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Neural system-enriched gene expression: relationship to biological pathways and neurological diseases

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Departments of [1]Cell Biology, Neurobiology and Anatomy, [2]Molecular Genetics, Microbiology and Biochemistry, [5]Environmental Health, and of [7]Pharmacology and Cellular Biophysics, University of Cincinnati College of Medicine, Cincinnati 45267; and Divisions of [3]Pediatric Informatics, [4]Pathology, and of [6]Molecular and Developmental Biology, Children's Hospital Research Foundation, Cincinnati, Ohio 45229

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microarray; nervous system; global context

THE MAMMALIAN NERVOUS SYSTEM consists of a complex network of neurons and supporting cells that are able to integrate internal and external signals and coordinate responses. To gain a comprehensive molecular description for its development and function, an increasing number of studies have used the genomics/microarray approach to compare gene expression in the nervous system during its development and aging (5, 25, 37, 53), among its subregions (6, 57, 68, 91, 92), or in its particular regions under different experimental or diseased conditions (50, 52, 59, 61, 64, 78). However, few studies addressed the critical question of how nervous tissues differ in their gene expression repertoire from peripheral tissues.

Obtaining an overview of nervous system genomic anatomy is critical for better understanding both normal nervous system function as well as the impact of gene expression on diseases. Although numerous single gene mutations, genetic polymorphisms, and alterations of gene expression have been found in various nervous system diseases (9, 30, 65, 85, 94), the molecular mechanisms of how genetic abnormalities lead to pathological consequences in the nervous system are poorly understood. One primary reason for the lack of understanding of disease mechanisms is the existence of and extensive interactions among nervous system-expressed gene products in influencing disease processes.

Warrington et al. (84) compared the expression of about 7,000 genes in 11 different human adult and fetal tissues and provided a glimpse of how normal tissues differ in their genetic constituents, especially regarding the expression of housekeeping genes. In another study, Penn et al. (58) discovered that 30% open reading frames from genome sequencing are novel genes and 29% are similar but not identical to known sequences using mRNA from 10 human tissues and cell types. Both the Warrington and Penn studies treated the brain as one single tissue. Because of the heterogeneity of brain regions, genes highly expressed in small regions of the brain would be masked and may not appear to be highly expressed in the whole brain. Treating the entire brain as one single tissue is therefore limited in providing comprehension of nervous tissue differentiation.

Miki et al. (51) provided a more comprehensive comparison of 49 adult and embryonic mouse tissues and provided evidence of neurogenesis and remodeling in the embryonic brain and postnatal cerebellum. The same group further described a detailed examination of expression profiles of enzymes in metabolic pathways and particularly glycolysis and illustrated

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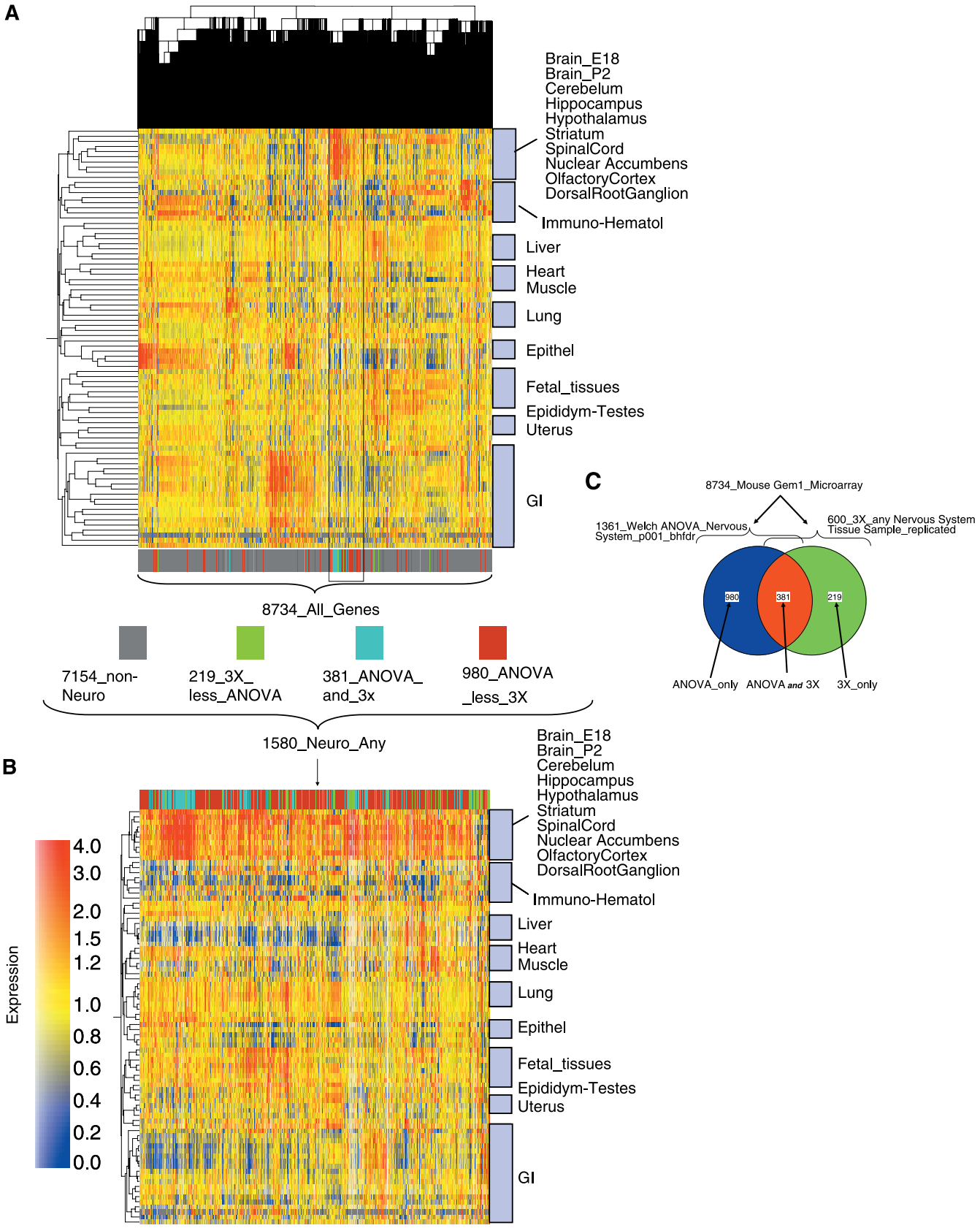
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SpinalCord
Nuclear Accumbens
OlfactoryCortex
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Immuno-Hematol
Liver
Heart
Muscle
Lung
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Fetal_tissues
Epididym-Testes
Uterus
GI
8734_All_Genes
7154_non-Neuro
219_3X_less_ANOVA
381_ANOVA_and_3x
980_ANOVA_less_3X
1580_Neuro_Any
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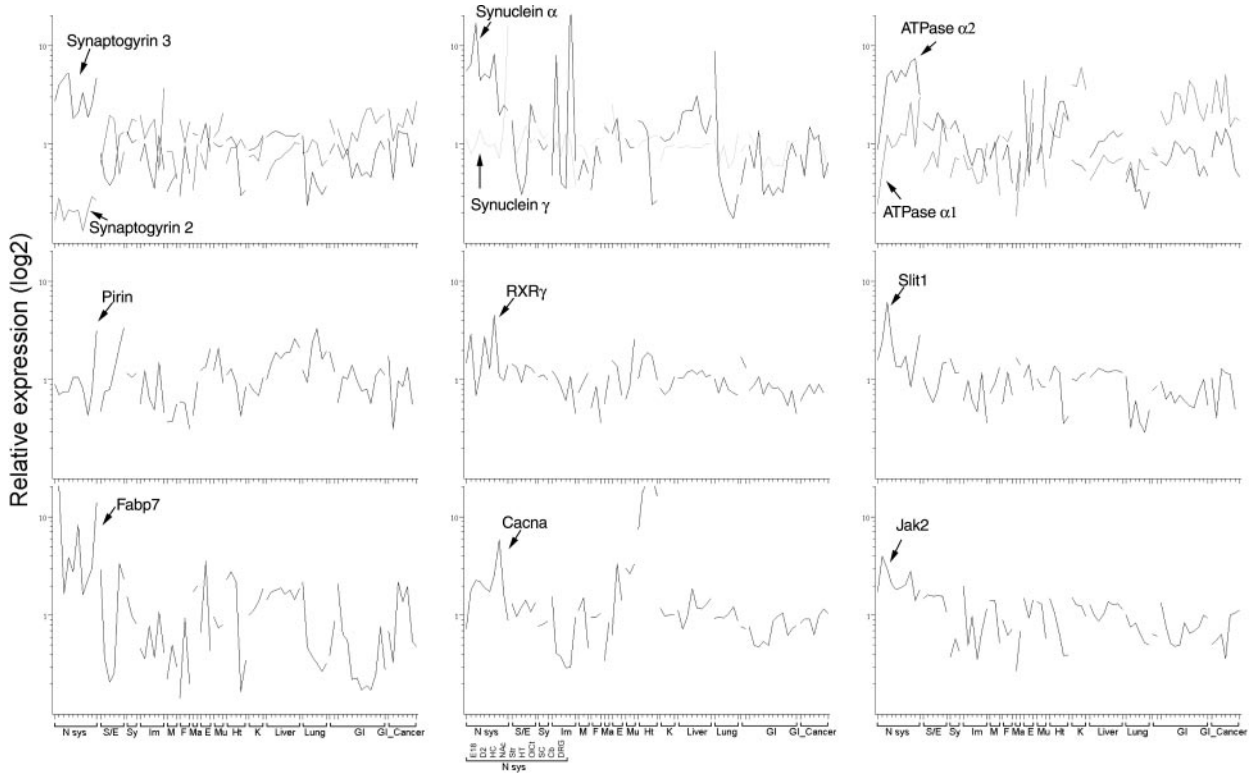

Fig. 2. The expression patterns of 12 selected genes in nervous and peripheral tissues exhibit outstanding correlation with those published in the literature. Synaptogyrin 3, α-synuclein, Na^+-K^+-ATPase α2, Slit1, Fabp7, and Jak2 are statistically highly expressed in the nervous tissues and are within the 381-gene list. Pirin, RXRγ, and Cacna are within the 600-gene list with high expression in at least one but not all nervous tissues and thus are not shared with the 381-gene list. Synaptogyrin 2, γ-synuclein, and Na^+-K^+-ATPase α1 are not highly expressed in the nervous tissues. N sys, nervous system; S/E, skin and epithelial tissues; Sy, synovial tissues; Im, immune system tissues; M, male reproductive system; F, female reproductive system; Ma, mammary gland; Mu, muscles; Ht, heart; K, kidney; GI, gastrointestinal tissues; *E18*, *E18* brain; *D2*, postnatal *day 2* brain; DRG, dorsal root ganglia; SC, spinal cord; Cb, cerebellum; HC, hippocampus; HT, hypothalamus; NAc, nucleus accumbens; Str, striatum; OlCt, olfactory cortex.

differences in energy utilization among tissues such as muscle, liver, testes, kidney, and whole brain (51). A report by Su et al. (75) provided an elegant analysis of genes expressed in different brain regions as well as peripheral tissues focusing on G protein-coupled receptors and kinases, genes containing a pituitary response element, and genes highly expressed in human prostate cancer compared with other normal tissues.

Although those studies described above did include individual brain regions as well as some peripheral tissues, the analyses were focused on very specific biological questions, for example, cancer or energy metabolism. To address the more broadly based question of how nervous tissues differ in genetic composition from peripheral tissues, and how genes abundantly expressed in the nervous tissues cater to particular need of the nervous system and influence susceptibility to nervous system diseases, we have here compared the gene expression profiles of 10 distinct mouse nervous system tissues vs. 72 other mouse tissues representing 30 developing and adult stage organs. We identified genes that are abundant in one or more nervous tissues, including genes of diverse functional categories and genes known to cause neurological diseases. Our data suggest that this global-context approach provides a powerful tool and a large-scale resource for nervous system gene repertoire profiling, pathway analyses, and identification of candidate genes for neurological diseases.

Fig. 1. Molecular signature of the mammalian nervous system. *A*: self-organizing map and Experiment Tree clustering of the expression of 8,734 cDNAs in all 82 tissues normalized against the median level of expression of each gene over all 82 tissues. Of these, 1,361 genes are highly expressed in the 10 nervous tissues vs. other tissues by Welch ANOVA. Six hundred genes are expressed at threefold or higher in at least one nervous tissue over their median expression in all 82 tissues. We found 1,580 are either in the 1,361-gene set or in the 600-gene set. We found 381 genes are in both the 1,361-gene set and the 600-gene set. *B*: self-organizing map and Experiment Tree clustering of the expression of 1,580 cDNAs in all 82 tissues normalized against the median level of expression of each gene over all 82 tissues. The red color indicates high expression, the yellow color indicates average expression, and the blue color indicates low expression. *C*: Venn diagram of the 1,361 genes highly expressed by nervous tissues according to Welch ANOVA and the 600 genes expressed at threefold or higher in at least one nervous tissue over the median of all 82 tissues. We found 980 genes are highly expressed in nervous tissue with statistical significance, while their expression in any nervous tissue is less than threefold over the median of all 82 tissues. We found 381 genes are both highly expressed in all nervous tissues over other tissues with statistic significance and expressed at threefold or higher in at least one nervous tissue over the median of all 82 tissues. We found 219 genes are expressed at threefold or higher in at least one nervous tissue over the median of all 82 tissues, while their expression are statistically higher in all nervous tissues vs. other tissues.

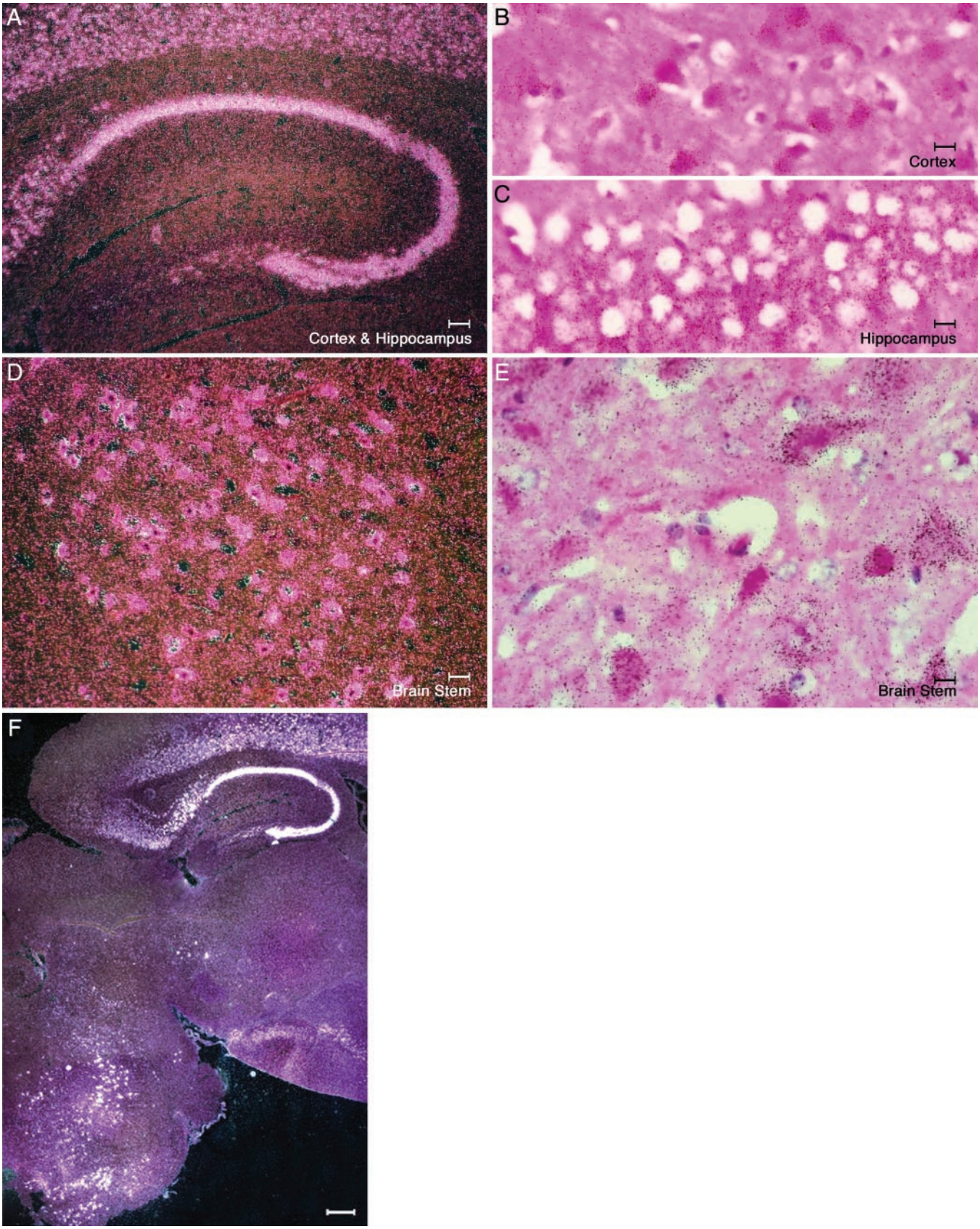

Fig. 3. Verification of Dkk3 (NM_015814) expression in the nervous system by in situ hybridization. Significant expression of Dkk3 was found in cortex, hippocampus, and brain stem. *A* and *D*: dark-field pictures of in situ hybridization signals in cortex and hippocampus, as well as in brain stem. *B*, *C*, and *E*: bright-field pictures of in situ hybridization signals in cortex, hippocampus, and brain stem. *F*: a dark-field picture of an overview of the brain. Scale bars: $A = 100$ μm; $D = 20$ μm; *B*, *C*, and $E = 10$ μm; $F = 400$ μm.

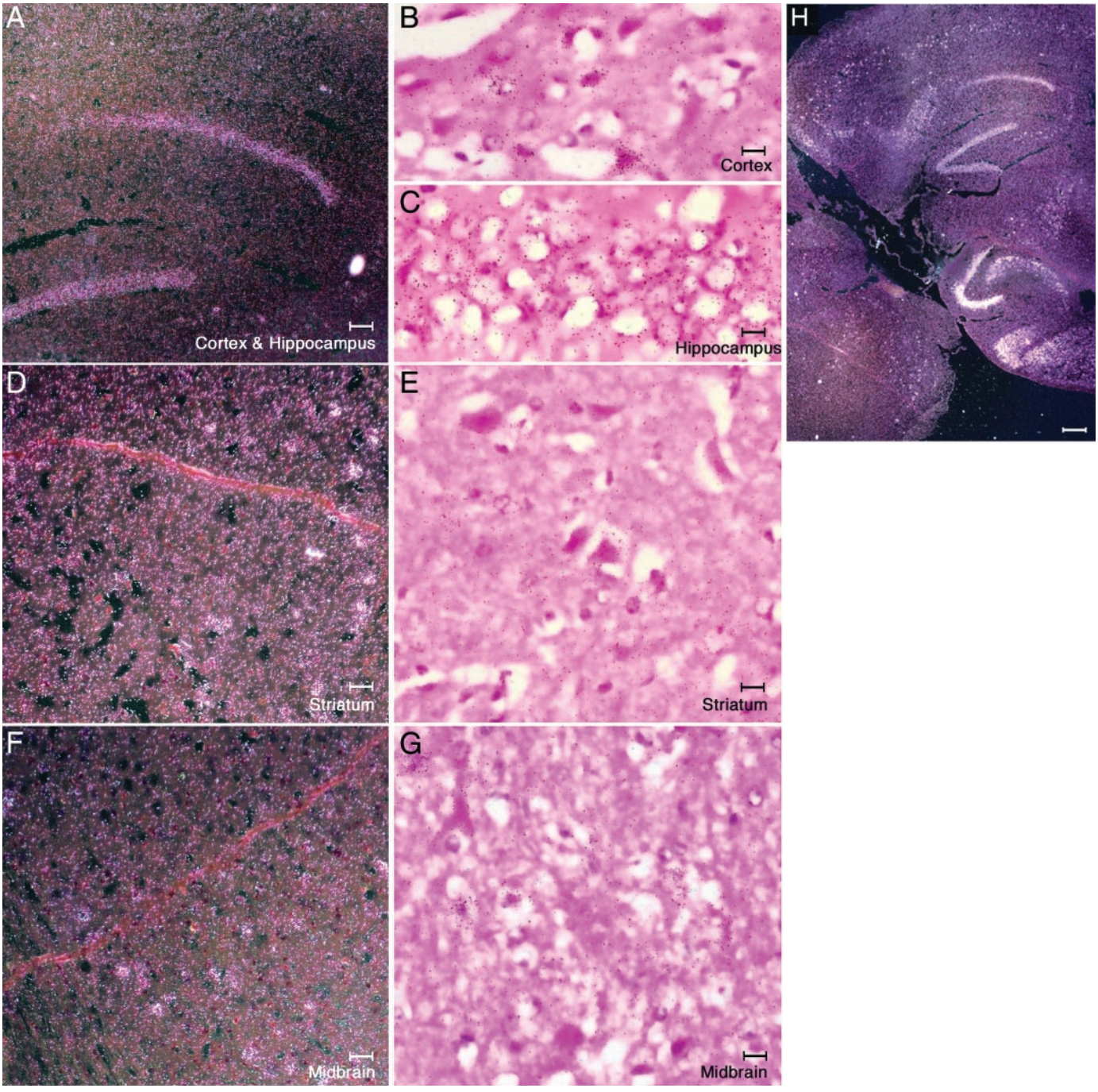

Fig. 4. Verification of a putative G protein-coupled receptor (AB041649) expression in the nervous system by in situ hybridization. Significant expression of this gene was found in cortex, hippocampus, striatum, and midbrain. *A*, *D*, and *F*: dark-field pictures of in situ hybridization signals in cortex and hippocampus, as well as in striatum and midbrain. *B*, *C*, *E*, and *G*: bright-field pictures of in situ hybridization signals in cortex, hippocampus, striatum, and midbrain. *H*: a dark-field picture of an overview of the brain. Scale bars: *A* = 100 μm; *D* and *F* = 40 μm; *B*, *C*, *E*, and *G* = 10 μm; *H* = 400 μm. *D–G* are from different brain sections as *H*.

MATERIALS AND METHODS

Preparing poly(A)$^+$ RNA from mouse tissues. All animal procedures were approved by the IACUC. We obtained C57BL/6 male mice of 8 mo of age from the Jackson Laboratory (Bar Harbor, ME) for all the adult tissues. In-house timed breeding was carried out to provide embryonic *day 18.5* (denoted *E18* for all text and figures for simplicity) and postanatal *day 2* (*P2*) brains. We dissected the 10 adult nervous tissues from 3–10 mice and immediately homogenized them in TRIzol reagent (Life Technologies, Rockville, MD). The 10 nervous tissues were: hippocampus (HC), nucleus accumbens (NAc), striatum (Str), hypothalamus (HT), cerebellum (Cb) and olfactory cortex (OlCt), spinal cord (SC), dorsal root ganglia (DRG), and the whole brain at *E18* and at *P2*. All 10 nervous tissues were dissected within clearly defined boundaries immediately after euthanizing the mice. We dissected all cervical, thoracic, and lumbar DRGs from 10 mice, HC from 4 mice, HT, NAc and Str from 6 mice, and other

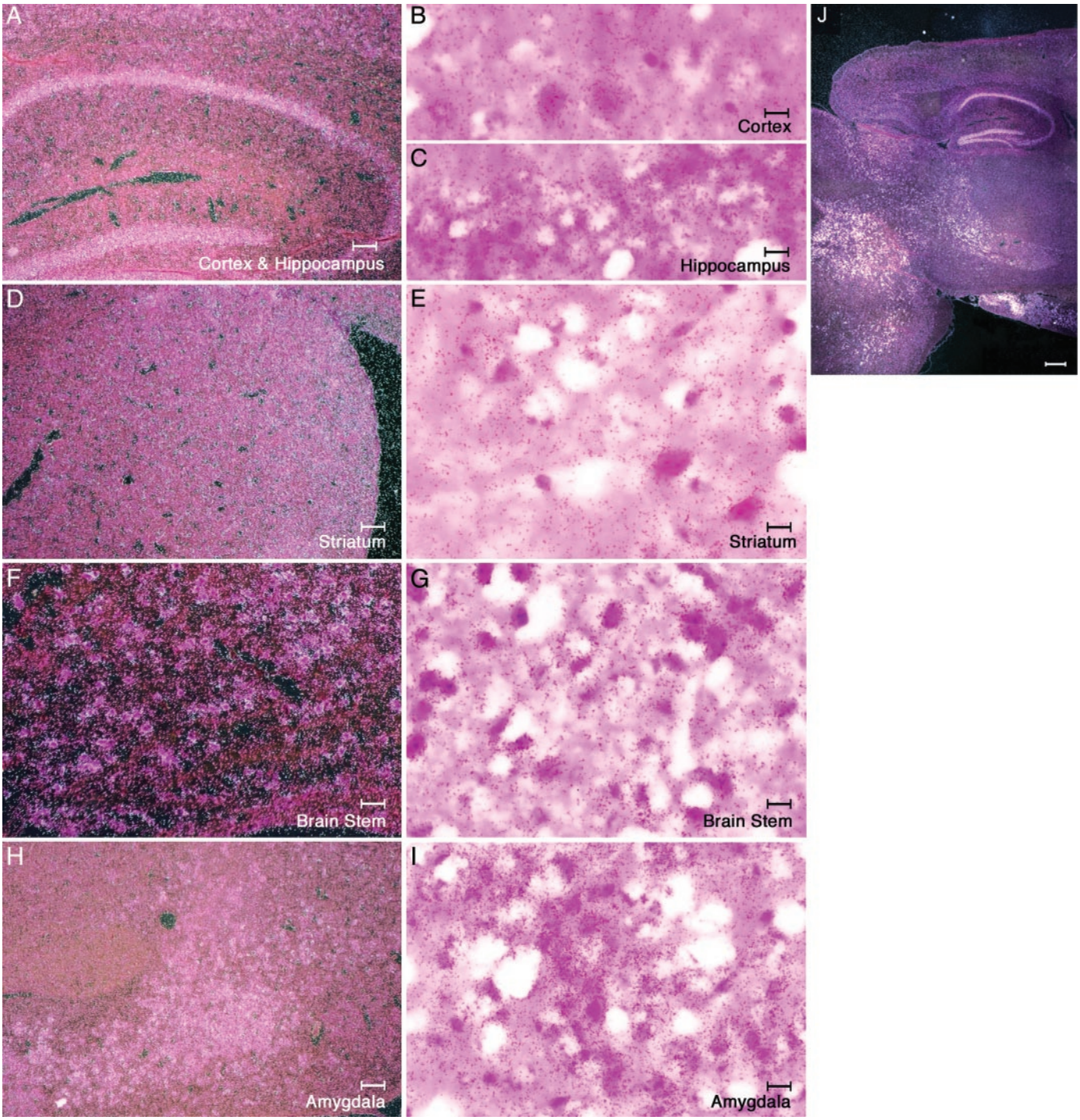

Fig. 5. Verification of a novel SH3-containing protein (W29432) gene expression in the nervous system by in situ hybridization. Significant expression of the novel gene was found in cortex, hippocampus, striatum, brain stem, and amygdala. *A*, *D*, *F*, and *H*: dark-field pictures of in situ hybridization signals in cortex and hippocampus, as well as in striatum, brain stem and amygdala. *B*, *C*, *E*, *G*, and *I*: bright-field pictures of in situ hybridization signals in cortex, hippocampus, striatum, brain stem, and amygdala. *J*: a dark-field picture of an overview of the brain. Scale bars: *A*, *D*, and *H* = 100 μm; *F* = 40 μm; *B*, *C*, *E*, *G*, and *I* = 10 μm; *J* = 400 μm. *D–I* are from different brain sections as *J*.

nervous tissues from 3 mice each. We then isolated total RNA following the manufacturer's protocol (Life Technologies) and purified poly(A)$^+$ RNA using Oligotex resin (Qiagen, Valencia, CA). RNA concentration was quantified using RiboGreen dye (Molecular Probes, Eugene, OR) and profiled for size distribution and ribosomal RNA contamination using an Agilent Bioanalyzer 2100. We submitted in duplicate 600-ng samples of poly(A)$^+$ RNA at 50 ng/μl per tissue to Incyte Genomics for cDNA labeling and microarray hybridization.

Microarray hybridization. Incyte Genomics (Palo Alto, CA) prepared labeled cDNA from poly(A)$^+$ RNAs using the GEMBright random primer reverse-transcription labeling kit (5′ dye-terminated random primers) and competitive hybridization to mouse GEM1

microarrays (14). We also confirmed the microarray data from Incyte with our in-house microarray facility using the identical Incyte mouse GEM1 clone set (**http://microarray.uc.edu/**). Duplicate arrays were hybridized for each poly(A)$^+$ RNA sample. Each hybridization was performed with Cy5-labeled poly(A)$^+$ RNA from nervous tissues in competition with the "universal reference," which was Cy3-labeled poly(A)$^+$ RNA from whole postnatal *day 1* (*P1*) mouse.

Data analyses. We used Incyte GEMTools software to analyze the quality of each hybridization using the parameters of signal to background fluorescence, spot geometry, the relative intensities of control genes "spiked" into the labeling reactions, and an assessment of dynamic range exhibited by each fluorescence channel. Spike controls exhibited low variation across the microarray series (data not shown). Data manipulation including normalization, filtering, and clustering was carried out using GeneSpring software (Silicon Genetics, Redwood City, CA). All selection and cutoff filters were applied to the mean expression ratio values based on the two replicate hybridizations (14).

We performed three types of normalization of the data. First, a per-array "whole mouse normalized" expression value for each gene was derived from the simple ratio of the sample to the whole mouse reference poly(A)$^+$ RNA. For each array, a single linear correction, "balance coefficient," was used to multiply the Cy5 channel to correct for the median Cy3-to-Cy5 intensity value ratios. We found no significant bias in using Cy3 and Cy5 ($P < 0.05$ for 10 random selected genes between dye reversal experiments), and we eliminated all dye preference by using the next normalization. Second, a "each gene normalized" value for each gene in each tissue was derived from dividing the "whole mouse normalized" expression value for each gene in that tissue by the median of all "whole mouse normalized" expression values for that gene in all 82 sampled tissues. The 82 sampled tissues include the 10 nervous tissue and 72 peripheral tissues representing 30 organs, such as skeletal muscle, male and female reproductive organs, and different regions of the gastrointestinal tract, as well as organs in a developmental context including lung, liver, kidney, and heart. Genes highly expressed in the nervous system were then selected according to their abundance in the nervous tissues over the median values across all 82 tissues.

Genes highly expressed in the nervous system were annotated by putative functions of encoded proteins based on classification data provided from GenBank (**http://www.ncbi.nlm.nih.gov**), GeneCards (**http://bioinformatics.weizmann.ac.il/cards**; Ref. 63), TIGR (**http://www.tigr.org**), and the MGI resource version 2.7 (**http://www.informatics.jax.org**). The functional categorization of these genes was verified from searching PubMed.

Statistic analysis. For analysis of genes expressed in the nervous tissues vs. peripheral tissues, analysis of variance was used based on Welch ANOVA (Benjamini and Hochberg false discovery rate, $P < 0.05$) provided by the GeneSpring software. We identified 1,361 genes that are significantly highly expressed in nervous tissues compared with peripheral tissues.

In situ hybridization. cDNA clones were purchased from Incyte Genomics. In situ hybridization was performed as previously described (89). Brains from C57BL/6 mice were dissected, fixed in 4% paraformaldehyde in PBS, pH 7.4, overnight at 4°C, saturated in 30% sucrose, and frozen in liquid nitrogen. ^{35}S-labeled UTP riboprobes to detect specific mRNA transcripts were synthesized from each cDNA cloned into BlueScript transcription vectors (Stratagene). Cryostat sections of the mouse brains cut at 8 to 10 μm were digested in proteinase K (0.1%) for 5 min at room temperature, acetylated in acetic anhydride, and dehydrated before being hybridized overnight at 45°C with 1×10^6 counts per slide. Following hybridization, the sections were digested with 50 μl/ml of RNase A and RNase T1, then stringently washed with 50% formamide in 0.1× SSC at 50°C. Slides were then dehydrated, dipped in NTB2 emulsion, exposed for periods ranging from 2–3 wk, and developed. Controls for riboprobe speci-

Table 1. *Functional categories of genes highly expressed in the nervous system: Intracellular signaling*

Common	GenBank	Description
		44 Intracellular signaling
Arhn	NM_009708	ras homolog N (RhoN)
Camk2g	AK078311	calcium/calmodulin-dependent protein kinase II gamma
Cnp1	NM_009923	cyclic nucleotide phosphodiesterase 1
Dcamkl1	NM_019978	double cortin and calcium/calmodulin-dependent protein kinase-like 1
Dkk3	NM_015814	dickkopf homolog 3 (*Xenopus laevis*)
Fgd1	NM_008001	faciogenital dysplasia homolog (human)
Fgfr1	BC010200	fibroblast growth factor receptor 1
Freq	NM_019681	frequenin homolog (*Drosophila*)
Gabarapl1	AB041648	GABA(A receptor-associated protein-like 1
Gdi1	BC037598	guanosine diphosphate (GDP) dissociation inhibitor 1
Gna11	NM_010301	guanine nucleotide binding protein, alpha 11
Gnao	AK047197	guanine nucleotide binding protein, alpha o
Gng10	NM_025277	guanine nucleotide binding protein (G protein), gamma 10
Gsk3b	NM_019827	glycogen synthase kinase 3 beta
Itsn	NM_010587	intersectin (SH3 domain protein 1A)
Jak2	NM_008413	Janus kinase 2
Lcn2	BG917714	lipocalin 2
LOC56795	NM_019968	ADP-ribosylation factor-like membrane-associated protein Arm1
Mapk1	NM_028991	mitogen activated protein kinase 1
Mapk9	BC028341	mitogen activated protein kinase 9
Nell2	NM_016743	nel-like 2 homolog (chicken)
Pdcl	NM_026176	phosducin-like
	AB041649	G protein coupled receptor
Pip5k2a	AK012196	phosphatidylinositol-4-phosphate 5-kinase, type II, alpha
Pla2g7	NM_013737	phospholipase A2, group VII (platelet-activating factor acetylhydrolase)
Plxna2	D86949	plexin A2
Pnck	NM_012040	pregnancy upregulated non-ubiquitously expressed CaM kinase
Ppp2r1a	NM_016891	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha
Ppp3ca	AK076570	protein phosphatase 3, catalytic subunit, alpha isoform
Ppp3r1	NM_024459	protein phosphatase 3, regulatory subunit B, alpha (calcineurin B, type I)
Prkacb	AK048319	protein kinase, cAMP dependent, catalytic, beta
Prkar1a	AK051068	protein kinase, cAMP dependent regulatory, type I, alpha
Prkar1b	BC011424	protein kinase, cAMP dependent regulatory, type I, beta
Rab3a	BC018451	RAB3A, member RAS oncogene family
Rab3b	AK082959	RAB3B, member RAS oncogene family
Rab6	AK083262	RAB6, member RAS oncogene family
Rab6ip1	AJ245569	Rab6 interacting protein 1
Rabj	NM_153082	Rab-related GTP-binding protein
Rac3	NM_133223	RAS-related C3 botulinum substrate 3
Rap1ga1	AK019076	Rap1, GTPase-activating protein 1
Stmn4	NM_019675	stathmin-like 4
Trim2	NM_030706	tripartite motif protein 2
Tyro3	U18343	TYRO3 protein tyrosine kinase 3
Xpr1	AK082643	xenotropic and polytropic retrovirus receptor 1

Tables 1–6 show functional categories of genes highly expressed in the nervous system. For each Table 1–6, known genes and GenBank/RefSeq accession numbers from the 381-gene list are shown in the following categories: intracellular signaling (Table 1); cytoskeleton function and cytoskeleton binding protein (Table 2); enzymatic activities (Table 3); RNA metabolism and transcription, and membrane structure and function (Table 4); cell differentiation, death, proliferation, and division, as well as protein metabolism, extracellular message, and secretory and secretion (Table 5); related to brain function and/or diseases, prion and regulation, and others (Table 6).

Table 2. *Functional categories of genes highly expressed in the nervous system: Cytoskeleton and cytoskeleton binding protein*

Common	GenBank	Description
	26 Cytoskeleton and cytoskeleton binding protein	
Add2	NM_013458	adducin 2 (beta)
Ank3	NM_146005	ankyrin 3, epithelial
Catna2	NM_009819	catenin alpha 2
Crym	NM_016669	crystallin, mu
Csrp3	NM_013808	cysteine-rich protein 3
Dbn1	NM_019813	drebrin 1
Epb4.1l3	NM_013813	erythrocyte protein band 4.1-like 3
Epb4.9	NM_013514	erythrocyte protein band 4.9
Ina	NM_010563	internexin neuronal intermediate filament protein, alpha
Kif1a	NM_008440	kinesin family member 1A
Kif21b	NM_019962	kinesin family member 21B
Kif2a	NM_008442	kinesin family member 2A
Kif5c	AB093244	kinesin family member 5C
Klc2	NM_008451	kinesin light chain 2
Mapre2	BC035254	microtubule-associated protein, RP/EB family, member 2
Mapt	NM_010838	microtubule-associated protein tau
Marcks	NM_008538	myristoylated alanine rich protein kinase C substrate
Mtap2	NM_008632	microtubule-associated protein 2
Nefl	BC029203	neurofilament, light polypeptide
Pfn2	NM_019410	profilin 2
Spnb3	BC033305	beta-spectrin 3
Stmn1	NM_019641	stathmin 1
Tagln3	NM_019754	transgelin 3
Tuba4	NM_009447	tubulin, alpha 4
Tubb4	NM_009451	tubulin, beta 4
Tubb5	NM_011655	tubulin, beta 5

See legend to Table 1 for details.

ficity included use of sense probe, as well as predigestion with RNases. Slides were counterstained with hematoxylin and eosin.

Data archive. Gene identities and expression data of the 381 genes that are highly expressed in the nervous tissues are available on our microarray database web server (**http://genet.cchmc.org**) in the mouse GEM1 genome, in the ZhangEtalBrain2004 subdirectory.

RESULTS

Identification of genes highly expressed in mouse adult nervous tissues. To gain a global view of the spectrum of genes highly expressed in the nervous system, we constructed a gene expression database in which we compared gene profiles in nervous tissues in relation to diverse mouse tissues using the C57BL/6 mouse strain. We have taken an approach based on a two-channel cDNA microarray technology in which a universal reference poly(A)$^+$ RNA was used to intercompare relative expression profiles of widely different mouse tissue samples. This approach allows for direct comparison of multiple tissues and the ability to add more tissues and experimental conditions to the database. The 8,734 cDNAs on the microarray were clustered according to their expression patterns using self-organizing map with Pearson correlation (76) as implemented in GeneSpring (Fig. 1*A*).

We selected genes that are highly enriched in the nervous system by two approaches. First, we used a Welch-ANOVA (Benjamini and Hochberg false discovery rate) analysis of the 10 nervous tissues vs. other tissues with a cutoff of $P < 0.05$. We identified 1,361 genes that are statistically higher in the 10 nervous tissues compared with other tissues. These genes represent 13.7% of all genes arrayed and may participate in common functions in nervous tissues. Second, from 8,734 genes, we identified 600 genes that exhibited threefold or greater expression levels in at least 1 of the 10 nervous tissues (*E18*, *P2*, DRG, Cb, HC, HT, SC, NAc, Str, and OlCt) over median values across all nervous and peripheral tissues. Therefore, a total of 1,580 genes fit the first or the second identification criteria. We clustered these 1,580 cDNAs according to their expression patterns using self-organizing map with Pearson correlation (76) and "Experiment Tree" analyses as implemented in GeneSpring (Fig. 1*B*). The 1,361 and the 600 genes have 381 in common, and represent a primary set of nervous tissue-enriched genes (Fig. 1*C*). The 219 genes left from the 600-gene list that also fail to be included in the 1,361-gene list may be genes exhibiting region-specific functions in the nervous system. See Supplemental Table S1 for detailed description of these gene lists, available at the *Physiological Genomics* web site.[1]

[1]The Supplementary Material for this article (Supplemental Table S1) is available online at **http://physiolgenomics.physiology.org/cgi/content/full/00220.2003/DC1**.

Table 3. *Functional categories of genes highly expressed in the nervous system: Enzymatic activities*

Common	GenBank	Description
	25 Enzymatic activities	
Adarb1	AF525421	adenosine deaminase, RNA-specific, B1
Aldo3	AK039267	aldolase 3, C isoform
Arf3	BC014778	ADP-ribosylation factor 3
Asns	AK076207	asparagine synthetase
B3galt3	NM_020026	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 3
B3gat1	BC034655	beta-1,3-glucuronyltransferase 1 (glucuronosyltransferase P)
Basp1	AK046868	brain abundant, membrane attached signal protein 1
Cpt1c	NM_153679	camitine palmitoyltransferase 1, brain
Ddah1	BC034505	dimethylarginine dimethylaminohydrolase 1
Dgkz	BC014860	diacylglycerol kinase zeta
Dpysl3	NM_009468	dihydropyrimidinase-like 3
Enpp2	NM_015744	ectonucleotide pyrophosphatase/phosphodiesterase 2
Enpp5	NM_032003	ectonucleotide pyrophosphatase/phosphodiesterase 5
Fabp7	NM_021272	fatty acid binding protein 7, brain
Gad1	NM_008077	glutamic acid decarboxylase 1
Gatm	NM_025961	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
Gcnt2	AK077598	glucosaminyltransferase, I-branching enzyme
Glul	NM_008131	glutamate-ammonia ligase (glutamine synthase)
Got1	NM_010324	glutamate oxaloacetate transaminase 1, soluble
Hk1	J05277	hexokinase 1
Hnk-1	NM_145142	HNK-1 sulfotransferase
Mat2a	NM_145569	methionine adenosyltransferase II, alpha
Pld3	NM_011116	phospholipase D3
Ptgds	BC038083	prostaglandin D2 synthase (21 kDa, brain)
	BU604722	ESTs, highly similar to O Chain O, beta-galactosidase (Chains I-P)

See legend to Table 1 for details.

Table 4. *Functional categories of genes highly expressed in the nervous system: RNA metabolism and transcription; and Membrane structure and function*

Common	GenBank	Description
	22 RNA metabolism and transcription	
Af1q-pending	NM_019914	ALL1-fused gene from chromosome 1q
AW060752	AK046510	a putative chromodomain helicase DNA binding protein 3
Bcl11a	NM_016707	B-cell CLL/lymphoma 11A (zinc finger protein)
Cirbp	NM_007705	cold inducible RNA binding protein
Cri1	NM_025613	CREBBP/EP300 inhibitory protein 1
Cugbp2	NM_010160	CUG triplet repeat, RNA binding protein 2
Gcn5l2	NM_020004	general control of amino acid synthesis-like 2 (yeast)
Hrmt1l1	NM_133182	heterogeneous nuclear ribonucleoprotein methyltransferase-like 1
Idb4	NM_031166	inhibitor of DNA binding 4
Klf9	AK028544	Kruppel-like factor 9
Lrrn1	NM_008516	leucine rich repeat protein 1, neuronal
Mef2c	BC037731	myocyte enhancer factor 2C
Myt1l	NM_008666	myelin transcription factor 1-like
Ndr3	NM_013865	N-myc downstream regulated 3
P37nb-pending	AK018071	37-kDa leucine-rich repeat (LRR) protein
Pcbp4	NM_021567	poly(rC) binding protein 4
Peg3	AB003040	paternally expressed 3
Rbm9	AK044929	RNA binding motif protein 9
Tcf1	NM_009327	transcription factor 1
Tcf4	NM_013685	transcription factor 4
Zfp238	NM_013915	zinc finger protein 238
Zfp261	NM_019831	zinc finger protein 261
	25 Membrane structure and function	
Abca2	NM_007379	ATP-binding cassette, subfamily A (ABC1), member 2
Ap1m1	NM_007456	adaptor-related protein complex AP-1, mu subunit 1
Atp1a2	BC025807	ATPase, Na^+-K^+ transporting, alpha 2 polypeptide
Atp1b1	AK010677	ATPase, Na^+-K^+ transporting, beta 1 polypeptide
Atp6v0c	NM_009729	ATPase, H^+ transporting, V0 subunit C
Atp6v1d	NM_023721	ATPase, H^+ transporting, V1 subunit D
Cacnb1	NM_031173	calcium channel, voltage-dependent, beta 1 subunit
Cacnb3	NM_007581	calcium channel, voltage-dependent, beta 3 subunit
Cdh13	NM_019707	cadherin 13
Cdh2	NM_007664	cadherin 2
Ddx26	NM_008715	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26
Evi2	NM_010161	ecotropic viral integration site 2
Gpsn2	NM_134118	glycoprotein, synaptic 2
Klhl2	BC031144	kelch-like 2, Mayven (*Drosophila*)
L1cam	NM_008478	L1 cell adhesion molecule
Mbp	AK040716	myelin basic protein
Necl1-pending	AK053077	nectin-like 1
Nptxr	NM_030689	neuronal pentraxin receptor
Nup88	NM_172394	nucleoporin 88 kDa
Pcdh13	AF464160	protocadherin 13
Pmm1	AK004631	phosphomannomutase 1
Scamp5	BC018613	secretory carrier membrane protein 5
Syngr3	NM_011522	synaptogyrin 3
Thy1	NM_009382	thymus cell antigen 1, theta
Vamp2	AK090178	vesicle-associated membrane protein 2

See legend to Table 1 for details.

Table 5. *Functional categories of genes highly expressed in the nervous system: Cell differentiation, death, proliferation, and division; Protein metabolism; Extracellular message; Secretory and secretion*

Common	Genbank	Description
	23 Cell differentiation, death, proliferation, and division	
3-Sep	NM_011889	septin 3
Agtpbp1	NM_023328	ATP/GTP binding protein 1
Al256814	BU504047	Mus musculus, clone IMAGE:1397659, mRNA
Bad	NM_007522	Bcl-associated death promoter
Bm88-pending	NM_021316	BM88 antigen
Deaf1	NM_016874	deformed epidermal autoregulatory factor 1 (*Drosophila*)
Dlgh4	NM_007864	discs, large homolog 4 (*Drosophila*)
Efnb3	AK048305	ephrin B3
Elavl4	AK014133	ELAV (embryonic lethal, abnormal vision)-like 4
Evl	NM_007965	Ena-vasodilator stimulated phosphoprotein
Fnbp1	NM_019406	formin binding protein 1
Fyn	NM_008054	Fyn proto-oncogene
Habp4	NM_019986	hyaluronic acid binding protein 4
Maged1	NM_019791	melanoma antigen, family D, 1
Mal	NM_010762	myelin and lymphocyte protein, T-cell differentiation protein
Meg3	NM_144513	maternally expressed gene 3
Mmd2	BC025064	monocyte to macrophage differentiation-associated 2
Ncdn-pending	NM_011986	neurochondrin
Ndr4	NM_145602	N-myc downstream regulated 4
Nelf	AK045384	nasal embryonic LHRH factor
Pak1	NM_011035	p21 (CDKN1A)-activated kinase 1
Slit1	AF144627	slit homolog 1 (*Drosophila*)
Usmg4	NM_031401	upregulated during skeletal muscle growth 4
	11 Protein metabolism	
Adam22	AB009674	a disintegrin and metalloprotease, domain 22
Cpe	NM_013494	carboxypeptidase
Cst3	NM_009976	cystatin C
Eef1a2	NM_007906	eukaryotic translation elongation factor 1 alpha 2
Gpm6a	NM_153581	glycoprotein m6a
Rpl27a	NM_011975	ribosomal protein L27a
Serpine2	NM_009255	serine (or cysteine) proteinase inhibitor, clade E, member 2
Slc3a1	NM_009205	solute carrier family 3, member 1
Timp2	NM_011594	tissue inhibitor of metalloproteinase 2
Uchl1	NM_011670	ubiquitin carboxy-terminal hydrolase L1
Vbp1	NM_011692	von Hippel-Lindau binding protein 1
	4 Extracellular message	
Gdf1	NM_008107	growth differentiation factor 1
Npy	NM_023456	neuropeptide Y
Ptn	NM_008973	pleiotrophin
Sst	NM_009215	somatostatin
	4 Secretory and secretion	
Psp	NM_008953	parotid secretory protein
Scg3	NM_009130	secretogranin III
Syngr3	NM_011522	synaptogyrin 3
Syt1	BC042519	synaptotagmin 1

See legend to Table 1 for details.

Table 6. *Functional categories of genes highly expressed in the nervous system: Related to brain function and/or diseases; Prion and regulation; and Others*

Common	GenBank	Description
	7 Related to brain function and/or diseases	
Aig1-pending	AF220355	acupuncture induced gene 1
Brp17	NM_019999	brain protein 17
Dscr1l2	NM_022980	Down syndrome critical region gene 1-like 2
Hap1	NM_010404	huntingtin-associated protein 1
Maged2	NM_030700	melanoma antigen, family D, 2
Sez6	NM_021286	seizure related gene 6
Ttc3	NM_009441	tetratricopeptide repeat domain
	5 Prion and regulation	
Apba2	L34676	amyloid beta (A4) precursor protein-binding, family A, member 2
Aplp1	NM_007467	amyloid beta (A4) precursor-like protein 1
App	NM_007471	amyloid beta (A4) precursor protein
Prnp	NM_011170	prion protein
Snca	NM_009221	synuclein, alpha
	6 Other	
Apg12l	NM_026217	autophagy 12-like (*S. cerevisiae*)
Elmo1	NM_080288	engulfment and cell motility 1, ced-12 homolog (*C. elegans*)
Igsf8	AF439263	immunoglobulin superfamily, member 8
Ly6a	NM_010738	lymphocyte antigen 6 complex, locus A
Ly6h	AK034884	lymphocyte antigen 6 complex, locus H
Mmp14	NM_008608	matrix metalloproteinase 14 (membrane-inserted)

See legend to Table 1 for details.

Data verification. To verify the quality of our results, we sampled 12 genes in our gene expression profile data to compare with work published in the literature (Fig. 2). We found outstanding agreements in all cases with genes encoding: synaptogyrins 2 and 3, α-synuclein and γ-synuclein, Na^+-K^+-ATPase α1 and α2, pirin, retinoid X receptor-γ (RXRγ), Slit1, Fabp7, calcium ATPase 2A (Cacna), and Jak2 (10, 12, 15, 17, 28, 47, 56, 87, 91). For example, synaptogyrin 3 is highly expressed in the nervous tissues in our study, whereas synaptogyrin 2 is expressed at a lower level in the nervous tissues compared with its expression in peripheral tissues. This finding agrees perfectly with and expands published work with Northern analysis that synaptogyrin 3 is highly expressed in the brain but almost nondetectable in heart, lung, liver, skeletal muscle, and kidney, whereas synaptogyrin 2 is highly expressed in the peripheral tissues and with very low levels in the brain (28). Similarly, we found that RXRγ is highly expressed in the *P2* brain, Str, and OlCt, a result in general agreement with the observation by in situ hybridization that RXRγ is highly expressed in the striatum and olfactory tubercle, but low in the hippocampus, hypothalamus, cerebellum, and spinal cord (91), consistent with the previous studies that RXRγ is important for dopamine D2 receptor expression (67). Furthermore, RXRγ is important in modulating locomotion and response of rodents to the addictive drug cocaine, functions related to the striatum, and dopamine receptor expression (33).

To further verify the quality of our microarray results and to provide neuroanatomical localization of some of the genes that are highly expressed in the nervous system, we confirmed the expression of three selected genes by in situ hybridization. All three genes were chosen because they are likely to be involved in signal transduction and neuroplasticity, and because they are highly expressed in the hippocampus as demonstrated by our microarray analyses. Two of the three genes were previously uncharacterized. These two genes have domains similar to G

Table 7. *Of 110 genes relevant to nervous system diseases: 21 common to the 381-gene list; 10 common to the 1,361-gene list but not in the 600-gene list; and 5 common to the 600-gene list but not in the 1,361-gene list*

Common	GenBank	Description
	21 Genes of the 110 Brain Disease genes also in the 381-gene list	
Apba2	L34676	amyloid beta (A4) precursor protein-binding, family A, member 2
Aplp1	NM_007467	amyloid beta (A4) precursor-like protein 1
App	NM_007471	amyloid beta (A4) precursor protein
ATP1A2	BC013561	H1 histone family, member 2
ATP1A2	BC013561	Similar to ATPase, Na^+-K^+ transporting, alpha 2 polypeptide
Dlgh4	NM_007864	discs, large homolog 4 (*Drosophila*)
Efnb3	NM_007911	ephrin B3
Fgd1	NM_008001	faciogenital dysplasia homolog
Fxh	NM_053104	fox-1 homolog (*C. elegans*)
Fyn	NM_008054	Fyn proto-oncogene
Gdi1	BC013758	guanosine diphosphate (GDP) dissociation inhibitor 1
Hap1	NM_010404	huntingtin-associated protein 1
Hrnbp3	AF229056	*Mus musculus* hexaribonucleotide binding protein 3 (Hrnbp3) mRNA, partial cds
Marcks	NM_008538	myristoylated alanine rich protein kinase C substrate
Marcks	NM_008538	myristoylated alanine rich protein kinase C substrate
Mapt	NM_010838	microtubule-associated protein tau
Ppp3r1	NM_024459	protein phospatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)
Prkacb	NM_011100	protein kinase, cAMP dependent, catalytic, beta
Prnp	NM_011170	prion protein
Snca	NM_009221	synuclein, alpha
Uchl1	NM_011670	ubiquitin carboxy-terminal hydrolase L1
	10 Genes of the 110 Brain Disease genes in the 1,361-gene list but not in the 600-gene list	
Bbs2	NM_026116	Bardet-Biedl syndrome 2 (human)
L1cam	NM_008478	L1 cell adhesion molecule
Mecp2	NM_010788	methyl CpG binding protein 2
Mecp2	NM_010788	methyl CpG binding protein 2
Msh2	NM_008628	mutS homolog 2 (*E. coli*)
Myo5a	NM_010864	myosin Va
Ncam1	X15052	neural cell adhesion molecule 1
Nckap1	X61453	NCK-associated protein 1
Ophn1	NM_052976	oligophrenin 1
Sorbs1	NM_009166	sorbin and SH3 domain containing 1
	5 Genes of the 110 Brain Disease genes in the 600-gene list but not in the 1,361-gene list	
Atp2a2	NM_009722	ATPase, Ca^{2+} transporting, cardiac muscle, slow twitch 2
Baiap2	NM_130862	brain-specific angiogenesis inhibitor 1-associated protein 2
C80751	BG066874	expressed sequence C80751
Cacna1a	NM_007578	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
Sncg	NM_011430	synuclein, gamma

For Tables 7 and 8, 21 genes are in the 381 (ANOVA + 3X_any) gene list, 10 are in the 1,361 (3X) but not the 600 (ANOVA) gene list, 5 are in the 600-gene list but not the 1,361-gene list (Table 7); and 74 are in the 7,154-gene list that are neither in the 1,361-gene list nor the 600-gene list (see Table 8).

protein-coupled receptors, and a SH3 domain, respectively, but their localization and function are unknown.

Dickkopf family of secreted proteins is involved in Wnt signaling, which is critical in many developmental processes (27). The SH3 domain-containing proteins and G protein-coupled receptors are involved in intracellular signal transduction (38, 69). We performed in situ hybridization with mouse dickkopf homolog 3 (Dkk3, NM_015814, Fig. 3, *A–E*), a putative G protein-coupled receptor (AB041649, Fig. 4, *A–G*), and an SH3 domain-containing protein (W29432, Fig. 5, *A–I*). Dkk3 and the putative G protein-coupled receptor are in the 381-gene list, but the SH3 domain-containing protein is in the 600-gene list but not in the 381-gene list. Our in situ hybridization results verified our microarray results and provided additional neuroanatomical description regarding the distribution of these gene products in the brain. Of particular interest, although all three genes express highly in the hippocampal formation, their expression patterns in the subregions of the hippocampal formation are different. Dkk3 expression is high in CA1–CA3 and low in dentate gyrus. The expression of the putative G protein-coupled receptor gene is high in the CA1 and the dentate gyrus, while low in CA3. The SH3 domain-containing protein gene is high in all regions of the hippocampal formation. Moreover, our results that Dkk3 is highly expressed in the cortex, hippocampus, and brain stem are consistent with a previously published work (34) and suggest a role of Dkk3 in neuroplasticity in the cortex and hippocampus. The putative G-protein-coupled receptor is also highly expressed in the cortex, hippocampus, striatum, and midbrain. The SH3 domain-containing protein is also expressed in the cortex and hippocampus, but in addition, it is expressed in the striatum and amygdala. Its exclusion from the 381-gene list may due to its low expression in the DRG and high expression in the testis as shown by our microarray study and previous Northern analyses (26).

Functional classification of genes highly expressed in adult nervous tissues. Neurons and supporting tissues in the nervous system are specialized in integrating internal and external stimuli and coordinating response. Neuronal excitability and synaptic transmission are important aspects of nervous system functions and require extensive signaling events and structural support, as well as constant synthesis of various intracellular and extracellular molecules. To investigate genomic commitment to nervous tissue functions, we classified known genes from the 381-gene list according to their involvement in different biological functions (Tables 1–6). Diverse functional categories were represented in the 381 genes, including those important in intracellular signaling (Table 1); cytoskeleton function (Table 2); enzymatic activities (Table 3); RNA metabolism and transcription, and membrane structure and function (Table 4); cell differentiation, death, proliferation, and division, protein metabolism, extracellular message, secretory and secretion (Table 5); brain function and disease, prion and regulation, and others (Table 6) (also see DISCUSSION). Many of the 381 genes have not been previously recognized as genes having nervous system-specialized functions. Finding the high levels of expression of these genes in the nervous system may help in identifying the roles they engage in nervous system differentiation, maintenance, and plasticity.

Abnormalities of genes that are highly expressed in the nervous tissues contribute to neurological diseases development. Determining tissue-specific and developmental stage-specific gene expression profiles in normal, healthy organisms is important for elucidation of mechanisms of pathogenesis of these diseases. We searched Online Mendelian Inheritance in Man (OMIM), University of California at San Francisco (UCSF), and the Human Gene Mutation Database (HGMD) sites and identified 110 known genes related to mental retardation, neurological disease, and neurodegeneration published in literature (Tables 7 and 8). These 110 genes were divided into four categories according to their expression patterns as shown in the Venn diagram in Fig. 1*C* (Tables 7 and 8).

We found that many of these 110 genes are highly expressed in nervous tissues. Twenty-one of the 110 genes are within the 381-gene list, much more than the predicted 5 that would be found purely by random sampling (Fig. 6). Because of the high representation of disease-relevant genes in the 381-gene list, and because high levels of expression infer relevance in function, the other genes in the 381-gene list may also be involved in aspects of neurological and psychiatric disease development and modulation.

The relatively large number of the genes expressed in the microarray also allowed us to identify genes that are coordinately expressed with those genes implicated in neurological diseases. For example, we found that genes for huntingtin-associated protein 1 (HAP1) and discs large homolog 4 are coordinately expressed. A few uncharacterized ESTs are coordinately expressed with genes implicated in diseases (AK018148 and amyloid-β precursor protein, AK013636 and ubiquitin carboxy-terminal hydrolase L1, AK018316 and α-synuclein). Since coordinately expressed genes may modulate one another's functions in the same tissues, the information provided by our database lays a foundation for further investigation of how the general and tissue-specific expression of genes in the nervous system contribute to the development of neurological diseases.

DISCUSSION

Using microarray technology, we have analyzed the expression profiles of 8,734 genes in 10 regions of the nervous system in the context of 30 peripheral organs. The large database of gene expression profiles in the nervous system and 30 peripheral organs is part of a continuing consortium effort of the University of Cincinnati and Children's Hospital Research Foundation to develop a microarray database in which all poly(A)$^+$ RNA samples were obtained and analyzed under identical experimental conditions and normalized to a single reference sample. Other studies using the University of Cincinnati-Children's Hospital Medical Center Mouse Tissue Specific Gene Expression Database described gene expression in liver development and regeneration (29), in anatomical segments of the gastrointestinal tract (2), and in the olfactory mucosa (14).

Gene expression varies among individuals and strains (8). We have used pools of mice to carry out the microarray experiments. We also used biological duplicate, i.e., poly(A)$^+$ RNA isolated from different sets of mice, for the duplicated microarray studies. Therefore, the genes expression differences we observe are not due to individual variations. In addition, we used C57BL/6 strains for all our analyses, thus eliminating

Table 8. *Of 110 genes relevant to nervous system diseases: 74 are common to the 7,154-gene list that are neither in the 1,361-gene list nor the 600-gene list*

Common	GenBank	Description
	74 Genes of the 110 Brain Disease genes not in the 600-gene list nor the 1,361-gene list	
A2m	NM_007376	alpha-2-macroglobulin
Abcd3	BC009119	ATP-binding cassette, subfamily D (ALD), member 3
Adsl	AH005408	ESTs, Weakly similar to NED4 MOUSE NEDD-4 PROTEIN [*M. musculus*]
Aldh3a2	NM_007437	aldehyde dehydrogenase family 3, subfamily A2
Als2	AB053307	amyotrophic lateral sclerosis 2 (juvenile) homolog (human)
Apaf1	NM_009684	apoptotic protease activating factor 1
Aplp2	NM_009691	amyloid beta (A4) precursor-like protein 2
Aplp2	NM_009691	amyloid beta (A4) precursor-like protein 2
Arhgef6	AK006635	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6
Bckdhb	L16992	branched chain ketoacid dehydrogenase E1, beta polypeptide
Blm	NM_007550	Bloom syndrome homolog (human)
Bsn	NM_007567	bassoon
Cebpa	BC011118	CCAAT/enhancer binding protein (C/EBP), alpha
Cebpa-rs1	NM_009882	CCAAT/enhancer binding protein alpha (C/EBP), related sequence 1
Cebpd	NM_007679	CCAAT/enhancer binding protein (C/EBP), delta
Cln2	NM_009906	ceroid-lipofuscinosis, neuronal 2
Cln2	NM_009906	ceroid-lipofuscinosis, neuronal 2
Cln3	NM_009907	ceroid lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease)
Cpo	NM_007757	coproporphyrinogen oxidase
Cpo	NM_007757	coproporphyrinogen oxidase
Cript-pending	NM_019936	postsynaptic protein Cript
Dhcr7	NM_007856	7-dehydrocholesterol reductase
Dia1	NM_029787	diaphorase 1 (NADH)
Dlgh1	NM_007862	discs, large homolog 1 (*Drosophila*)
Dnajb1	NM_018808	DnaJ (Hsp40) homolog, subfamily B, member 1
Eif2ak3	NM_010121	eukaryotic translation initiation factor 2 alpha kinase 3
Ercc2	NM_007949	excision repair cross-complementing rodent repair deficiency, complementation group 2
Fmr1	NM_008031	fragile X mental retardation syndrome 1 homolog
Gart	NM_010256	phosphoribosylglycinamide formyltransferase
Gart	NM_010256	phosphoribosylglycinamide formyltransferase
Gba	NM_008094	acid beta glucosidase
Hprt	NM_013556	hypoxanthine guanine phosphoribosyl transferase
Igf1	NM_010512	insulin-like growth factor 1
Itm2b	NM_008410	integral membrane protein 2B
LOC231691	NM_145565	similar to L-serine dehydratase; L-threonine deaminase
Lrp10	NM_022993	low-density lipoprotein receptor-related protein 10
Magel2	NM_013779	melanoma antigen, family L, 2
Man2b1	NM_010764	mannosidase 2, alpha B1
Msh2	NM_008628	mutS homolog 2 (*E. coli*)
Ntf3	NM_008742	neurotrophin 3
Nufip1	NM_013745	nuclear fragile X mental retardation protein interacting protein
Ogdh	BC013670	oxoglutarate dehydrogenase (lipoamide)
Pah	NM_008777	phenylalanine hydroxylase
Pccb	NM_025835	propionyl CoA carboxylase, beta polypeptide
Pccb	NM_025835	propionyl CoA carboxylase, beta polypeptide
Pdha1	BC007142	pyruvate dehydrogenase E1 alpha 1
Prkar1b	BC011424	protein kinase, cAMP dependent regulatory, type 1 beta
Prps1	NM_021463	phosphoribosyl pyrophosphate synthetase 1
Psen1	NM_008943	presenilin 1
Psen2	NM_011183	presenilin 2
Psen2	NM_011183	presenilin 2
Psen2	NM_011183	presenilin 2
Pts	NM_011220	6-pyruvoyl-tetrahydropterin synthase
Rad23a	NM_009010	RAD23a homolog (*S. cerevisiae*)
Rb1	NM_009029	retinoblastoma 1
Sap	NM_011318	serum amyloid P-component
Sds	NM_133902	serine dehydratase
Six3	D83144	sine oculis-related homeobox 3 homolog (*Drosophila*)
Slc1a2	NM_011393	solute carrier family 1, member 2
Slc25a15	NM_011017	solute carrier family 25 (mitochondrial carrier; ornithine transporter), member 15
Smarca5	NM_053124	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5
Smpd1	NM_011421	sphingomyelin phosphodiesterase 1, acid lysosomal
Sod1	BC002066	superoxide dismutase 1, soluble
Sox3	NM_009237	SRY-box containing gene 3
Tcn2	NM_015749	transcobalamin 2
Tgif	NM_009372	TG interacting factor

Continued

Table 8.—*Continued*

Common	GenBank	Description
	74 Genes of the 110 Brain Disease genes not in the 600-gene list nor the 1,361-gene list (Continued)	
Timm8a	NM_013898	translocase of inner mitochondrial membrane 8 homolog a (yeast)
Timm8a	NM_013898	translocase of inner mitochondrial membrane 8 homolog a (yeast)
Tsc2	NM_011647	tuberous sclerosis 2
Ubh1	AK006739	ubiquitin hydrolyzing enzyme 1
Usp14	NM_021522	ubiquitin specific protease 14
	BI692155	ESTs, highly similar to T42731 atrophin-1 related protein-rat [*R. norvegicus*]
	BI526719	ESTs, moderately similar to CABI_RAT calcineurin-binding protein Cabin 1 (CAIN)
	BI526719	ESTs, moderately similar to CABI_RAT calcineurin-binding protein Cabin 1 (CAIN)

See legend to Table 7 for details.

strain variations. Our result may not be identical to results from other strains of mice.

We established a working group of 381 genes that are the most highly expressed in the nervous system based on statistical significance between nervous and peripheral tissues and based on their levels of expression in at least 1 nervous tissue over the mean expression levels. The entire set of 8,734 genes can be found in *Data archive* in MATERIALS AND METHODS. Of all the genes in the NIH Mouse Brain Molecular Anatomy Project (BMAP), 9,237 genes had RefSeq or RepAccNum identification numbers that can be used to compare with the Incyte cDNA microarray gene set. Of the 9,237 genes, 1,683 are present in the mouse GEM1 Incyte cDNA microarray used in this study. Of those 1,683 genes, 370 of these are expressed at a level that is at least twofold or higher in at least one nervous tissue, and 124 are in the 381-gene list. The lists of 1,683, 370, and 124 genes can be found in our microarray database, ZhangEtalBrain2004 subdirectory. Of note, many of the BMAP genes are not nervous system enriched. And many of the genes in the Incyte cDNA mouse GEM1 array that are highly enriched in the nervous system are not contained in the BMAP. Compared with the BMAP genes, we have identified 257 new genes (381 minus 124) that are both statistically highly expressed in the nervous system compared with other organs and expressed at least threefold in one or more regions of the nervous system compared with other organs.

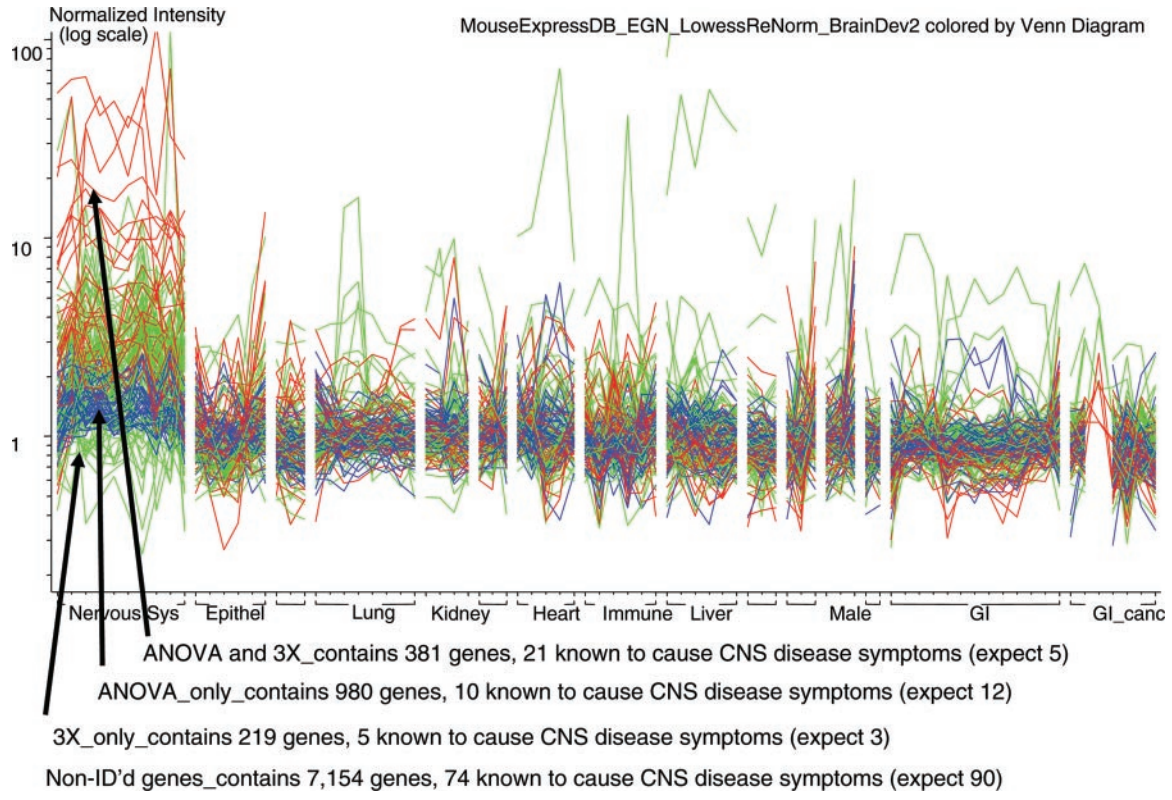

Fig. 6. Genes highly expressed in the nervous system are likely to be relevant to nervous system diseases. The color is in accordance with that in Fig. 1*C*. Of a total of 110 genes suggested to be relevant to nervous system diseases, 21 are in the group of 381 (red). Five genes were expected to be within the 110-gene list for 381 random genes. Ten of the 110 disease-relevant genes are within the 980 gene group (blue). Twelve genes were expected to be within the 110-gene list for 980 random genes. Five of the 110 disease-relevant genes are within the 219 gene group (green). Three genes were expected to be within the 110-gene list for 219 random genes. Not shown in the charts are 74 of the 110 disease-relevant genes that are not in the nervous system-enriched gene list. Ninety genes were expected to be within the 110-gene list for 7,154 random genes.

Table 9. *Evolutionarily conserved genes within the 381-genes list that are highly expressed in nervous tissues*

Common	GenBank	Description
Apg12l	NM_026217	autophagy 12-like (*S. cerevisiae*)
Deaf1	NM_016874	deformed epidermal autoregulatory factor 1 (*Drosophila*)
Dkk3	NM_015814	dickkopf homolog 3 (*Xenopus laevis*)
Dlgh4	NM_007864	discs, large homolog 4 (*Drosophila*)
Elavl4	AK014133	ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 4 (Hu antigen D)
Elmo1	NM_080288	engulfment and cell motility 1, ced-12 homolog (*C. elegans*)
Freq	NM_019681	frequenin homolog (*Drosophila*)
Gcn5l2	NM_020004	general control of amino acid synthesis-like 2 (yeast)
Hrmt1l1	NM_133182	heterogeneous nuclear ribonucleoprotein methyltransferase-like 1 (*S. cerevisiae*)
Klhl2	BC031144	kelch-like 2, Mayven (*Drosophila*)
Nell2	NM_016743	nel-like 2 homolog (chicken)
Slit1	AF144627	slit homolog 1 (*Drosophila*)
Ttyh1	AK018148	tweety homolog 1 (*Drosophila*)
	BU604722	ESTs, highly similar to O Chain O, beta-galactosidase (chains I-P) [*E. coli*]

Genes from diverse functional categories are represented in the nervous system. Within the group of genes encoding intracellular signaling, RhoN, Rab3a, Rab3b, Rab6, and RabJ are highly expressed in the nervous system, consistent with a role of Ras signaling pathway in neuronal development, synaptic plasticity, and growth and differentiation of oligodendrocyte progenitors, as well as amyloid protein secretion (4, 31, 46). Protein kinase A subunits (Cβ, Rα, and Rβ) are highly expressed in the nervous system, and are involved in synaptic plasticity, neurite initiation, and ligand and voltage-gated channel function, as well as regulation of apoptosis (11, 22, 45, 60, 83). Frequenin homolog is a neuronal calcium sensor protein that may be involved in synaptic plasticity (13).

In the family of cytoskeleton and cytoskeleton binding proteins, adducin, drebrin, tau, Marcks, profilin, and tubulin are highly expressed in the nervous system, consistent with a crucial role in neuroplasticity (1, 21, 32, 43, 48, 66, 71, 74). Furthermore, these are particularly highly expressed in postnatal *day 2* brains (Tables 1–6), suggesting that these gene products are involved in rapid expansion of neurons in the nervous system at early developmental stages (24). Neurofilament light chain protein is particularly highly expressed in the DRG, consistent with a role in nerve regeneration after injury (3, 81).

In the family of genes involved in protein metabolism, Von Hippel-Lindau binding protein (Vbp1) participates in the formation of an active E3 ubiquitin ligase complex, which is important for hypoxia-inducible factor-1 protein degradation (23, 49). Vbp1 may be involved in neuronal differentiation of central nervous system progenitor cells (Moseley A, Jegga AJ, Gupta A, Sartor M, Williams SS, Ley-Ebert C, Coolen L, Egnaczyk G, Genter MB, Lehman M, Lingrel J, Maggio J, Parysek L, Walsh R, Xu M, Zhang J, and Aronow BJ; unpublished observations), as well as oxygen sensing in ischemia/hypoxia-induced brain conditions (7, 72, 80).

In the category of genes encoding secreted extracellular messengers, growth differentiation factor 1 (Gdf1), neuropeptide Y, pleiotrophin, and somatostatin are highly expressed in the nervous system. Gdf1 is a member of the transforming growth factor-β (TGF-β) superfamily capable of regulating cell growth and differentiation and was previously shown to be highly expressed in the nervous system (39, 62). Neuropeptide Y and somatostatin are neuropeptides important for modulating neurotransmission (82). Pleiotrophin is a heparin binding protein important for neurite outgrowth, neuroplasticity, and neuronal survival (20, 42).

Genes that are highly conserved in evolution may be required for viability or other fundamental functions of the cell. We identified 17 of the 381 genes that have highly conserved homologs in nonmammalian organisms and show higher levels of expression in the nervous system compared with most peripheral tissues in mammals (Table 9). Consistent with their high conservation, these genes encode proteins of fundamentally important biological functions, such as signal transduction, RNA metabolism, cell differentiation, intercellular communication, and transcription. The expression profiles of these genes in the nervous system, along with their possible cellular functions open up new avenues for studying the functions of these evolutionarily conserved genes. For example, Gcn5l2 is also known as p300/CBP-associated factor (P/CAF), which has a lower eukaryotic homolog. P/CAF has an intrinsic histone acetylase activity, binds to E1A and Twist, and plays a role in regulation of gene expression (18). Furthermore, P/CAF is required for mouse embryogenesis (90). Whether histone acetylase activity is required for various adult neuronal activities in the mammalian nervous system will be interesting to investigate.

We observed high representation of genes involved in neurological diseases in the 381-gene list. Twenty-one genes of the 381 were within the 110-gene list known to cause neurological disease symptoms, whereas the expected number from the 381-gene list to overlap with the 110-gene list is only 5. The 21 genes include amyloid-β precursor protein and amyloid-β precursor binding protein, which are involved in Alzheimer disease (70); huntingtin-associated protein 1, which is involved in Huntington disease (40, 41); and ubiquitin carboxy-terminal hydrolase L1 and α-synuclein, which are involved in Parkinson disease (36, 44). Furthermore, many genes in the 381-gene group may physically and functionally interact with those genes whose mutation or mal-expression directly predispose to diseases, thus contributing to disease mechanisms. Alternatively, a dysregulation of genes in the 381 list may provide new understanding of mechanisms in disease development and progression. For example, genes in the Ras signaling pathway are involved in development of neurofibroma by regulating neurofibromatosis type 1 (NF1) function (4). The cytoskeletal drebrin and the secreted pleiotrophin are involved in Alzheimer disease and Down syndrome by regulating synaptic plasticity (19, 20, 42, 73, 89). Maged2 is in a chromosomal hot spot for involvement in mental retardation (35). Acupuncture-induced gene 1 may be involved in regulation of acute and chronic pains. Analysis of the remaining of the 381 genes will help identify more neurological disease-associated genes and enhance our understanding of disease development.

Some of the genes implicated in neurological diseases are expressed in multiple tissues, including some in olfactory epithelium or peripheral blood (77, 78, 79). For example, we have confirmed that α-synuclein and Thy1 are two proteins expressed highly both in nervous tissues and in peripheral

blood. Profiling genes implicated in nervous system diseases in multiple nervous tissues and in various peripheral tissues where easier sampling and gene profiling can be obtained will aid the development of better strategies for diagnosis.

Our study provides a unique opportunity to examine genes that are highly expressed in nervous tissues compared with other peripheral tissues. We provide in this study a foundation of information for many researchers to use to investigate many diverse neurobiological problems. This is particularly relevant to the study of neurological disease in both humans as well as in animal models. Further study is warranted to seek the identification of region-specific gene regulation in the nervous system and transcription regulatory elements involved in region-specific gene expression.

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