Viral vectors as tools for neuroscience

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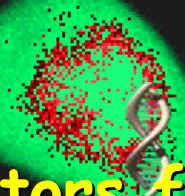

Lecture 2 (AGT):

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

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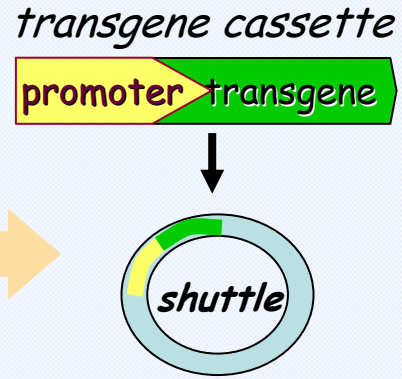
Viral Vectors for Cell Type-Specific Transgene Expression in the Brain

- Introduce the transgene into brain cells
  - Make the appropriate delivery vehicle:
    - Adenoviral vector (AVV)
    - Lentiviral vector (LVV)

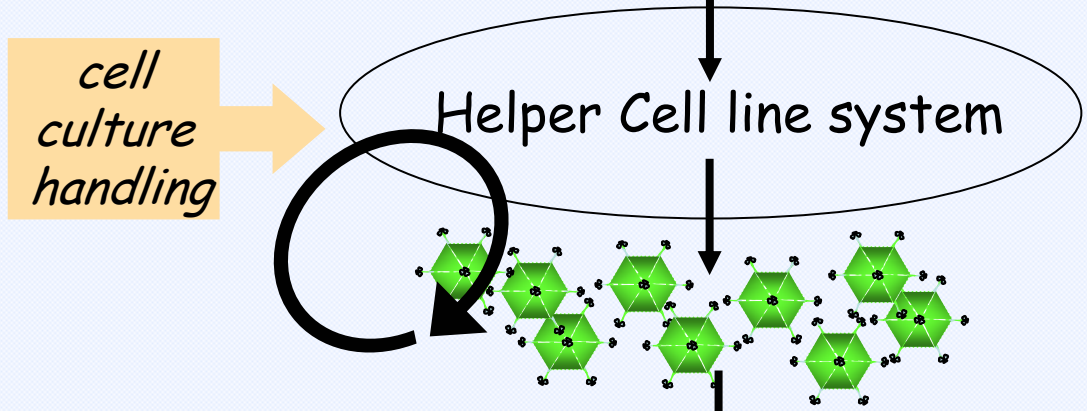
- Control expression to the desired cell type

Viral Vector Production

- Shuttle plasmid (design of expression cassette)

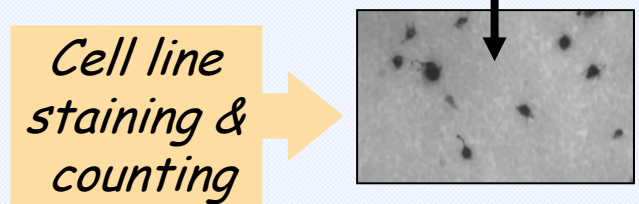


- VV construction
- VV proliferation *(AVV only)*



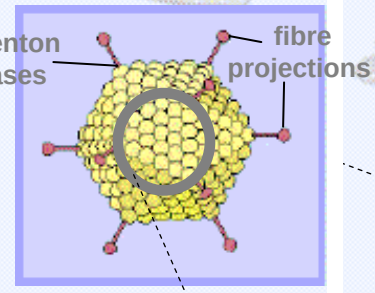
- VV purification

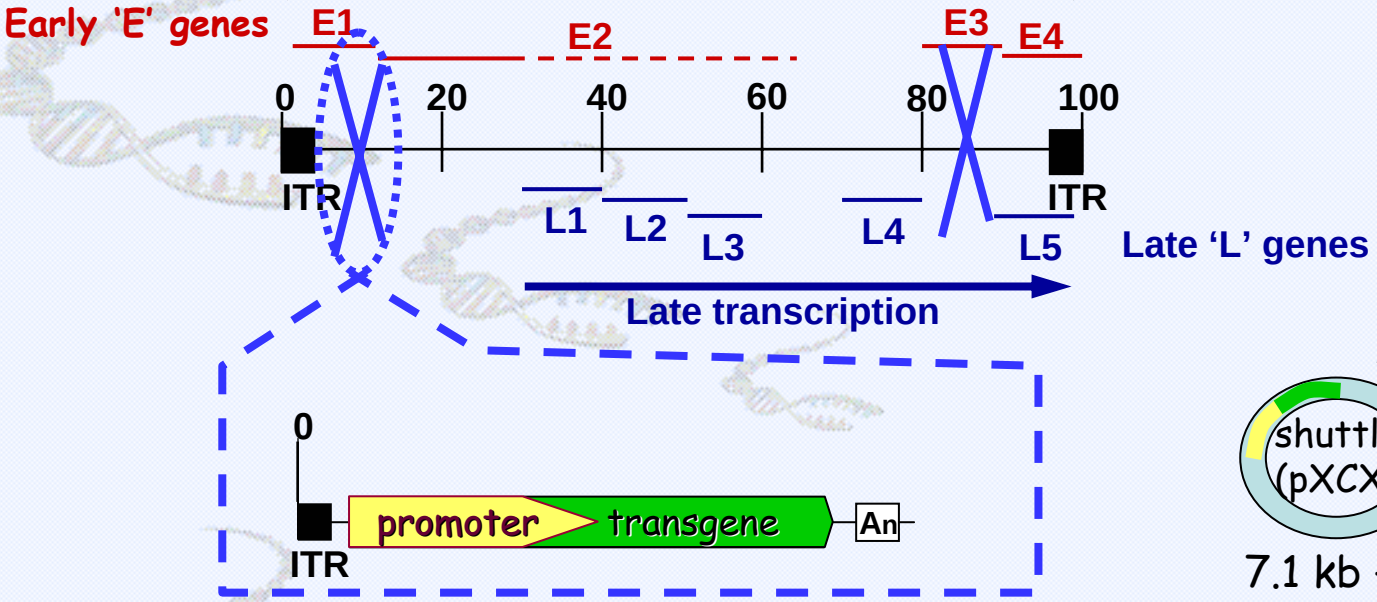
- Titer evaluation



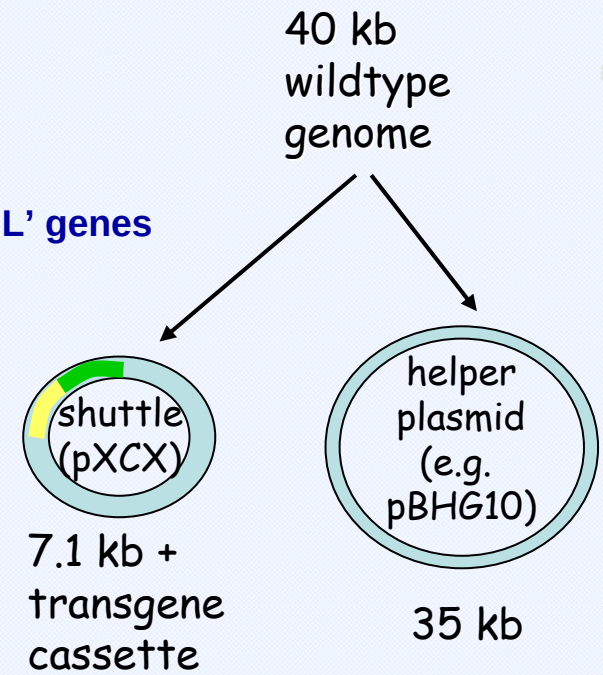
AVV - background

- Derived from a non-enveloped double stranded DNA virus which causes mild human infections
- Receptor-dependent uptake (CAR)
- Genome remains episomal in host cell nucleus



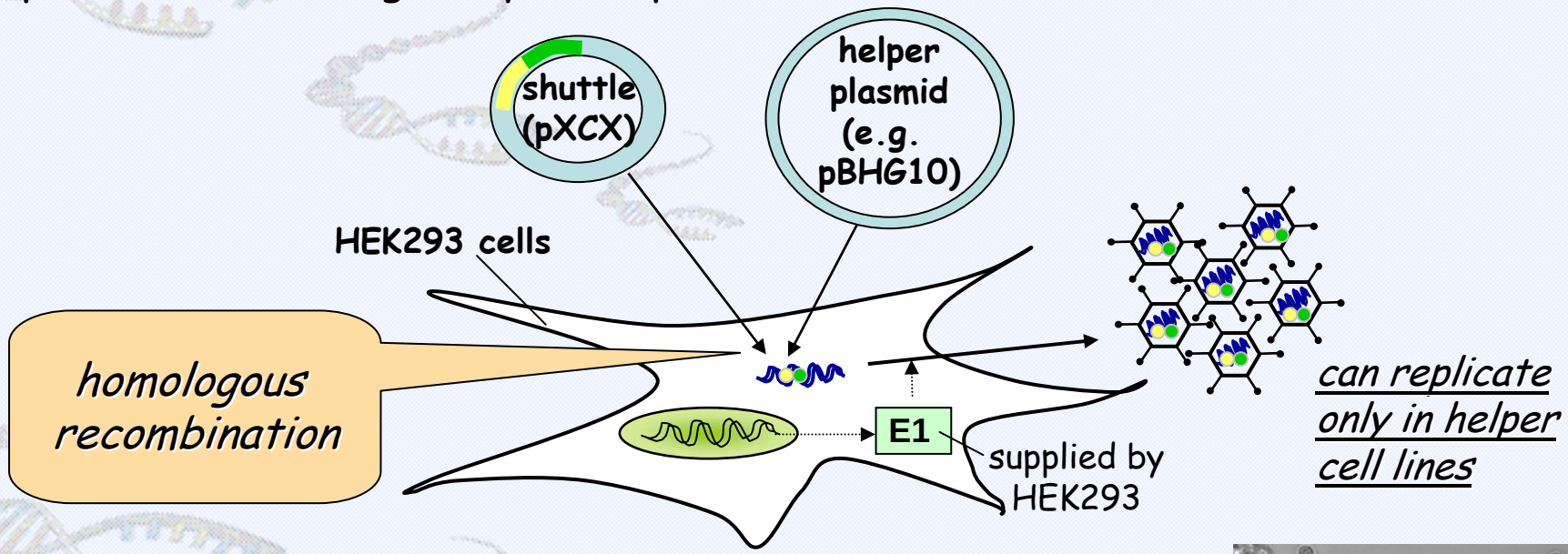


Bett et al (1994) PNAS 91(19):8802-8806

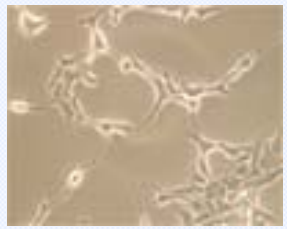


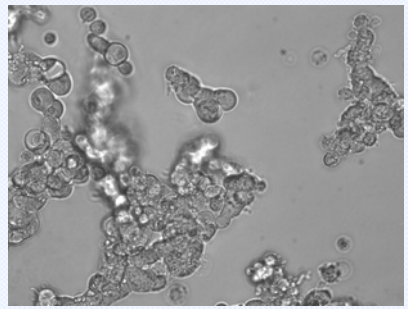
AVV – construction & proliferation

- Co-transfection of shuttle & helper plasmid into HEK 293 cells which provide the lacking Early Gene products



- Viral production followed by host cell death

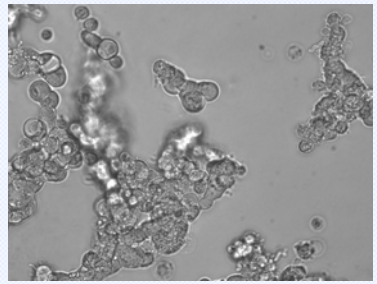




- Enrichment steps (re-infection)
- Verification
  - Genome restriction digest analysis
  - Functional testing
- AVV clone can now be used to re-infect more HEK293 cells – mass production
- *Standard sterile cell line handling & transient transfection*

AVV - purification

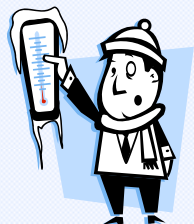
- Collect dying infected HEK293 cells (*cpe* = cytopathic effect)
- Rupture plasma membranes to free viral particles (freeze-thaw; ultrasonication)



- CsCl ultracentrifugation gradient (2x)
- De-salting column to remove Cs

Alternative: commercially available adenoviral vector purification kit

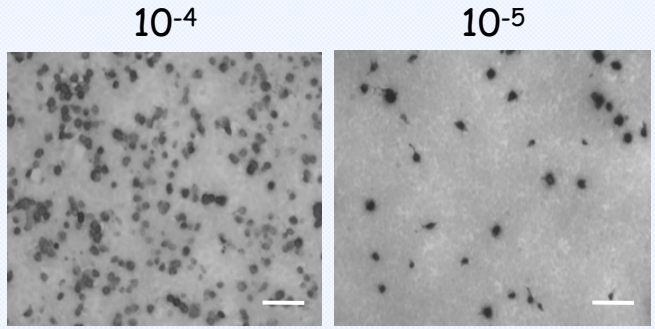
- Sterile filter
- Aliquot, snap-freeze in liquid N_2
- Storage: -80°C



AVV - titration

- Infect HEK293 cells with serial dilutions of AVV
  - wait 2 days for AVV proliferation to start but not spread (no *cpe*)

- Fix cells and carry out immuno-cytochemistry against hexons (adenoviral capsid protein)
  - Secondary HRP-coupled antibody, followed by DAB reaction



adapted from: Bewig, B. & Schmidt, W. E. (2000) *Biotechniques* 28, 870-873.

AVV - summary

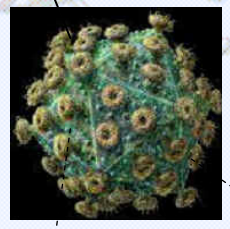
Advantages

- ability to infect a wide variety of non/dividing cell types: pituitary, thyroid gland, neurones, blood vessel cells
- high efficiency and speed of gene transfer (expression may start already after 12 hours)
- capacity of standard vectors ~ 8kB (there are large capacity "gut-less" adenoviral vectors but they are very difficult to make)
- easy to produce in large quantities
- high titres up to 10^{12} TU/ml (standard types)

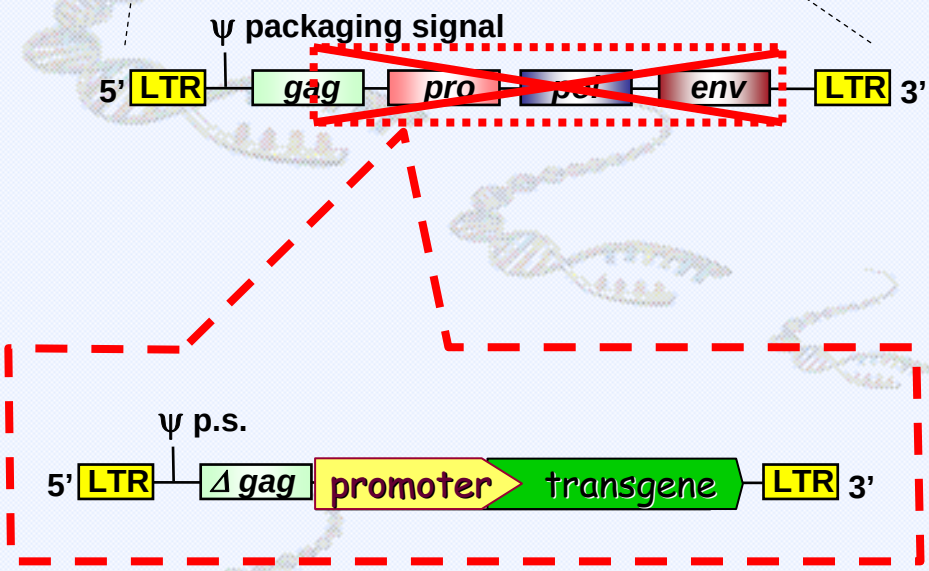
Disadvantages

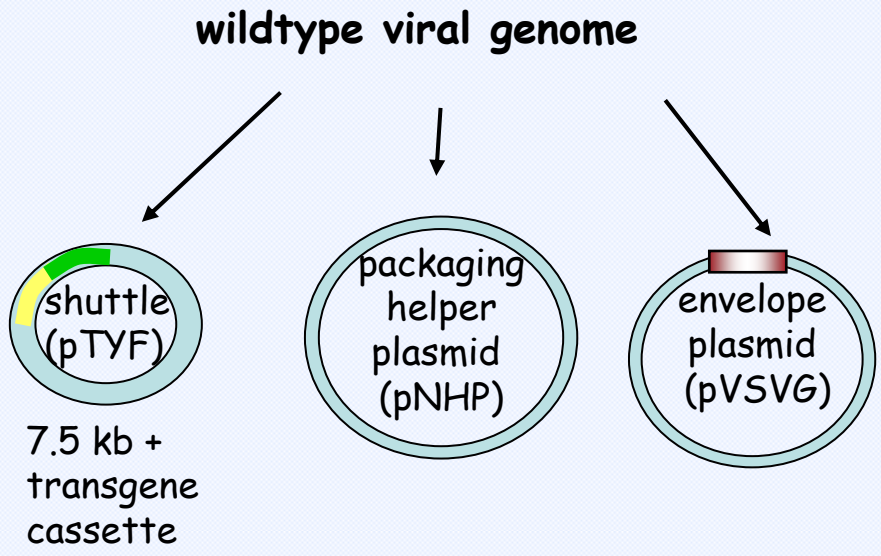
- immune response to viral capsid proteins – especially problematic in peripheral tissues
- Lack of integration + immune response = transient expression (re-administration may be needed)

LVV - background



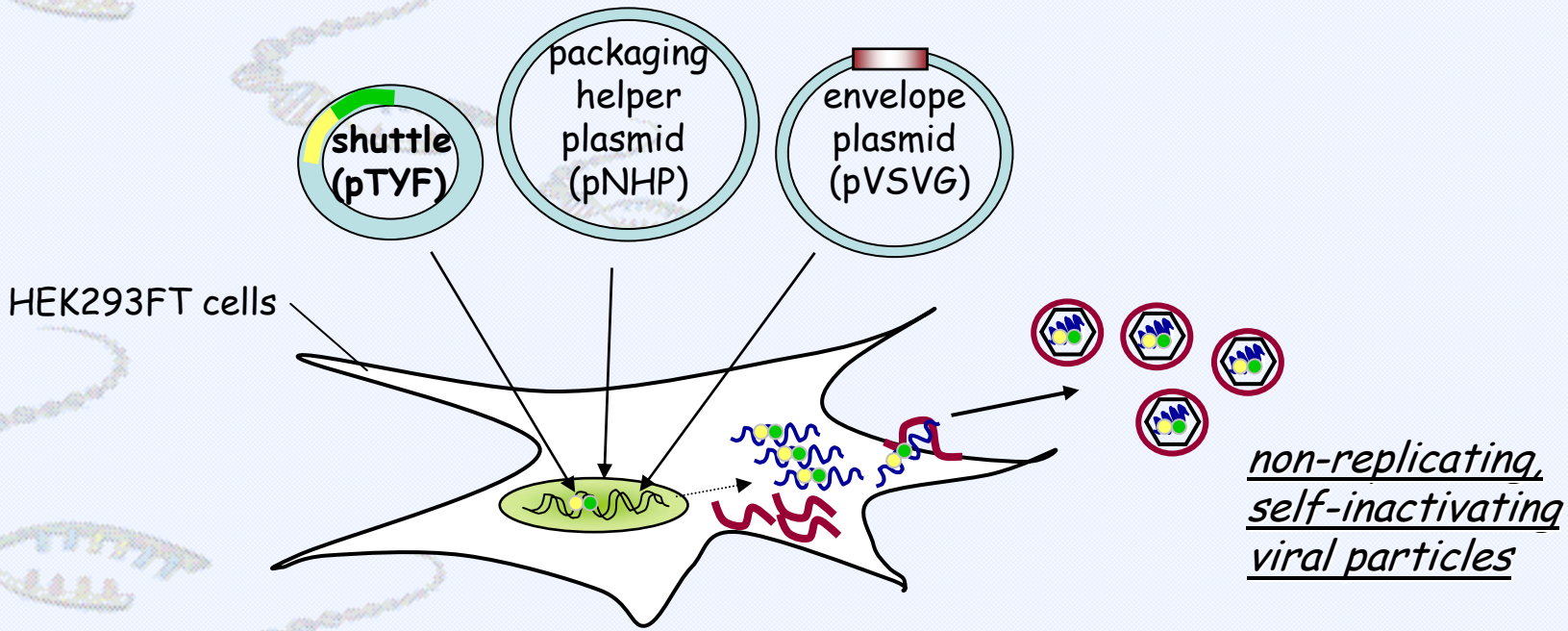
- Derived from HIV, i.e. enveloped, single-stranded RNA viruses
- Pseudotyped with vesicular stomatitis virus (VSVG) coat for receptor-independent transduction
- reverse transcription followed by integration of DNA into host genome





LVV – construction

- Co-transfection of shuttle & helper plasmids into HEK293FT cells

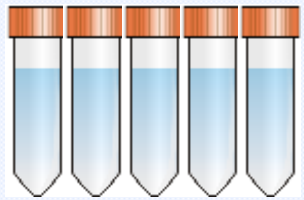


- Secretion of viral particles into media (no cell death)
- *Standard sterile cell line handling & transient transfection*

Coleman, Huentelman et al (2003) Physiological Genomics, 12:221-228

LVV - purification

- Collect media containing LVV at optimal time window



- Concentrate LVV - pelleting by ultra-centrifugation (iodixanol 2x)

Alternative: commercially available lentiviral vector purification kit

- Aliquot, snap-freeze in liquid N_2
- Storage: -80°C

LVV - titration

- mix into your LVV-containing media a small fraction of PLAP-expressing LVV, and purify together
- infect TE671 cells with serial dilutions of LVV
  - wait 3 days for transgene expression
- fix cells and incubate at 75°C (*this kills all alkaline phosphatase except for PLAP)*
- stain for PLAP activity
- alternatively, immuno-cytochemical titration can be carried out

adapted from: Coleman, Huentelman et al. (2003) *Physiological Genomics* 12: 221-228.

LVV - summary

Advantages

- can transfect and express genes in a wide range of both dividing and non-dividing cells including neurones *in vivo*
- capacity up to 12 kB (?) – can take complicated constructs
- integrate into host chromosomes – expression more stable
- lack of immunogenic viral proteins – no immune aggression against transduced cells – expression more stable
- easy and quick to produce

Disadvantages

- cannot be obtained in large quantities (for example a typical yield currently is in the range of 50 ul of 10^9 TU/ml per purification procedure; this compares to 2,5 ml of 10^{11} of adenoviral vectors per procedure).
- risk of mutagenesis if integration occurs into "wrong" host genes (more relevant for gene therapy applications)

The choice between AVV and LVV

criteria:	**AVV**	**LVV**
transgene capacity	up to ~8 kb	up to ~12 kb
production time	initially: ~2-4 months later: ~2 weeks *	~2 weeks
titer and quantity	10^{10} - 10^{11} TU/ml 2.5 ml	10^{9} - 10^{10} TU/ml 50 ul
efficient transduction of cell type in exp. system of choice	glia, neurones, other brain cells *in vitro* and *in vivo*	prefers neurones *in vivo* not immunogenic

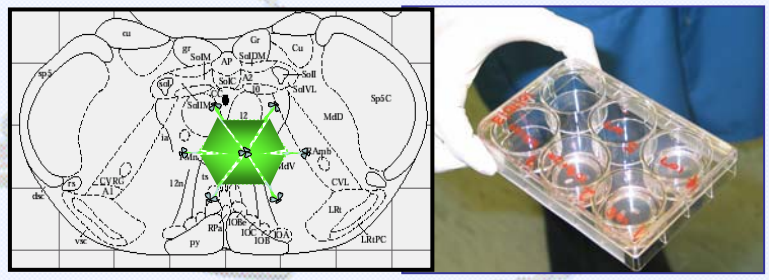
Teschemacher et al (2005) *Advanced Drug Delivery Reviews* 57:79-93
Duale et al (2005) *Experimental Physiology* 90(1):71-78.
Teschemacher et al (2005) *Experimental Physiology* 90(1):61-69.

Transgenesis of brain cells

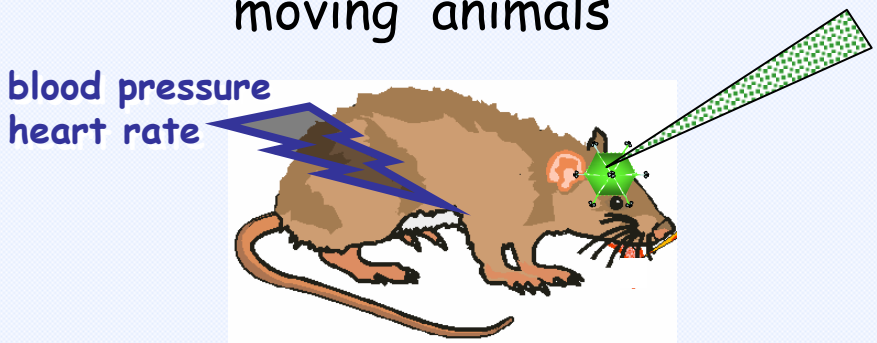
AVV

LVV
or AVV

in vitro: VV transduction in organotypic brainstem slice culture



in vivo: VV microinjections, outcome evaluated in freely moving animals



see:

Kasparov et al. (2004) *Prog Biophys Mol Biol* 84:251-277

Teschemacher et al (2005) *Advanced Drug Delivery Reviews* 57:79-93.

Teschemacher et al. (2005) *Experimental Physiology* 90(1):61-69.

see:

Waki et al. (2003) J Physiol 546: 233-242

Duale et al (2005) *Experimental Physiology* 90(1):71-78.

Duale et al (*in press*) *Cardiovascular Research*

Safety issues

- AVV and LVV will not replicate in *your* cells, nor will they survive in the environment
- *however*, in principle, they could deliver transgenes into your cells:

treat as biohazard:

- gloves, lab coat, flow hood, autoclave or incinerate disposable waste
- treat (potentially) contaminated surfaces with viricidal disinfectant (Virkon or bleach)
- observe local genetically modified organisms (GMO) regulations

Standard Equipment !

cell culture handling

- flow hood for sterile work
- CO^2 incubator
- centrifuge (50 ml tubes; up to 2000 rmp)
- cell line consumables

centrifugation and column purification

- rod sonicator (optional)
- ultracentrifuge (or kits)
- -80C freezer
- consumables, e.g. de-salting columns

Cell line staining & counting

- basic histology facilities
- oven (75C)

Viral Vectors for Cell Type-Specific Transgene Expression in the Brain

- Introduce the transgene into brain cells
  - Make the appropriate delivery vehicle:
    - Adenoviral vector (AVV)
    - Lentiviral vector (LVV)

- Control expression to the desired cell type
  - Choice of Promoter, e.g.:
    - "constituitive" (hCMV)
    - "pan-neuronal" (Synapsin, EF1a, Ta1, NSE)
    - neuronal phenotype-specific:
      - Derived from endogenous promoter (GAD67(3.7))
      - Rational design (PRSx8)
    - activity-dependent (mFOS)
    - glia-specific
    - endothelial-specific

transgene cassette

Targeting transgene expression to a specific cell type

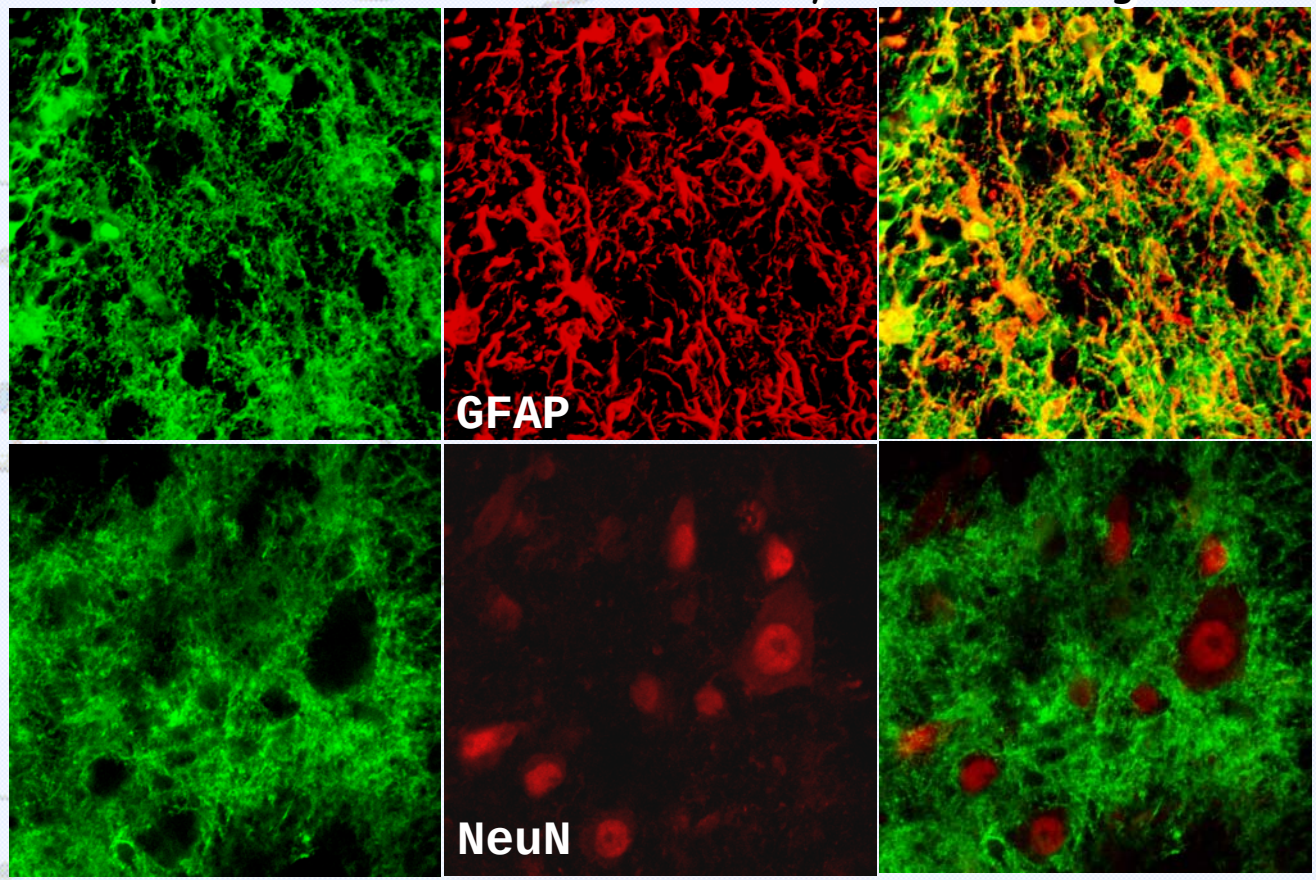
- What is special or unique about gene expression in this cell type? For example:
  - Noradrenergic neurones express dopamine-β-hydroxylase (DBH)
  - GABAergic neurones express glutamate decarboxylase GAD65 or GAD67)
  - Seronergic neurones express tryptophane hydroxylase (TPH)
  - Many neurones express neuronal markers, e.g. synapsin (Syn), neurone-specific enolase (NSE), etc.
  - Astrocytes express glial fibrillary acidic protein (GFAP)

- Make use of the endogenous transcription machinery of the cell type: to express your transgene,

  - use a short version of an endogenously active promoter, e.g. GAD67(3.7)- or GFAP-promoters
  - be clever and design a promoter that is switched on by endogenous transcription factors, e.g. PRSx8

Example 1: Selective transgene expression in astroglia

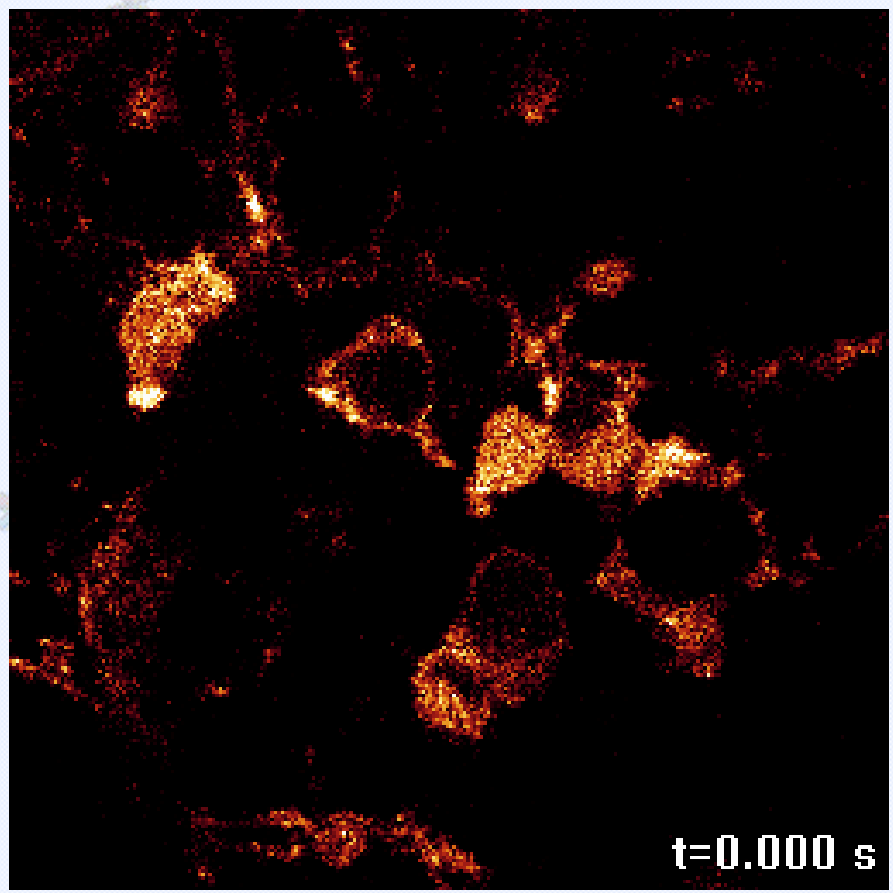
superGFAP → enhanced green fluorescent protein (EGFP)

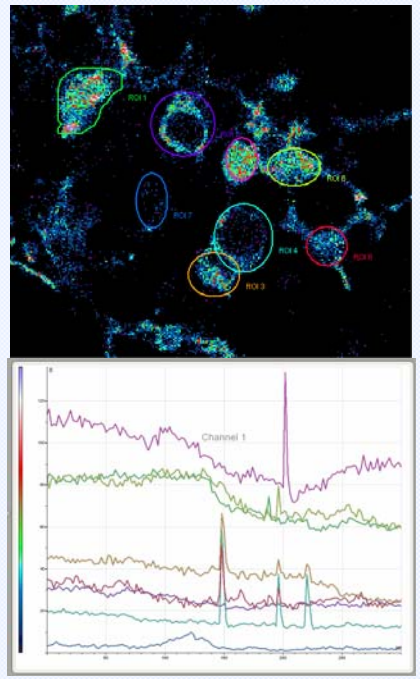


Liu BH et al. (2006) *Molecular Therapy* 14(6):872-882

Example 1: Selective transgene expression in astroglia

superGFAP → Ca^{2+}-sensitive fluorescent protein (cps2r1)

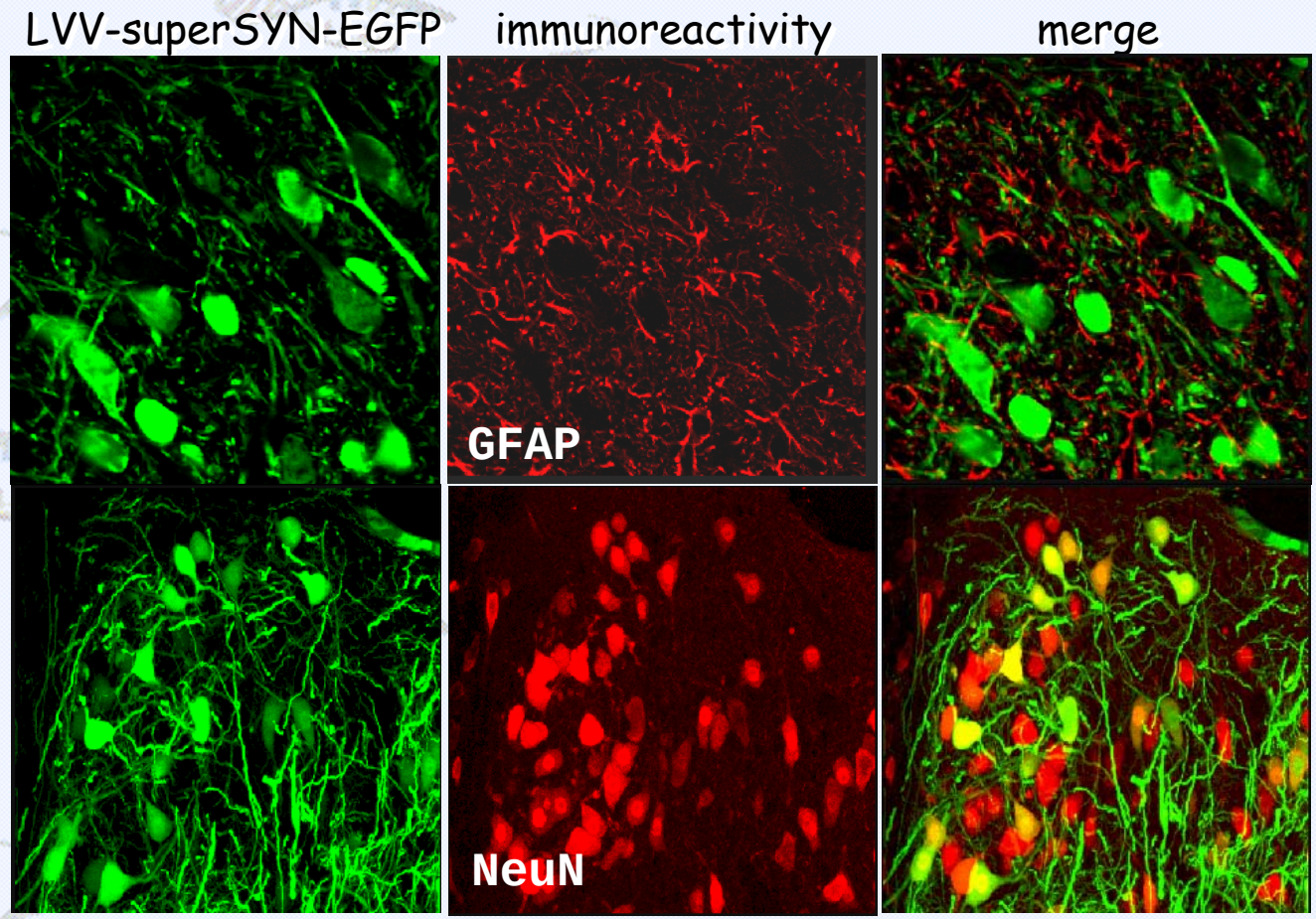




Souslova,E.A. et al, BMC Biotechnology, 2007,7,37

Example 2: Selective transgene expression in neurones

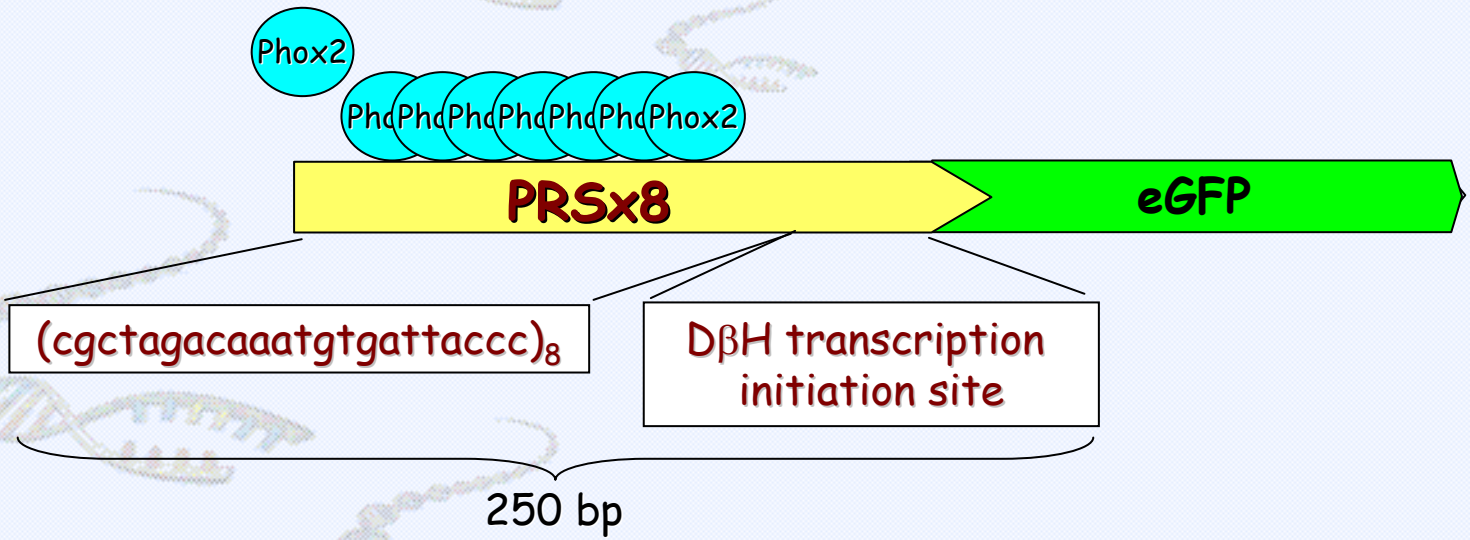
superSYN → enhanced green fluorescent protein (EGFP)



Liu BH et al. (2006) *Molecular Therapy* 14(6):872-882

Example 3: Selective transgene expression in noradrenergic neurones

⇨ In nor/adrenergic neurones,
the transcription factor Phox2 is abundantly expressed.



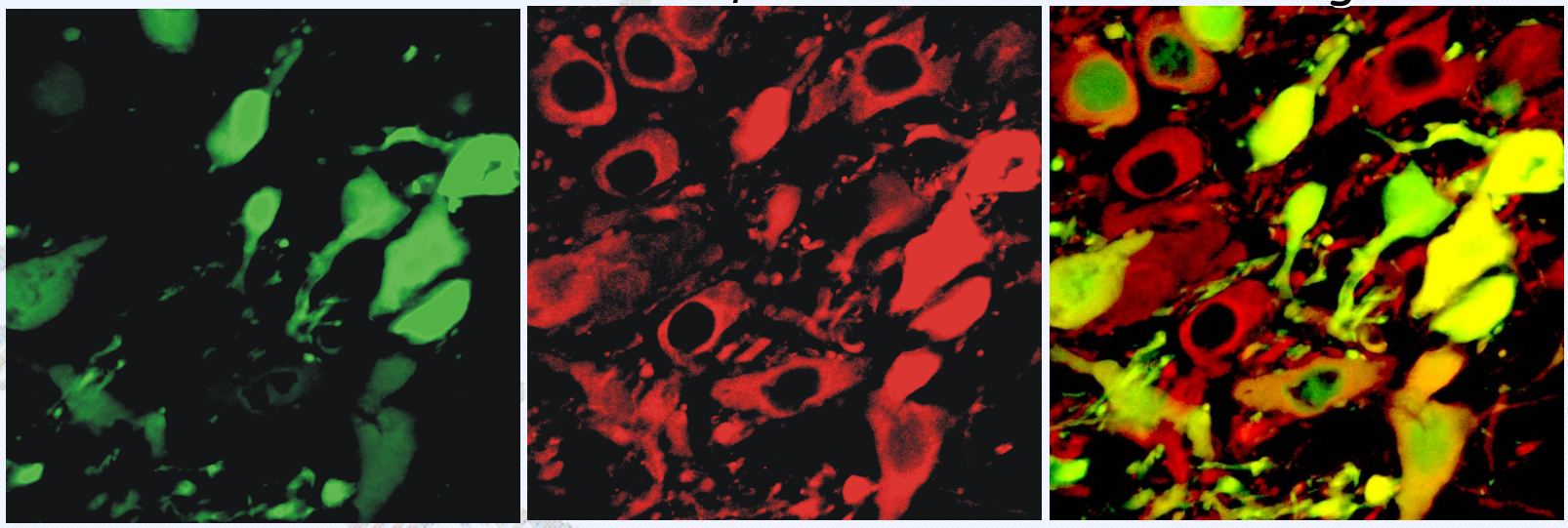
⇨ PRSx8 is a short, powerful and specific artificial promoter for VV transgenesis

Teschemacher et al (2005) *Advanced Drug Delivery Reviews* 57:79-93
Hwang et al. (2001) *Human Gene Therapy* 12(14):1731

Example 3: Selective transgene expression in noradrenergic neurones

locus coeruleus (A6):

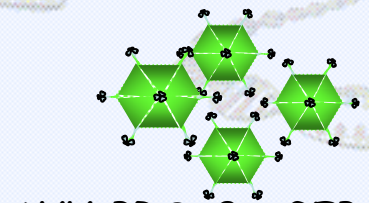
AVV-PRSx8-EGFP | DβH-IR | merge



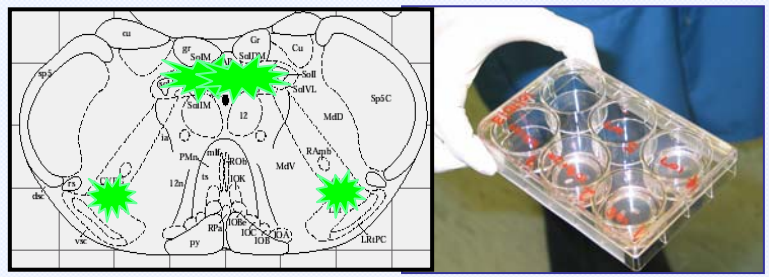
… while other 'constitutive' or 'neurone-specific' promoters (hCMV, SYN, Ta1-EGFP,NSE) are inactive in noradrenergic neurones!

Lonergan et al (2005) *Physiological Genomics* 20(2):165-72

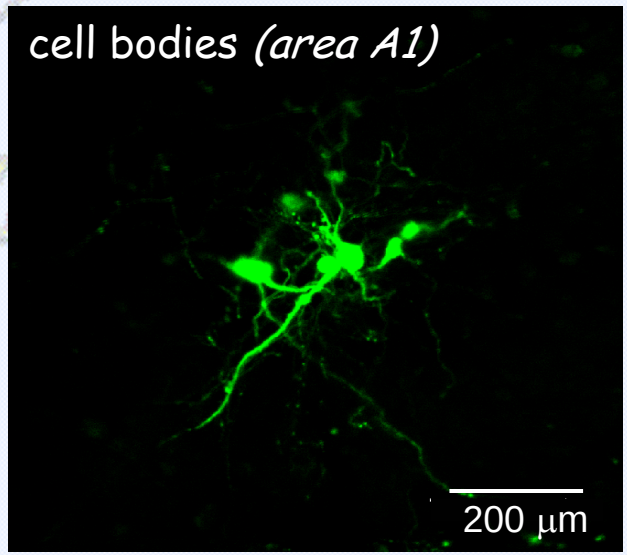
Example 3: Selective transgene expression in noradrenergic neurones

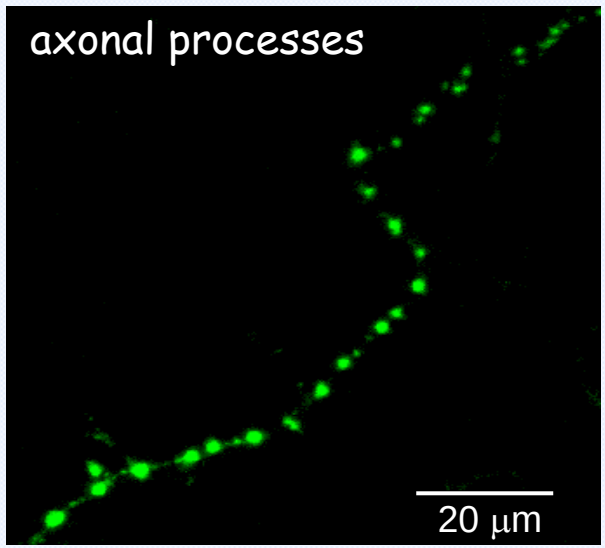


AVV-PRSx8-eGFP



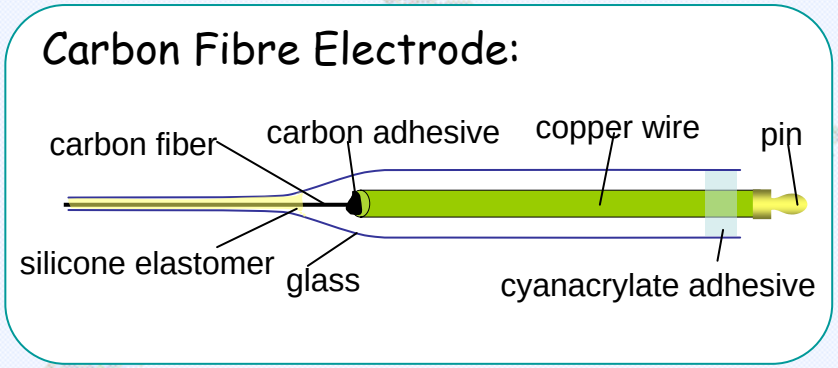
organotypic brainstem slice culture

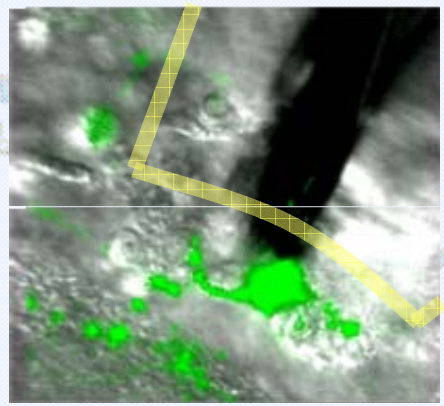


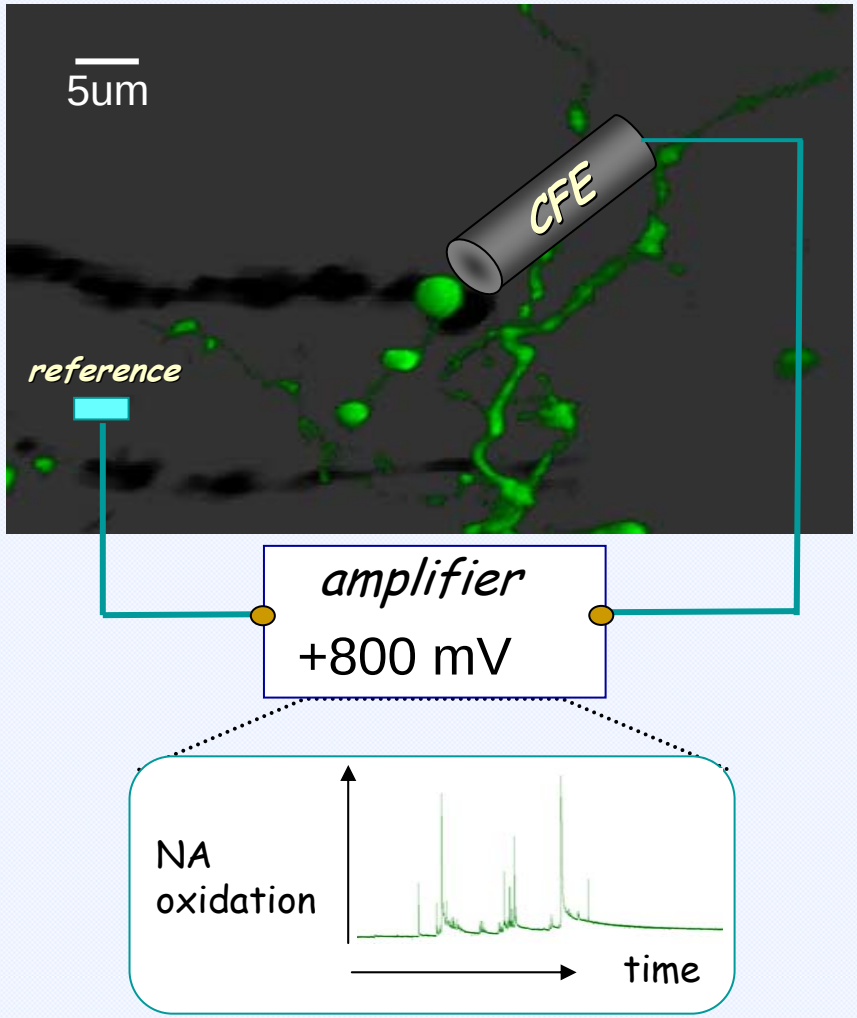


Teschemacher et al (2005) *Advanced Drug Delivery Reviews* 57:79-93
Teschemacher et al (2005) *Experimental Physiology* 90(1):61-69.

Example 3: Noradrenaline release from brainstem neurones in vitro: Micro-amperometry





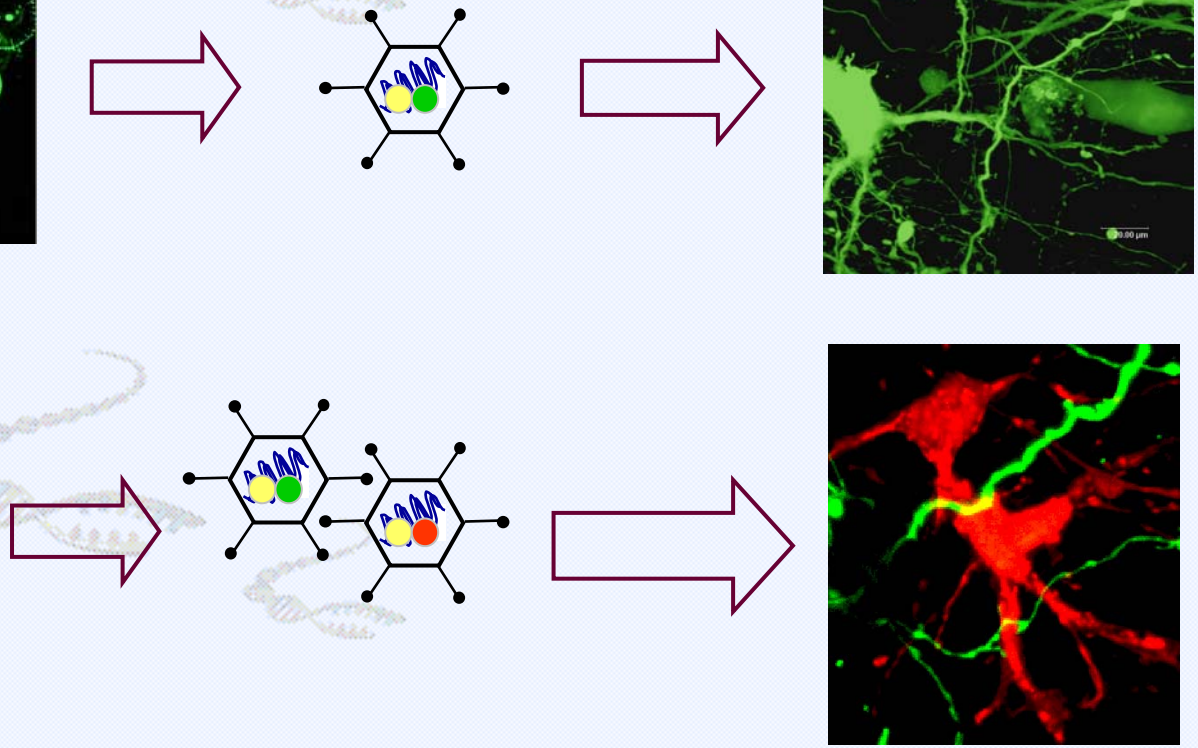


Teschemacher (2005) *Autonomic Neuroscience: Basic and Clinical* 117:1-8.
Chiti & Teschemacher (2007) *FASEB Journal* 21(10):2540-2550

Example 4: Co-expression of cell-specific transgenes for study of cell-cell interactions

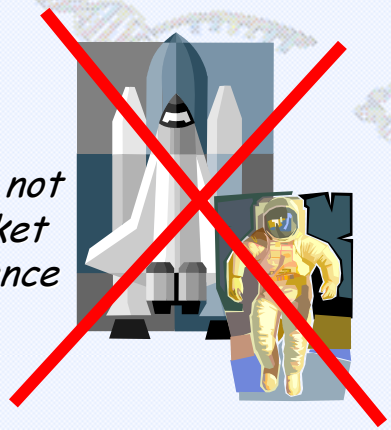
Green Fluor-escent Protein

Red Fluorescent Protein



Conclusions

- AVV and LVV are useful tools for cell-specific targeting of brain cells
  - to visualise them for cellular studies (electrophysiology / -chemistry, morphology)
  - To alter their function and investigate their roles in physiology *in vivo*
- They are easy to use and require only a little additional equipment and training



CAN DO IT!

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Useful terms:

Gene expression – the process of formation of messenger RNA (mRNA) of a DNA template which then is translated into the sequence of amino acids 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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- Jeff Croker
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Royal Society
BBSRC
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