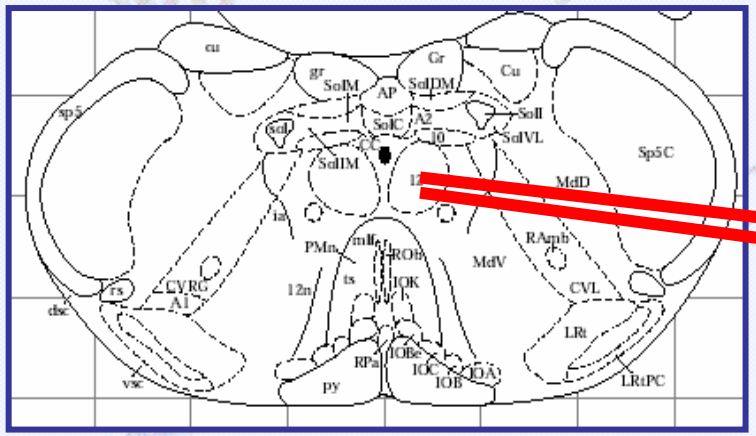


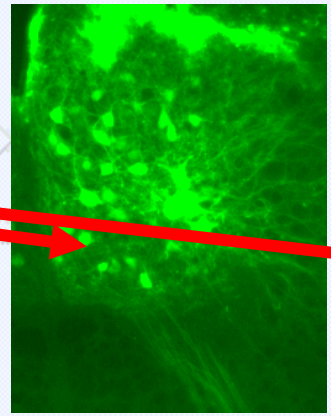




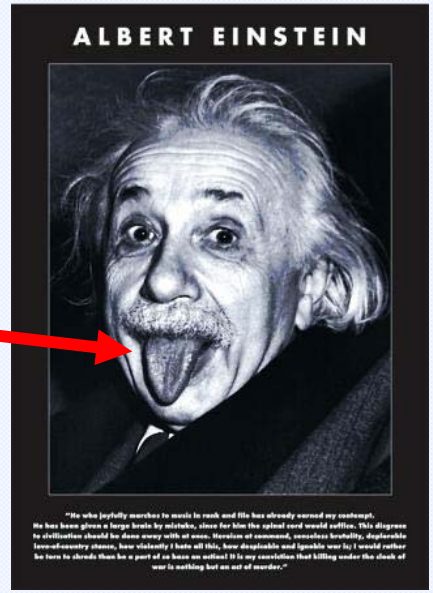


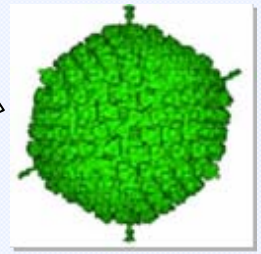
Example 2:
Retrograde transduction of hypoglossal motor neurones by injecting AVV into a tongue





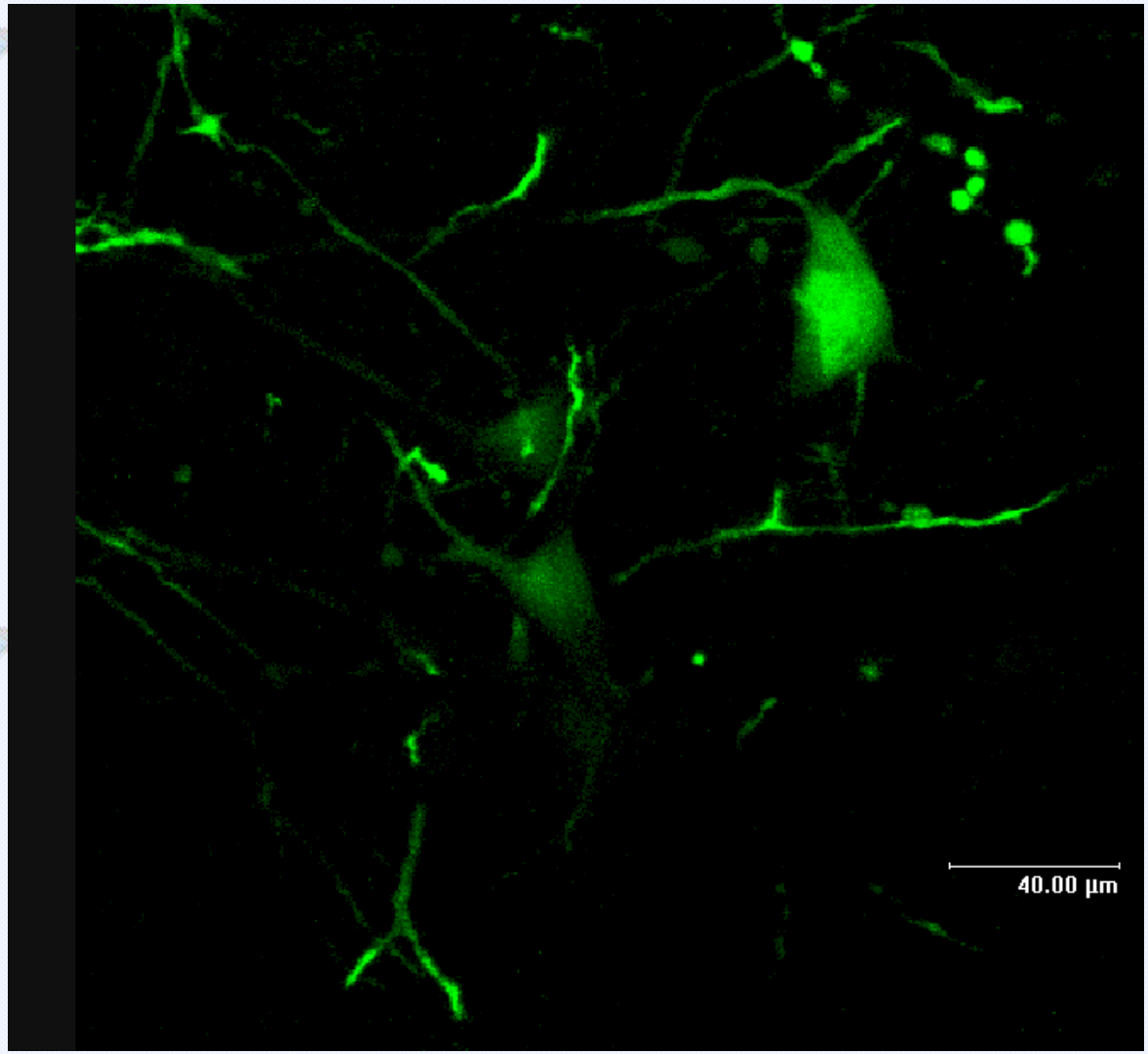
AVV hCMV-EGFP drives high level of expression when injected directly into the hypoglossal nucleus



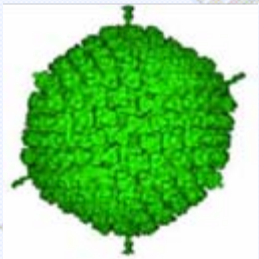


Adenoviral vectors to express nNOS and EGFP

Retrogradely transduced hypoglossal motor neurones

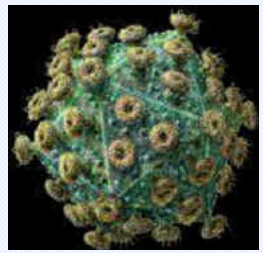


Adenoviral vectors may travel retrogradely in <u>some types</u> of neurones



Adenoviral vector

Lentiviral vectors with VSVG coat do not travel retrogradely



Lentiviral vector (VSVG-coated)

TAKE HOME MESSAGE:

Take this feature into account. Other types of vectors also differ in their ability to travel retrogradely.

"Dosing" your effect

Drugs

Effect depends on:

1. Concentration
2. Volume – how large is the area where the concentration will be sufficient to cause an effect

Viral vectors

Effect depends on:

1. Titre (concentration)
2. Volume (how large is the transduced area, the number of transduced cells)
3. How strong is your expression system

General "rule of thumb": The stronger – the better… as you will need lower MOI

VIRAL GENE TRANSFER vs DRUGS

VECTORS

Offer unique opportunities of studying cell function, in many cases not achievable by any other means

It is possible to express a transgene in a particular cell type using cell-specific promoters

Allow studies of long-lasting effects which is closer to most physiological situations

Hard to control the concentration of the transgene in the cells

In peripheral tissues ADV cause immune reactions and transduced cells get killed by immune response. Seems not to be a problem in the brain.

DRUGS

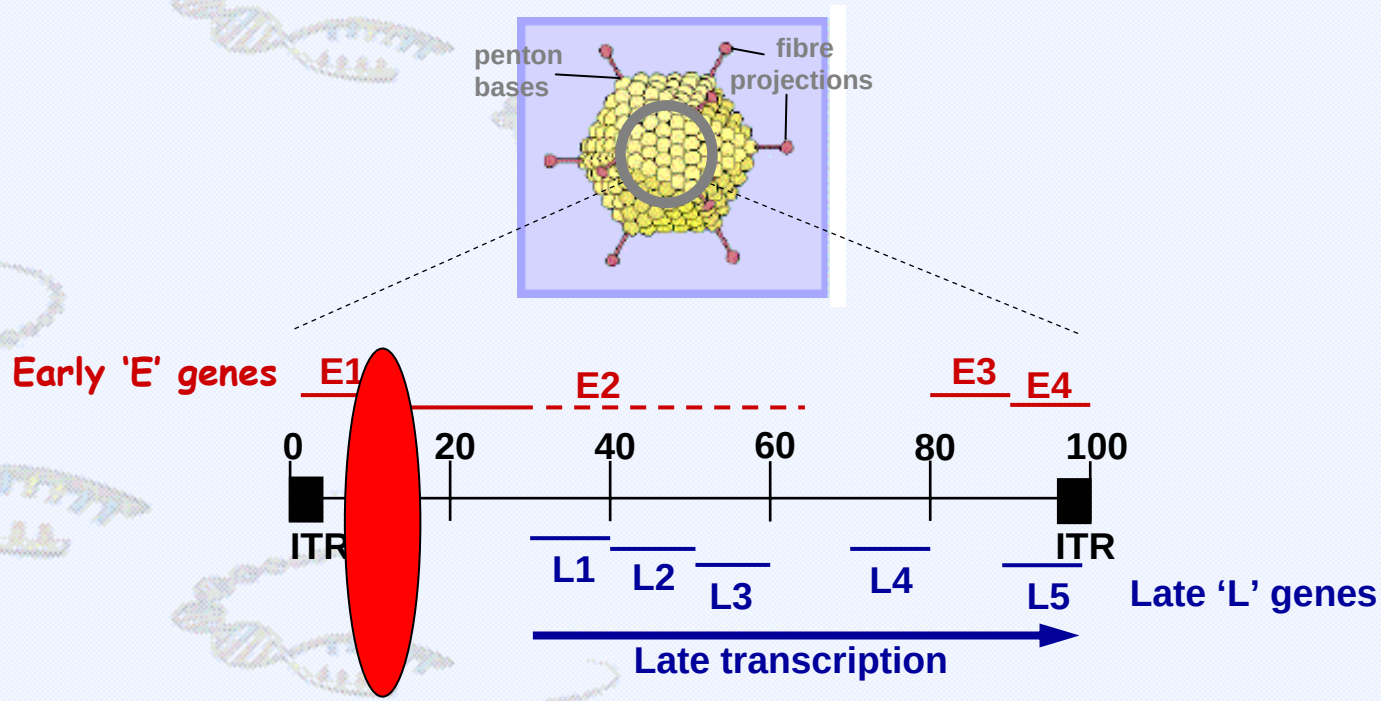
Pharmacological tools are often better characterised and their side-effects are known

Drugs spread better in the tissue and affect *all* cells where applied. Concentration may be precisely controlled in some types of experiments

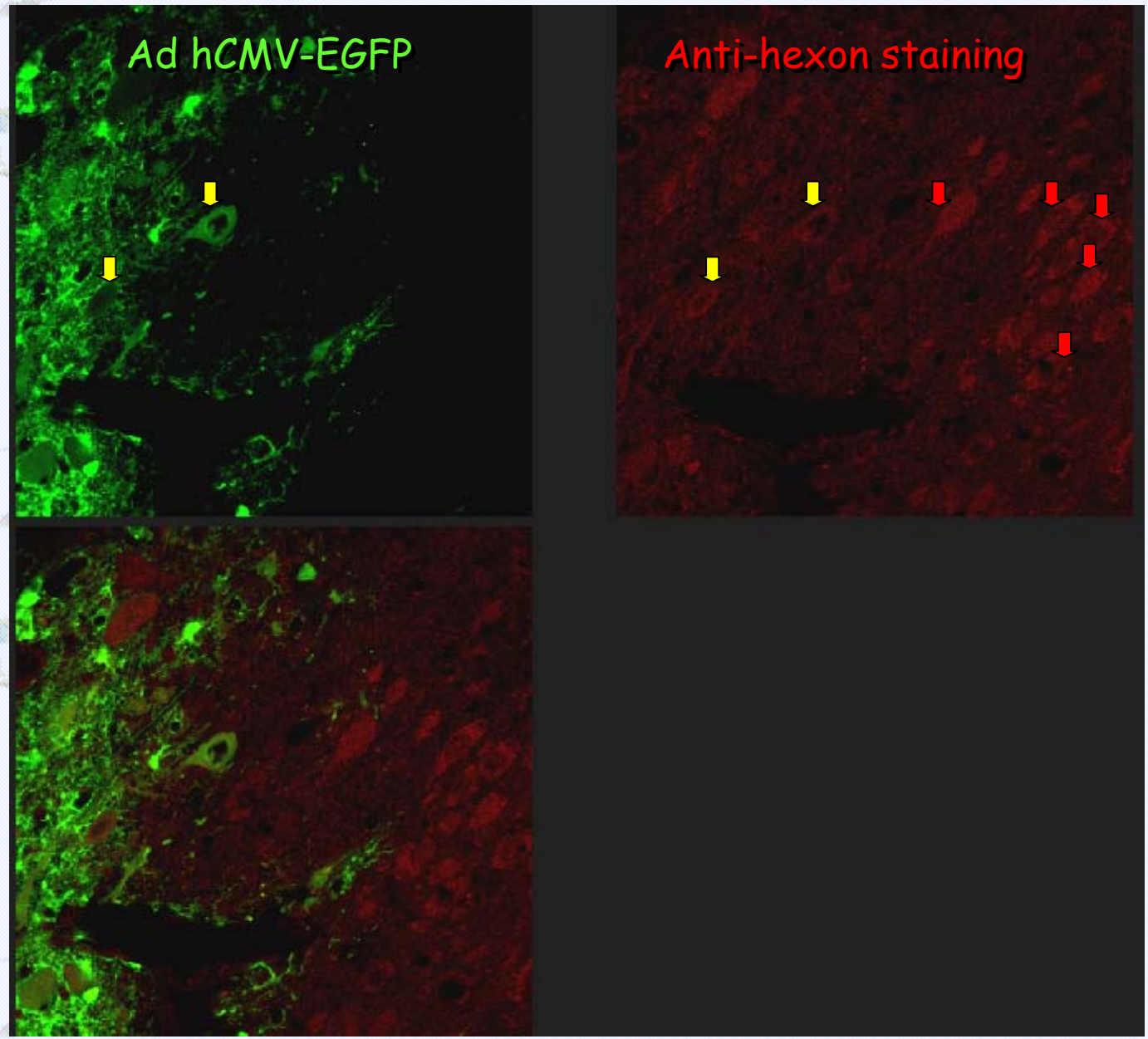
Virtually every drug has more than one action. For many targets there are no really specific drugs.

Chronic delivery of drugs in a defined brain area is technically very difficult, if not impossible

Immune response caused by adenoviral vectors



Hexon staining after cmv-EGFP ADV injection



Generally it is better to have as potent expression system as possible because:

a)You will get a stronger effect
b)You will save your vectors
c)It will help to avoid immune response if you use AVV

See: Liu et al 2006 Mol Therapy

For advanced users:

Tetracycline-controllable expression system

Vectors for gene knock-down using miRNA

"Gene transfer studies using adenoviral vectors" BBSRC JREI grant (2001)
S. Kasparov, A.G. Teschemacher, J.F.R. Paton

Viral constructs

Cell-specific expression of indicator proteins

Wang,S., Teschemacher, A.G., Paton, J.F.R.., & Kasparov, S. (2006). The mechanism of nitric oxide action on inhibitory GABAergic signaling within the nucleus tractus solitarii, FASEB Journal Express, 9, 1537-1539

EGFP in NA neurones

Z. Chiti and A. G. Teschemacher
Exocytosis of norepinephrine at axon varicosities and neuronal cell bodies in the rat brain. FASEB J 2007

EGFP in NA neurones

Teschemacher A.G., Wang,S., Paton,J.F.R. and Kasparov,S. Differential modulation of catecholaminergic A2 and C1 neurones in normotensive and hypertensive rats by angiotensin II. Circ. Res. In preparation

EGFP in NA neurones

PW Howorth, AG Teschemacher and AE Pickering. Retrograde adenoviral vector targeting of nociresponsive pontospinal noradrenergic neurones in the rat in vivo.

J Comp Neurol (2007) in preparation.

EGFP in NA neurones

Expression of functional constructs to modify cell's function

Duale,H., Waki,H., Howorth,P., Kasparov, S., Teschemacher,A.G.and Paton,J.F.R.
Restraining infulence of A2 neu in chronic control of blood pres in SHR, Cardiovascular Research, i revision

Kir2.1 & EGFP in NA neurones

H.Waki, S. Kasparov, B. Liu, M. Miyake, K.Katahira, D.Murphy, J.F.R. Paton.
Junctional adhesion molecule-1 is up regulated in the spontaneously hypertensive rat: evidence for a pro hypertensive role within the brainstem. Hypertension 2007.

hCMV-driven JAM-1

Expression of constructs to antagonise a pathway

Waki, H., Kasparov, S., Wong, L.-F., Murphy, D., Shimizu, T., & Paton, J. F. R., 2003. Chronic inhibition of eNOS activity in NTS enhances baroreceptor reflex in conscious rats. Journal of Physiology 546, 233-242

hCMV-driven eNOS DomNeg

H. Waki, D. Murphy, S. T. Yao, S. Kasparov, and J. F. R. Paton. (2006). Endothelial nitric oxide synthase activity in nucleus tractus solitarii contributes to hypertension in spontaneously hypertensive rats. Hypertension 48 (4):644-650

hCMV-driven eNOS DomNeg

C. P. Bailey, S. Oldfield, L. Hull, C. J. Caunt, A. G. Teschemacher, S. Kasparov, C. A. McArdle, F. L. Smith, W. L. Dewey, E. Kelly, and G. Henderson. Agonist-Selective Mechanisms of m-Opioid Recepto Desensitisation in Mature Brain Neurons: Role of PKCa and GRK2. In preparation for submission

PKC DomNeg in NA neurones

Our viral lab WEBsite:

http://www.bris.ac.uk/Depts/Physiology/Staff/Pysk/virallab/index.htm