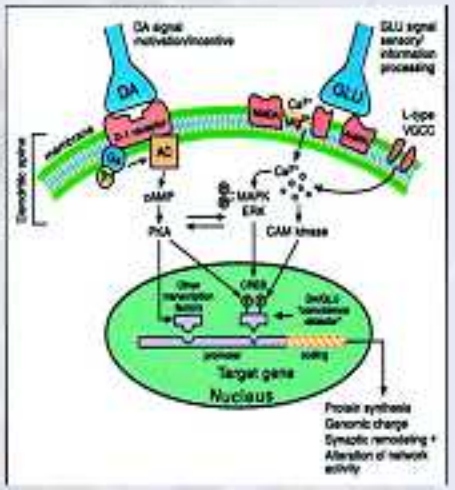
Examining Brain Reactivity Using Real-Time Quantitative PCR (qPCR)



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Outline of Talk

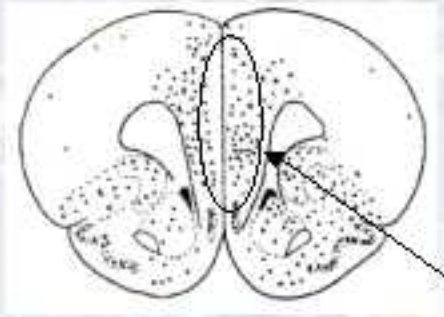
- 1. Measuring Brain Reactivity
- 2. Dealing with Variability

Drawbacks of IC/ISH Analysis

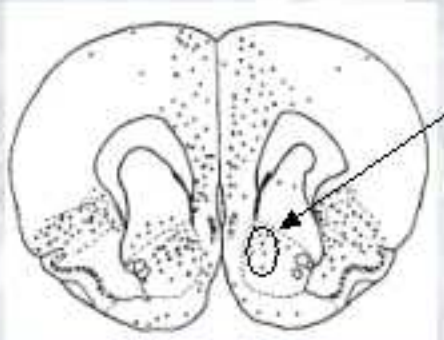
- Time-consuming
- Laborious
- Single Marker

Reactive Brain Areas and Stress: A Fos study

Stressed rat on Elevated Plus Maze (EPM)

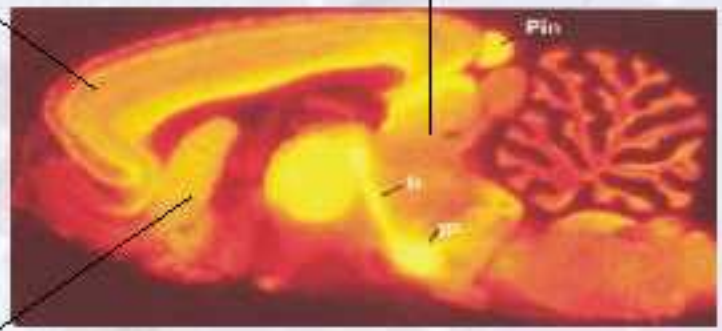


Prefrontal Cortex (PFC)



Nucleus Accumbens (NAC)

Other brain areas, other activity markers?



Duncan et al., 1996

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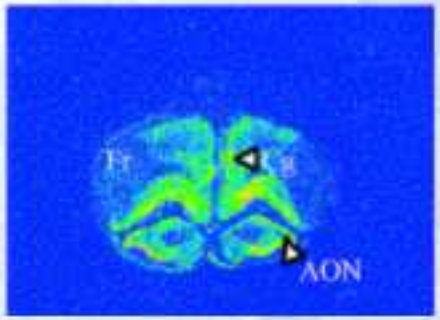
Detecting Neuronal Reactivity

- Rodents

  -*in situ* hybridization (ISH)
  (*c-fos)*
  -Immunocytochemistry (IC)
  (Fos, or activated kinases)

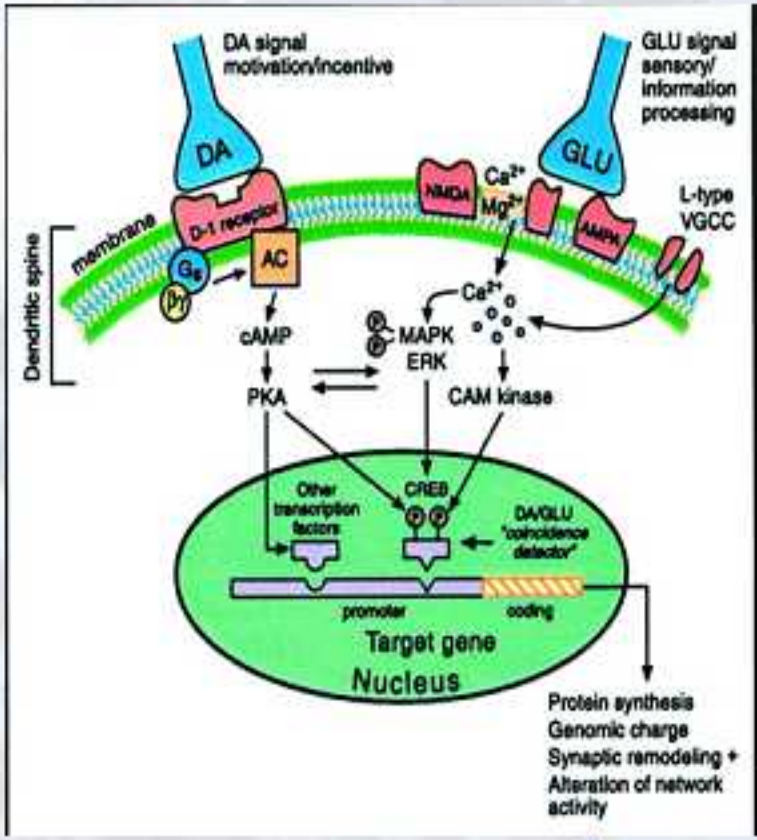


- Humans
  - PET imaging

Indices of Neuronal Reactivity

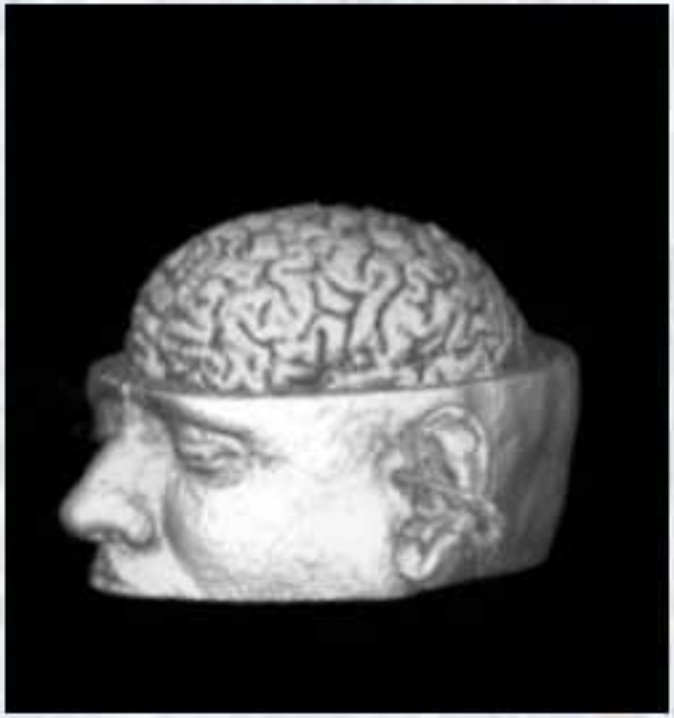
Changes in levels of:

- Glucose utilization
- Protein phosphorylation
- Activity Regulated Genes (ARG's)
- Protein synthesis



1. Central Questions in Neuroscience

Stimulus → (e.g. stress)



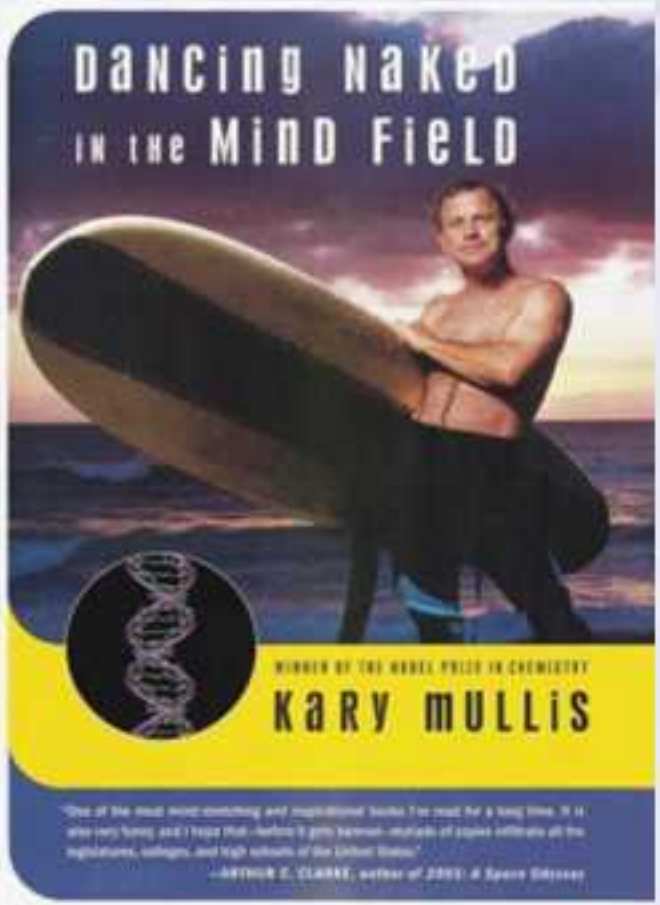
→ Reactivity:

- When?
- Where?
- How?

PCR: Born in the Brain

DNA chains coiled and floated. Lurid blue and pink images of electric molecules injected themselves somewhere between the mountain road and my eyes."-- Opening words,

Dancing Naked in the Mind Field, © 1998, by Dr. Kary Mullis, Pantheon Books.



Objective

Examine stress-related brain reactivity in more detail using qPCR by measuring *multiple ARG'S in several brain areas*

Advantages:

1)Fast

2)Increased sensitivity of detection

3)Allows simultaneous measurements of multiple ARG's

	ISH/IC	RT-qPCR
time	12weeks	6weeks
# of Markers	1	10 to 20
Spatial Res.	Yes	No

Examined ARG's

ARG	Induction Mechanism/Function
arc	synapse modification
BDNF	facilitation of synaptic transmissior
CRH	stress modulation
sgk	glucocorticoid administration
c-fos	transcriptional regulation
egr-1	transcriptional regulation
egr-2	transcriptional regulation
Nr4a3	transcriptional regulation , CRH

Concluding Remarks: Using qPCR in Neuroscience

- More ARGs: Increased chance of finding reactivity
- Unique ARG expression patterns suggestive of unique input
- Facilitates probe selection for ISH
- qPCR: High Resolution, Low variability
- Biological variability is gene specific
- <1.5 fold difficult to resolve

- ***qPCR: rapid, powerful tool to determine brain region reactivity!***

Points of Concern: Biological Variation

- Resolution between 1.1-1.4 fold difficult

- Stimulus should lead to robust changes (1.5 fold or more) to detect differences

Recommended Sample Size and Fold-Regulation

(30% CV for both exp., cont,
power=0.80, alpha=0.05, two-tailed test)

Fold Difference	# of Units (exp, cont)
1.25	33; 27
1.50	11; 7
1.75	7; 4
2.00	6; 3

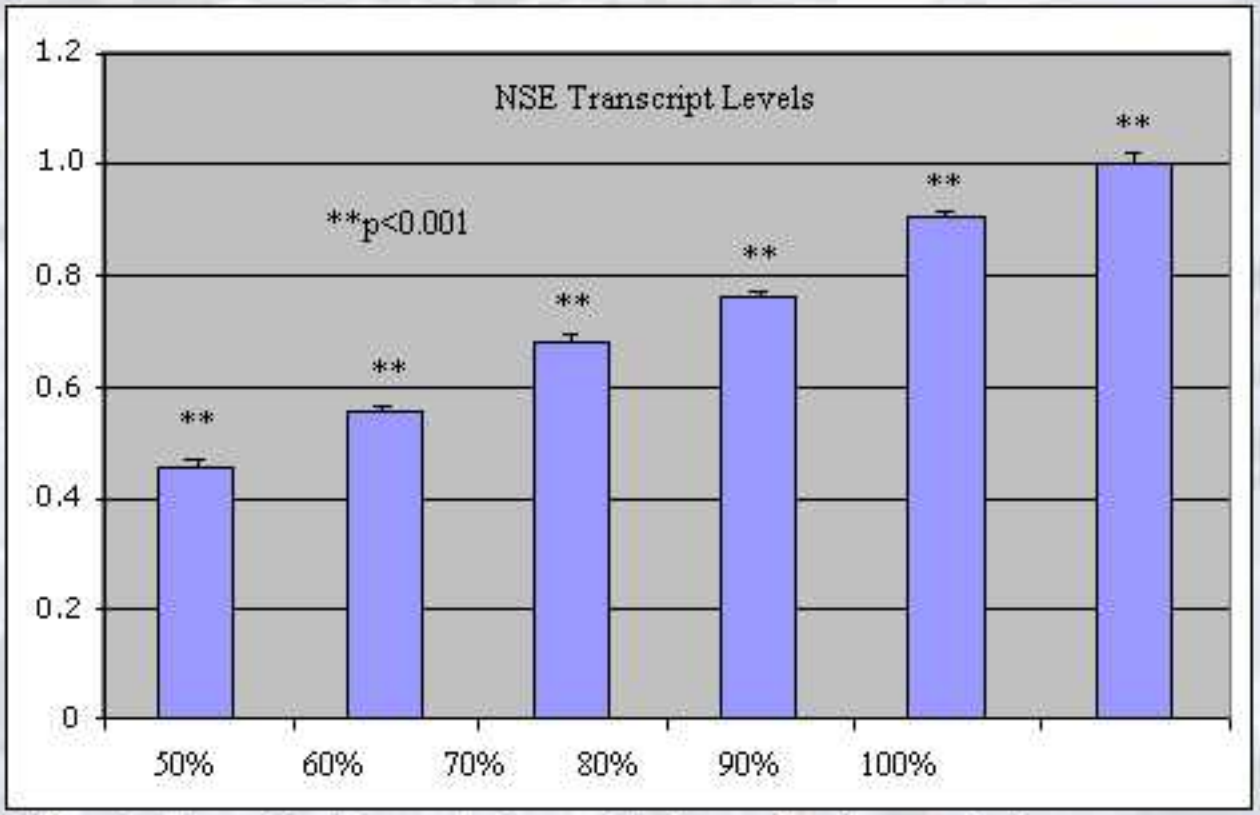
UCLA On-line Power Calculator
http://calculators.stat.ucla.edu/powercalc/

Stress Sensitive ARG's Have High Variability

Coefficient of Variation (CV) % from control group (n=12-14)

		mPFC	NAS	VTA
ARG's	arc	66	35	70
	BDNF	66	71	73
	crh	19	64	70
	SGK	70	16	53
	c-fos	83	54	63
	egr-1	23	22	70
	egr-2	46	64	n/a
	Nr4a3	40	33	50
House-keeping genes	B-actin	11	7	36
	NSE	6	5	12
	HPRT	7	6	14

qPCR: High Resolution, Low Variability



- CV(%) 8.5 5.5 4.2 2.3 3.1 4.3
- Resolution: 1.1fold (10%) (n=6 per diln)

Spijker et al., 2004 FASEB

2. Variety: The spice of life?

- 1. Technical
- 2. Biological

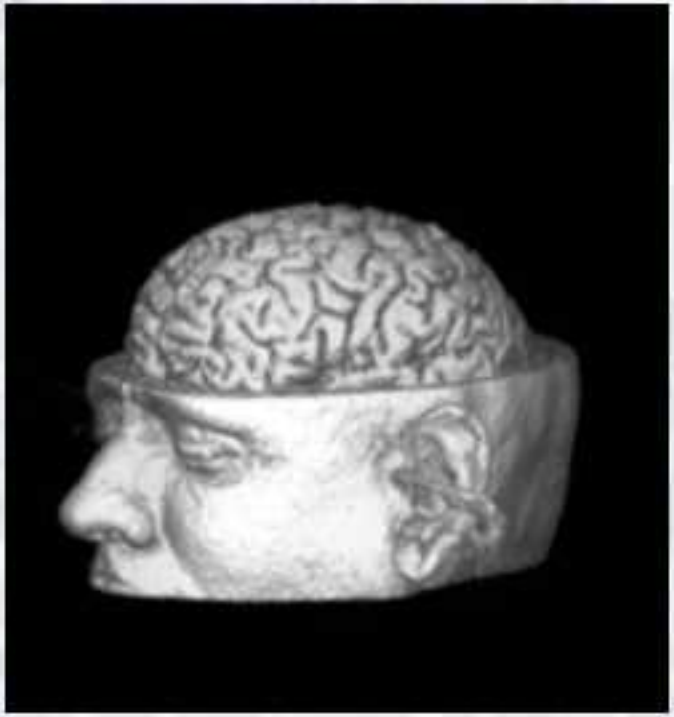
The Next Step

- Design ISH probe based on qPCR data

	PFC	NAC	VTA
arc	2.5***	1.4*	1.1
BDNF	1.1	2.5**	1.1
crh	1.2	2.5**	1.1
SGK	0.9	1.0	2.1*
c-fos	1.9***	1.4*	1.2
egr-1	1.6***	1.3*	1.2
egr-2	2.9***	2.3**	n/a
Nr4a3	1.0	2.2**	1.1

Central Questions in Neuroscience

Stimulus → (e.g. stress)



→ Reactivity:

- When?
- Where?
- ***How?***

Each Brain Region Has a Unique ARG Expression Profile

	PFC	NAC	VTA
arc	2.5***	1.4*	1.1
BDNF	1.1	2.5**	1.1
crh	1.2	2.5**	1.1
SGK	0.9	1.0	2.1*
c-fos	1.9***	1.4*	1.2
egr-1	1.6***	1.3*	1.2
egr-2	2.9***	2.3**	n/a
Nr4a3	1.0	2.2**	1.1

***p<0.001

** p<0.01

* p<0.05

N=12-14

(exp., cont.)

Normalization Procedure

For Transcript of interest (X):

- **Normalized amount=E^{-CtX}/(NF)**

  E= PCR Efficiency (~2copies/cycle)

  E^{-CtX}=Absolute Amount of transcript of interest

  NF =Normalization factor

  (geometric mean of multiple reference genes)

Vandesompele et al., 2002

My Time Point Advisor

“A lot can happen in 45 minutes!”

Experimental setup

Stressor
(Elevated plus maze)

45min.

Tissue Removal
(PFC, NAC, VTA)

↓

RNA isolation

↓

cDNA synthesis

↓

qPCR Analysis
(SYBR-Green)

Acknowledgments

Dept. of Medical Pharmacology
Vrije Universiteit Med. Center
ANM Schoffellmeer
TJ de Vries
JR Homberg

Dept. of Mol. And Cell. Neurobiology
Vrije Universiteit Amsterdam
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