Hippocampal NPY gene transfer in subjects with intractable MTLE (mesial temporal lobe epilepsy)

Phase I Trial sponsored by Neurologix, Inc.

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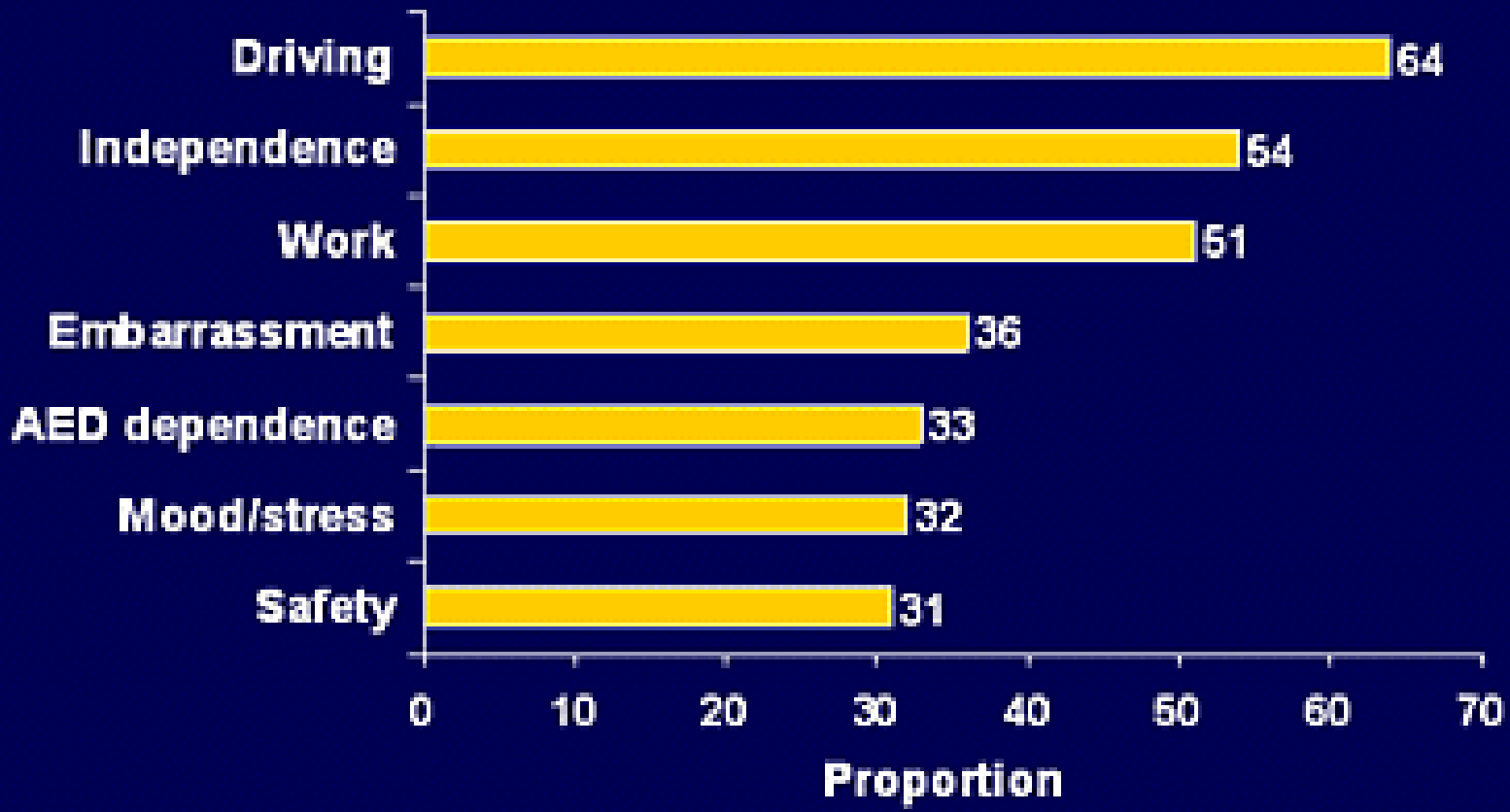
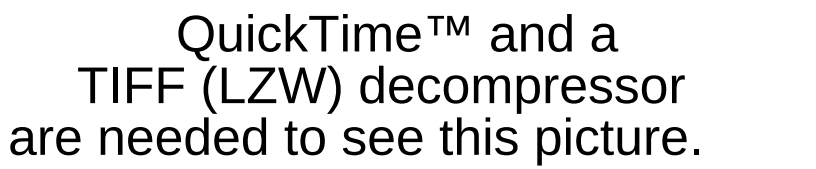
The Burden of TLE

- Temporal Lobe Epilepsy is the most common and most refractory form of epilepsy
  - Semah et al., >25% of all patients, 11% seizure-free
  - Stephens and Brodie, most common, 46% seizure-free
- 2.5 Million people with epilepsy in the USA
  - 30% (750,000) pharmacoresistant
  - Half with mesial temporal lobe epilepsy (350,000)
- $12.5 Billion = cost of epilepsy in the USA
  - 80% ($10 billion) due to intractable epilepsy
  - $5 billion may be due to intractable TLE

Concerns of Patients With ≥1 Seizure in Last 6 Months



Gilliam F. *Neurology*. 2002.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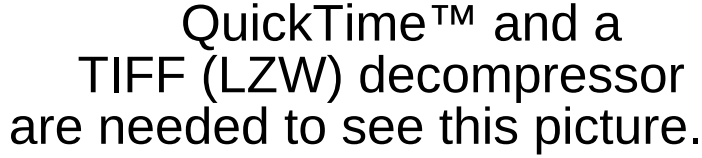
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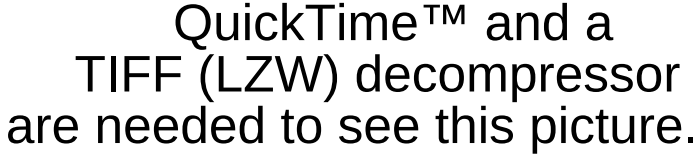
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State of Epilepsy Surgery Today

- Temporal lobectomy highly efficacious
- 100 - 200 thousand Americans with intractable MTLE could benefit from surgery, yet current estimates suggest only 3,000 surgical cases per year in the US - Large Unmet Need
- Lack of awareness of state of intractability, anxiety and perceived deficits relating to resective surgery and cost
- "studies should also be designed to find more cost-effective approaches to surgical therapy that would not compromise efficacy or safety to ensure existing health care resources will be readily available for this important alternative treatment modality" Consensus Statement AAN, AES, AANS, Engel et al., *Neurology 2003, 60, 538.*

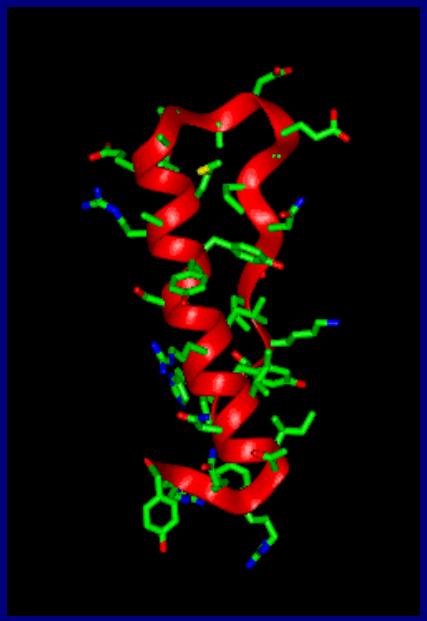
Why Gene Therapy? Anatomically favorable target

- Focal pathology, i.e. by definition TLE involves the temporal lobe
- Optimal medical treatment fails, and resistance to resective surgery results in an unmet need
- Success of surgery (anteromedial temporal lobectomy) in leading centers (~70% seizure-free one year) supports rationale that gene transfer leading to altered physiology in structures that are typically resected should be sufficient

Clinical Precedent and Rationale

- Tissue resection represents the most extreme form of cellular “silencing”
- Pharmacological and anesthetic agents can also silence, but lack selectivity/anatomical specificity - doses sufficient to completely dampen hippocampal activity lead to stupor and coma
- Local silencing could be obtained by local drug delivery: gene transfer one method to obtain local and sustained release of an inhibitory molecule

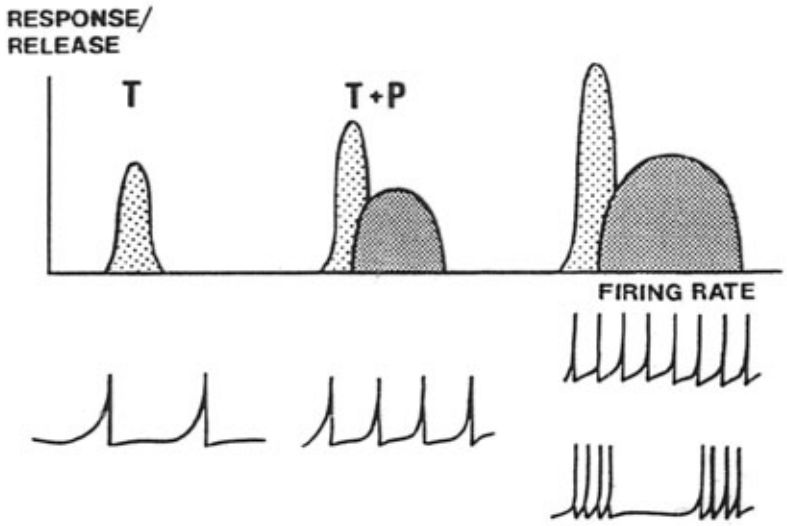
Why NPY?

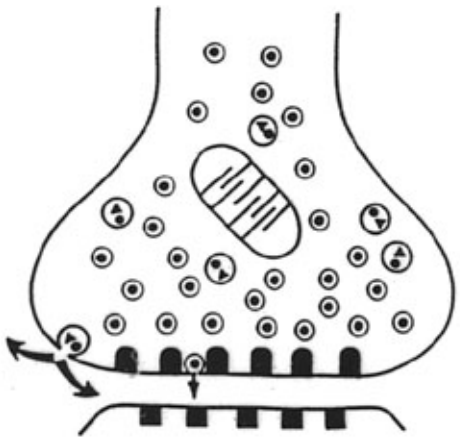


Neuron, Vol. 7, 867–879, December, 1991, Copyright © 1991 by Cell Press

Neuropeptides in Perspective: The Last Ten Years

Tomas Hökfelt





Neuropeptide Y and Seizures

Changes in NPY expression in neurons in experimental models of seizures and epileptogenesis and in human epileptic tissue

Changes in NPY release and in its receptor subtypes

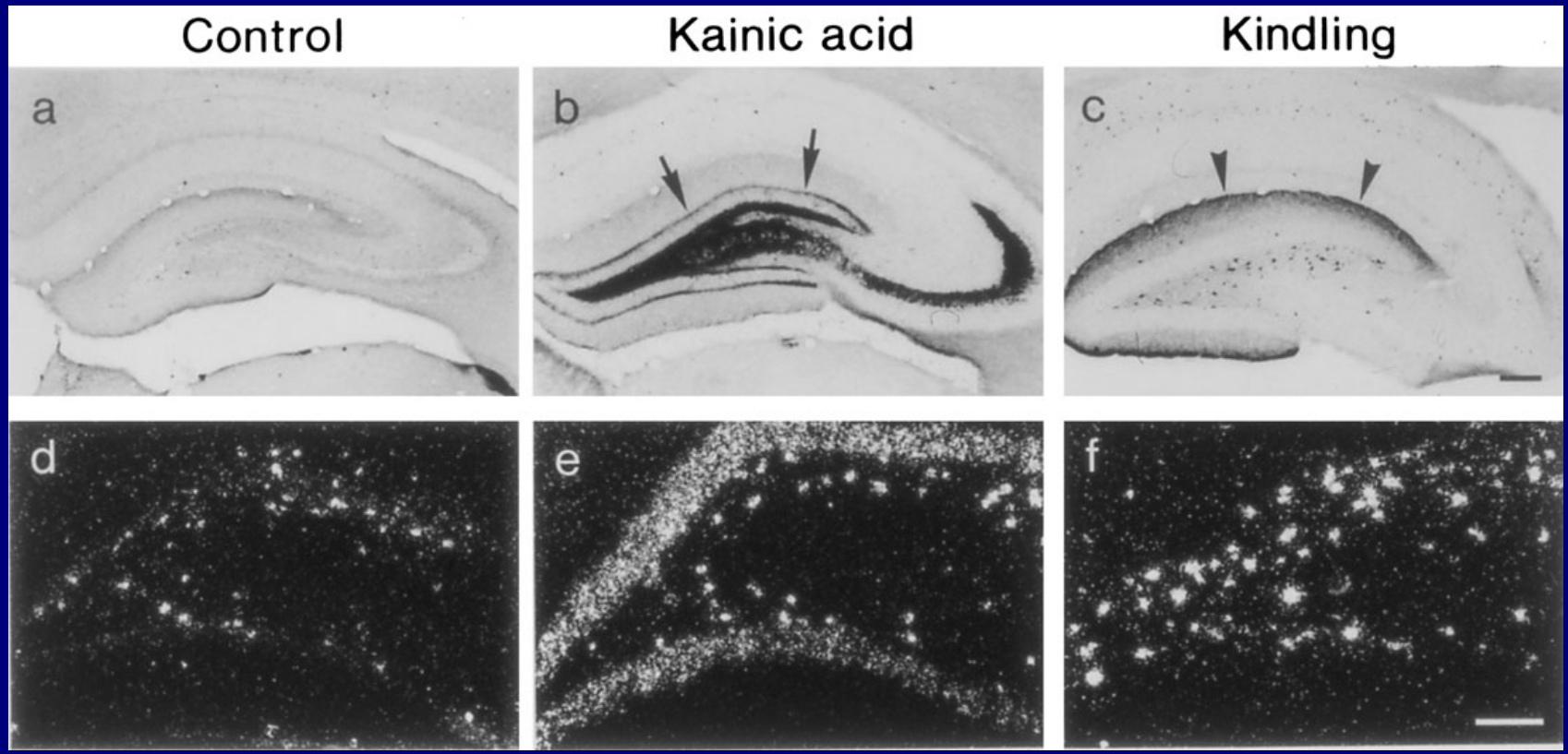
NPY-mediated neurotransmission is altered by seizures

NPY significantly inhibits excitatory synaptic transmission and seizure activity

NPY Y2 /Y5 receptors mediate inhibitory actions
NPY Y1 receptors mediate excitatory actions

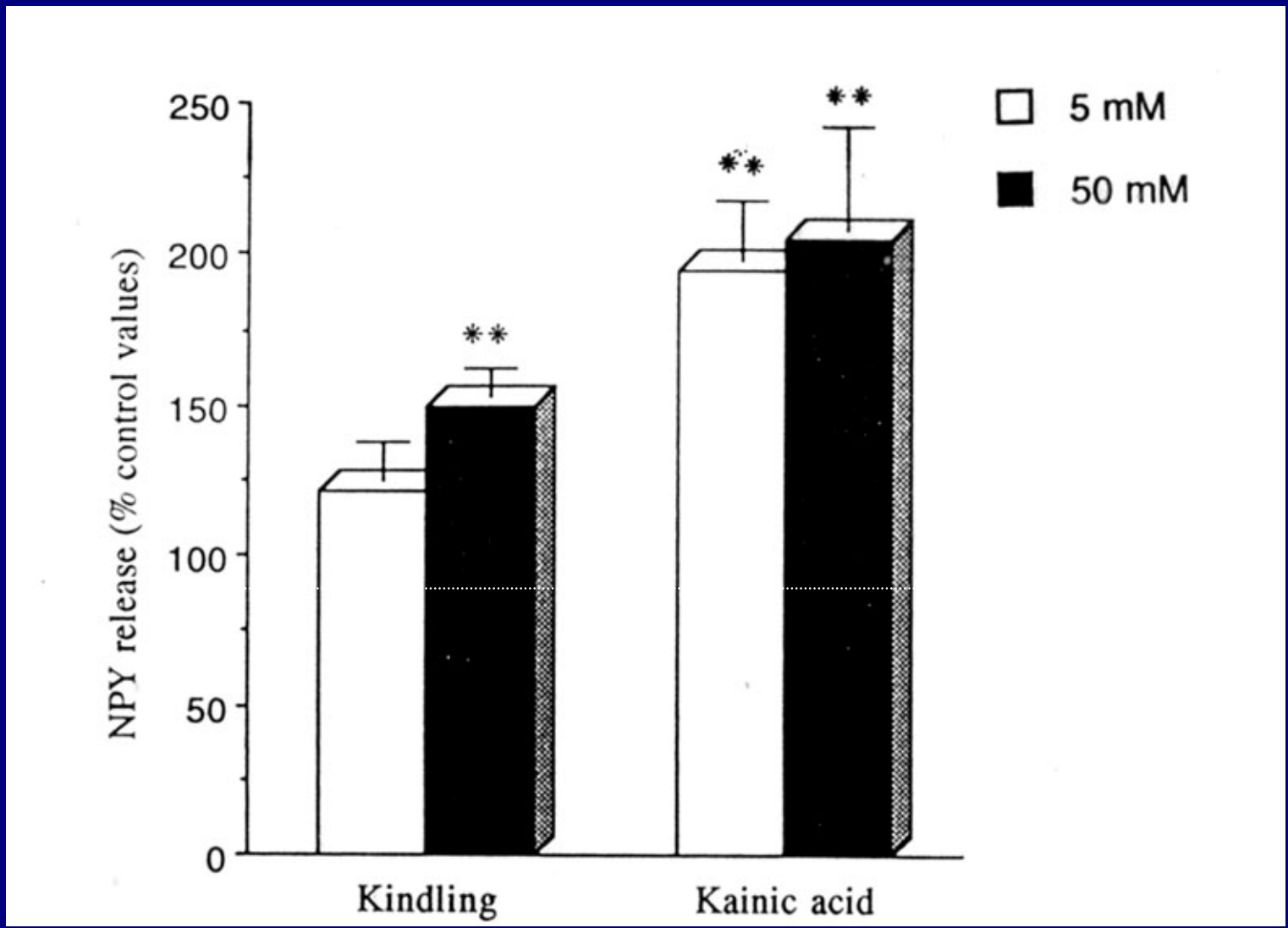
For review see Vezzani et al, *TINS*, 1999; Redrobe et al, *Brain Res*, 1999

NPY overexpression in rat epileptic tissue



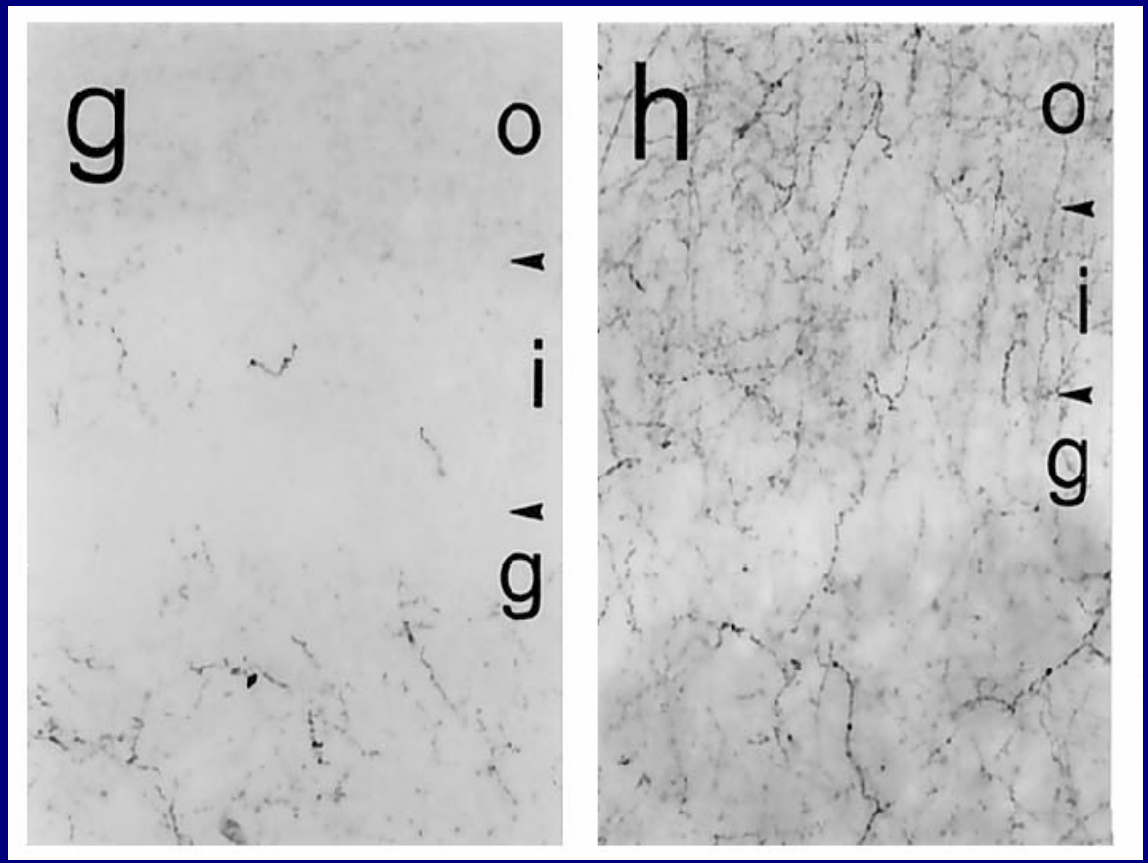
Vezzani et al, Trends Neurosci, 1999

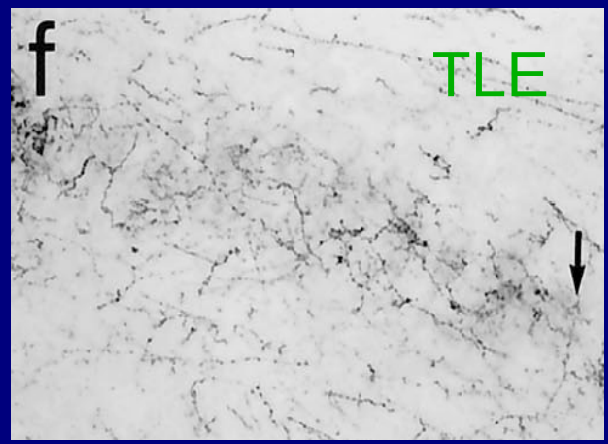
NPY release from rat hippocampal slices



Rizzi et al, Eur J Neurosci, 1993; Vezzani et al, Brain Res, 1994

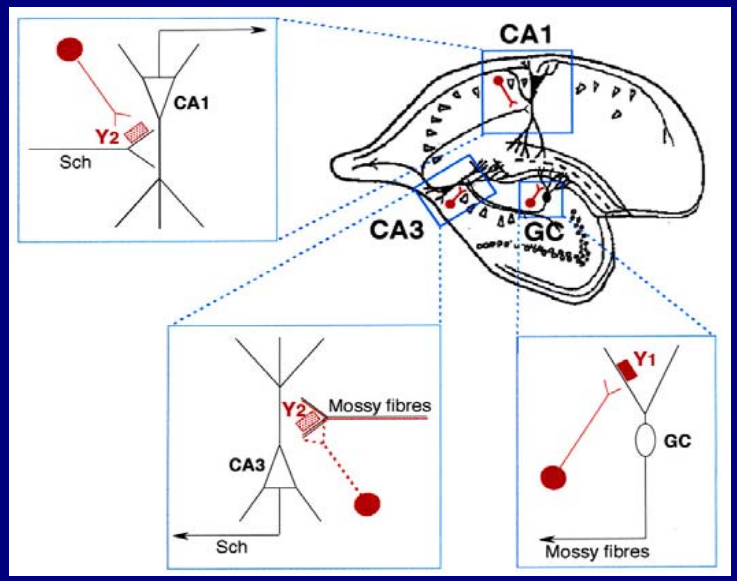
Sprouting of NPY-IR fibers in human brain



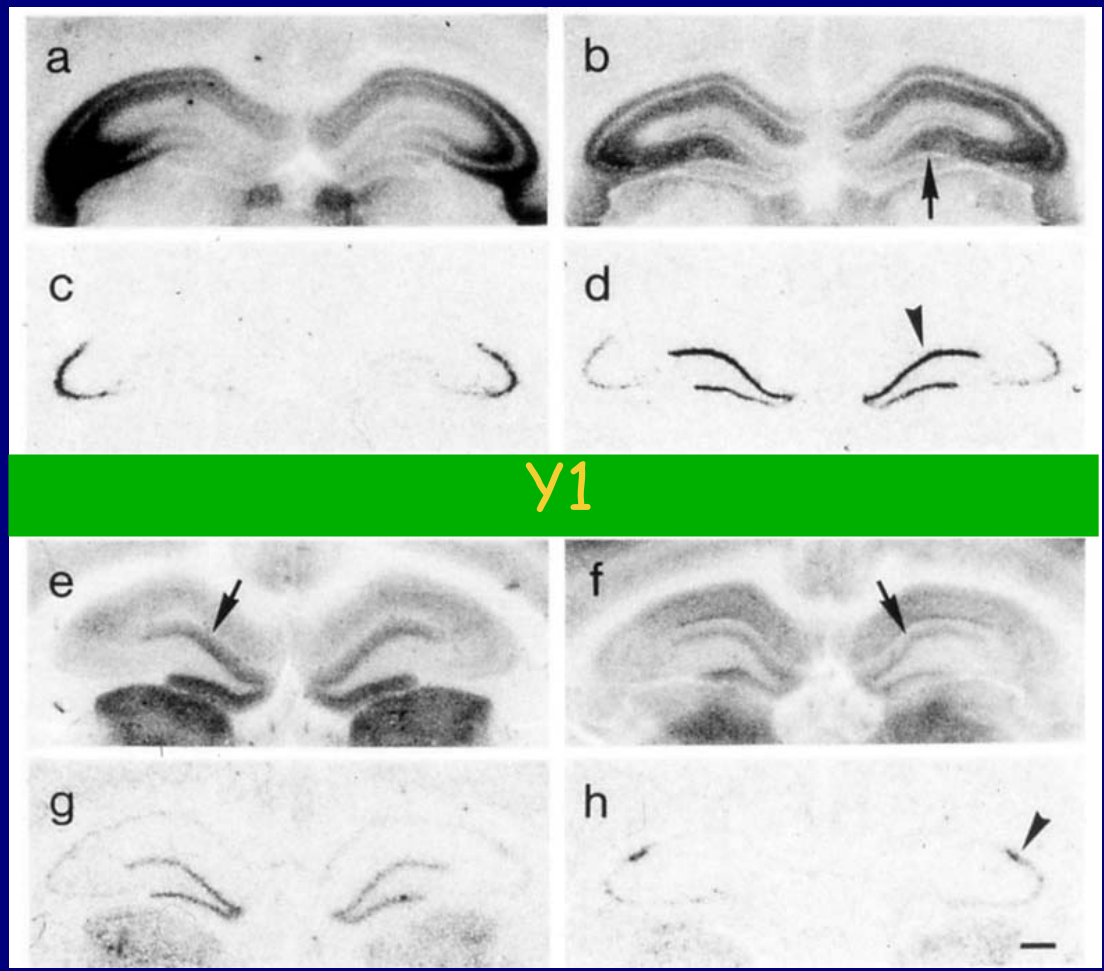


Control TLE (Sclerotic)

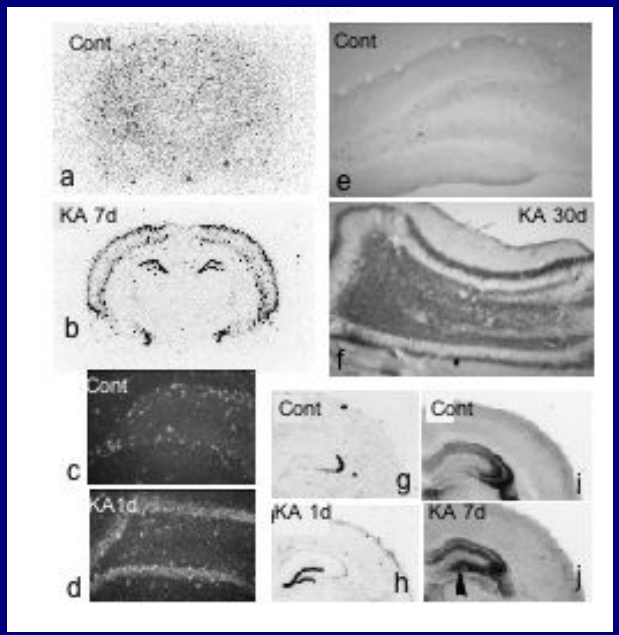
Furtinger et al, J Neurosci, 2001



Y2

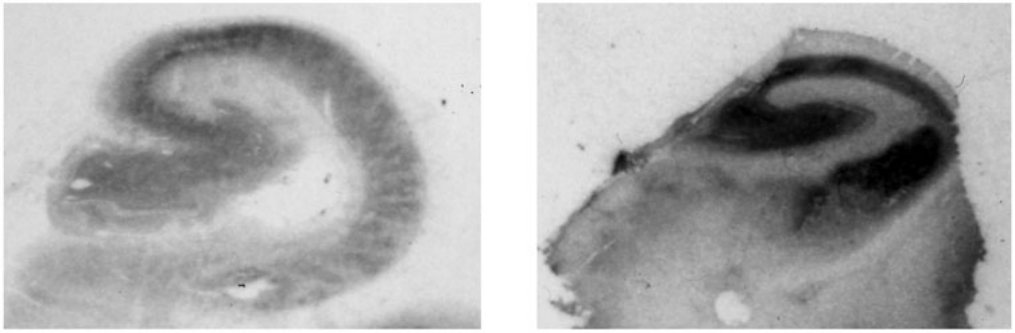


Y1



Kofler et al, Neurosci Lett, 1997; Scharzer et al, 1998; Gobbi et al, J Neurochem, 1998

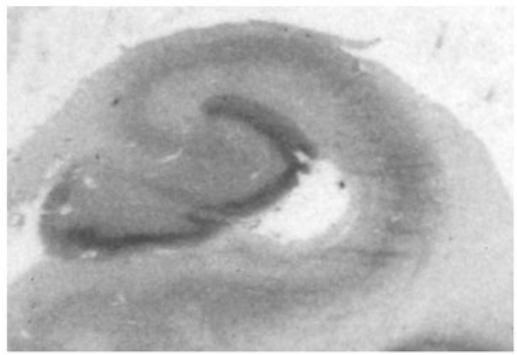
Y2 Receptor in Human TLE



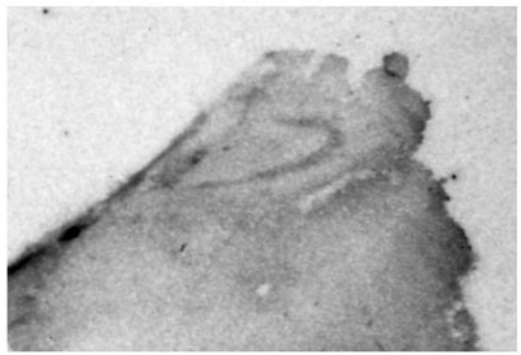
Control

Epileptic

Y1 Receptor in Human TLE



Control



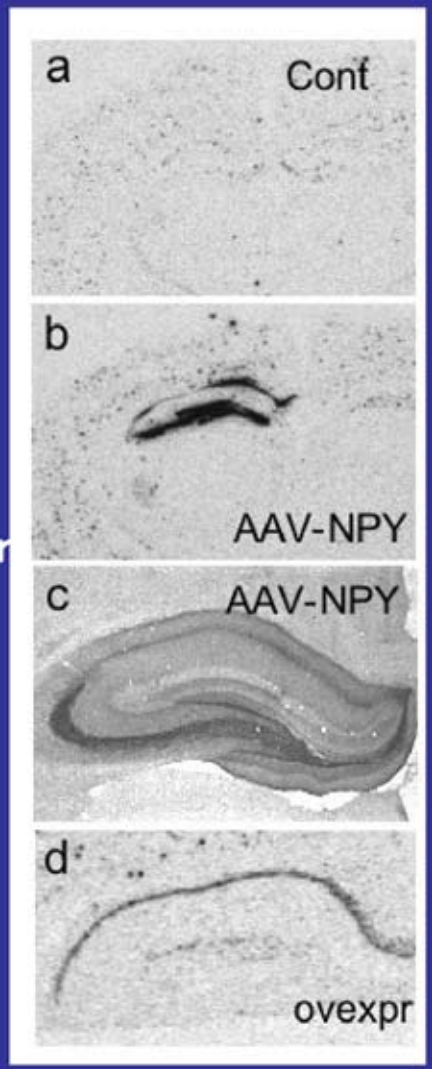
Epileptic

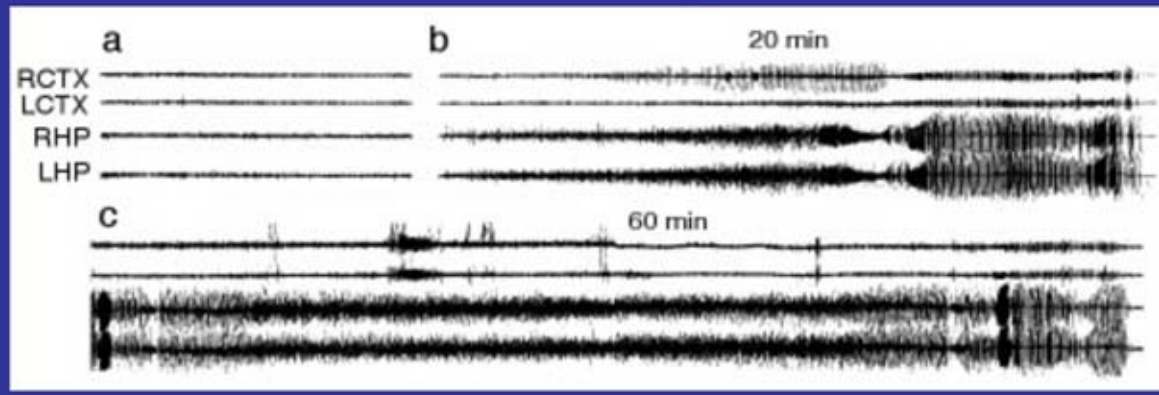
Furtinger et al, J Neurosci, 2001

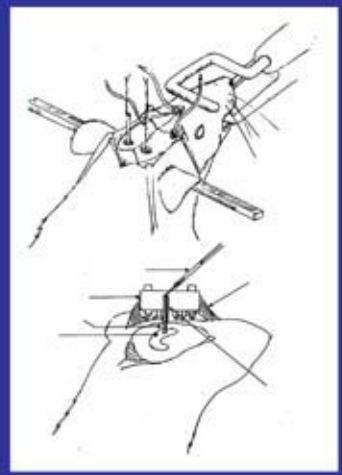
Effects of NPY overexpression

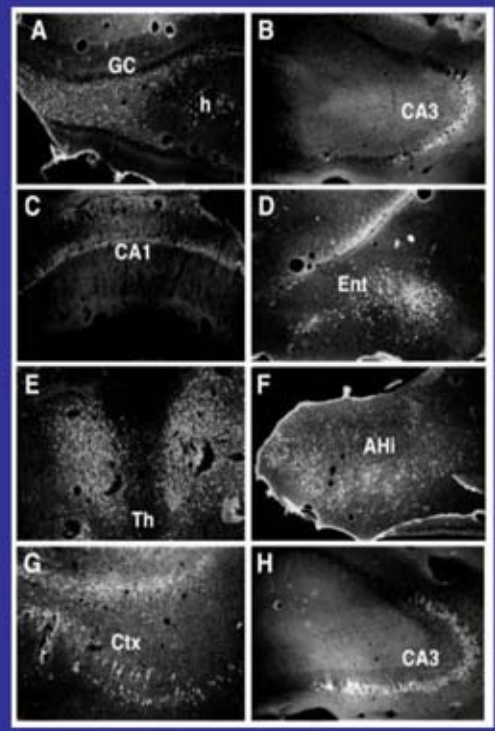
Gene transfer

Tg rats

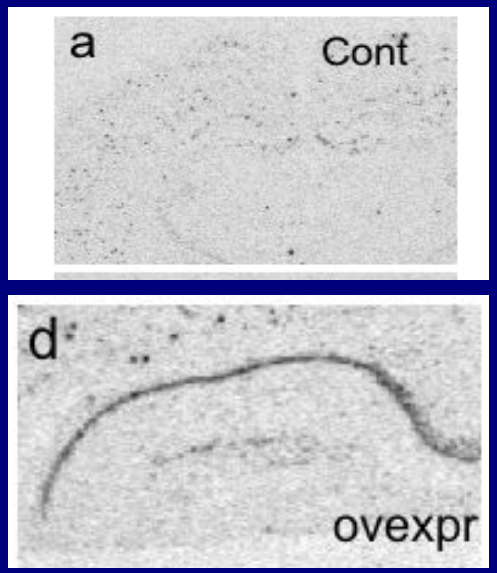


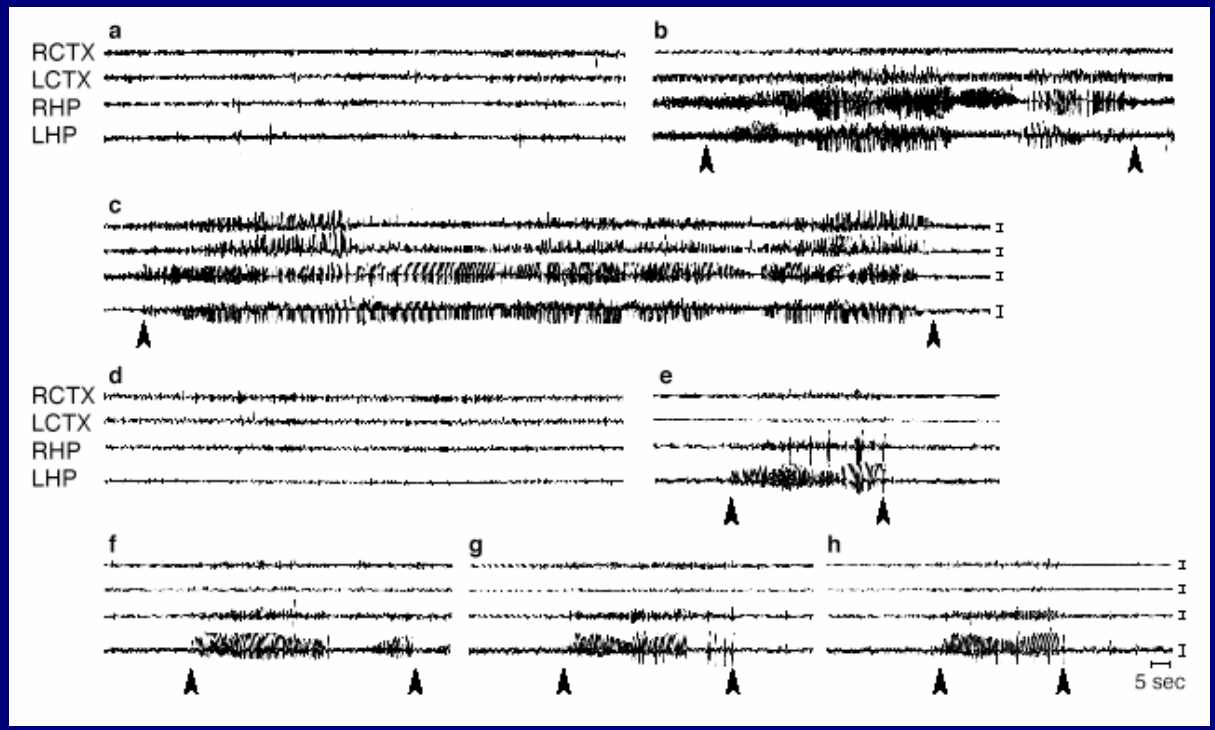






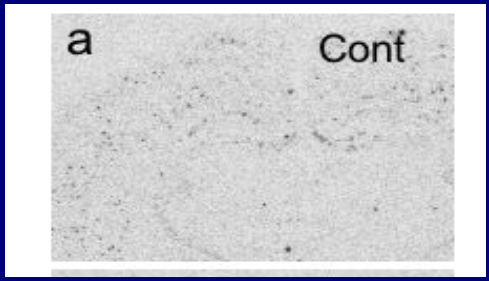
Seizure susceptibility in NPY transgenic rats

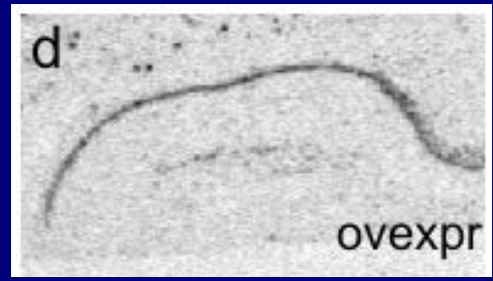




	SE	ONSET (min)	Number of seizures	Time in seizures (min)
WT	9/12	5.4 ± 1.1	27.0 ± 4.0	78.0 ± 9.6
Tg	2/9[§]	19.3 ± 9.7*	19.0 ± 3.0*	35.0 ± 13.4**

*p<0.05, **p<0.01 vs wt

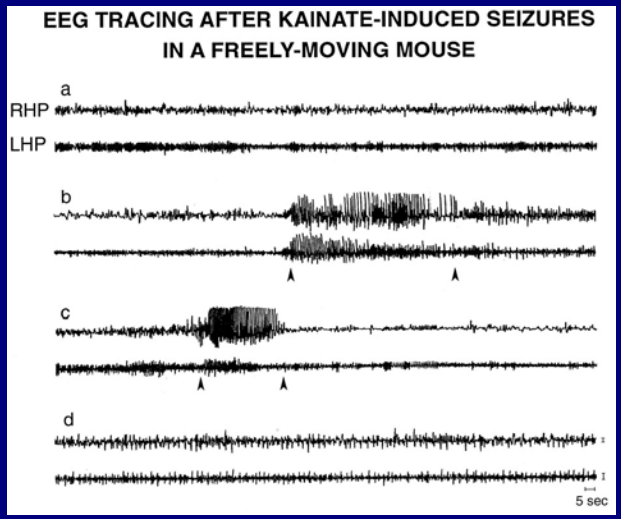




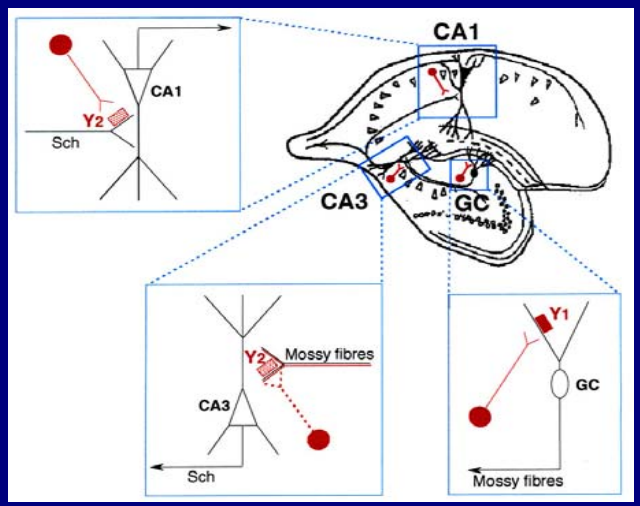
Kindling epileptogenesis

	Wt (n=9)	Tg (n=9)
μA	149 ± 20	120 ± 11
Stage 1-2	4 ± 1	5 ± 1
Stage 3	10 ± 2	15 ±3
Stage 4-5	23 ± 3	38 ± 4**
AD (sec) at st 2	11 ± 2	3 ± 1*
AD (sec) at St 5	29 ± 3	27 ± 1

Generalization of seizures is impaired



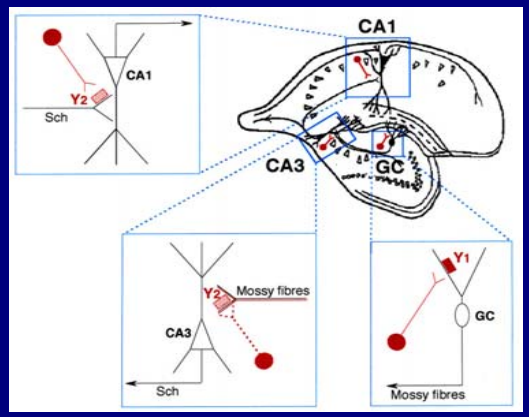
Enhanced seizure susceptibility in Y2 receptor KO mice



	ONSET (min)	Number of seizures	Time in seizure activity (min)
WT	9.6 ± 0.9	9 ± 1	30.0 ± 3.7
Y2 -/-	7.7 ± 1.2	12 ± 1	65.1 ± 6.2**

Data are mean ±SE (n=15-18). Kainic acid, 5 ng/0.5 μl intrahippocampally, * * p<0.01 vs WT Student's test

Antagonism of NPY Y1 receptors inhibits seizures



EFFECT OF BIBP 3226 AND BIBO 3304 ON EEG SEIZURES INDUCED BY INTRAHIPPOCAMPAL ADMINISTRATION OF 0.2 nmol KAINIC ACID IN FREELY-MOVING RATS

Drug	Dose (nmol/rat)	Onset (min)	No. of seizures	Time in seizures (min)
BIBP3435 (inactive enantiomer)	**18** (n=13)	14.8 ± 2.3	15.0 ±4.0	28.9 ± 6.0
BIBP3226	**9** (n=8)	23.0 ± 5.0	9.0 ± 2.0	10.0 ± 2.3*
	18 (n=10)	33.4 ± 5.4**	4.0 ± 1.0**	6.1 ± 1.3**
BIBO3457 (inactive enantiomer)	**15** (n=15)	13.1 ± 2.8	26.0 ± 4.0	28.9 ± 5.0
BIBO3304	**1.5** (n=8)	14.8 ± 1.9	10.0 ± 2.0*	10.0 ± 2.6*
	15 (n=7)	13.8 ± 3.5	15.0 ± 3.0	17.4 ± 3.9*

Data are the mean ± SE (n=No rats). BIBP and BIBO were dissolved in 25%PEG. Drugs were unilaterally infused in the hippocampus (1 μl) 15 min before kainic acid (0.2 nmol/0.5 μl). *p<0.05; **p<0.01 vs respective controls by Tukey's test.

Garibaldi et al, Eur J Neurosci, 1998

NPY Y2 Agonist shows anti-epileptic activity

EFFECT OF [Ala^{24},Ala^{27},Leu^{28},Leu^{31}]NPY24-36 (GW) ON EEG SEIZURES INDUCED BY INTRACEREBRAL ADMINISTRATION OF KAINIC ACID IN FREELY-MOVING RATS

Drug	nmol/rat	Onset (min)	No of seizures	Time in seizures (min)
		Intrahippocampally		
Control (n=8)		9.4 ± 0.9	20.0 ± 1.0	30.7 ± 2.8
GW (n=8)	10	12.4 ± 2.3	12.0 ± 2.0**	16.0 ± 3.7*
		Intracerebroventricularly		
Control (n=6)		14.1 ± 2.3	15.0 ± 2.0	22.0 ± 3.2
GW Protected (n=6 out of 12)	10	25.9 ±10.0	8.0 ± 1.0*	9.8 ± 0.9*

Data are the mean ± SE. GW was injected unilaterally in the hippocampus (0.5 μl) or icv (5 μl) in PBS (pH 7.4) 10 min before applying 0.2 nmol kainic acid in the hippocampus. Controls received PBS 10 min before kainic acid. *$p<0.05$, **$p<0.01$ vs respective controls by Student's t- test.

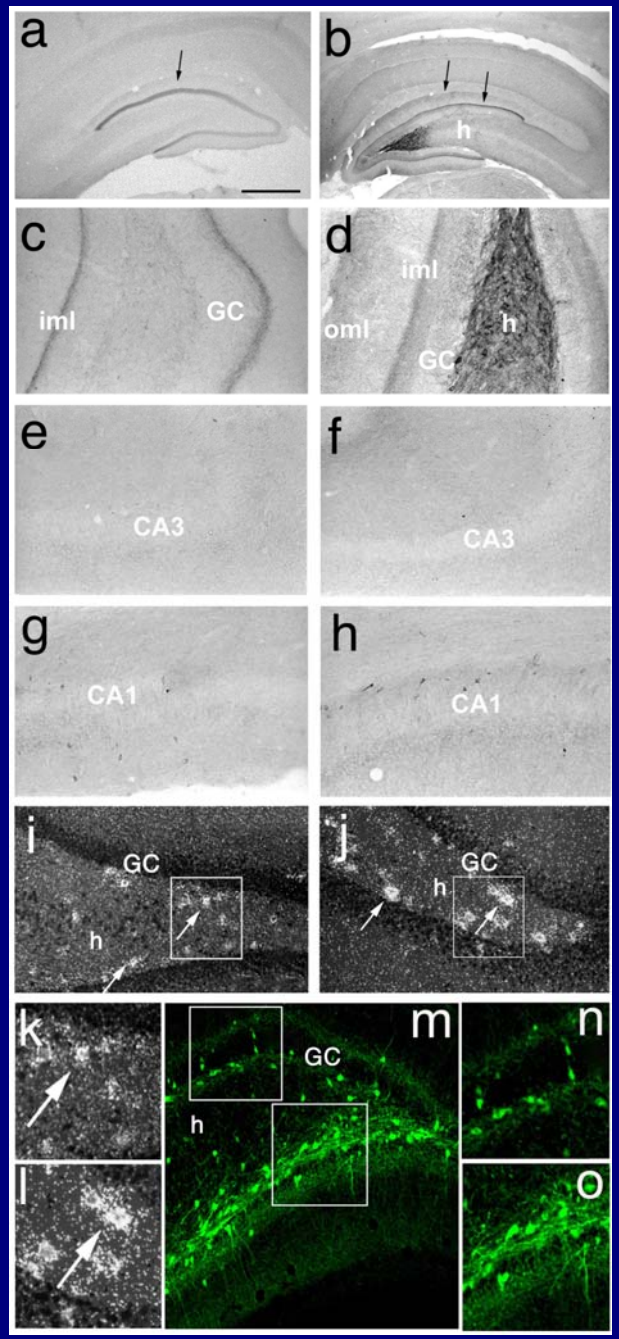
rAAV-NSE-NPY serotype 2

8 WEEKS AFTER rAAV VECTOR DELIVERY

NPY is synthesized and transported in fibres

NPY mRNA is overexpressed specifically in interneurons

The vector spreads for ~1.5 mm around the injection site

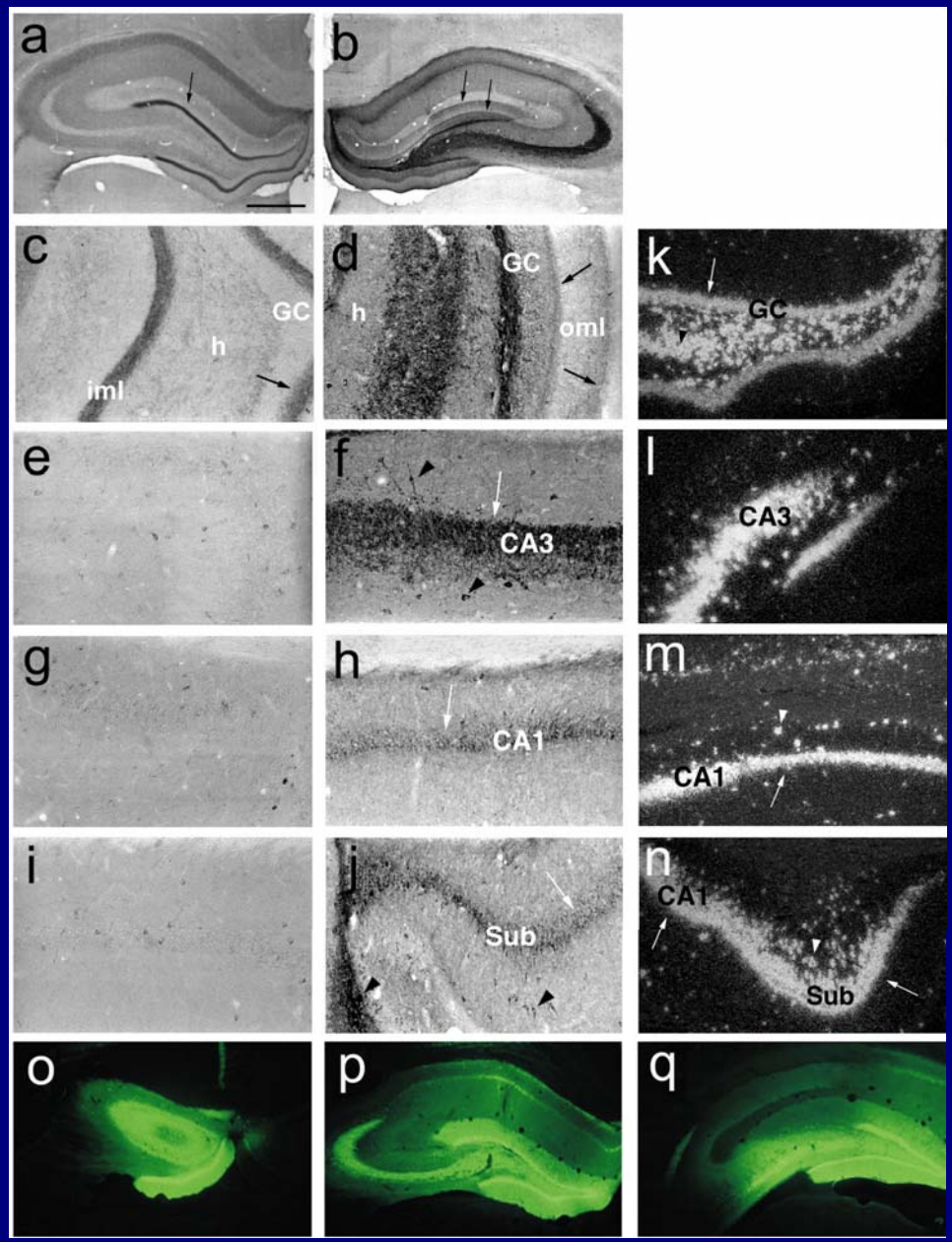


rAAV-NSE-NPY serotype 1/2

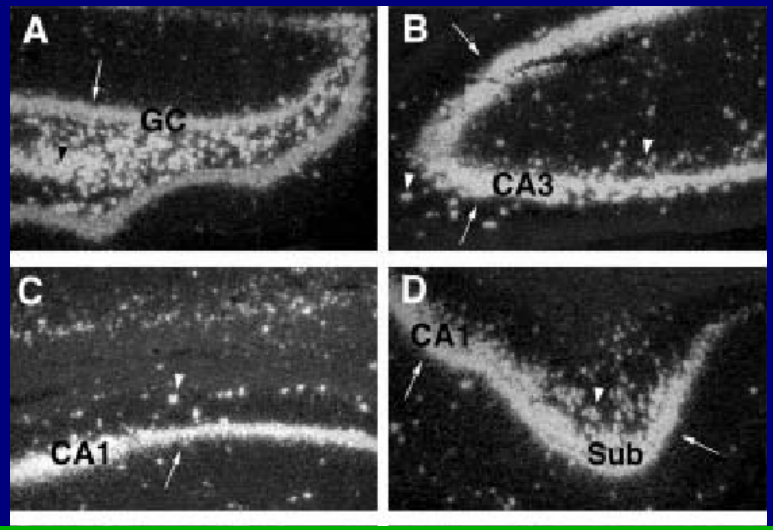
8 weeks after vector delivery

- The peptide is increased in hilar interneurons, granule cells, in pyramidal cells and subiculum

- The vector spreads for ~2.5 mm around the injection site.

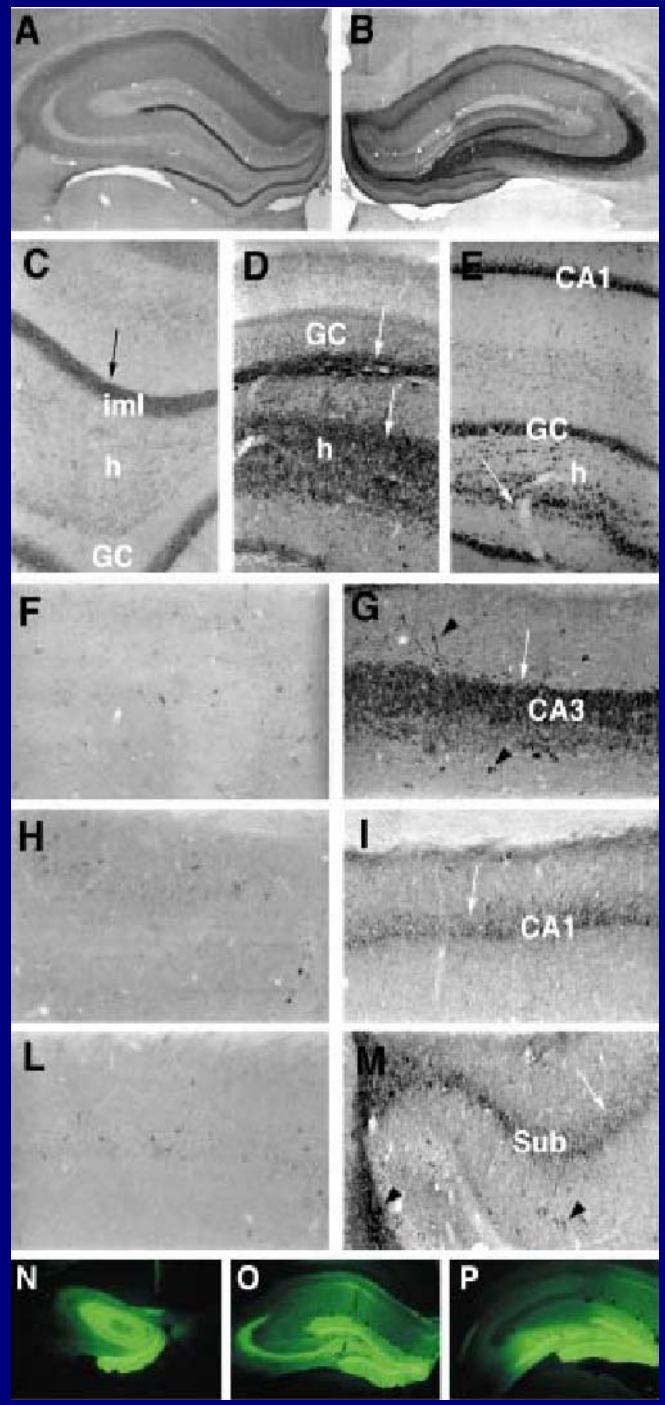


rAAV-NSE-NPY serotype 1/2

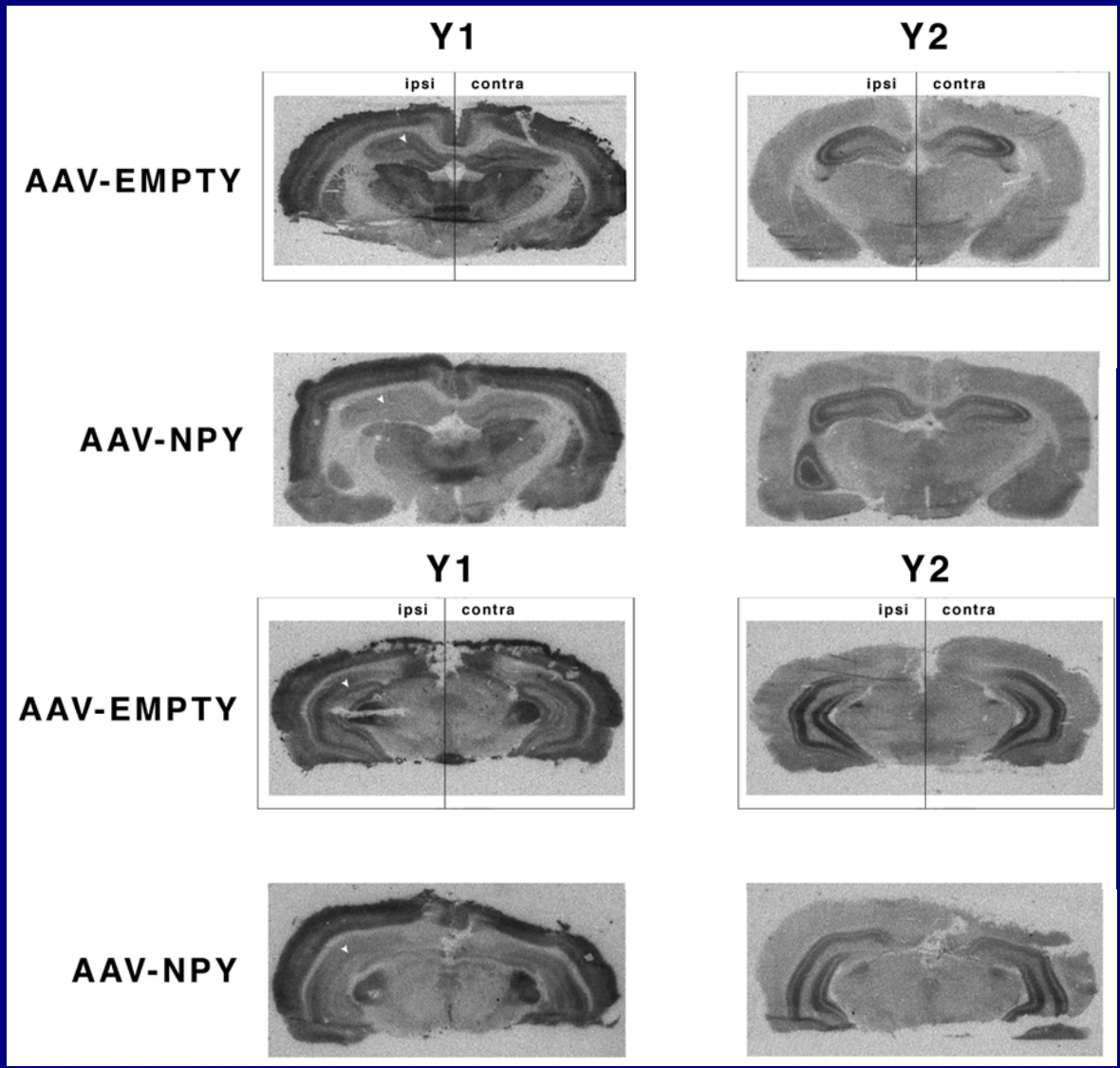


- The vector spreads for ~2.5 mm around the injection site.
- The peptide increases in hilar interneurons, granule cells as well as pyramidal cells.

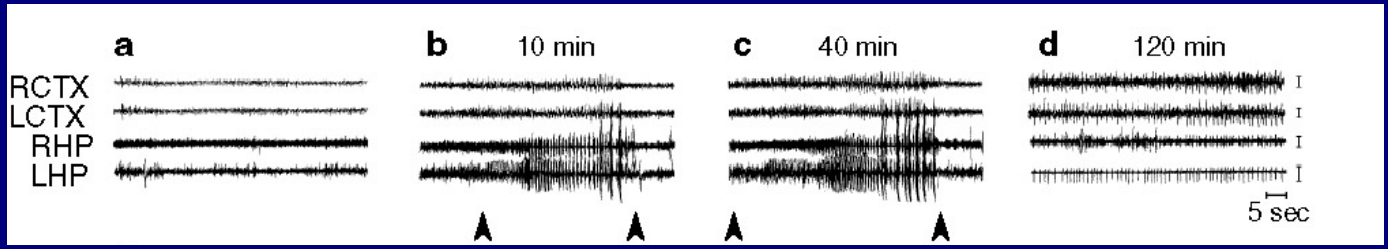
8 weeks after rAAV injection

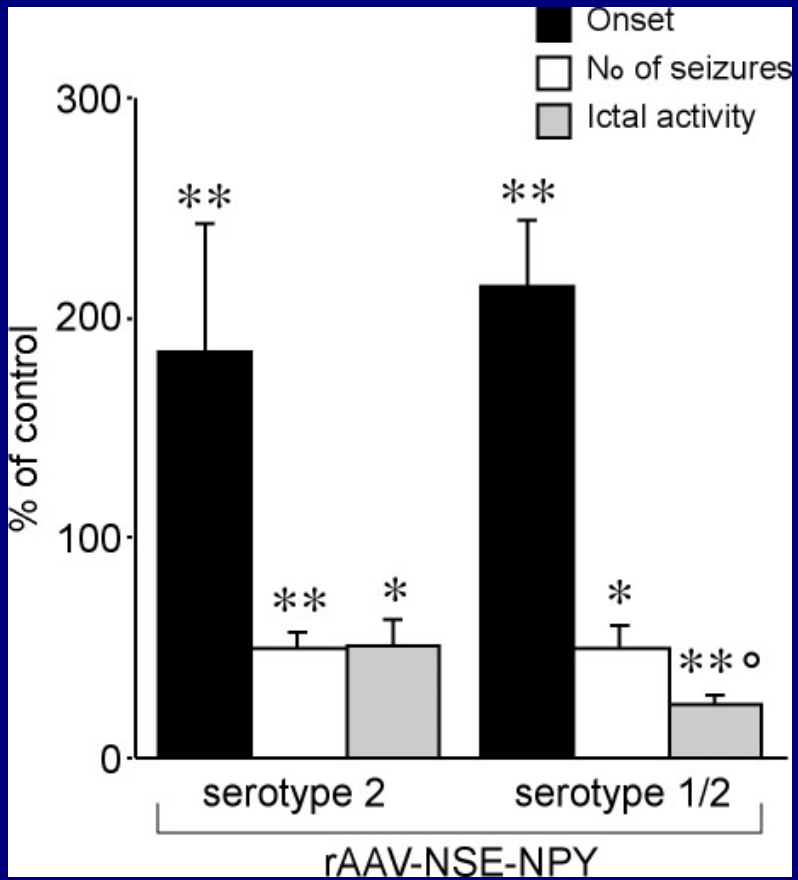


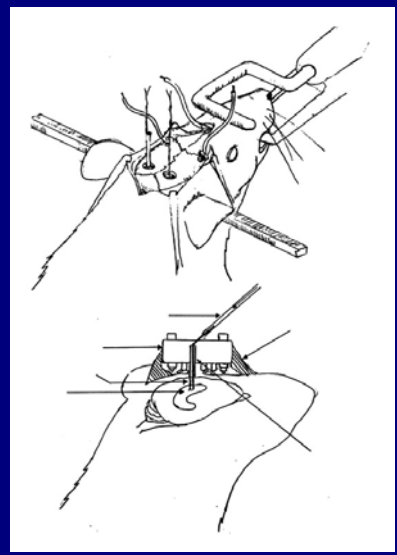
AAVNPY leads to downregulatioin of Y1 but maintenance of inhibitory Y2 receptors



EEG seizures induced by intrahippocampal kainic acid

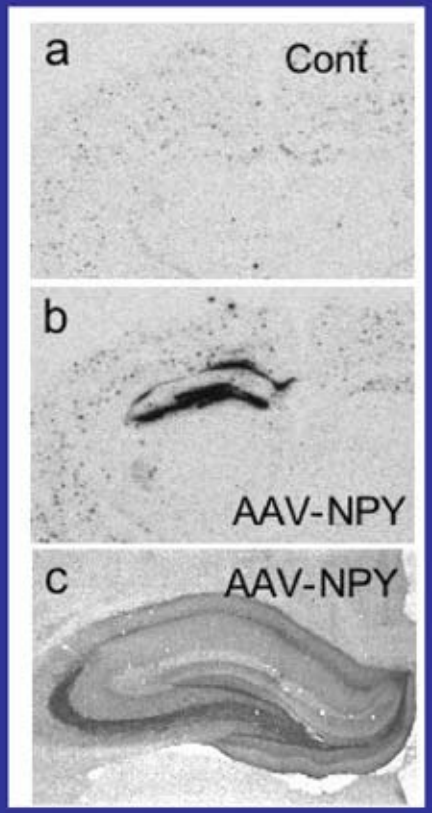


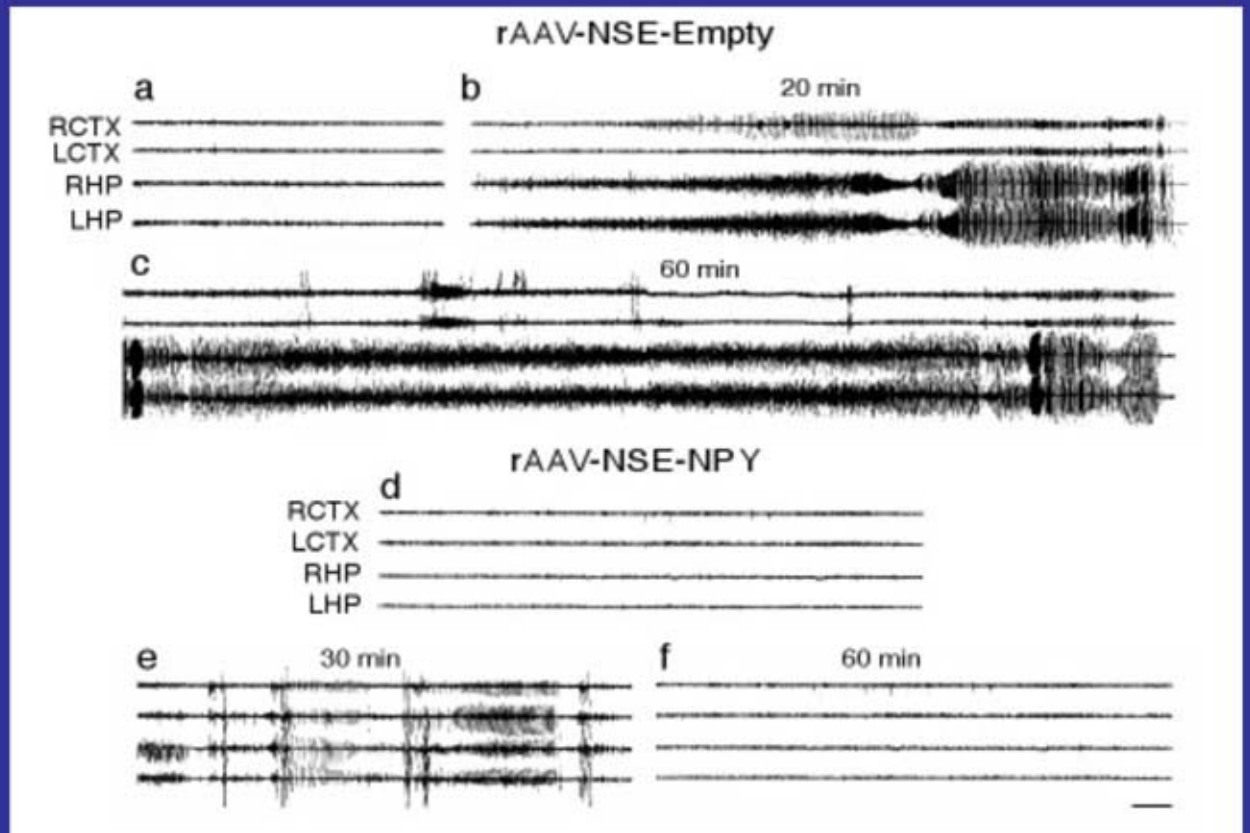




Serotype 1/2 rAAV-NSE-NPY is more effective than serotype 2 in inhibiting hippocampal seizures

Protection from status epilepticus in rats overexpressing NPY



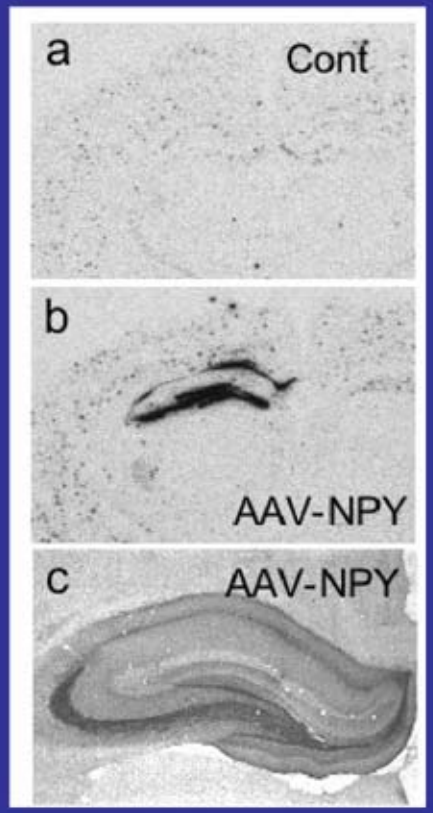


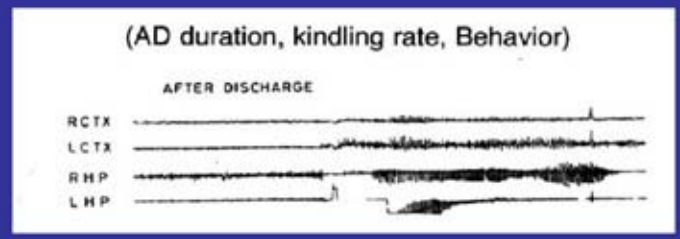
Seizure activity in rats overexpressing NPY in the hippocampus 8 weeks after rAAV-NSE- NPY injection

Treatment	Onset (min)	Number of seizures	Time in discrete seizures (min)	Time in status epilepticus (min)	Total time in seizures (min)
rAAV-NSE-Empty	6.2 ± 0.3	18.0 ± 1.0	53.5 ± 6.0	86.9 ± 10.1	137.0 ± 7.9
rAAV-NSE-NPY (serotype 1/2)	11.5 ± 1.8**	23.0 ± 6.0	53.4 ± 9.7	0	53.4 ± 9.7**

Data are the mean ± SE (n=6-10). Kainic acid was injected intracerebroventricularly (250 ng in 0.5 µl), 8 weeks after vector injection. **p<0.01 vs rAAV-NSE-Empty by Tukey's test.

Kindling epileptogenesis



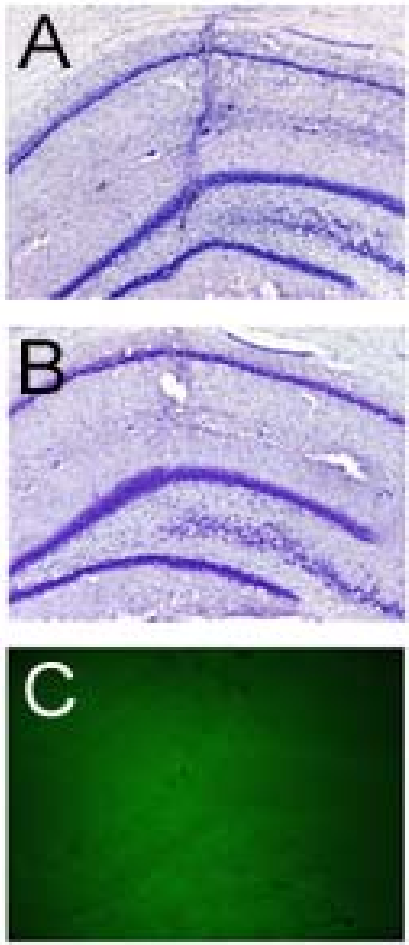


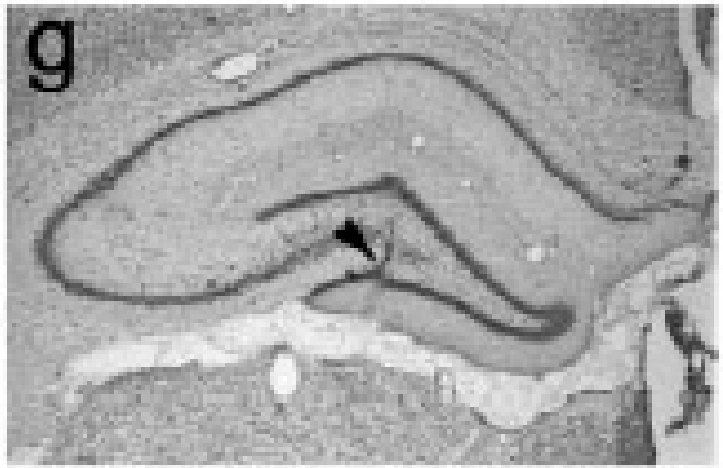
	Empty-vector (n=9)	rAAV-NPY (n=7)
μA	127 ± 4	180 ± 21*
Stage 1	1.1 ± 0.1	1.3 ± 0.1
Stage 2	1.4 ± 0.3	2.4 ± 0.7
Stage 3	6.2 ± 1.8	12.8 ± 2.1*
Stages 4-5	11.4 ± 2.5	25.5 ± 4.2**
AD (min)	14.9 ± 2.0	9.9 ± 1.0*

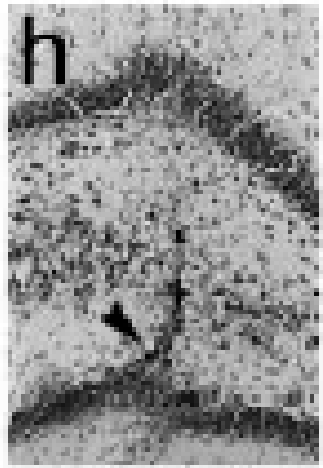
Local excitability is reduced

Generalization of seizures is impaired

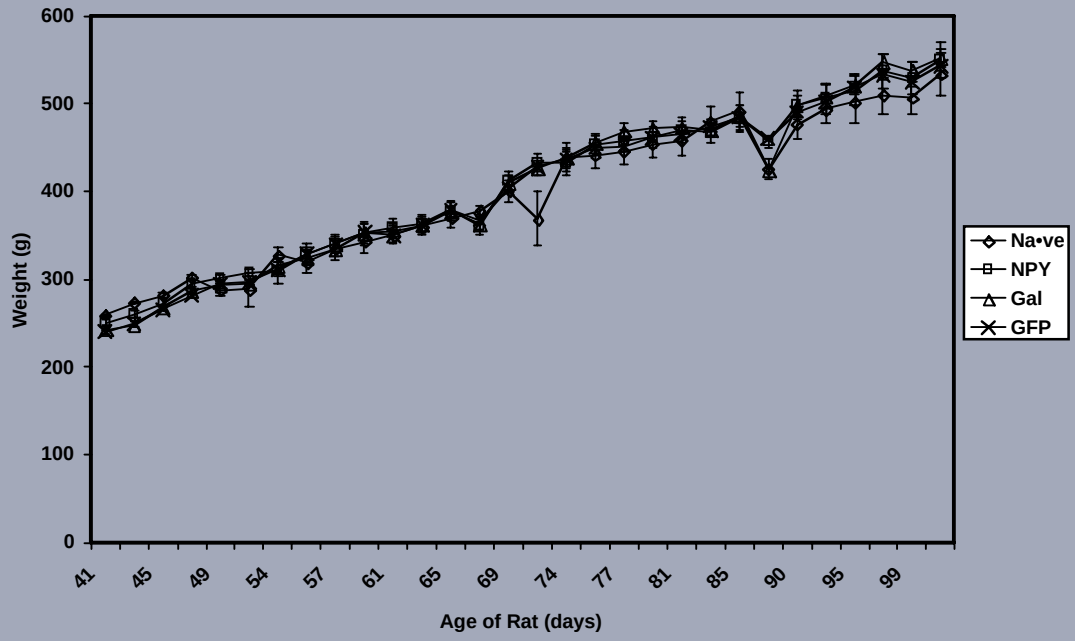
Insertional injury only at the injection site

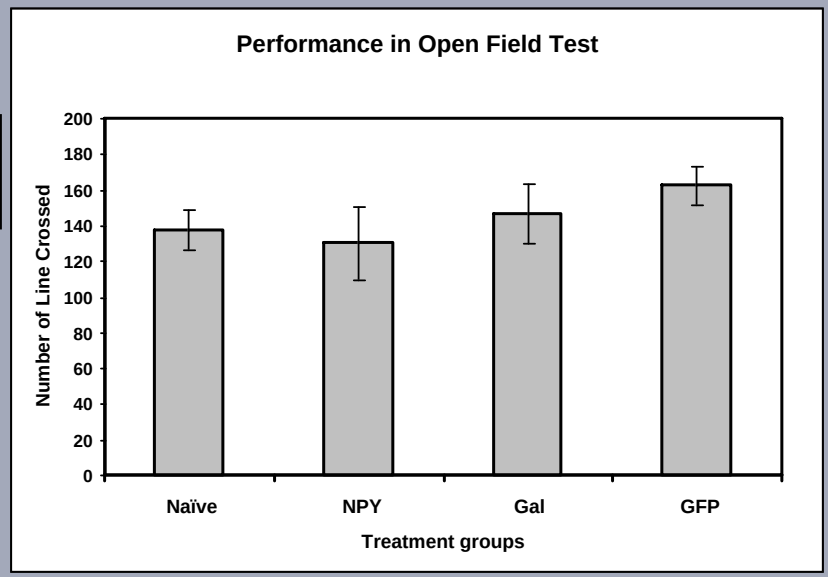




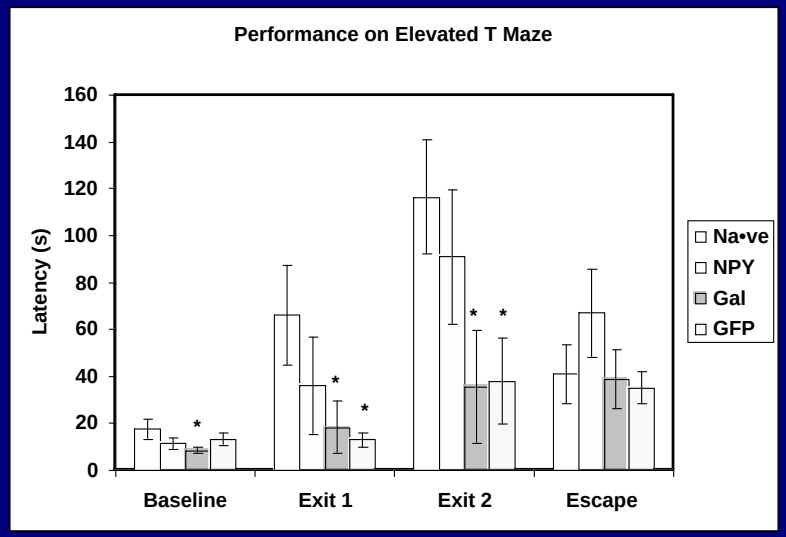


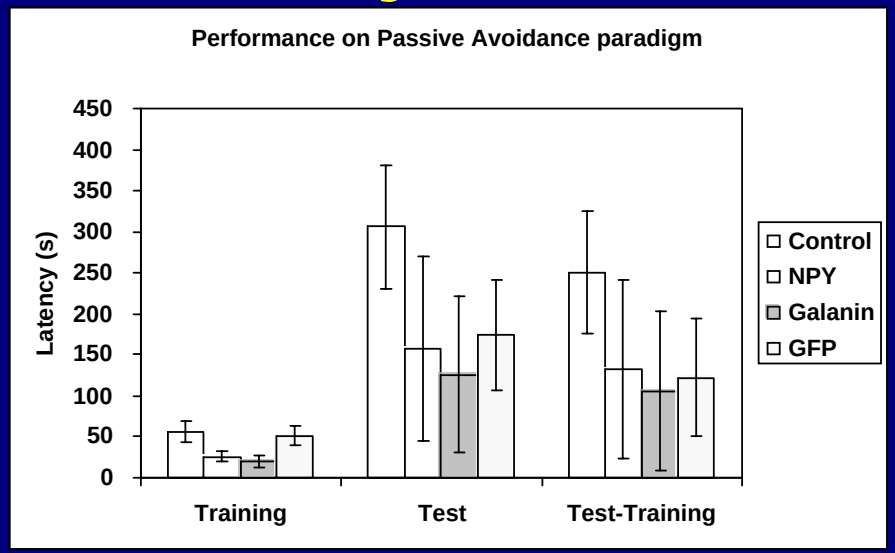
Effects on weight and locomotion

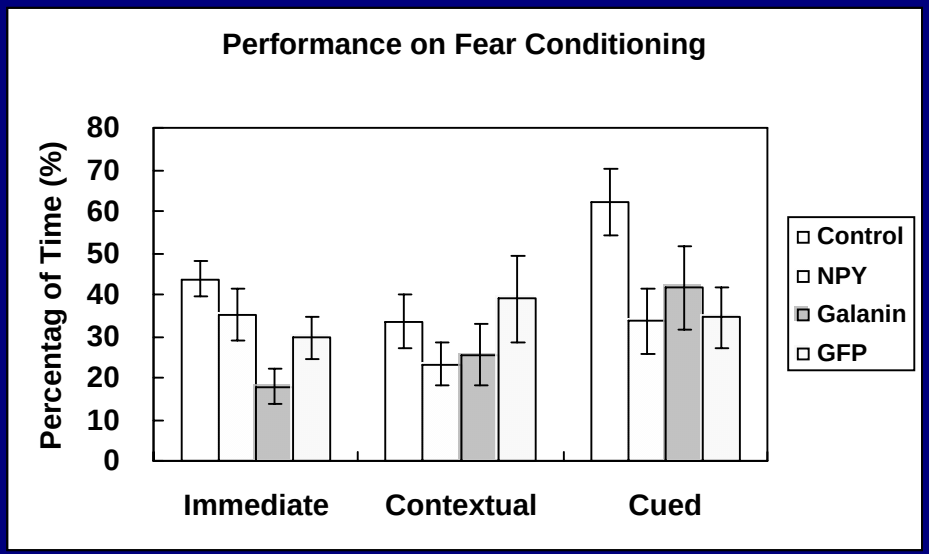




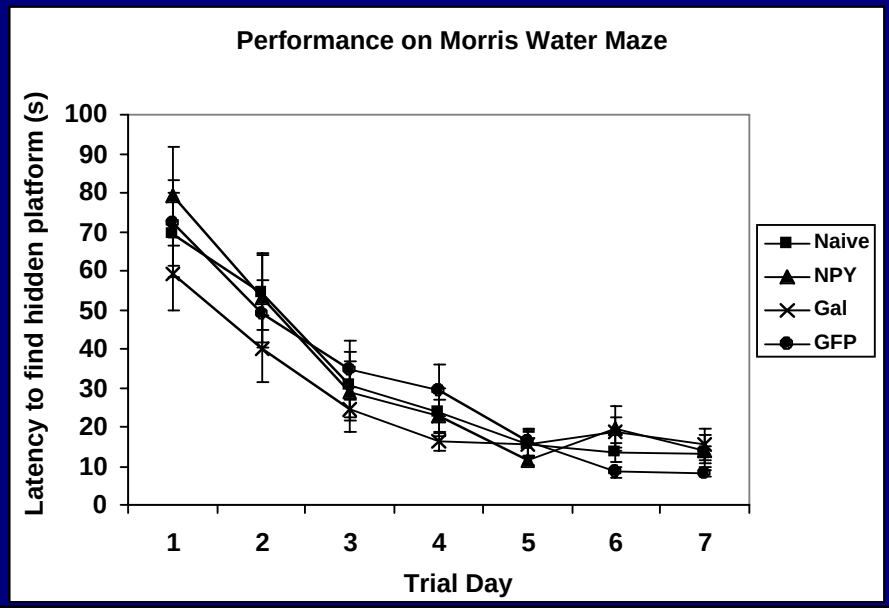
Effects on anxiety and hippocampal-dependent memory

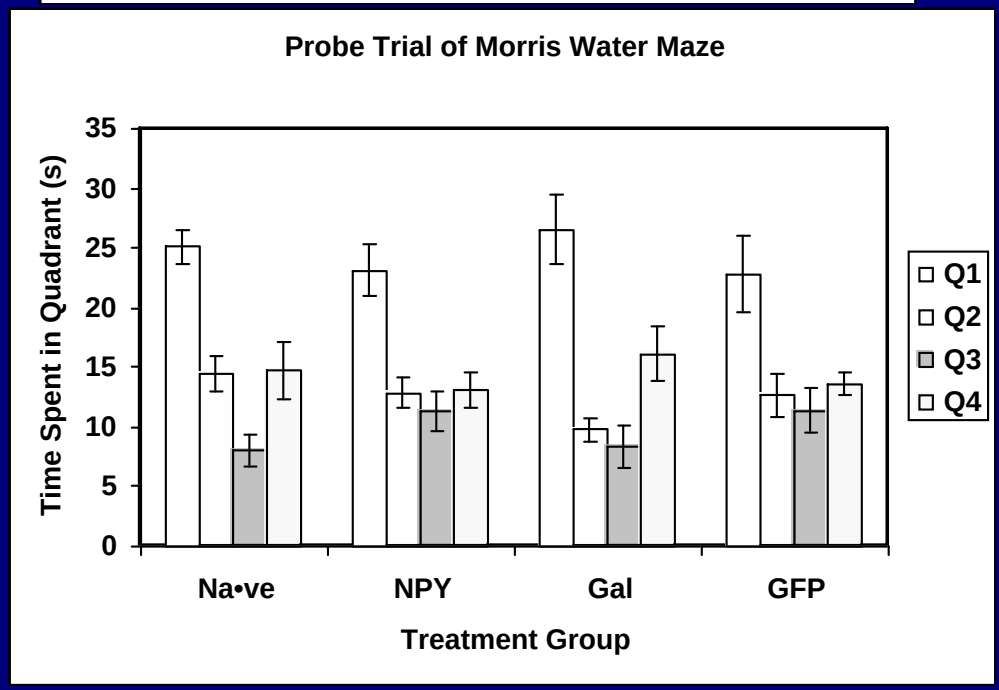




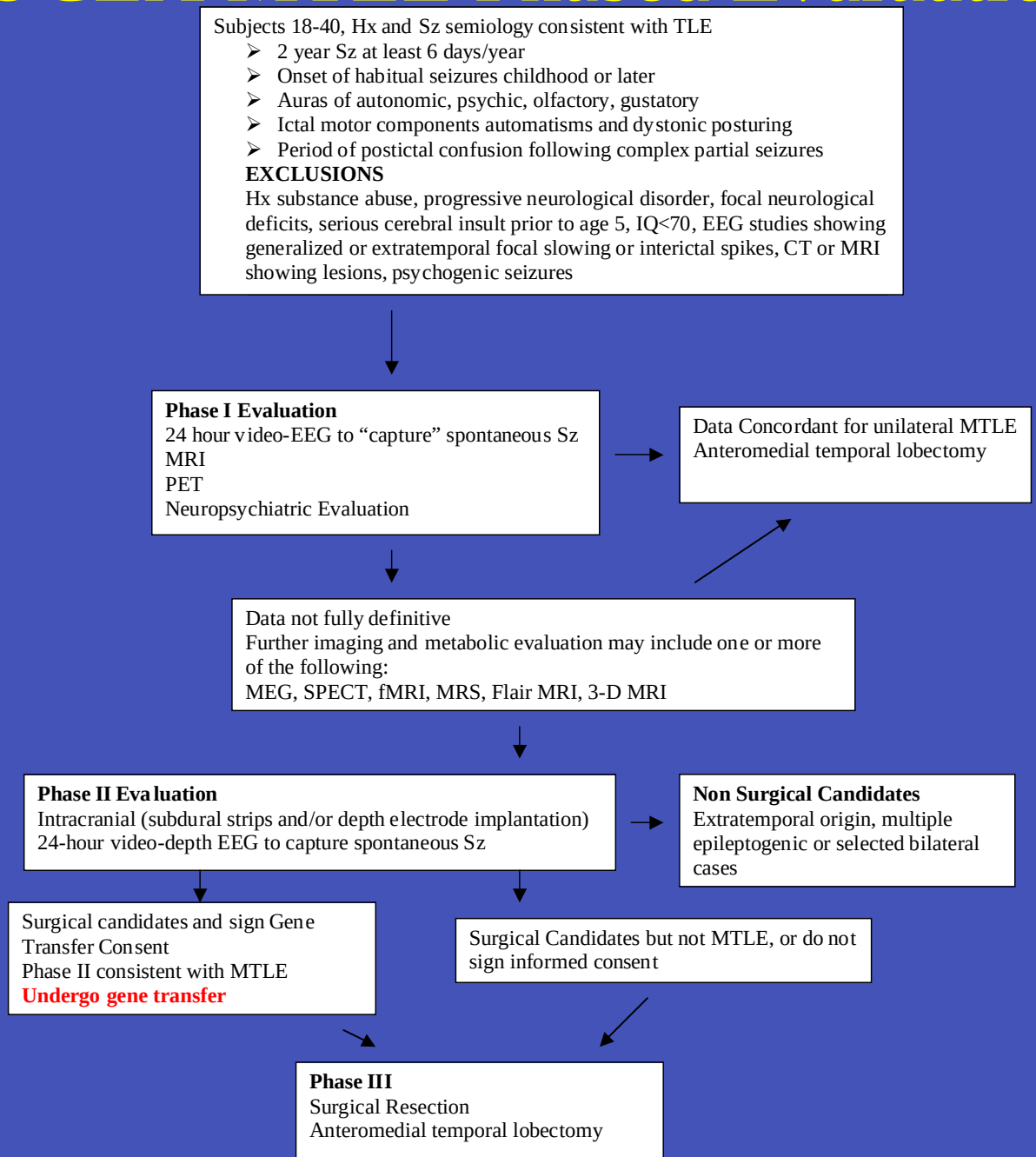


Effects on Morris Water Maze





UCLA MTLE Phased Evaluation



Assessment Timeline

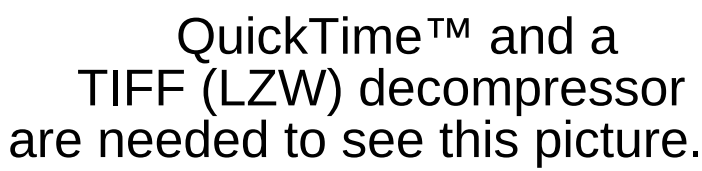
Baseline
Seizure Log
Liverpool Severity Scale (MLSS)
QOLIE89/ESI-55
Neuropsych/Social/Global Rating
MRI
FDG-PET

1 and 3 months
Seizure Log
MLSS
QOLIE89/ESI-55
Neuropsych
MRI

6 Months
Seizure Log
Liverpool Severity Scale (MLSS)
QOLIE89/ESI-55
Neuropsych/Social/Global Rating
MRI, including volumetrics
FDG-PET

Best standard of care not obviated by current protocol but built into study design

- Only subjects who meet criteria for temporal lobectomy are eligible
- Surgical resection postponed up to six months
- Results from Wiebe et al. show that long-term outcome and QOL essentially identical if surgery delayed for one year (small risk of SUD)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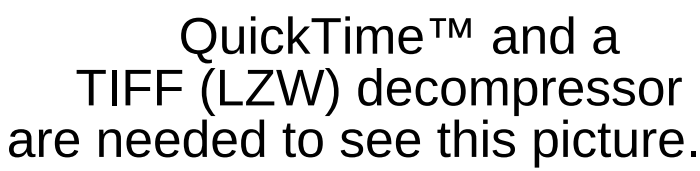
TIFF (LZW) decompressor
are needed to see this picture.

Potential advantages of NPY gene transfer

- Current surgery leads to significant adverse events including permanent major neurological deficits
- Cognitive impairment based on sensitivity of assessment with >5% having major cognition and/or language problems, a majority having more subtle deficits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Current Protocol designed to minimize risk to the patient

- Piggy-backed on an indicated invasive procedure with no further interventional procedure necessary
- All subjects will meet criteria for Phase II evaluation of intractable TLE
- Phase I is non-invasive EEG, MRI and PET evaluation. If data non-concordant, then a subgroup undergoes Phase II, or invasive, intracranial electrode implantation. This group may include patients with unilateral MTLE with hippocampal sclerosis, although MRI changes minor, bilateral MTLE, as well as subjects with primary entorhinal cortex origin of seizures.

Current protocol has a built in rescue procedure as default

- At 6 months post gene transfer, an anteromedial temporal lobectomy, as routinely performed at UCLA for MTLE will be carried out
- Subjects may elect not to have resection. The protocol now accommodates for this option, and any such subject will have ongoing follow-up and evaluation and can have Sx scheduled at any time

Major Comments Raised by RAC Members and Marc Dichter, M.D., Ph.D.

- Conflicts of Interest/Funding/Sponsor - Neurologix, Inc
- NSE vs. CBA promoter
- Lack of non-human primate safety data for specific construct/vector
- Hippocampal sclerosis alter gene expression/efficacy & need therefore to study models more reflective of HS
- Potential failure of rescue procedure by vector spread
- Rationale behind dose and two cohorts only
- Subjects undergoing Phase II atypical, and few eligible MTLE
- Potential for subject to ‘violate’ protocol by electing not to have resective surgery

Conflicts of Interest

- Neurologix, Inc. will sponsor and fund the trial
- Dr. During is a founder and consultant. He will have no involvement in patient recruitment, clinical care and management, or data collection
- Drs Fried and Stern have no relationship with Neurologix.

Lack of primate and specific construct safety/toxicity data

- Additional safety data will support the IND submission
- Rodents using high titer ~10^{13}/ml AAV-CBA-NPY
- Collaboration with Dr. Luiz Mello - Depto. de Fisiologi, Universidade Federal de Sao Paulo to use high titer virus in marmosets

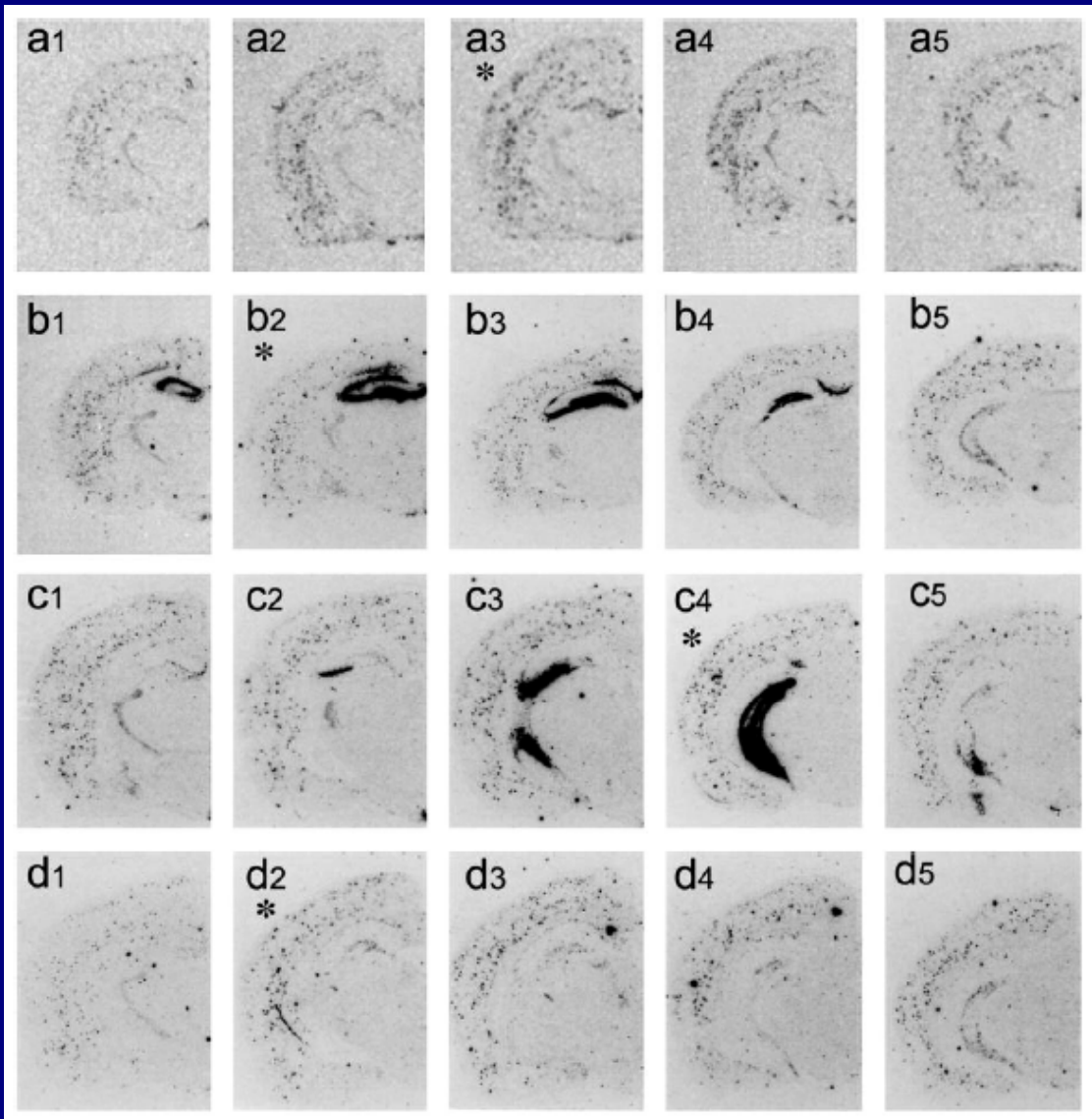
Hippocampal sclerosis may alter gene expression/efficacy & need therefore to study models more reflective of HS

- Previously published on AAV gene transfer into human tissue: Freese A, Kaplitt MG, O'Connor WM, Abbey M, Langer D, Leone P, O'Connor MJ, During MJ. Direct gene transfer into human epileptogenic hippocampal tissue with an adeno-associated virus vector: implications for a gene therapy approach to epilepsy. Epilepsia. 1997 Jul;38(7):759-66.
- Ongoing collaboration with Dr. Pitkanen - SSSE leading to spontaneous seizures with hippocampal neuronal loss and mossy fiber sprouting: Nissinen J, Halonen T, Koivisto E, Pitkanen A. A new model of chronic temporal lobe epilepsy induced by electrical stimulation of the amygdala in rat.Epilepsy Res. 2000 Feb;38(2-3):177-20.

Potential failure of rescue procedure by vector spread and dosing rationale

- Relative (bilateral) hippocampal volumes: 7718mm^3 in man vs. 80mm^3 in Wistar Rats. West MJ. Stereological studies of the hippocampus: a comparison of the hippocampal subdivisions of diverse species including hedgehogs, laboratory rodents, wild mice and men. *Prog Brain Res.* 1990;83:13-36.
- Hence, in our icv kainate and kindling experiments, AAV-NPY was injected into both a dorsal and ventral hippocampal site, 3ul(1-2x10^{12}/ml) each site for a total of ~10^{10} AAV genomic particles with no extrahippocampal expression. Dose equivalent to 600ul and ~10^{12} genomic particles in humans -leading to rationale for proposed clinical starting dose).
- Resection area includes adjacent cortical regions & amygdala - if not removal of all transduced cells, will be very small number remaining based on lack of spread in rat studies

Localized Transgene Expression



Subjects undergoing Phase II atypical, and few eligible MTLE

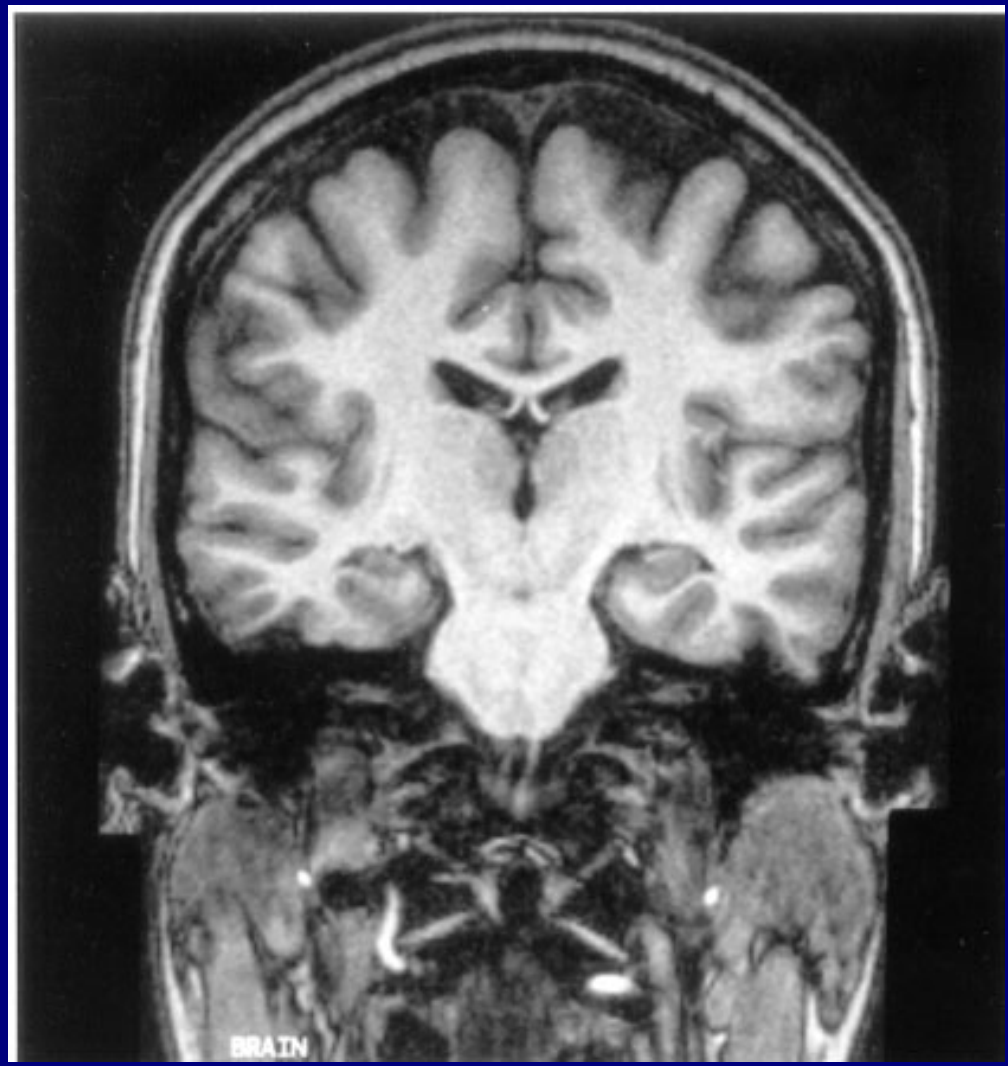
- Garden-variety MTLE typically do not need intracranial EEG because imaging and surface EEG definitive
- Phase II patients more complicated with a minority having MTLE with hippocampal sclerosis. Hence, subjects relatively rare and therefore may consider broadening eligibility criteria to include bilateral TLE subjects and those who fail the Wada test (ultimately perhaps “ideal” gene transfer candidates as resective surgery not always indicated)

Subjects electing to forgo temporal lobectomy a protocol violation?

- All subjects told of efficacy and limited adverse events associated with anteromedial temporal lobectomy and undergo risks of Phase II because of benefits associated with resective surgery
- Those that defer Sx likely to perceive significant benefit, and no major adverse events from the gene transfer, i.e. least likely to require “rescue”
- The built in rescue procedure is perhaps one of the most attractive components of this epilepsy study, but largely from a safety issue, expression analysis and any functional studies a bonus
- Subjects who no longer consent to resective Sx, not protocol violators, but will be followed prospectively by the investigators

International Research and Collaborators Acknowledgements

- Annamaria Vezzani - Mario Negri, Milan, IT
- En-Ju Lin - University of Auckland, NZ
- Herbert Herzog - Garvan Institute, Sydney
- Luiz Mello - São Paulo, Brazil
- Asla Pitkanen - Kupio, Finland
- Marcus Heilig - Stockholm, Sweden
- Ross Bland/Helen Fitzsimons - Neurologix, NY


BRAIN