Use of microarray to monitor gene expression profiles over time

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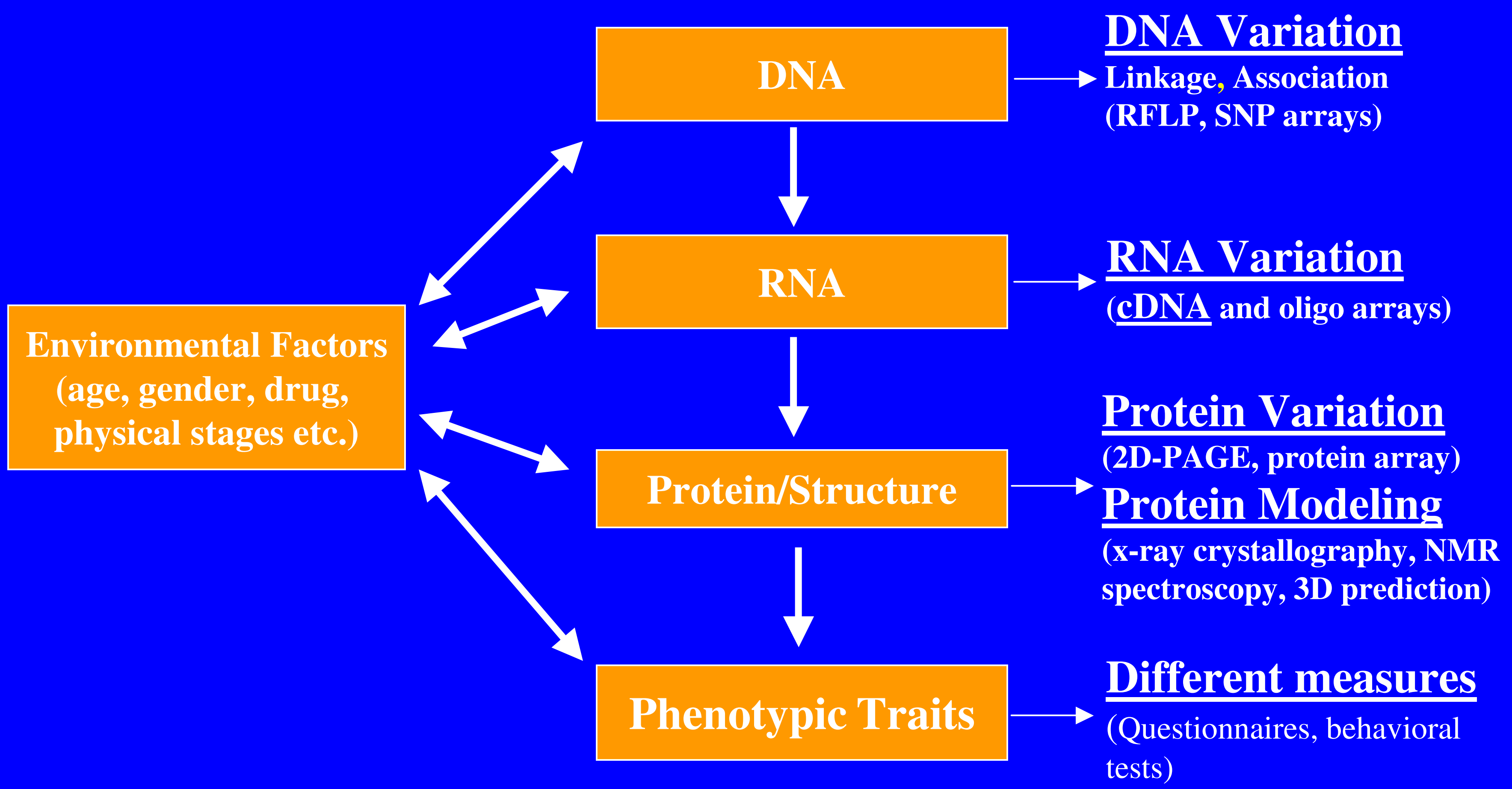
Relationship of genetics, environments, phenotype and their interactions at different regulatory levels



P-values for gene-ethnicity and gene-gender interaction of individual nicotinic receptor α4 subunit SNPs with three nicotine dependence measures *(Li et al. 2005)*

SNP ID	Pooled samples			African-American samples			European-American samples		
	SQ	HSI	FTND	SQ	HSI	FTND	SQ	HSI	FTND
rs2273505	0.38	0.73	0.69	0.71	0.82	0.85	0.087	0.27	0.37
rs2273504	0.49	0.60	0.56	0.25	0.092	0.074	0.094	0.064	0.029[r]
rs2229959	0.54	0.66	0.59	0.58	0.53	0.36	0.37	0.49	0.57
rs1044397	0.30	0.39	0.31	0.15	0.20	0.12	0.024[r] 0.025[d]	0.092	0.23
rs3787137	0.18	0.19	0.079	0.044[a]	0.081	0.041[a]	0.11	0.31	0.41
rs2236196	0.21	0.15	0.097	0.066	0.033[r] 0.034[d]	0.020[r] 0.022[d]	0.18	0.24	0.26

1). The model used for the heterogeneity test is:

Pooled samples: E (phenotype) = $\mu + \alpha$*marker + $\beta1$*age + $\beta2$*ethnicity + $\beta3$*gender + γ*ethnicity*marker;

Each ethnic sample: E (phenotype) = $\mu + \alpha$*marker + $\beta1$*age + $\beta2$*gender + γ*gender*marker.

2). SQ = smoking quantity per day; HSI = Heaviness of Smoking Index; FTND = Fagerstrom Test for Nicotine Dependence

Measurement of gene expression at RNA and protein levels

- **Traditionally**, we use Northern blotting and/or quantitative real-time RT-PCR for RNA or Western blotting for protein. However, these methods can only handle a limited number of genes.
- **Currently**, we use microarray (either cDNA or oligonucleotide arrays) to monitor RNA expression or 2-dimentional gel electrophoresis or protein array for protein. With these techniques, we can measure expression levels of thousands of genes at a time.

What can we learn from microarray analysis?

- **Determine how a specific treatment affects expression levels of multiple genes simultaneously**
  - over time
  - across different developmental stages or tissues

  (This is equivalent to study gene-environment interaction)
- Determine biochemical regulatory network or co-regulation of genes by an environmental factor

  (This is equivalent to study gene-gene interaction)

Transcriptional response to nicotine over different time in five rat brain regions *(Li et al. 2004)*

Rats → **Treated with <u>nicotine</u> or <u>saline</u> by *ip* injection for <u>3</u>, <u>7</u>, or <u>14</u> days** →

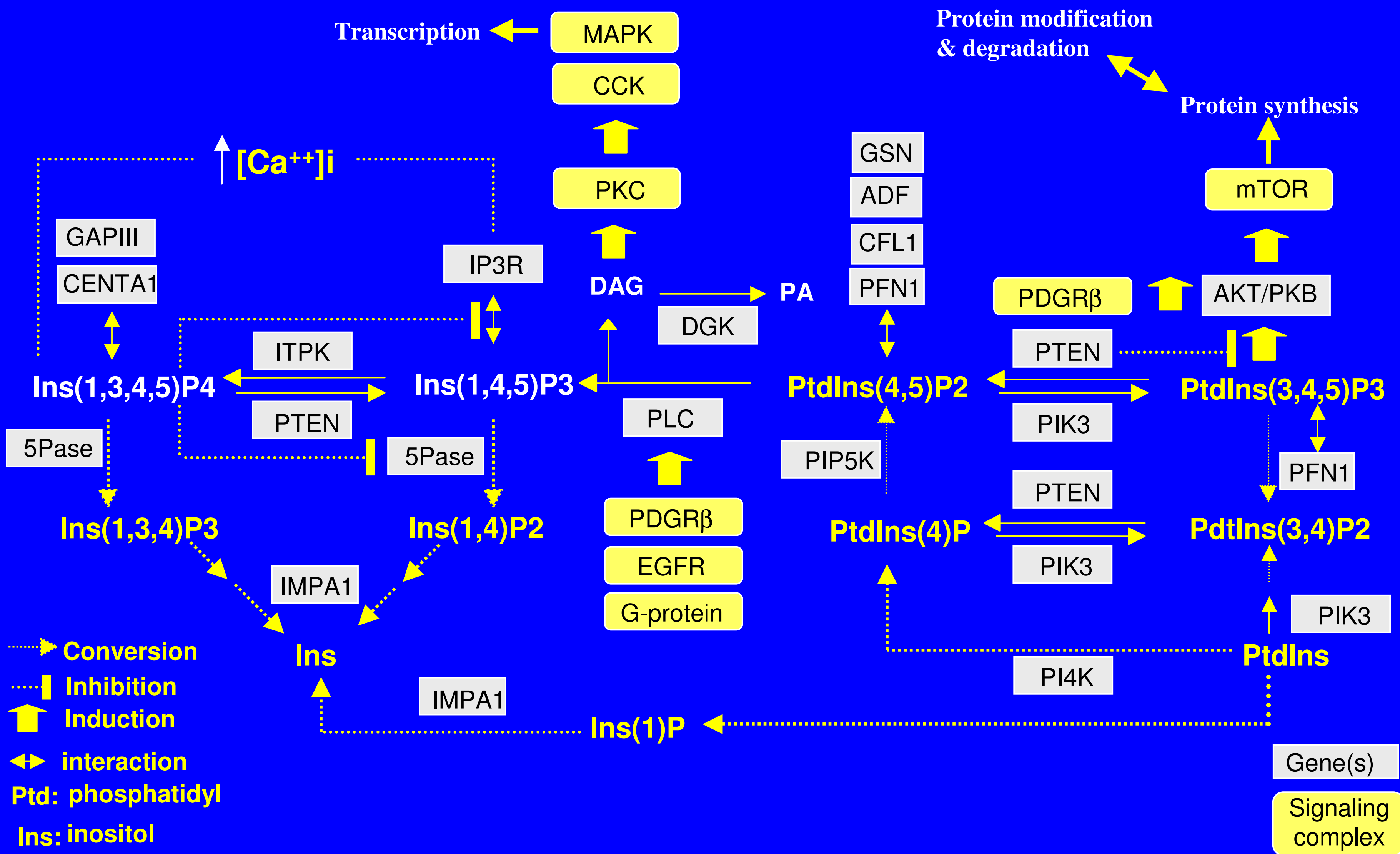
<u>Collected Brain Regions:</u>
Amygdala (Amyg)
Medial Basal Hypothalamus (MBH)
Prefrontal Cortex (PFC)
Striatum (ST)
Ventral tegmental Area (VTA)

5-7 animals per experimental group
5 regions collected from each animal
6 experimental groups
Total: >150 RNA samples

General categorization of the pathway-focused microarray, with examples given for each category *(Konu et al. 2004)*

Functional Category	Example	Number
Signal transduction	cAMP, calcium, phosphatidylinositol, growth factors/receptors, MAPK	244
Protein modification and degradation	ubiquitin/proteasome, heat-shock proteins and chaperonins	78
Transcription factors	general transcription factor IIH, transcription elongation factors	56
Transporters	ion channels and small molecule transporters	55
Protein synthesis	ribosomal protein subunits	51
Cell division	cyclins, histones, histone deacetylases	47
Neuronal structure and transmission	neuron enriched proteins, neurotransmitters and receptors	38
Cell structure	laminins, tubulins	23
Metabolism	cytochrome p450	22
Other	Miscellaneous function	24
Total		638

Overview of the phosphatidylinositol (PI) signaling based on pathway-focused cDNA microarray *(Konu et al. 2004)*



Summary of number of genes that were up- or down-regulated by nicotine in 5 rat brain regions across three 3 points of treatment (5% FPR)

Day	Direction	Amyg	MBH	PFC	ST	VTA	Total
D3	Up	77	4	0	8	32	121
	Down	5	39	9	68	35	156
D7	Up	50	69	17	81	3	220
	Down	9	38	15	97	34	193
D14	Up	15	82	24	17	5	143
	Down	16	87	23	7	0	133
Total		172	319	88	278	109	966

FPR: False positive rate

Number of differentially expressed genes co-modulated by nicotine over time within each brain region at 5% FDR

Brain Region	Day 3	Day 7	Day 14	Days 3 & 7	Days 3 & 14	Days 7 & 14	Days 3, 7 & 14
Amygdala	67	47	16	7	10	7	2
Medial Basal Hypothalamus	25	40	99	11	14	63	7
Prefrontal Cortex	8	25	41	1	0	6	0
Striatium	48	144	16	28	2	8	2
Ventral Tegmental Area	60	33	2	4	3	0	0

Summary of number of genes that were modulated by nicotine in at least 1 rat brain regions across 3 time points (1% FPR)

Day	1 region	2 regions	3 regions	4 regions
Day 3	175	40	2	0
Day 7	275	117	42	4
Day 14	188	60	4	0

A partial list of genes that were altered in at least two brain regions after 3 days of nicotine treatment

Symbol	Gene Name and function	Amyg	MBH	PFC	ST	VTA
Rab2	Rat ras-related protein mRNA, clone NTRAB2R	55.6 ↑	-38.4 ↓	-5.4	-28.9 ↓	7
Rplp1	ribosomal protein, large, P1	54.0 ↑	-6.3	-34.0 ↓	-31.4 ↓	-14.4
Sap30	cyclin-dependent kinase 2	39.6 ↑	-4.2	6.7	23.2	-23.2 ↓
Jun	Jun oncogene	85.9 ↑	-41.2 ↓	29.6	1.9	-8.2
Borg3-pending	Cdc42 effector protein 5	30.6 ↑	-24.6 ↓	13.7	2.4	-21.3
Nckap1	NCK-associated protein 1	26.4 ↑	11.9	2.8	-26.0 ↓	-22.7
M6pr	mannose-6-phosphate receptor, cation dependent	27.0 ↑	-32.7 ↓	-7.8	0.7	-10.8
Gabrd	GABA-A receptor, subunit delta	1.7	-23.4 ↓	17.2	-26.9 ↓	-27.6
Lrp4	Rattus norvegicus mRNA for MEGF7	30.5	-3.1	12.6	-29.7 ↓	-25.5 ↓
Ptph6	Rat protein-tyrosine phosphatase (SHP-1) mRNA	32.4 ↑	-15	19.1	11.9	-25.7 ↓
Il1b	Rat interleukin 1-beta mRNA, complete cds	-2.3	-26.9 ↓	10.7	-15.1	-23.7 ↓
Pomc	Rat proopiomelanocortin (POMC) gene	2.7	-24.6 ↓	43.6	4.2	-44.6 ↓
Cnr1	Cannabinoid receptor 1	30.2 ↑	5.9	-15	-30.6 ↓	27.4
Kcnc1	Potassium channel gene 1	28.7 ↑	18.4	10.7	1.8	38.9 ↑
Ppp3ca	Calcineurin subunit A alpha	13.1	196.7↑	6.8	-53.6 ↓	-3.1
Tuba1	Rat mRNA encoding alpha-tubulin	12.9	-24.1 ↓	37.9	-13.2	40.4 ↑
Gabarapl2	Rat GABA(A) receptor-associated protein like 2 (Gabarapl2)	27.4 ↑	10.6	17.7	-22.9 ↓	4.6
Bid3	Rat brain mRNA for neuronal death protein	35.0 ↑	-1.1	2.7	-39.3 ↓	-3.4
Atp1b1	ATPase Na+/K+ transporting beta 1 polypeptide	84.7 ↑	101.0 ↑	37.1	-26.1	11.9
Fez1	Rat synaptotagmin binding zyginl mRNA	32.0 ↑	21.5	8.1	-29.2 ↓	-2.5
Adra1d	Rat alpha-1A-adrenergic receptor mRNA	-35.2 ↓	1.3	12.7	-21.1	-25.5 ↓
Map2k1	Rat MAP kinase kinase mRNA	31.2 ↑	1.1	-25.4	-35.0 ↓	-19.1

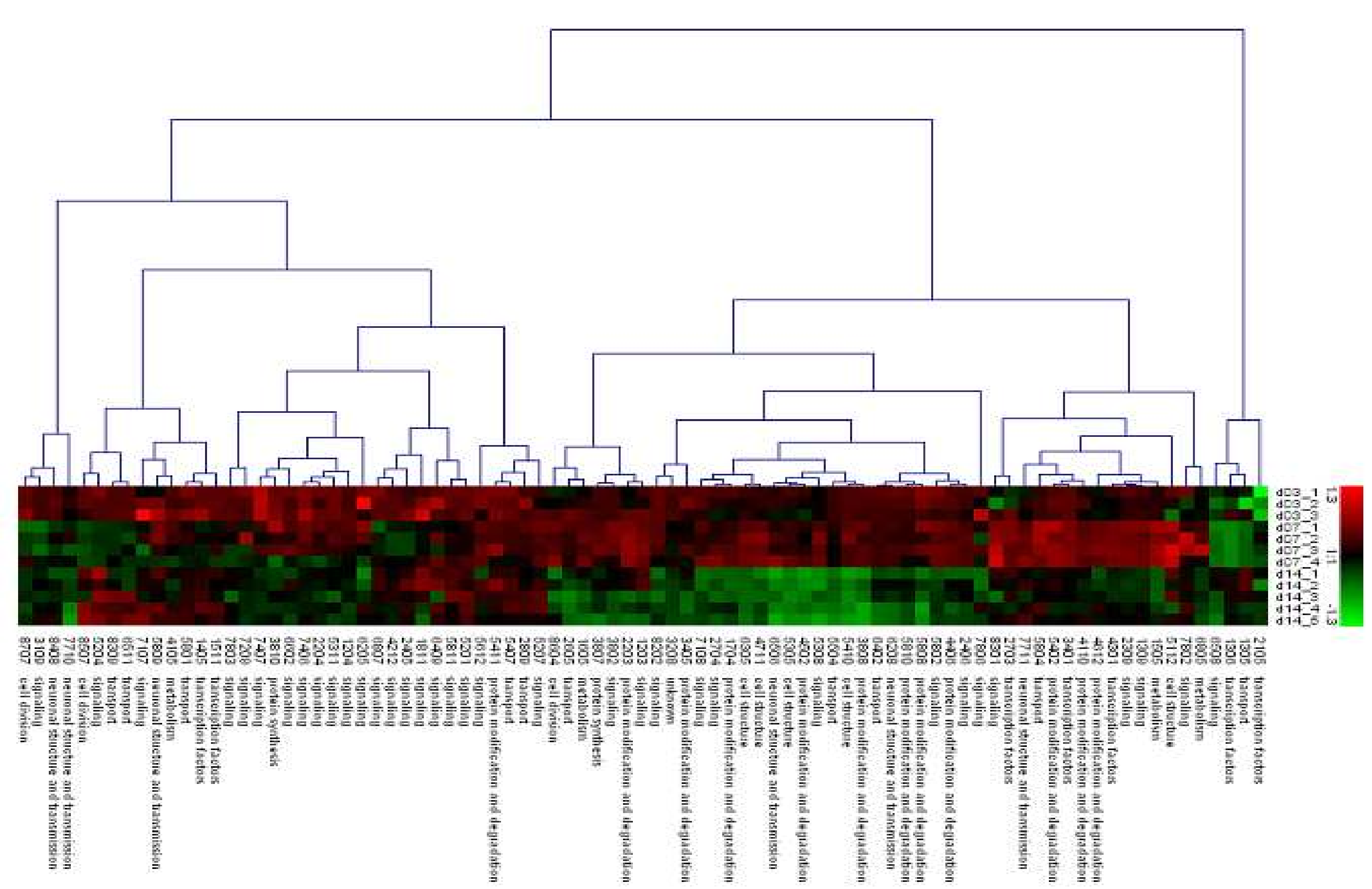
A partial list of genes that were altered in at least two brain regions after 7 days of nicotine treatment

Symbol	Gene name and function	Amyg	MBH	PFC	ST	VTA
App	amyloid beta (A4) precursor protein	43.3 ↑	43.0 ↑	-13.9 ↓	-27.0 ↓	17.4
Ubc	ubiquitin C	27.9 ↑	48.9 ↑	-14.2 ↓	-43.8 ↓	13.4
Gapd	Mus musculus glyceraldehyde-3-phosphate dehydrogenase (Gapd), mRNA	62.9 ↑	41.1 ↑	-1.4	-35.5 ↓	99.0 ↑
calmodulin 2	calmodulin 2	40.2 ↑	27.2 ↑	-37.6 ↓	-34.0 ↓	-15.6
Tnk2	Cdc42 GTPase-inhibiting protein	12.4	30.5 ↑	19.4 ↑	-71.9 ↓	21.9
Ccnb2	cyclin B2	-11.6	-21.4 ↓	6.0	128.1 ↑	-35.4 ↓
Ccnb1-rs1	cyclin B1, related sequence 1	-15.7	-21.2 ↓	9.3	158.2 ↑	-25.0 ↓
Ppp1cc	Rattus norvegicus Protein phosphatase 1, catalytic subunit, gamma isoform 1	73.4 ↑	36.8 ↑	4.1	-30.5 ↓	32.1
Melk	Mus musculus maternal embryonic leucine zipper kinase (Melk), mRNA	-5.8	-45.4 ↓	43.7 ↑	-70.0 ↓	5.0
Calcrl	Calcitonin receptor-like receptor	0.5	-32.0 ↓	-2.1	49.9 ↑	-64.5 ↓
Eef1a1	eukaryotic translation elongation factor 1 alpha 2	24.0 ↑	38.5 ↑	-6.6	-41.0 ↓	12.9
Prkcb1	Protein kinase C beta	42.3 ↑	32.1 ↑	-15.7	-24.1 ↓	15.6
A2a	R.norvegicus nonerythroid alpha-spectrin gene, 3'' end	15.1	31.9 ↑	-18.2 ↓	-30.1 ↓	12.9
Gabt1	Rat GABA transporter protein mRNA, complete cds	18.3 ↑	23.7 ↑	-2.2	-24.0 ↓	13.7
Tuba1	Rat mRNA encoding alpha-tubulin	37.6 ↑	31.4 ↑	-4.7	-45.1 ↓	27.3
Gabarapl2	Rattus norvegicus GABA(A) receptor-associated protein like 2 (Gabarapl2)	19.9 ↑	39.4 ↑	-19.0	-22.3 ↓	15.0
Atp2b2	ATPase isoform 2, Na+K+ transporting, beta polypeptide 2	136.9 ↑	32.5 ↑	34.4	-29.8 ↓	40.1
Atp1b1	ATPase Na+/K+ transporting beta 1 polypeptide	19.3	48.2 ↑	-16.8 ↓	-35.7 ↓	1.4
Fez1	Rattus norvegicus synaptotagmin binding zyginI mRNA, complete cds	37.7 ↑	-2.2	-17.0 ↓	-27.8 ↓	4.1
Map2k1	Rattus norvegicus MAP kinase kinase mRNA, complete cds	31.2 ↑	10.0	-13.9 ↓	-22.5 ↓	8.9

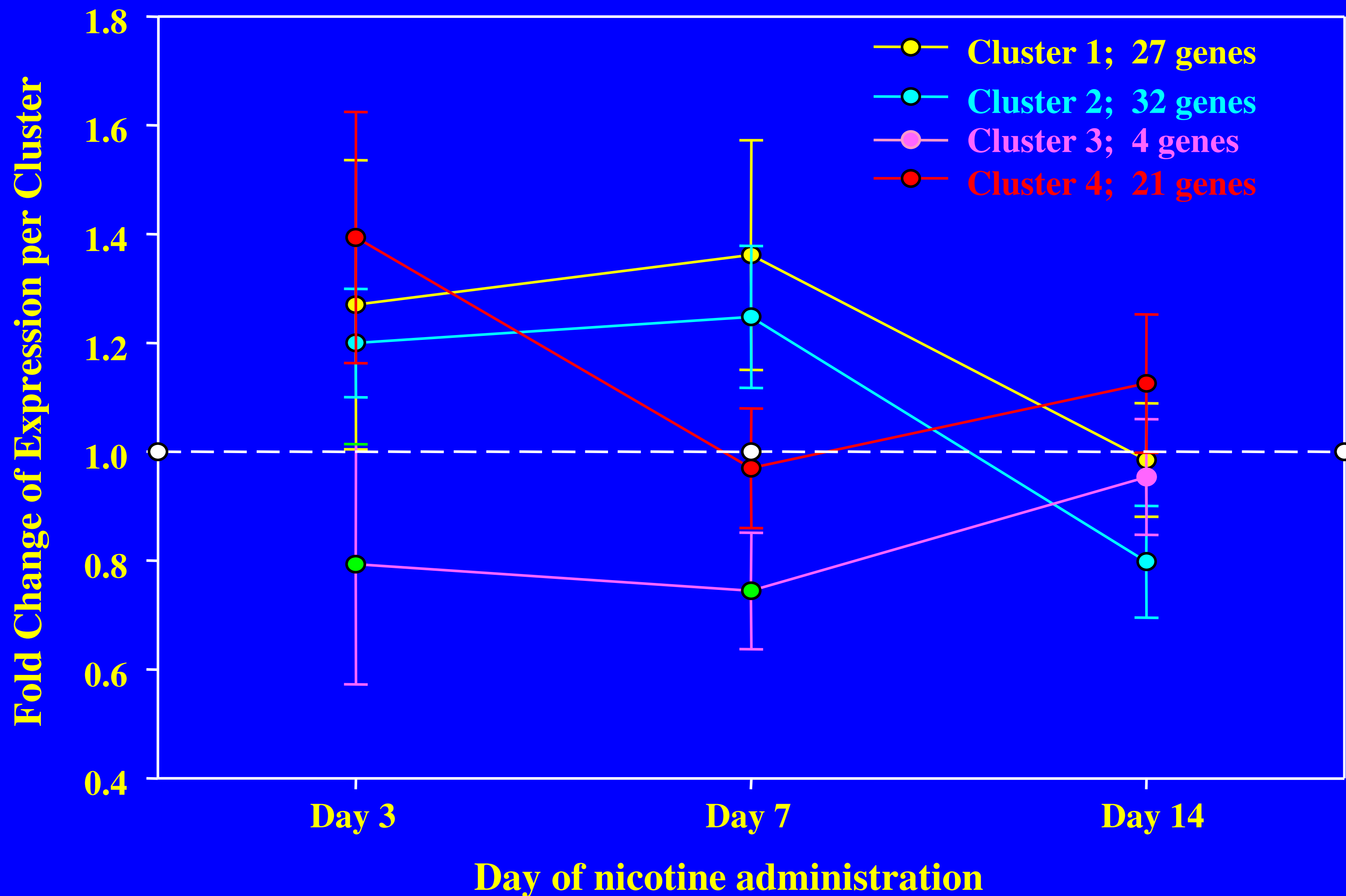
A partial list of genes that were altered in at least two brain regions after 14 days of nicotine treatment

Symbol	Gene name and function	Amyg	MBH	PFC	ST	VTA
Ap2m1	Rat assembly protein (AP50) associated with clathrin-coated vesicles mRNA,	-29.6 ↓	-29.4 ↓	47.3 ↑	12.5	34.2
Gnas	Guanine nucleotide-binding protein G-s, alpha subunit, Genbank no U51565	-34.9 ↓	-35.5 ↓	-12.3	20.2 ↑	-83.9
Rpl41	ribosomal protein L41	-9.4	-28.8 ↓	-39.7 ↓	20.2 ↑	12.3
Csnk2a2	casein kinase II, alpha 2, polypeptide	-21.9	-36.8 ↓	65.7 ↑	-0.4	80.8 ↑
Rbbp7	retinoblastoma binding protein 7	-12.2	-17.2 ↓	55.0 ↑	-13.2	-6.7
Chek1	checkpoint kinase 1 homolog (S. pombe)	28.0	26.8 ↑	-31.8 ↓	12.9	1.6
Cdc25c	cell division cycle 25 homolog C (S. cerevisiae)	13.0	27.6 ↑	-3.9	33.3 ↑	11.3
Ccnh	Mus musculus cyclin H (Ccnh) mRNA	19.3	25.8 ↑	66.2 ↑	-1.4	-10.9
Hap1	Rattus norvegicus huntingtin associated protein (rHAP1-B) mRNA	-25.8 ↓	-36.2 ↓	19.2	8.4	16.5
Gria2	glutamate receptor, ionotropic, AMPA2 (alpha 2)	9.5	27.5 ↑	-34.8	-27.4 ↓	30.8
Ptprj	Protein tyrosine phosphatase, receptor type, J	5.5	31.9 ↑	60.0 ↑	9.4	7.1
Bmp6	Bone morphogenetic protein 6	-3.8	-13.2 ↓	49.0 ↑	11.4	10.9
Rb1	Retinoblastoma 1 (including osteosarcoma)	8.0	16.5 ↑	-17.9	17.8 ↑	1.2
Capon	Rattus norvegicus carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase	-0.3	16.9 ↑	-0.4	10.6 ↑	13.5
Cdh2	Rattus norvegicus mRNA for N-cadherin	39.9	255.1 ↑	-3.5	90.5 ↑	-0.6
Itgp	integrin-associated protein	0.6	103.2 ↑	6.0	267.1 ↑	7.2
Sod2	Superoxide dimutase 2, mitochondrial	-15.0	16.6 ↑	-21.7	-20.0 ↓	-1.6
Slc18a2	Solute carrier family 18 A2 (vesicular monoamine transporter 2)	67.2 ↑	3.6	-23.0	21.0 ↑	12.5
Kcnc1	Potassium channel gene 1	11.6	122.5 ↑	-11.7	-37.0	159.6 ↑
Eno2	Rattus norvegicus neuron-specific enolase (NSE) mRNA	-36.6 ↓	-34.2 ↓	22.6	9.3	28.2
Prlpa	Prolactin-like protein A	1.4	14.2 ↑	5.7	15.9 ↑	-5.0
Tuba1	Rat mRNA encoding alpha-tubulin	-27.4 ↓	-44.3 ↓	-2.7	-6.2	13.1

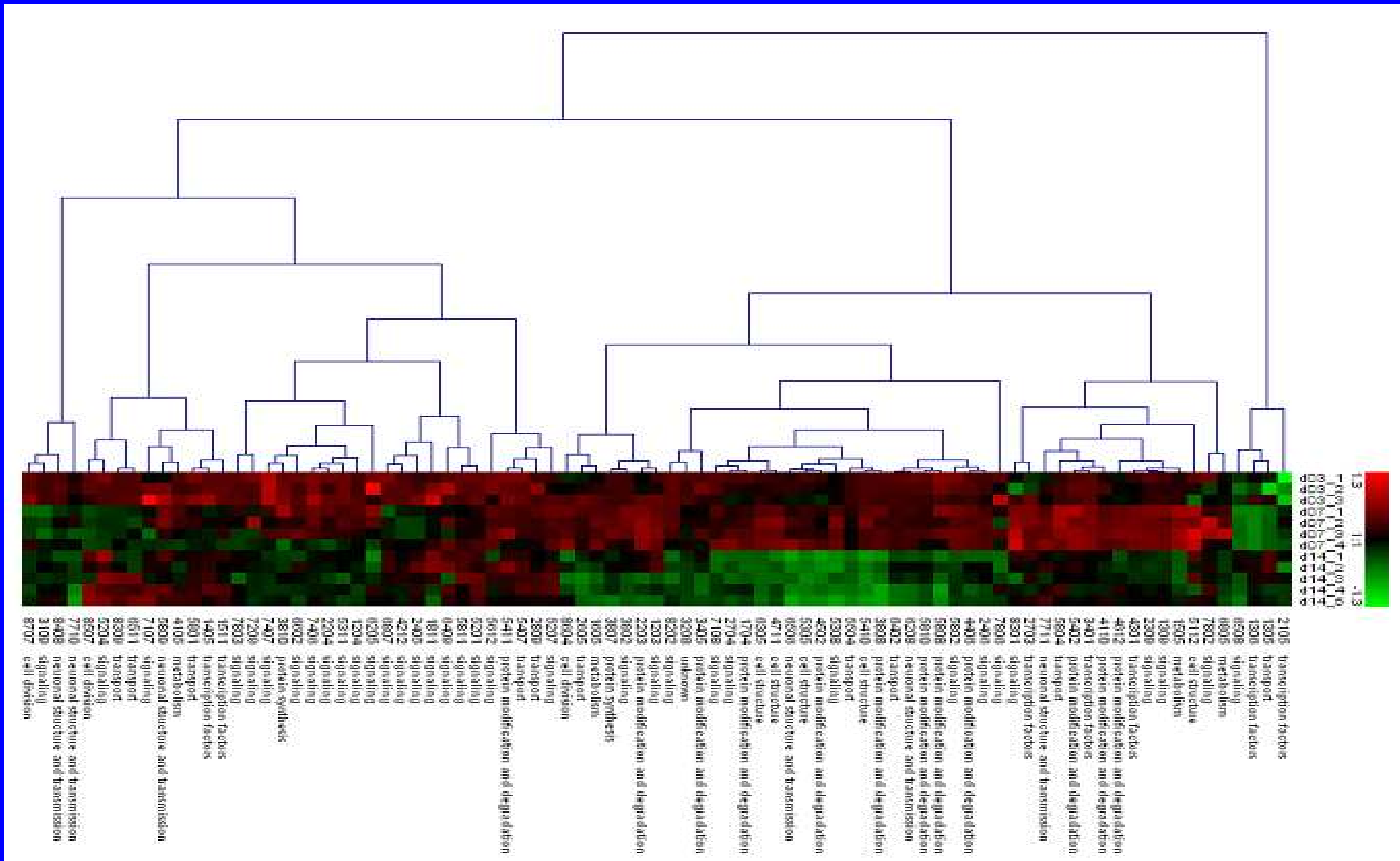
Hierarchical clustering of differentially expressed genes in the amygdala over three time points



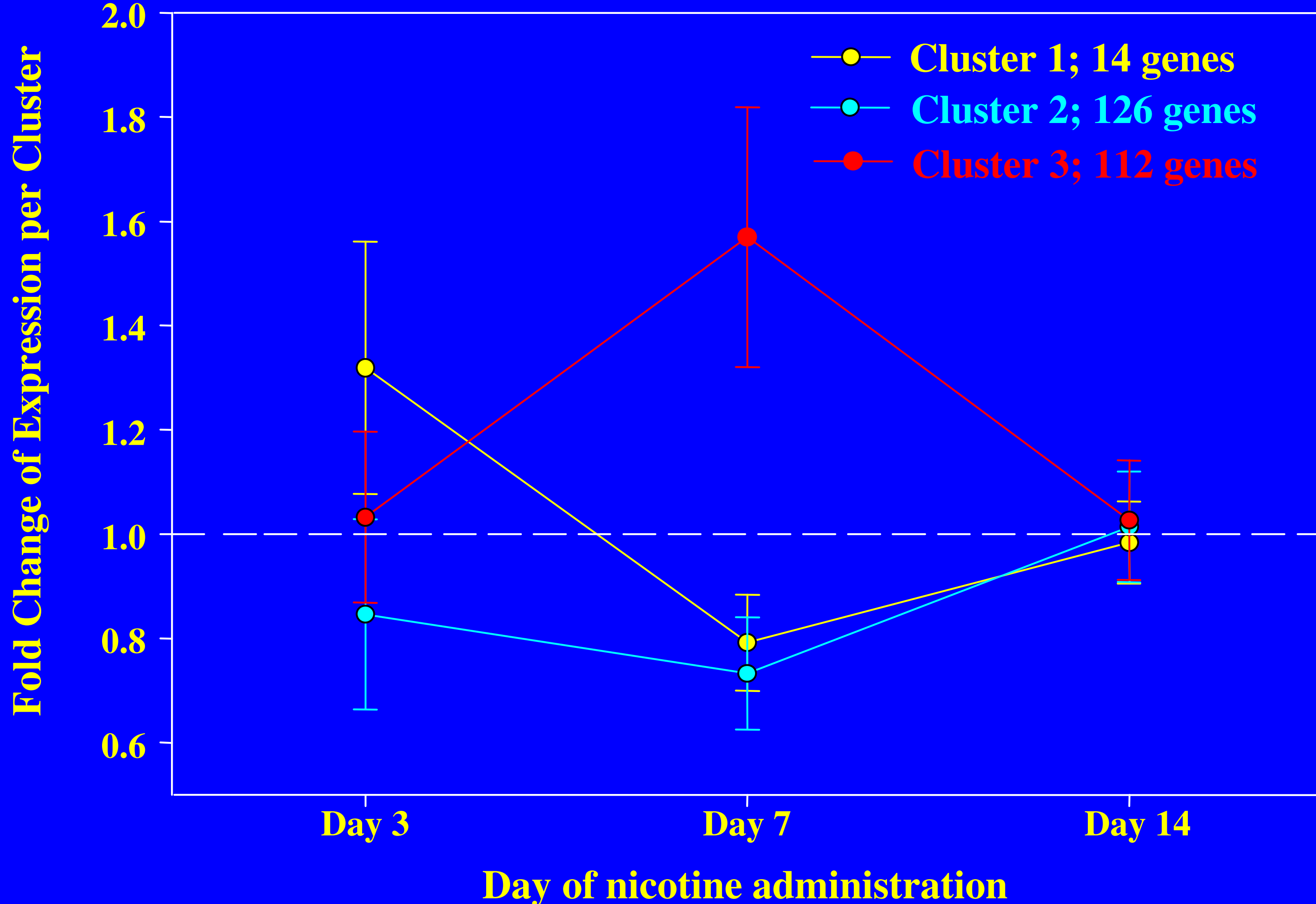
Comparison of average expression levels among clusters in the amygdala over three time points



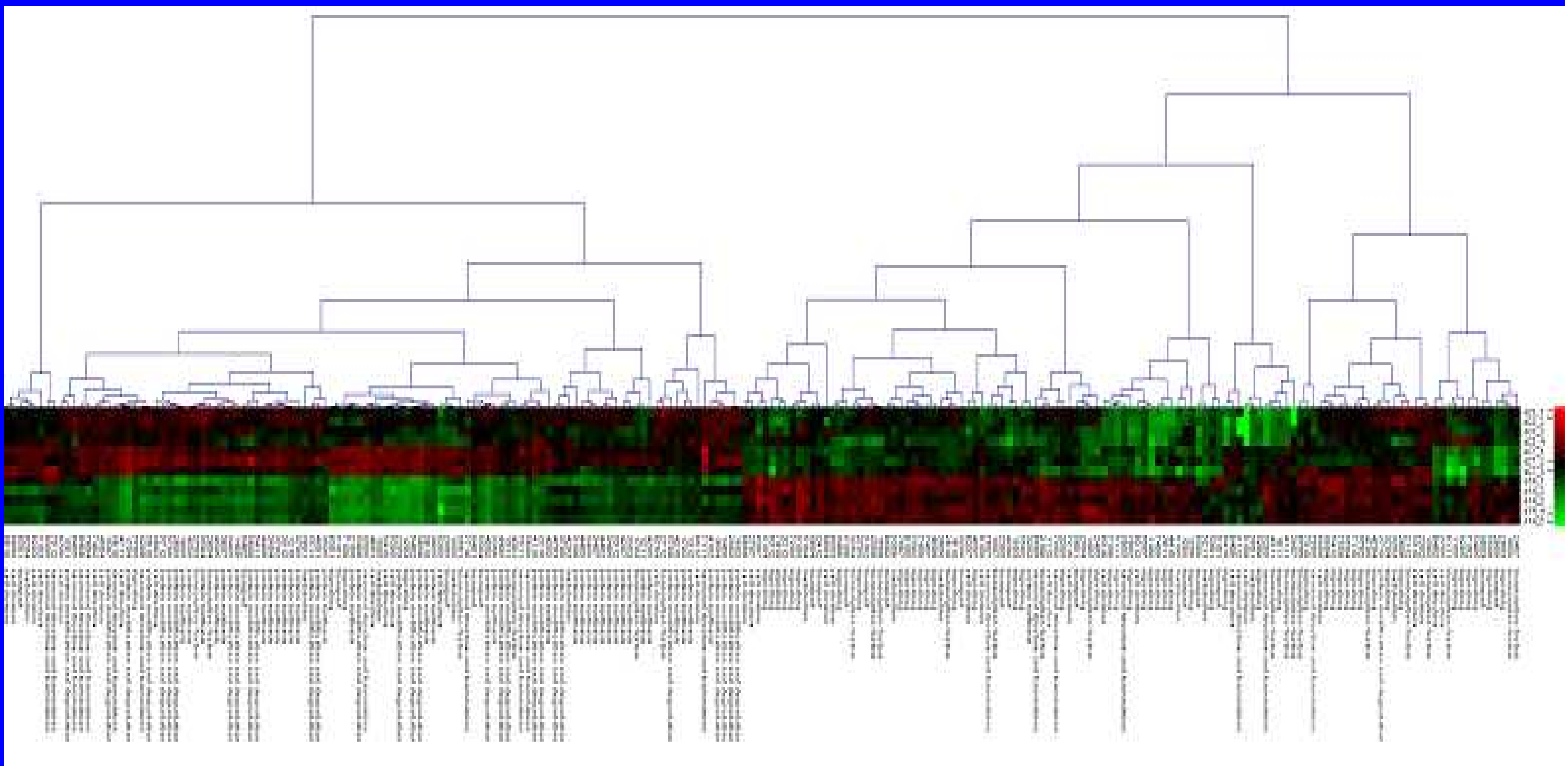
Hierarchical clustering of differentially expressed genes in the striatum over three time points



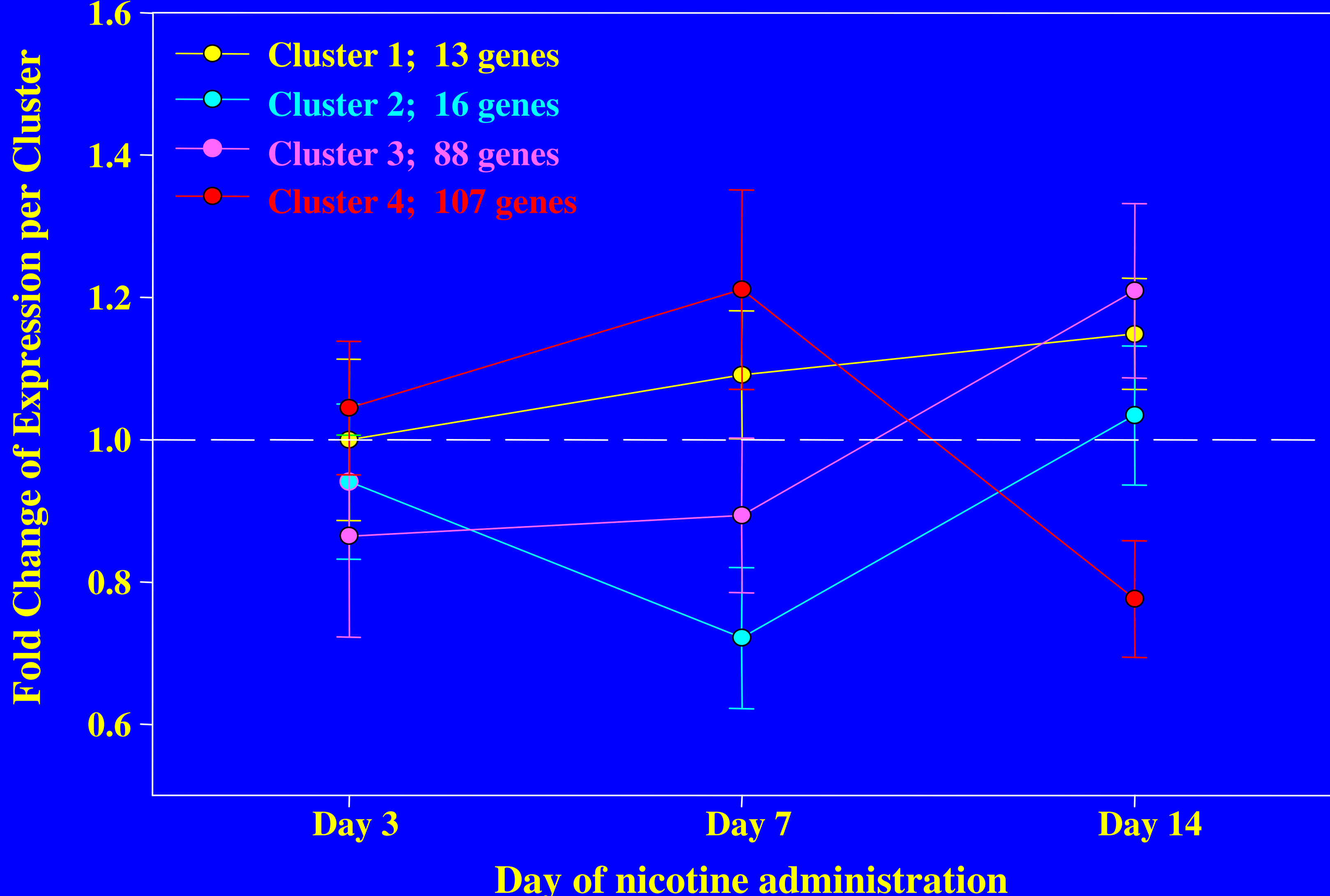
Comparison of average expression levels among clusters in the striatum over three time points



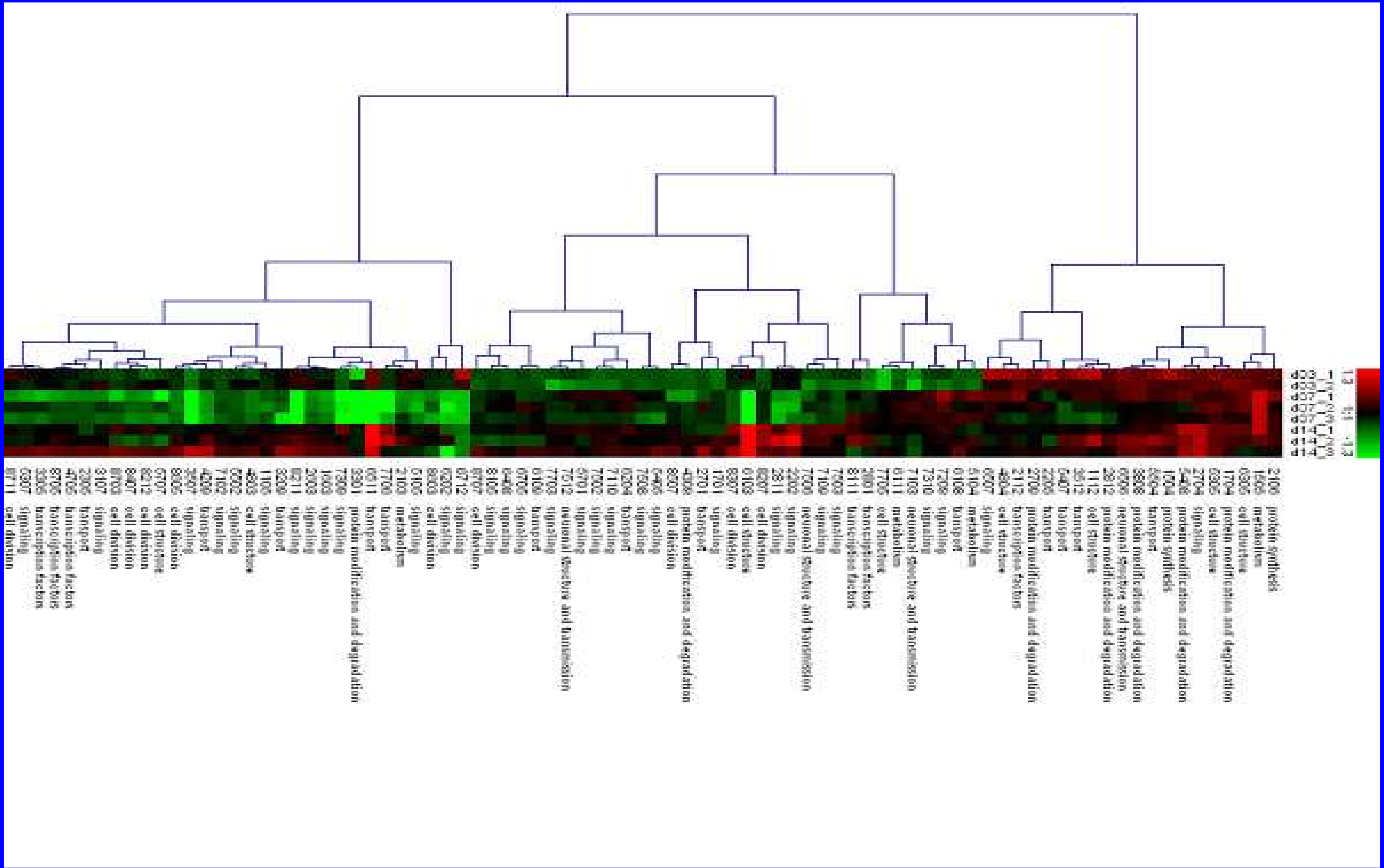
Hierarchical clustering of differentially expressed genes in the hypothalamus over three time points



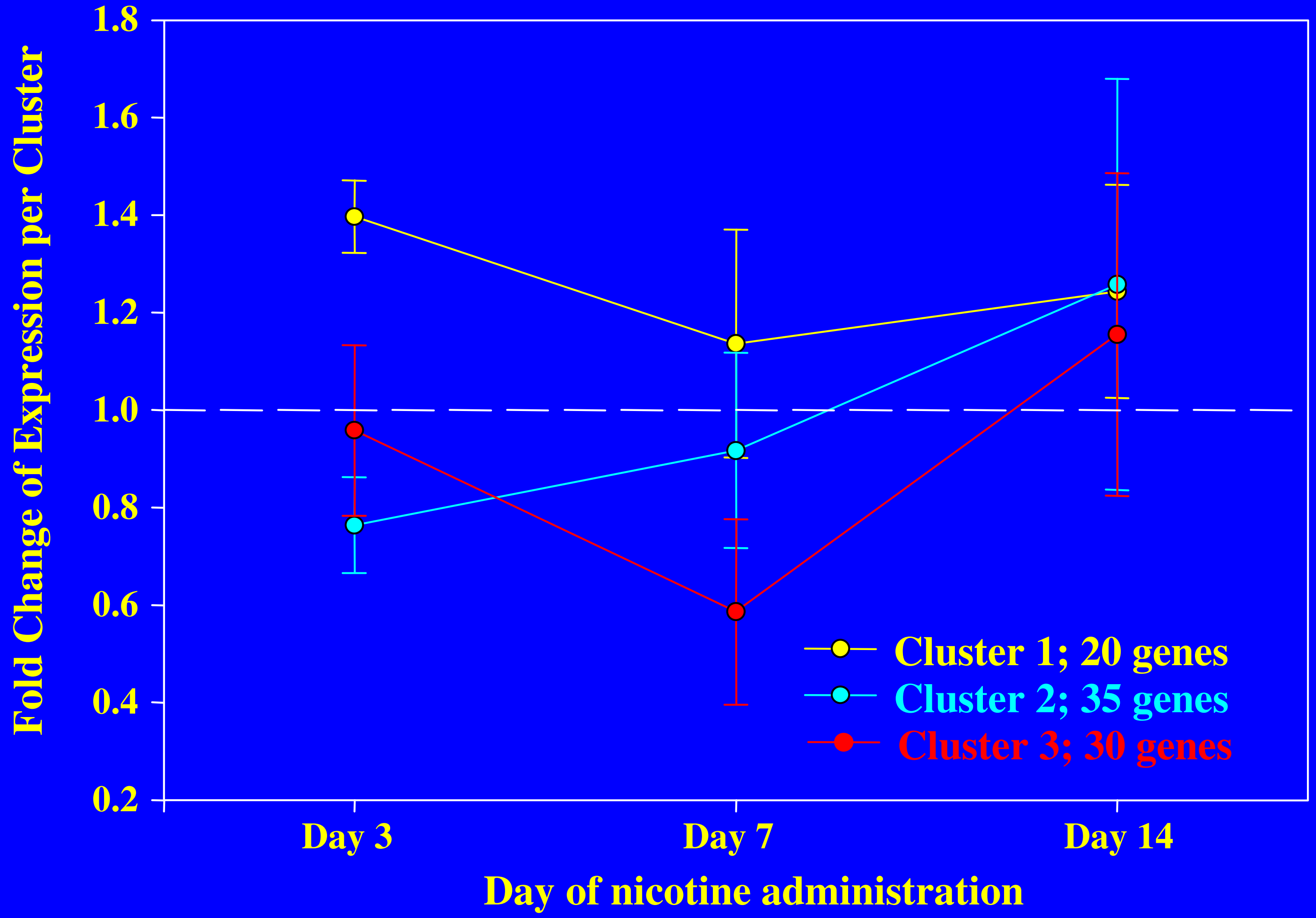
Comparison of average expression levels among clusters in the hypothalamus over three time points



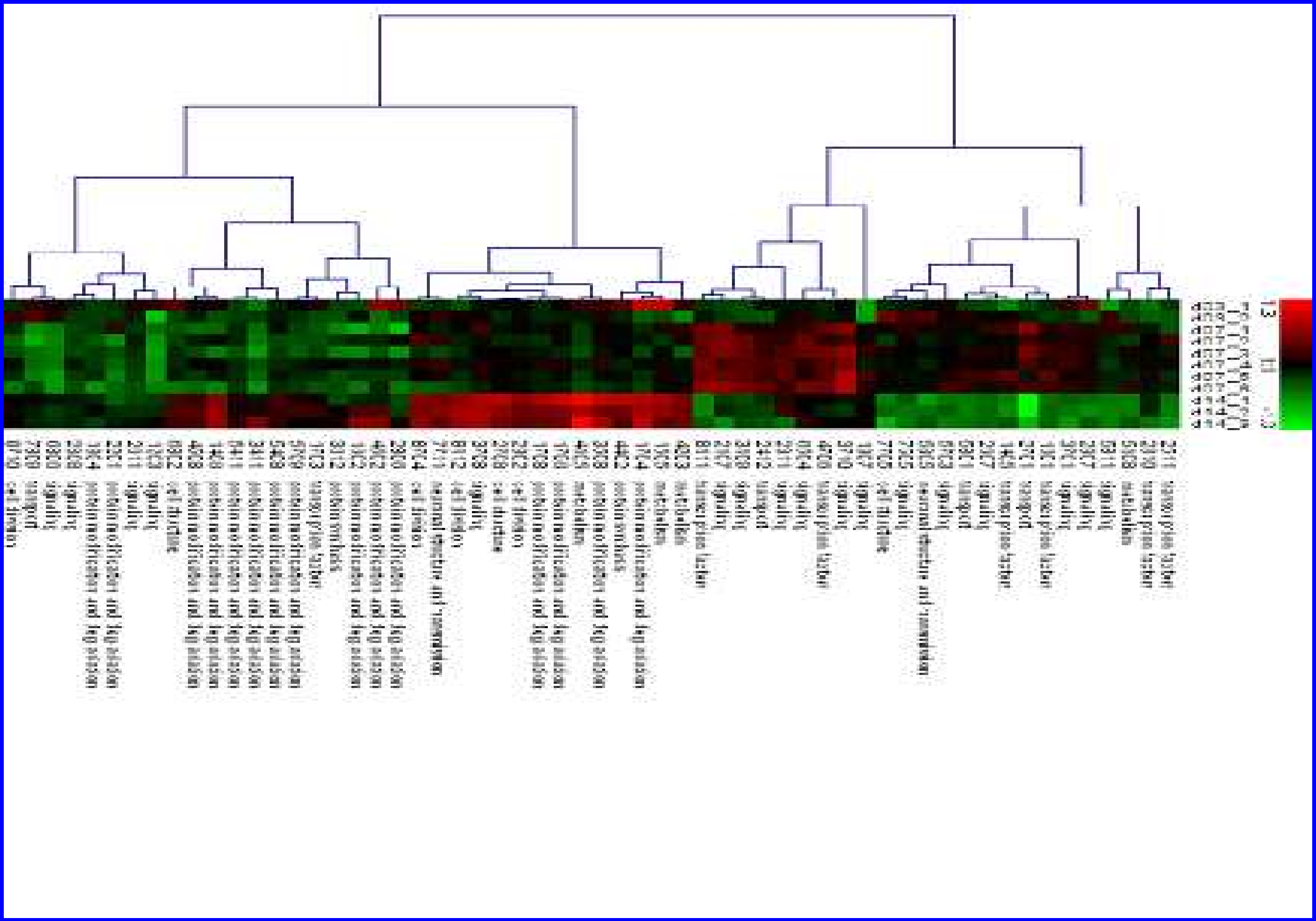
Hierarchical clustering of differentially expressed genes in the <u>ventral tegmental area</u> over three time points



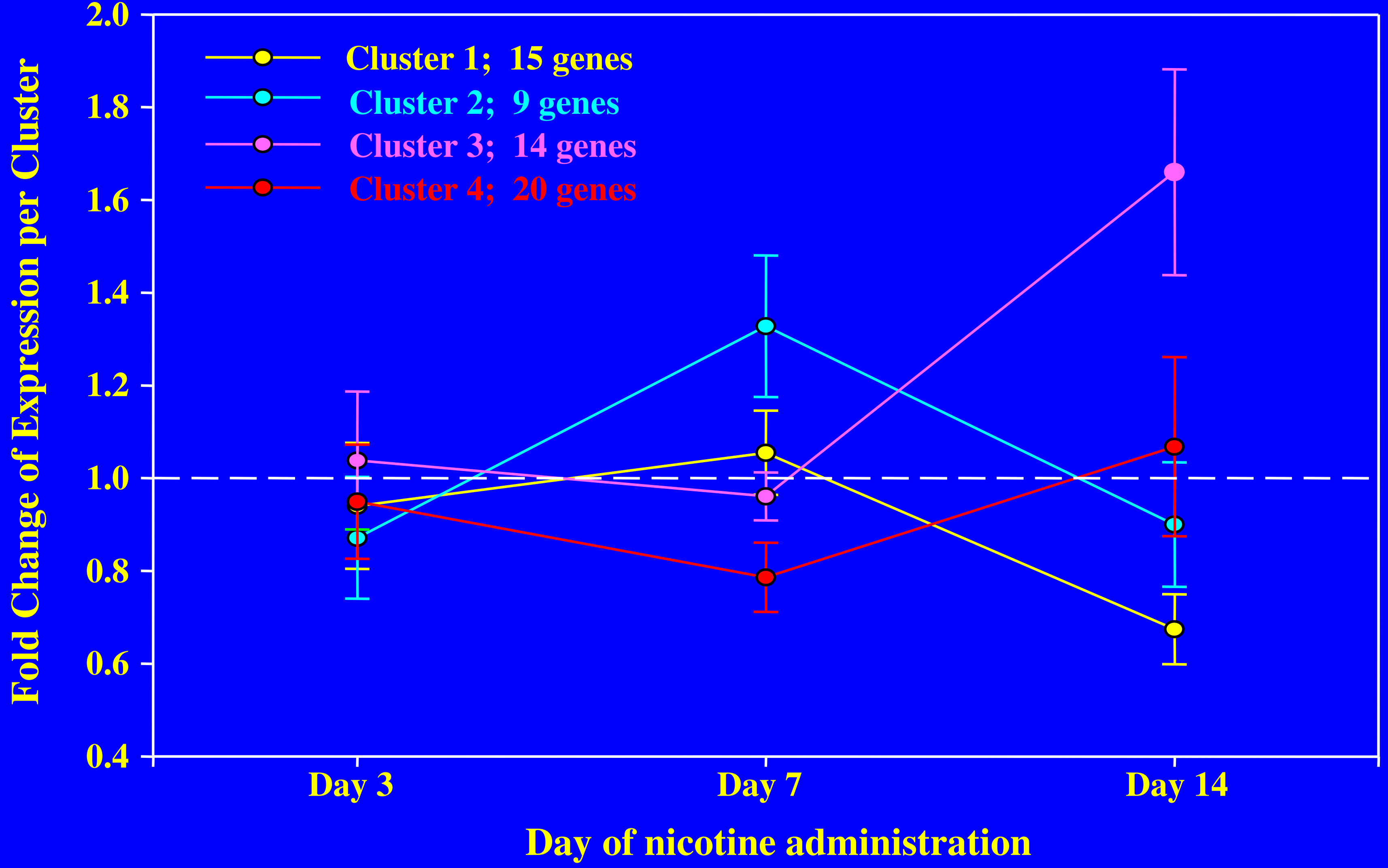
Comparison of average expression levels among clusters in the VTA over three time points



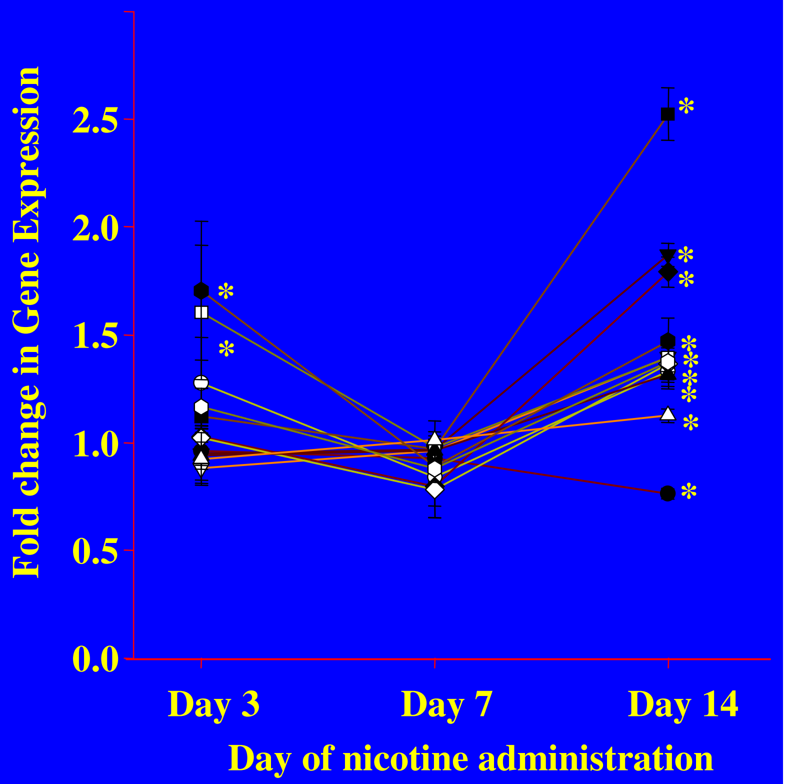
Hierarchical clustering of differentially expressed genes in the prefrontal cortex over three time points



Comparison of average expression levels among clusters in the prefrontal cortex over three time points

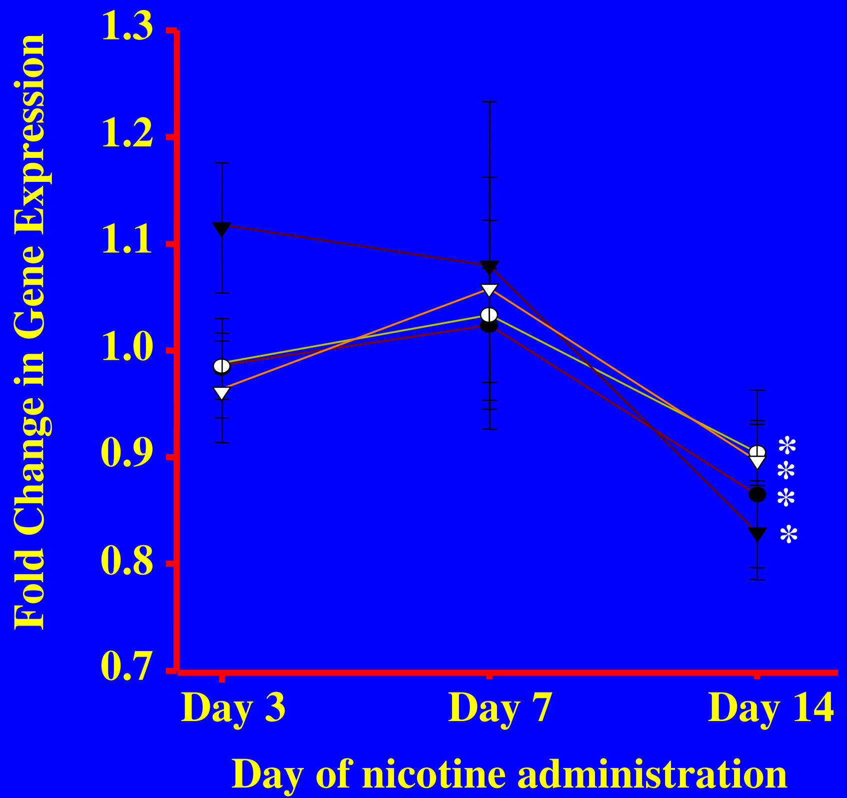


Upregulation of ubiquitin and small ubiquitin-related modifiers after 14 days of nicotine treatment in the prefrontal cortex (Kane et al. 2004)



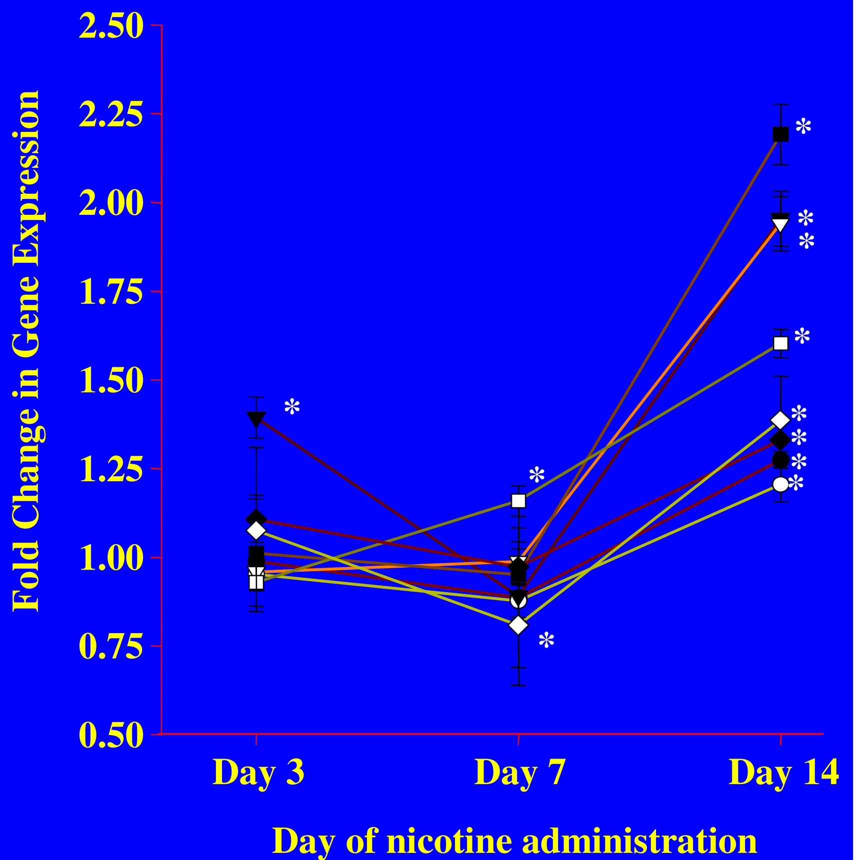
Symbol	Abbrev	Unigene #	Gene Name
	Ufd1l	Mm.1830	Ubiquitin fusion degradation 1
	Usp5	Mm.3571	Ubiquitin specific protease
	Ubl1	Mm.7353	Ubiquitin-like 1 (SUMO1)
	Ubc	Mm.331	Ubiquitin C
	Usp8	Mm.36569	Deubiquitinating enzyme
	Uba52	Rn.4300	Fusion protein of ubiquitin & ribosomal L40
	Loc19	Rn.1253	Polyubiquitin
	Ube2a	Mm.27541	Ubiquitin-conjugating enzyme HR6A
	Ubb	Mm.235	Ubiquitin B
	Ube4b	Mm.21634	Ubiquitination factor E4B
	Usp2	Mm.12914	Ubiquitin specific protease
	Ube2e1	Mm.4429	Ubiquitin-conjugating enzyme 5

Down-regulation of ubiquitin-conjugating enzymes after 14 days of nicotine treatment in the hypothalamus



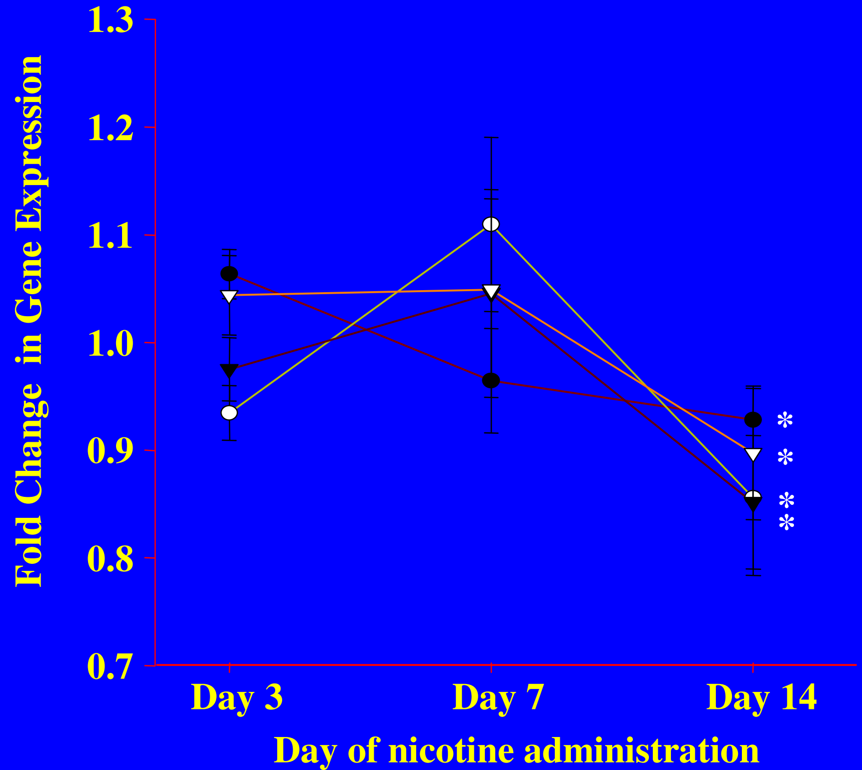
Symbol	Unigene #	Gene Name
●	Mm.4429	Conjugating enzyme 5
○	Rn.2274	Conjugating enzyme E2I
▼	Mm.396	Ubiquitin protease 9
▽	Rn.3530	Conjugating enzyme

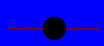
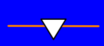
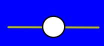
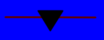
Upregulation of *Proteasome*-related genes after 14 days of nicotine treatment in the prefrontal cortex



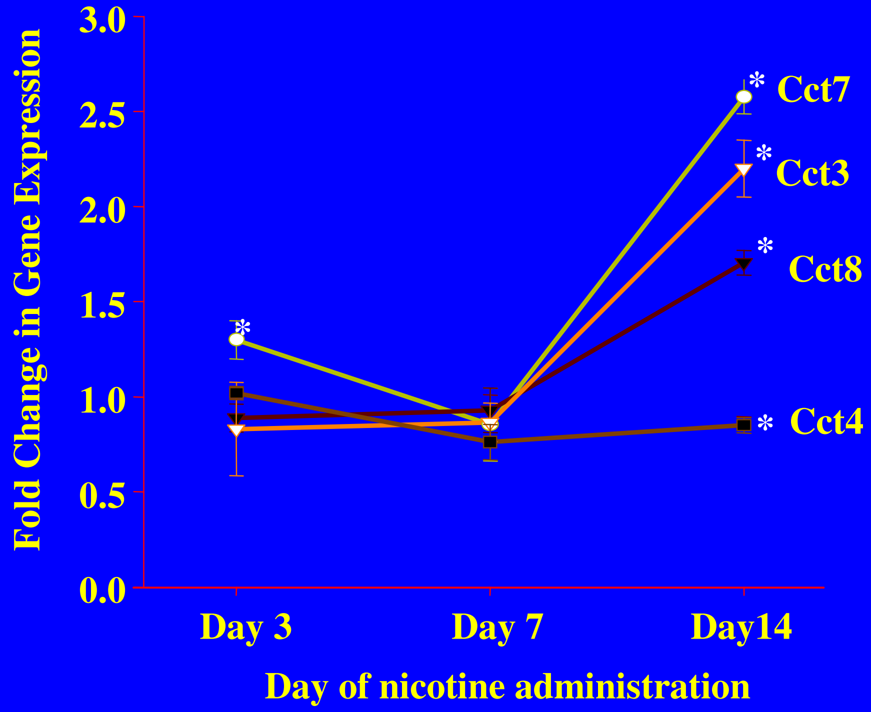
Symbol	Abbrev	Unigene	Gene Name
	Psmc3	Mm.20946	Proteasome 26S, ATPase 3
	Psmd13	Mm.29760	Proteasome 26S, non-ATPase
	Psmb1	Mm.42197	Proteasome subunit, beta type 1
	Psmd4	Mm.2261	Proteasome 26S, non-ATPase, 4
	Psmb5	Mm.8911	Proteasome beta type sub 5
	LOC818	Rn.10972	Proteasome p45/SUG
	Rc5	Rn.6016	Proteasome subunit RC5
	Psmb2	Mm.22233	Proteasome subunit, beta type 2

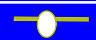
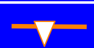
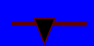
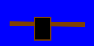
Down-regulation of *Proteasome*-related subunits after 14 days of nicotine treatment in the hypothalamus



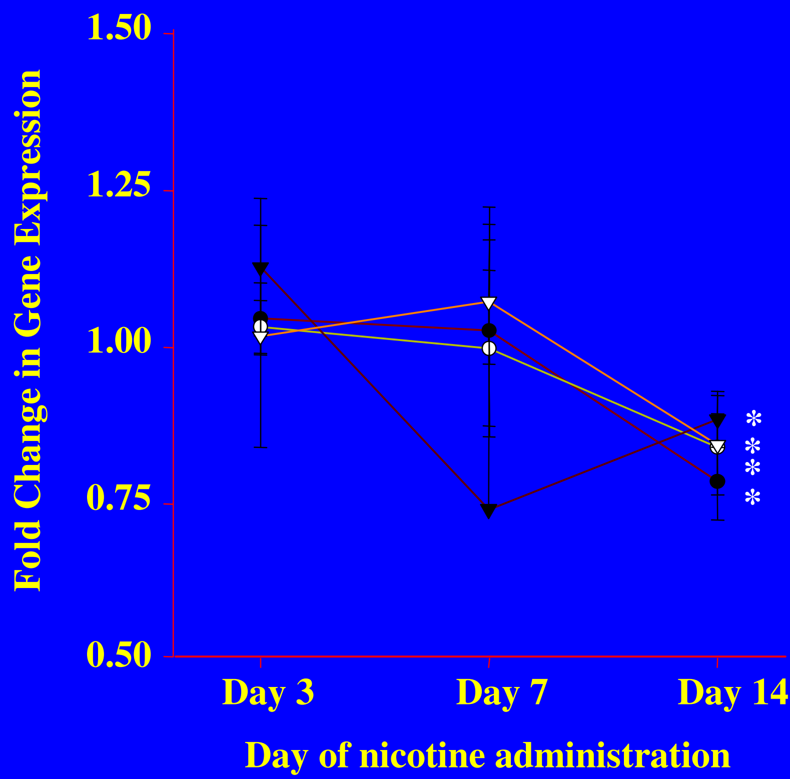
Symbol	Abbrev	Unigene #	Gene Description
	Psma6	Mm.30210	Proteasome subunit, alpha type 6
	Poh1	Mm.27933	26S proteasome-associated pad1 homolo.
	Rpl24	Mm.3428	Proteasome subunit, alpha type 2
	Psma3	Mm.1007	Proteasome subunit, alpha type 3

Up-regulation of *Chaperonin* subunits after 14 days of nicotine treatment in the prefrontal cortex



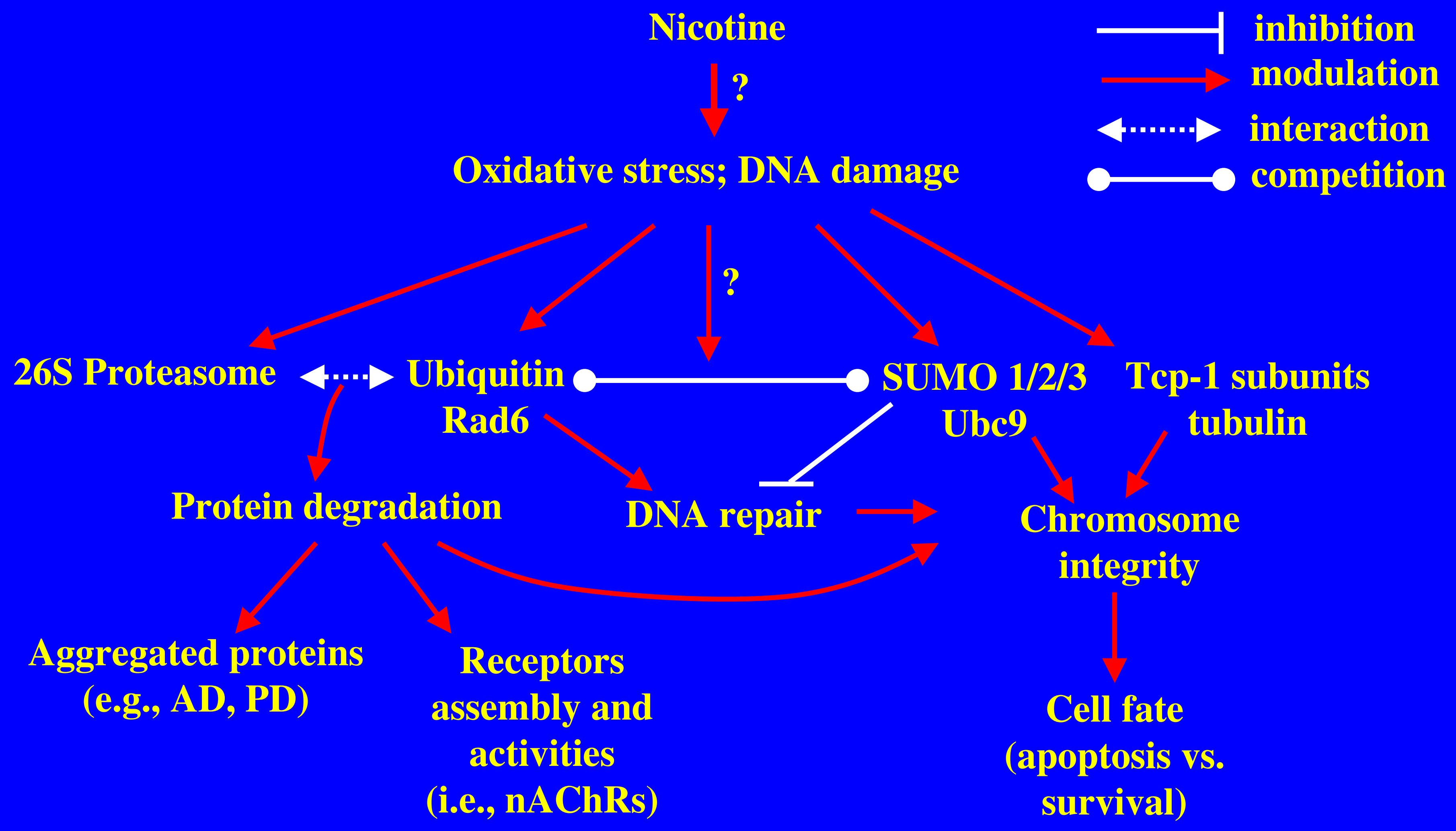
Symbol	Unigene #	Abbrev.	Gene Name
	Mm.914	Cct7	Chaperonin subunit 7 (theta)
	Mm.3576	Cct3	Chaperonin subunit 3 (gamma)
	Mm.17989	Cct8	Chaperonin subunit 8 (theta)
	Mm.6821	Cct4	Chaperonin subunit 4 (delta)

Down-regulation of *Chaperonin* subunits after 14 days of nicotine treatment in the hypothalamus



Symbol	Abbrev.	Unigene #	Gene Description
	Cct3	Mm.3576	Chaperonin subunit 3 (gamma)
	Cct4	Mm.6821	Chaperonin subunit 4 (delta)
	Cct2	Mm.3670	Chaperonin subunit 2 (beta)
	Cct8	Mm.17989	Chaperonin subunit 8 (theta)

Biological implication of co-regulation of ubiquitin and proteasome signaling pathways by nicotine in rat brain



A comparison of gene expression profiles in response to chronic nicotine in C57BL/6 and C3H mouse brain

Mice (C57BL/6 & C3H → **Received nicotine (100 ug/mL) via drinking water or saline for 14 days** →

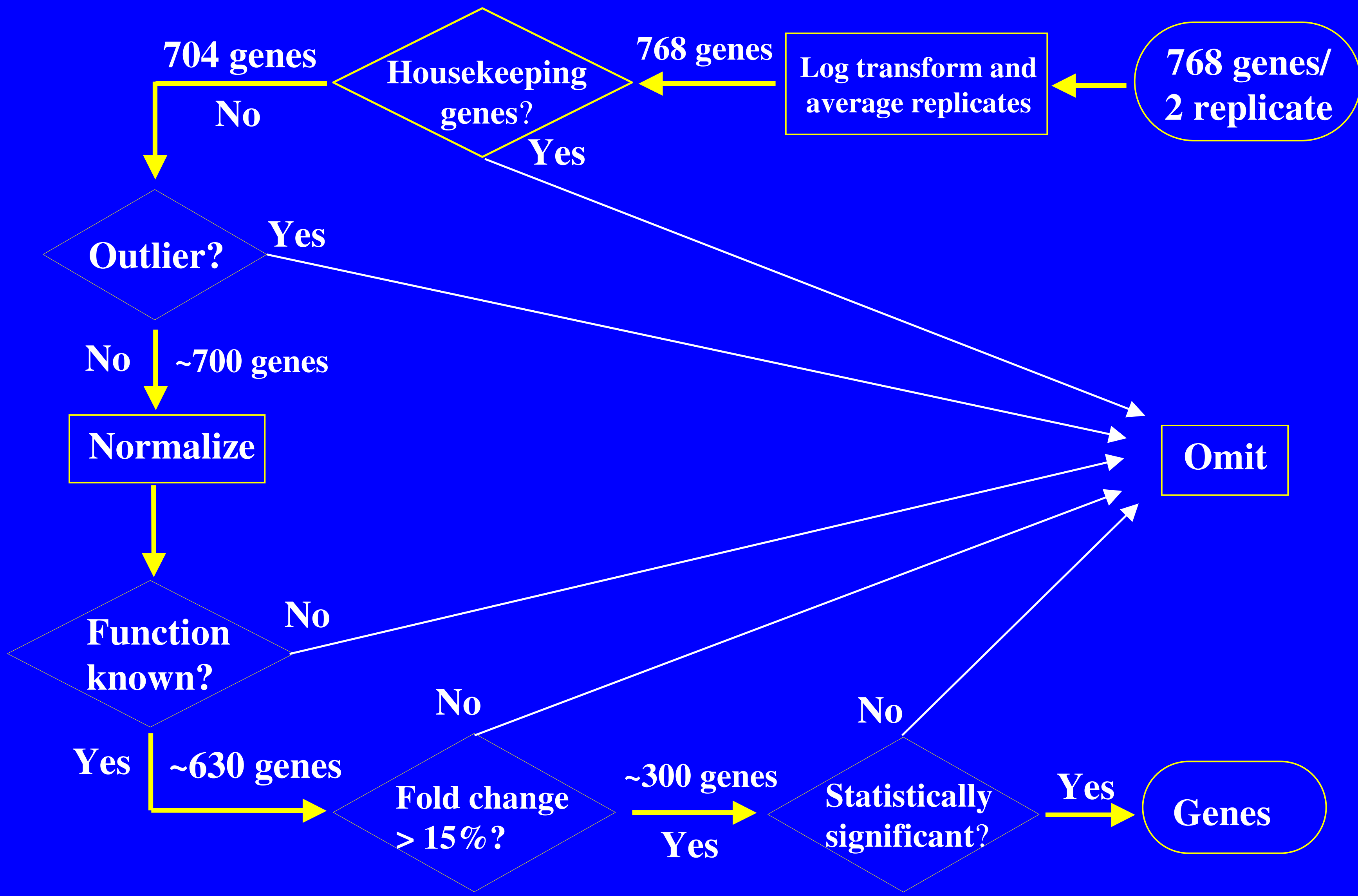
Collected Brain Regions:
Amygdala (Amyg)
Hippocampus (HP)
Prefrontal Cortex (PFC)
Nucleus Accumbens (NA)
Ventral tegmental Area (VTA)

5-7 animals per experimental group
5 brain regions collected from each animal
2 mouse strains: C57BL/6, C3H
4 experimental groups
Total: >140 RNA samples

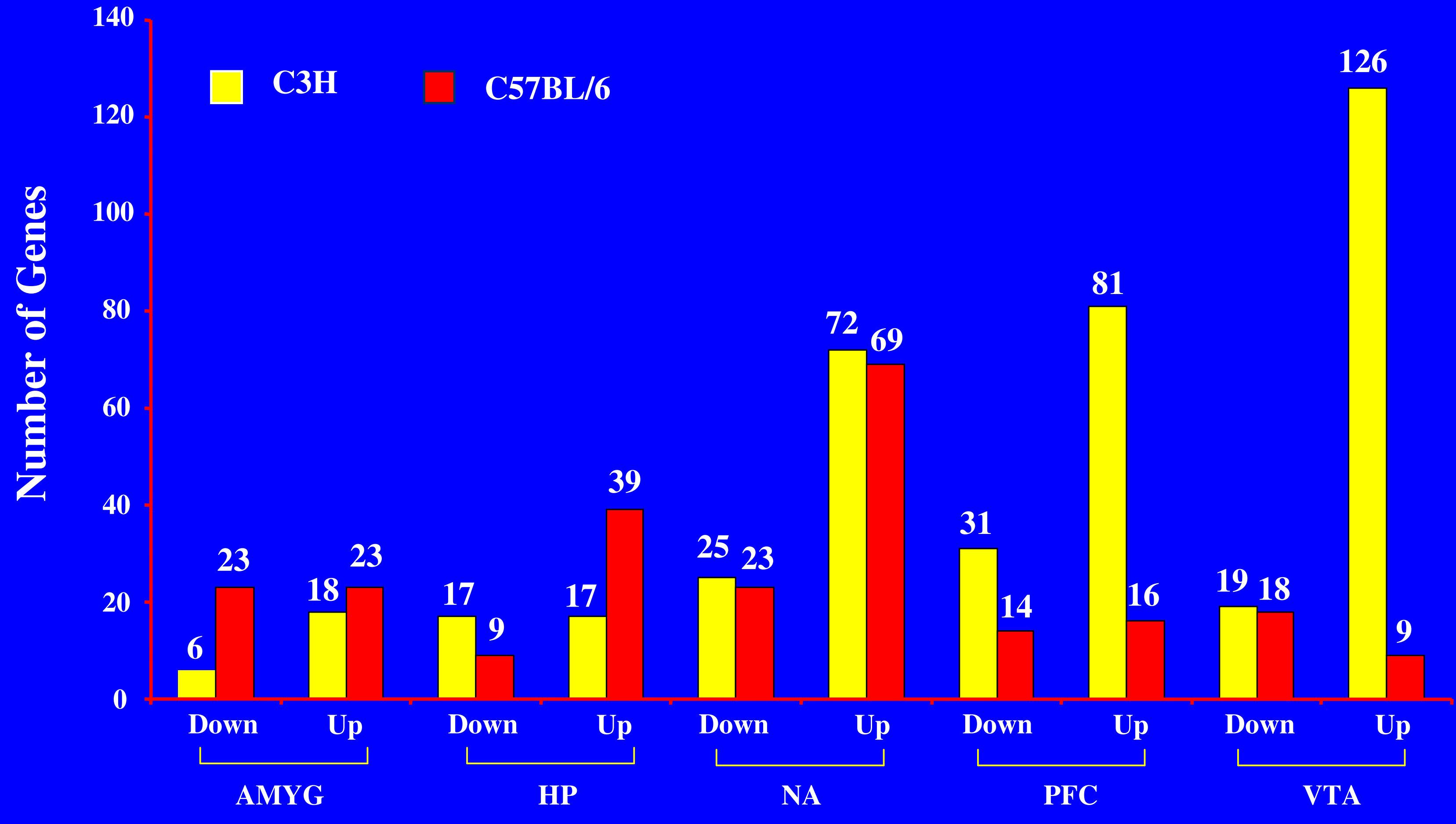
Why are we interested in these two mouse strains?

- Because these two mouse strains show very different behavioral responses to nicotine. For example,
  - C3H mice develop tolerance at a high dose while C57BL/6 develop tolerance at a much low dose of nicotine (3.93 vs 1.12 mg/kg per hour).
  - C3H mice consume much less nicotine than that of C57BL/6 (4.8 vs 11.7 mg/kg/day).
  - low dose of nicotine increases locomoter activity in C3H while nicotine suppressed locomoter activity in C57BL/6.

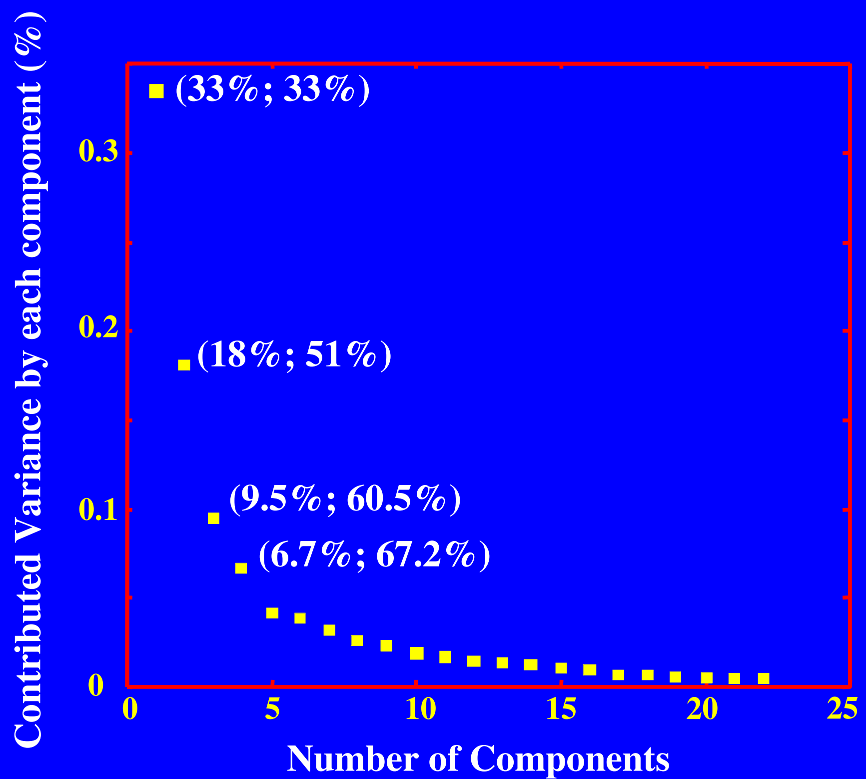
Data analysis procedure used in identification of significantly differential expressed genes



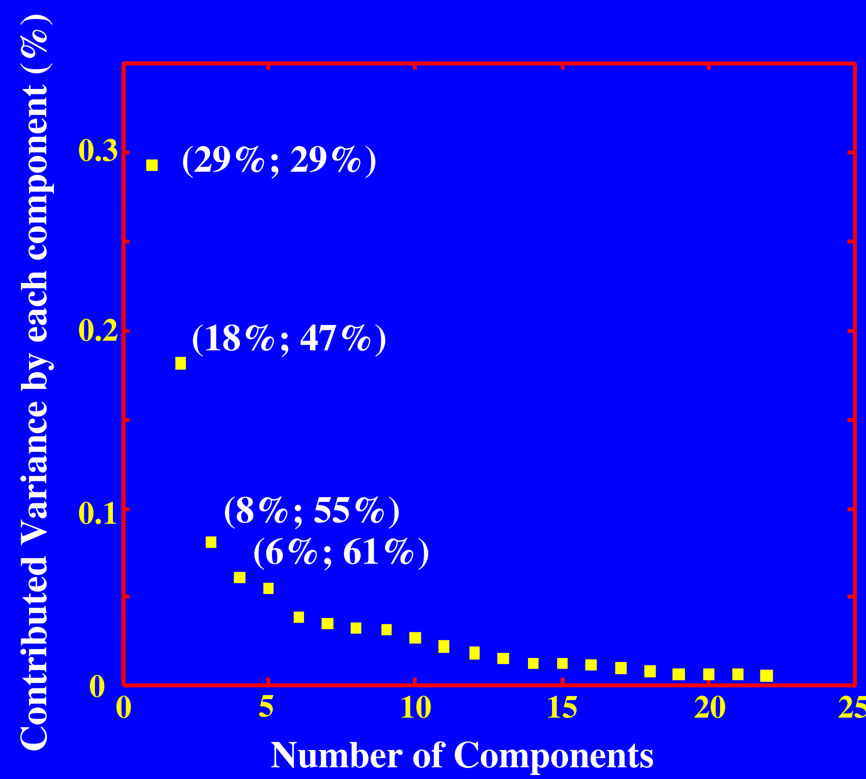
A comparison of number of up-regulated or down-regulated by nicotine between C57BL/6 and C3H in five brain regions



Determination of unique and common biological processes between C57BL/6 and C3h using principal component analysis on differentially expressed genes

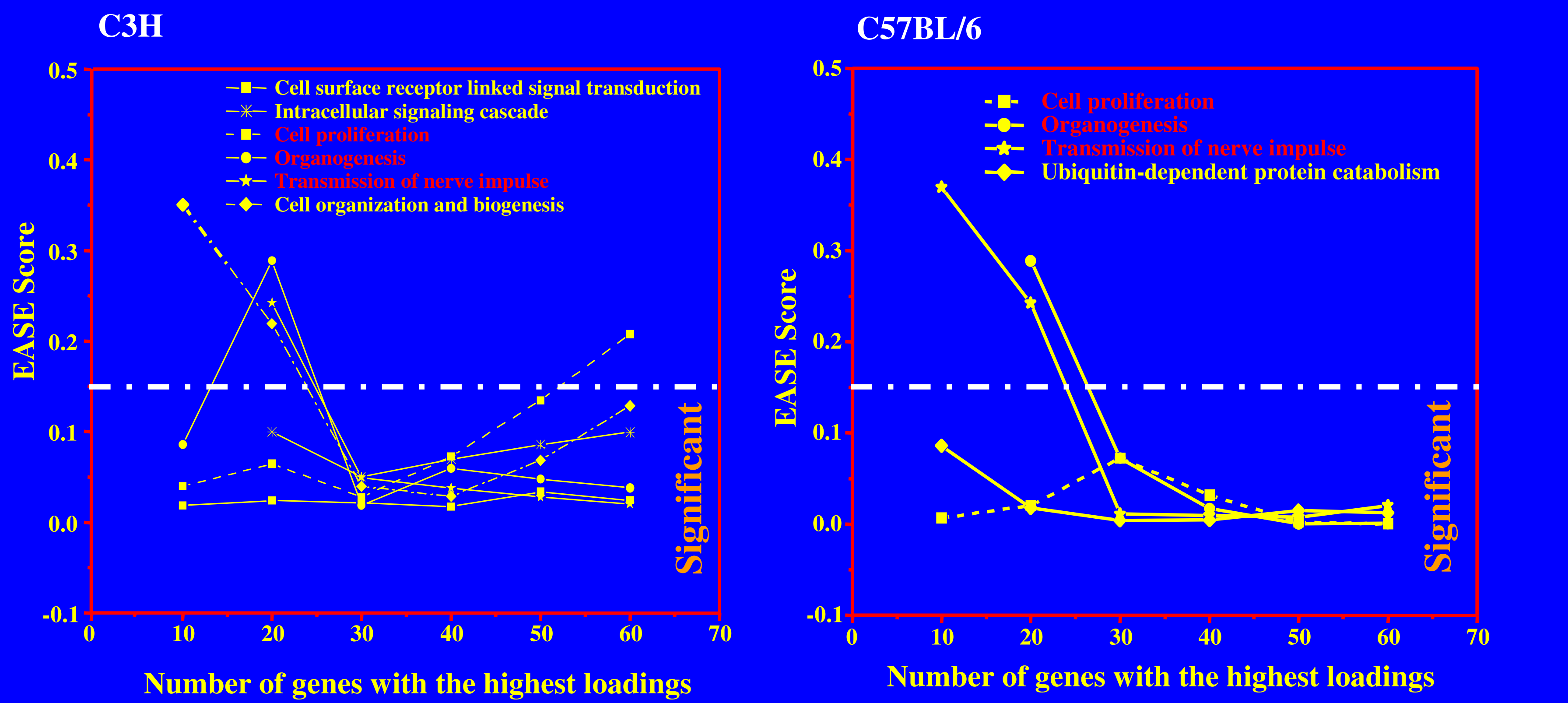


C3H



C57BL/6

Identification of overrepresented biological processes by EASE analysis of differentially expressed genes with the highest loadings of the first three components from principal component analysis



Summary

1. There exist significant differences in transcriptional response to nicotine between different treatment time, brain regions, and animal stains.
2. Regulation of gene expression may have more influence on individual differences than genetic variations.
3. Microarray is a powerful technology to study gene-gene (co-modulation) and gene-environmental interaction.

Summary

4. **Statistical analysis of microarray data has been challenging. So far, there is no consensus on which method is better than the others and it depends on the purpose of the study and dataset per se. Many statistical methods have been used in microarray data analysis, which include cluster analysis, t-test or modified t-test, ANOVA, mixed linear model, and various multivariate analysis like principal component analysis.**
5. **Because of many potential variables associated with microarray technique, data normalization within and between chips is the key to derive meaningful biological conclusions.**

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