Regional and strain-specific gene expression mapping in the adult mouse brain

Rickard Sandberg*†, Rie Yasuda†‡, Daniel G. Pankratz*, Todd A. Carter*, Jo A. Del Rio§, Lisa Wodicka§, Mark Mayford‡, David J. Lockhart§, and Carrolee Barlow*¶

*The Salk Institute for Biological Studies, The Laboratory of Genetics, 10010 North Torrey Pines Road, La Jolla, CA 92037; ‡Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093-0691; and §Genomics Institute of the Novartis Research Foundation, 3115 Merryfield Row, San Diego, CA 92121

Edited by Charles F. Stevens, The Salk Institute for Biological Studies, La Jolla, CA, and approved July 12, 2000 (received for review June 6, 2000)

To determine the genetic causes and molecular mechanisms responsible for neurobehavioral differences in mice, we used highly parallel gene expression profiling to detect genes that are differentially expressed between the 129SvEv and C57BL/6 mouse strains at baseline and in response to seizure. In addition, we identified genes that are differentially expressed in specific brain regions. We found that approximately 1% of expressed genes are differentially expressed between strains in at least one region of the brain and that the gene expression response to seizure is significantly different between the two inbred strains. The results lead to the identification of differences in gene expression that may account for distinct phenotypes in inbred strains and the unique functions of specific brain regions.

seizure | C57BL/6 | 129SvEv | oligonucleotide array | amygdala

Neurobehavioral studies have advanced substantially through the use of mouse genetics. Many studies have shown that inbred mouse strains exhibit significant variation in several central nervous system (CNS) phenotypes. For example, despite similar seizure susceptibility, inbred strains exhibit large differences in neuronal cell death after seizures (1) vary greatly in their behavioral response to drugs of addiction, and show marked differences in behavioral testing (for a review see ref. 2). With the advent of highly parallel gene expression studies using DNA arrays (3–5), it is now possible to ask the questions: what is the interacting set of genes that account for the differences between inbred mouse strains and which genes are responsible for the unique structures and functions of specific brain regions? We have applied gene expression profiling of multiple brain regions in two commonly used inbred strains that differ in their neurobehaviorial phenotypes, the 129SvEv and C57BL/6 strains (for a review see ref. 6 and for revised nomenclature of 129 strains see ref. 7).

We determined the number and pattern of genes that are differentially expressed in multiple brain regions in these strains of mice and in response to seizure.

Materials and Methods

Animal Use and Tissue Collection. All animal procedures were performed according to protocols approved by The Salk Institute for Biological Studies Animal Care and Use Committee. Male C57BL/6 and 129SvEv mice were purchased from Taconic Farms at an age of 7 weeks and housed individually for 1 week before death. Two samples were prepared and analyzed from different mice for each strain. For animals used in the seizure analysis, pentylenetetrazol solution was administered s.c. at a dose of 50 mg/kg. All animals had a similar seizure response as assessed by using standard criteria (8). Animals were killed 60 min after seizure. Dissections were done between 14.00–17.00 h on wet ice covered with parafilm. Cortical dissections included the entire cortex except olfactory bulbs. The midbrain consists of the brain dissected free of cortex, pons and medulla. Cortex, cerebellum, midbrain, and hippocampus were prepared in duplicate from two different mice of each strain. To obtain sufficient tissue from amygdala and entorhinal cortex, the microdissected regions of seven animals were pooled. Dissected tissue was directly frozen on dry ice and stored at −80°C. Mouse embryonic fibroblasts were prepared for each strain according to standard protocols from six embryos at day 13.5 (9).

RNA Preparation/Northern Blot Analysis. Tissues were placed into TRIzol (GIBCO/BRL) (added to the frozen tissues at approximately 1 ml per 100 mg tissue) and homogenized (Polytron, Kinematica, Lucerne, Switzerland) at maximum speed for 90–120 sec. Subsequent steps were done according to the manufacturer's instructions. Labeling of samples, hybridization, and scanning were performed as described (4). Northern blot analysis was performed by using 20 μg of total RNA, and probes were derived from random priming of 500–700 base pair fragments derived from expressed sequence tags available from I.M.A.G.E. consortium. Blots were scanned with a PhosphorImager (Molecular Dynamics).

Gene Expression Analysis. Two different arrays (GeneChip, Affymetrix, Santa Clara, CA) were used that together represent 13,069 probe sets corresponding to more than 10,000 genes and expressed sequence tags (Mu11KsubA and Mu11KsubB). Data analysis was performed by using GENECHIP version 3.1 (Affymetrix) and NFUEGGO 2.1C (Lockhart and Lockhart, San Diego). We used the GENECHIP software global scaling algorithm to compare all 24 samples (48 total arrays, 24 SubA and 24 SubB arrays). We scaled all samples to a target intensity of 200. A target intensity of 200 has been shown to correspond to ≈3–5 transcripts per cell (4).

All strain variation analyses were done by comparing C57BL/6 to 129SvEv. To generate data for Fig. 1 and Table 1, all C57BL/6 samples were compared with all 129SvEv samples (24 experiments, six regions per strain prepared and performed in duplicate used to generate 12 comparison files). The criteria used to detect differences in gene expression were a fold change of 1.8 or greater and a difference call (as described in the GENECHIP software) of increase, marginal increase, decrease, or marginal decrease and a signal change greater than 50 in 8/12 comparisons. For data in Table 3, which is published as supplemental data on the PNAS web site, www.pnas.org, the duplicate C57BL/6 samples were compared with those from the corresponding duplicate 129SvEv brain region and the same criteria were used (for 3/4 comparisons). To determine expression differences in response to seizure the same criteria (for 4/4 comparisons) were used.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: CNS, central nervous system; MEF, mouse embryonic fibroblast.

See commentary on page 10676.

†R.S. and R.Y. contributed equally to this work.

¶To whom reprint requests should be addressed. E-mail: barlow@salk.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

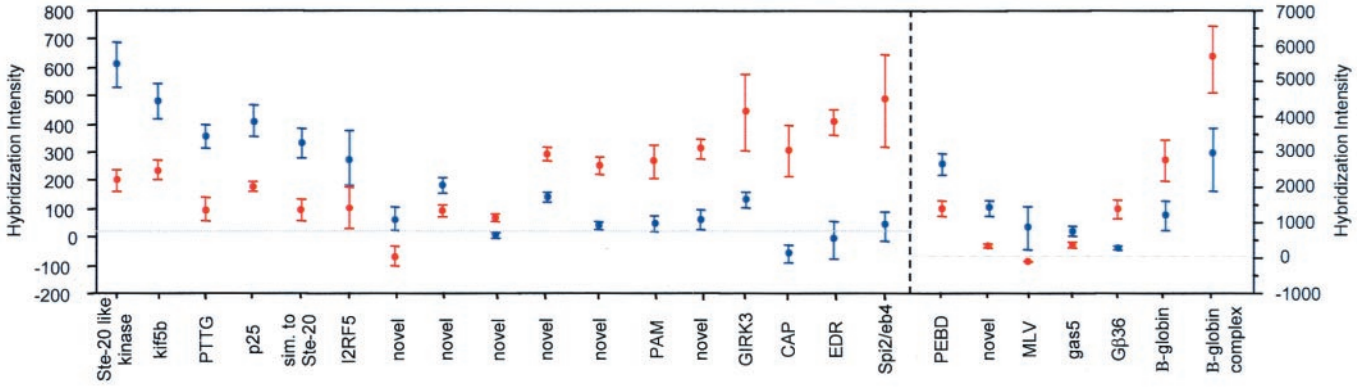

Fig. 1. Gene expression differences between C57BL/6 and 129SvEv mouse strains. Shown are the hybridization signals of the 24 genes differentially expressed in all brain regions between C57BL/6 and 129SvEv mouse strains. Each gene is represented by a mean value based on the hybridization intensity from the 12 individual samples for each strain (six brain regions in duplicate) (blue circles represent C57BL/6 and red circles 129SvEv). The *y* axis is labeled with the hybridization intensities ranging from −200 to 800 (*Left*) and −1,000 to 7,000 (*Right*) separated by a hatched vertical line. The horizontal line indicates the noise level. Error bars represent the 95% confidence interval derived from the 12 values for the six different brain regions for each strain.

Brain Region Gene Identification. To identify genes with region-restricted expression patterns, genes were classified as present in a region if the gene had a call of present in at least three of four samples. Similarly, to classify genes as clearly not detected, we used a call of absent in four of four brain samples (absent or expression at levels below the threshold of detection). The signal from one brain region was compared with all other brain regions and genes with significant differences were included ($P < 0.05$ by using a Student's t test). These data were used to generate Venn diagrams representing overlapping and nonoverlapping gene expression patterns (see Fig. 4).

To detect region-specific variation (both restriction and enrichment), the standard criteria above were used, with the additional criterion that the gene must be scored as present in at least 80% of the comparisons (e.g., in comparison of amygdala to cerebellum, midbrain, hippocampus, entorhinal cortex, 14 of the total 16 samples had to be in agreement). Genes were classified as (*i*) restricted/highly enriched if they were called absent in all other regions, (*ii*) enriched if detected in all other regions but with higher levels in the region in question, (*iii*) decreased if detected in all other regions but lower in the region in question, and (*iv*) not detected if scored as absent in all four samples but present in another region. Note that the number of genes in Table 2 is less than the number represented in the Venn

Table 1. Strain-specific variation across all brain regions

Gene name	FC	B6	129	Accession no.	MEF
Murine leukemia virus (*pol*)	~40	12P	0P	AA097626	A
Novel	~9.0	2P	0P	C77761	A
Pituitary tumor transforming gene protein (*PTTG*)	~8.5	12P	2P	AA711028	=
Sim. Ste20-like kinase	5.4	8P	0P	W51229	A
Potassium channel β-2 subunit (*I2RF5*)	~5.0	12P	9P	U31908	A
Novel	3.8	12P	12P	AA409826	*
Ste20-like kinase	3.4	12P	9P	AA120636	*
Novel	~3.0	12P	3P	W35693	*
Dynactin subunit p25 (*p25*)	2.3	12P	12P	AA110732	*
Phosphatidylethanolamine binding protein (*PEBD*)	2.1	12P	12P	AA049118	*
Kinesin heavy chain (*kif5b*)	2.1	12P	12P	AA072168	*
Growth arrest-specific protein-5 (*Gas5*)	1.9	12P	9P	X59728	*
Erythroid differentiation regulator (*EDR*)	~−17	0P	1P	AA538477	A
Spi2 proteinase inhibitor (*spi2/eb4*)	~−17	0P	10P	M64086	A
Adenylyl cyclase-associated protein (*CAP*)	~−12	0P	12P	L12367	*
Novel	~−10	9P	12P	AA138388	*
Peptidylglycine α-amidating monooxygenase (*PAM*)	~−8.4	0P	10P	U79523	A
Novel	~−5.8	0P	9P	AA689927	*
Novel	−4.9	10P	12P	AA114725	*
G protein β 36 subunit (*Gβ36*)	−4.7	12P	12P	U29055	=
G protein coupled inward rectifier K+ channel 3 (*GIRK3*)	−2.9	2P	12P	U11860	A
β-1 globin	−2.6	12P	12P	V00722	A
β-globin complex DNA	−2.3	12P	12P	X14061	A
Novel	−2.3	12P	12P	AA674148	*

Average-fold change (FC) indicates the mean ratio of expression levels in C57BL/6 relative to 129SvEv in all comparisons (positive indicates a higher level of expression in C57BL/6, a negative number, a higher level in 129SvEv). ≈ indicates an approximation because the numerator or denominator in one of the comparisons was small relative to the noise. B6 represents the number of times a gene scored as present in the analysis of C57BL/6 samples; 129 indicates the number of times a gene scored as present in the analysis of 129SvEv samples; MEF indicates the results of the expression pattern when comparing C57BL/6 to 129SvEv fibroblast samples. ''A'', absent; *, trend in MEFs similar to brain; =, no change in expression level between the two samples.

Table 2. Genes with restricted expression or not detectable in specific brain regions

Accession no.	Gene name	Accession no.	Gene name
Cerebellum restricted/highly enriched		Cerebellum absent	
AA183544	Novel	AA183623	Novel
AA212550	Novel	AA220788	Novel
135029	NMDA receptor subunit NR2C (NMDA2C)	AA607353	Novel
M21532	PCD-5 mRNA/PCP-2	142463	Rho-GDI3
m32299	D-amino acid oxidase	m83749	Mouse D-type cyclin (CYL2)
M90388	Protein tyrosine phosphatase (70zpep)	N28171	Novel
M60596	GABA-A receptor delta-subunit	U06483	Telencephalin precursor
Z38118	Synaptonemal complex protein 1	u28217	Protein tyrosine phosphatase STEP61
X80417	MB-IRK2 mRNA	U36760	Brain factor-1 (Hfhbf1)
M90365	Plakoglobin mRNA, partial cds	U39738	P21 activated kinase-3 (mPAK-3)
L00919	Protein 4.1	u92565	Fractalkine
X61397	Carbonic anhydrase-related polypeptide	u92565	Fractalkine
W63974	Sim. B-reg. subunit of protein phosphatase 2A	U56649	Cyclic nucleotide phosphodiesterase (PDE1A2)
D13266	Glutamate receptor channel delta 2 subunit	AA017811	C kinase calmodulin binding protein (RC3)
Cortex restricted/highly enriched		Cortex absent	
u68058	Frezzled (fre)	D78572	Membrane glycoprotein
L13171	Myocyte spec. enhancer factor 2 (MEF-2C)	U61751	Vesicle associated membrane prot VAMP-1
W64596	Novel	AA002979	Na/K-ATPase beta 3 subunit
		W13136	Angiotensinogen
Midbrain restricted/highly enriched		Hippocampus absent	
AA106347	Angiotensinogen	W09664	Ca/calmodulin-dependent kinase II (e-88)
X70393	Inter-alpha-inhibitor H3 chain.	W30289	Ca/calmodulin-dependent kinase II delta (e-158)
Amygdala restricted/highly enriched		Entorhinal cortex absent	
x76653	ARP-1	U81317	Myelin-associated/oligodendrocyte basic protein
		U64572	Myelin/oligodendrocyte glycoprotein

Genes classified as restricted/highly enriched (*Left*) or not detected (absent, *Right*). Fractalkine and angiotensinogen are listed twice because they are both represented by two different probe sets on the array.

diagrams in the restricted and absent categories because stricter criteria were used. However, all genes identified in Table 2 also were identified in the analysis used to generate the Venn diagrams.

Results

Gene Expression Differences Between C57BL/6 and 129SvEv Mice. Gene expression profiles were measured for multiple brain regions in two different mouse strains (C57BL/6 and 129SvEv). The regions studied were cortex, hippocampus, amygdala, entorhinal cortex, midbrain, and cerebellum plus passage 1 mouse embryonic fibroblasts (MEFs). In total, 24 samples from six brain regions and four samples from MEFs were analyzed. Duplicate samples were prepared from different animals from each region for each strain. Of the 13,069 probe sets analyzed, 7,169 (55%) gave a hybridization signal consistent with a call of present (refs. 3 and 4 and *Materials and Methods*) in at least one brain region. This finding indicates that at least 55% of the genes covered on the murine arrays are detected in one or more areas of the adult male mouse brain.

To estimate experimental reproducibility within a strain and brain region, the number of genes that scored as differentially expressed in comparisons of all duplicate brain samples from the same strain was determined. On average, only two genes of 13,069 (0.017%) met the criteria as differentially expressed in replicate measurements (see *Materials and Methods*). To determine which genes were differentially expressed between C57BL/6 and 129SvEv mice, all C57BL/6 brain samples were compared with all corresponding 129SvEv samples by using similar conservative criteria. Twenty-four genes were identified that were differentially expressed in all six brain regions of C57BL/6 compared with 129SvEv (see Fig. 1 and Table 1). We found that the mRNA for the murine leukemia virus gene (derived from the endogenous retrovirus isolated from a C57BL/6-derived cDNA library) was detected only in C57BL/6 (10), as expected. In addition, the mRNA abundance for the *Gas5* gene was lower in the 129SvEv strain. It is known that the *Gas5* gene in 129 strains harbors mutations that alter mRNA stability (11): this stability difference likely accounts for the ≈2-fold decrease in steady-state mRNA abundance in 129SvEv compared with C57BL/6. These findings validate the approach for identifying biologically relevant gene expression differences.

We next determined which genes were differentially expressed in specific brain regions between the two strains of mice (see *Materials and Methods* for analysis criteria). A total of 73 genes were differentially expressed in at least one brain region between the two strains (73 of the 7,169 expressed genes or ≈1.0% of genes expressed in the adult male mouse brain). Twenty-four of these 73 genes were already identified and described above. The remaining 49 are listed at the web site (ftp://ftp.gnf.org/pub/papers/brainstrain/) and Table 3. A similar comparison by using MEFs showed that 115 genes were differentially expressed between the strains (0.88% of all measured genes or 1.2% of the genes expressed in MEFs). In general, genes differentially expressed between the strains in one brain region showed either a consistent trend in all other regions or were not detected in other regions in either strain (see Table 3). Only two of the 73 genes showed a pattern that was different in different regions. The level of glutathione peroxidase mRNA was lower by approximately 9-fold in the midbrain of C57BL/6 compared with the 129SvEv midbrain. By contrast, in the cerebellum the level of glutathione peroxidase was higher by a factor of more than 1.5-fold in C57BL/6. The mRNA abundance for the other gene of unknown function (Table 3) was lower by approximately 8-fold in the entorhinal cortex of C57BL/6 compared with 129SvEv. In contrast, the mRNA was more abundant by more than 1.5-fold in the cerebellum of C57BL/6. This finding suggests that the majority of genes identified as differentially expressed in one brain region between the two strains did not meet the strict criteria used in Table 1, but did show a similar trend in all other brain regions.

Northern Blot and Reverse Transcription–PCR Analysis. To test the accuracy in detecting differentially expressed genes and to

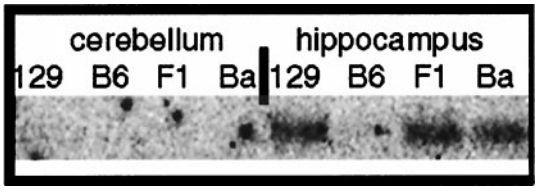

Fig. 2. Northern blot analysis. Northern blot for *spi2/eb4*. 129 indicates 129SvEv, B6 indicates C57BL/6, F1 indicates a first-generation offspring from a 129SvEV and C57BL/6 cross and Ba indicates BALB/c.

determine whether differences were unique to one or many strains, Northern blot analysis was performed on total RNA from hippocampus and cerebellum from C57BL/6, 129SvEv, first-generation offspring of C57BL/6 and 129SvEv, and BALB/c strains. The analysis was performed for genes shown in Table 1 that scored as absent in one strain but present in the other. These included *CAP*, *PAM*, *spi2*, and the gene similar to *ste20-like* kinase. We found that *spi2* was detectable in the hippocampus of the 129SvEv mouse brain but was absent in the C57BL/6 hippocampus consistent with the array results (see Fig. 2). However, this gene is expressed in the hippocampus of all other strains tested, indicating that the lack of expression in C57BL/6 is unique to this strain. Northern blot analysis was not sufficiently sensitive to detect mRNA for *CAP, PAM*, or the gene highly similar to *ste-20*. Therefore, semiquantitative reverse transcription–PCR was done by using RNA from C57BL/6 and 129SvEv. Consistent with the array results, *CAP and PAM* were more highly expressed in 129SvEv, and *ste20-like* kinase was more abundant in C57BL/6 (data not shown).

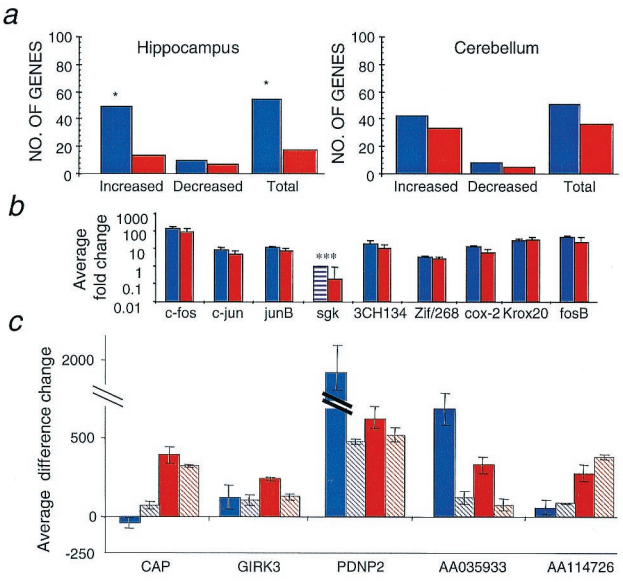

Fig. 3. Gene expression changes in response to pentylenetetrazol-induced seizure. (*a*) The total number of genes that were increased and decreased in response to seizure in C57BL/6 (blue) and 129SvEv (red) hippocampus and cerebellum. (*b*) Average fold change values for immediate-early genes in C57BL/6 (blue) and 129SvEv (red) hippocampus. The *y* axis is a log scale. Hatch indicates decreased expression. (*c*) Baseline (filled boxes) and seizure-induced hybridization signal (hatched boxes) for C57BL/6 (blue) and 129SvEv (red) hippocampus. Error bars indicate standard error. *, $P < 0.001$; ***, $P < 0.04$ using a χ^2 test.

Gene Expression Differences in Response to Seizure. The cellular response to neurotoxic insults varies between inbred strains of mice (1), and the C57BL/6 strain is resistant to seizure-induced hippocampal cell death. To test whether gene expression analysis could detect differences that correlate with known phenotypic variability in CNS response, the expression profiles of hippocampus and cerebellum in the two strains 1 h after seizure induction using pentylenetetrazol were determined. As shown, the C57BL/6 mice had a significantly greater overall transcriptional response to seizure induction (Fig. 3*a*). This was evident mainly as an increase in the number of genes induced in the hippocampus (49 in C57BL/6 compared with 12 in 129SvEv, $P < 0.001$). The transcriptional response of several known immediate-early genes was compared between the two strains (Fig. 3*b*), including members of the *fos* and *jun* family, serum and glucocorticoid-regulated kinase (*sgk*), growth factor inducible immediate early gene (3CH134), *cox-2*, and the transcription factors *KROX20* and *zif/268*. All but one of these genes, *sgk,* showed a similar level of postseizure induction in the two strains (Fig. 3*b*). Therefore, the immediate-early response to seizure is similar between the two strains whereas the overall transcriptional response is blunted in 129SvEv. It will be important to determine which of these genes account for the differential response in these strains (for the complete list of genes see ftp://ftp.gnf.org/pub/papers/brainstrain/).

To test whether genes differentially regulated at baseline might contribute to variation in seizure response, the level of induction of the genes that were differentially expressed between the two strains (for hippocampus) was assessed. Of the 32 genes differentially expressed in the hippocampus (24 from Table 1 and eight from Table 3), only seven showed a greater than 1.8-fold change in response to seizure in at least one strain (*CAP*, *GIRK3*, *MEF-2C*, and *PDNP2*, and novels AA114725, AA048853, and AA035993) and the response between the two strains was different for *CAP*, *GIRK3*, *PDNP2*, and two novel genes AA035992 and AA114725 ($P < 0.05$, Student's *t* test, Fig. 3*c*). Most of these genes, although different at baseline and differentially responsive, showed a similar directional response (Fig. 3*c*). *CAP* was the only gene repressed in 129SvEv but induced in C57BL/6. This pattern of expression suggests that changes in response to seizure for specific genes may be similar between the two strains, but that baseline differences dictate what type of transcriptional response is required.

Brain Region-Specific Differences in Gene Expression. Finally, we identified genes that were uniquely expressed or highly enriched in one brain region. To determine the likelihood of error caused by dissection inconsistency, we compared four independently obtained samples from the same brain region. No genes met the criteria for differential gene expression, indicating that mouse-to-mouse differences and dissections did not contribute significant variability in the array measurements. We next compared the expression profiles of cortex, cerebellum, and midbrain within the same strain and found that, on average, a relatively small number of genes (70/13,069 or 0.54%) showed clear differences (see *Materials and Methods* for analysis criteria). In contrast, 13.6% (1,780/13,069) of the monitored genes were differentially expressed between brain and fibroblasts, even though the two very different types of cell populations express a similar overall number of genes.

A further analysis was used to identify genes expressed uniquely in particular brain regions (see *Materials and Methods* and Fig. 4). An additional analysis was used to detect region-specific variation (both restriction and enrichment) (see Table 2 and ftp://ftp.gnf.org/pub/papers/brainstrain/), which indicates that the cerebellum appears to be the most unique region of those tested. Twenty-three genes were expressed in the cerebellum that were not detected in other regions (Fig. 4*a*) and another 28 were not expressed in cerebellum but were present in other brain regions (Fig. 4*a*). Importantly, genes such as *PCP-2*, a known cerebellar-specific gene, and *NMDA NR2C*, a known

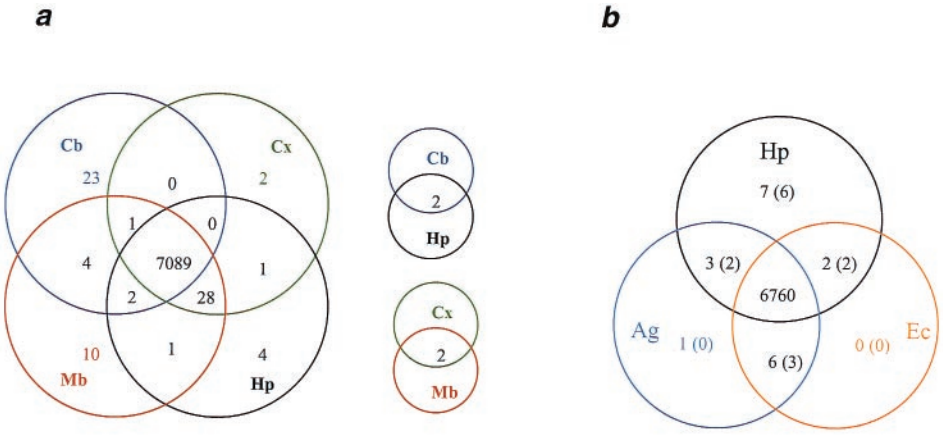

Fig. 4. Overlapping and nonoverlapping gene expression in a subset of adult mouse brain regions in both strains of mice. (*a*) Region-dependent expression patterns for cerebellum (blue-Cb), cortex (green-Cx), midbrain (red-Mb), and hippocampus (black-Hp) are represented as color-coded circles. The diagram shows the number of genes with the indicated expression patterns. For clarity, extra circles for areas not captured in the main diagram are shown on the right. (*b*) A separate Venn diagram from an analysis of hippocampus (Hp), amygdala (Ag), and entorhinal cortex (Ec). The values in parentheses represent the subset of genes identified that also were expressed in midbrain and/or cerebellum.

cerebellar-specific *N*-methyl-D-aspartate receptor subunit, were identified as being specifically expressed in the cerebellum, providing further validation of the approach. In contrast to the cerebellum, the structures of the medial temporal lobe (hippocampus, amygdala, and entorhinal cortex) showed extremely similar expression profiles. Only eight genes were unique to one of the three regions (Fig. 4*b*). Of the seven genes present in hippocampus but not amygdala or entorhinal cortex, six also were expressed outside of the medial temporal lobe (Fig. 4*b*). There was only one gene uniquely expressed in the amygdala and none in the entorhinal cortex. This finding suggests that forebrain structures, despite some functional differences, are highly similar at the molecular level. Finally, the midbrain was interesting in that, although 10 genes were uniquely expressed, no genes were exclusively absent.

The level of consistency between our expression data and published results was considerable. As shown in Table 2, 14 genes were highly enriched or restricted to the cerebellum. Of the known genes, the regional expression patterns were entirely consistent with published findings for 10 of 11 genes. Only *MB-IRK2* was inconsistent in that we were unable to detect mRNA for *IRK2* in any region except the cerebellum, whereas published reports found expression in the cortex and hippocampus, with higher levels in the cerebellum (12). The greater than 90% concordance with published results suggests that the gene expression patterns are being accurately measured in the highly parallel array-based experiments.

Discussion

We have generated a catalogue of brain region-specific gene expression differences that might contribute to the unique neurobehavioral phenotypes of these commonly used strains of mice. We determined which genes are consistently differentially expressed between these strains and also found that the two strains differ markedly in their transcriptional response to seizure. Finally, we used these data to determine brain region-specific differences in gene expression. Our findings suggest that gene expression profiling of inbred strains may be a useful tool for dissecting the molecular mechanisms of behavioral variation.

Candidate Gene Analysis. Although these data are correlative, candidate genes were identified for further study to determine their role in mediating strain-specific phenotypes. Virtually all of the known genes observed to be differentially expressed have previously defined roles in the CNS. It is interesting to speculate that the resistance to some forms of neurotoxic insults in C57BL/6 (1) is caused by the combination of a decrease in the expression of genes involved in mediating neuronal damage (*GluR1*) (13) and (*spi2/eb4*) (14, 15) and an increase in the expression of a gene that augments the cellular response to stress (*ste-20*) (16).

Gene Expression Profiling as a Method to Augment Quantitative Trait Loci Analysis. Several of the differentially expressed genes are encoded in chromosomal regions thought to harbor genes important for strain differences in CNS phenotypes (8, 17–19). Although quantitative trait loci analysis is powerful for mapping susceptibility loci to chromosome intervals, many genes reside in these large intervals, and extensive additional work is required to identify the specific gene or genes involved. Our findings suggest that an expression-based strategy is useful in identifying candidate genes responsible for quantitative traits. For example, *GIRK3* (more highly expressed in 129SvEv) is located on chromosome 1 in a region that has been shown to contain one or more of the genes that contribute to strain differences for free running period and locomotor activity (20), aspects of fear conditioned response (cued and contextual) (21, 22), open field emotionality (23), and acute pentobarbital-induced seizures (24). This gene plays a role in maintaining resting potential and in controlling excitability of the cell (25) and should be considered a candidate for involvement in modulating multiple CNS phenotypes. *PAM* (more abundant in 129SvEv) is a key bifunctional enzyme in the activation of neuropeptides (26). The gene encodes two different enzymes, peptidylglycine α-hydroxylating monooxygenase and peptidyl-α-hydroxyglycine α-amidating lyase. These enzymes function sequentially in a two-step pathway of peptide amidation. This gene maps to chromosome 1 at 57.5 cM, and an ethanol-induced loss of righting reflex locus has been mapped to chromosome 1 between 43 and 59 cM (27). Interestingly, changes in several neural peptides, such as neurotensin, have been linked to ethanol sensitivity, providing a potential link between PAM and modifications of peptides involved in mediating ethanol responses (28). Another two genes differentially expressed between the strains, *I2RF5* and a G-protein subunit, are located on distal mouse chromosome 4. This region of chromosome 4 has been linked to quantitative trait loci for alcohol drinking preference, saccharin and sucrose preference (29–32), and methyl β-carboline-3-carboxylate seizure susceptibility (33). These genes are good candidates for further study and suggests that gene expression profiling may be a useful and more rapid approach for identifying or establishing the role of a set of genes involved in a particular complex trait.

Strain Variation in Seizure Response. The seizure experiments were designed to determine which genes might contribute to strain-specific responses. Several of the genes identified as different between the two strains were genes whose expression was differentially altered in response to seizure. In addition, the marked difference in transcriptional response between these strains sug-

gests that changes in expression may account for strain variation in the cellular consequences of seizure response. We also note that very few genes were repressed (nine genes, see ftp://ftp.gnf.org/pub/papers/brainstrain/) 1 h after seizure induction. It will be interesting to study the function of the genes repressed in response to seizure at early as well as later time points and to further define their role in seizure response.

Gene Expression Profiling in Nonisogenic Strains. It is important to consider the implications of these results for studies that use nonisogenic strains of mice. Many laboratories have generated mice with targeted mutations in genes (knockouts) or overexpressed genes (transgenics) and reported novel behavioral phenotypes. The neurobehavioral phenotype of a particular mouse results not only from the specific alteration induced by a targeted mutation, but also from the effects of modifiers, which may differ significantly based on genetic background (34). We have estimated that the 129SvEv gene expression profile is significantly different ($\approx$1% of expressed genes) from that of other strains commonly used in transgenic experiments, such as C57BL/6. Use of nonisogenic mouse strains is therefore likely to produce situations where differences may be identified, but it will be difficult to know with certainty whether the differences are caused by the specific perturbation or are heavily influenced by other differences caused by variation in genetic background among nonisogenic littermates. By considering the results presented here, it should be possible to exclude genes that differ because of genetic background alone and to identify modifiers that modulate phenotypes in different genetic backgrounds.

Brain Region Gene Expression Profiling. The high concordance between our data and published results indicates that array-based gene expression profiling can be used to determine which genes are expressed and where. However, we found that it was important to use stringent analysis criteria coupled with statistical tests to ensure that the expression profiles are interpreted appropriately. As it becomes possible to use this technology for nuclei or even small cell populations in the CNS, higher-resolution, region-specific, and cell-type specific information will be gained. Studies of the regulatory elements for the uniquely expressed genes may be useful in identifying promoters that could be used to drive expression in specific cell types or tissues in animal models. By making the complete data sets available on the web, we encourage others to investigate the data to uncover more candidates for further study.

This study demonstrates the feasibility and utility of brain region expression profiling and lays the foundation for asking system-level questions. The expression results serve as a framework to begin to understand the factors responsible for the variation in phenotypes involving behavior, drug sensitivity, and neurotoxic-induced cell death. There is no doubt that advances in gene targeting technology, robust behavioral analysis, and global gene expression measurements will provide new avenues for studying the brain and further our ability to understand the interplay between the genes that give rise to complex behaviors and unique brain functions.

We thank P. G. Schultz for inspiration and support, Daniel Lockhart for the development of the NFUEGGO software, J. Hogenesch, R. Vega, and members of the Lockhart laboratory for assistance with gene expression profiling, J. A. Greenhall for assistance with PCR, K. G. Xanthopoulos, L. Tarantino, and A. Wynshaw-Boris for reviewing the manuscript, and members of the Barlow laboratory for helpful discussions. This work was supported in part by funds to C.B. from the Frederick B. Rentschler Developmental Chair and the Esther A. and Joseph Klingenstein Fund.

1. Schauwecker, P. E. & Steward, O. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 4103–4108.
2. Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., Hitzemann, R. J., Maxson, S. C., Miner, L. L., Silva, A. J., *et al*. (1997) *Psychopharmacology* **132,** 107–124.
3. Lockhart, D. J., Dong, H., Byrne, M. C., Follettie, M. T., Gallo, M. V., Chee, M. S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H. & Brown, E. L. (1996) *Nat. Biotechnol.* **14,** 1675–1680.
4. Wodicka, L., Dong, H., Mittmann, M., Ho, M. H. & Lockhart, D. J. (1997) *Nat. Biotechnol.* **15,** 1359–1367.
5. Lipshutz, R. J., Fodor, S. P., Gingeras, T. R. & Lockhart, D. J. (1999) *Nat. Genet.* **21,** 20–24.
6. Simpson, E. M., Linder, C. C., Sargent, E. E., Davisson, M. T., Mobraaten, L. E. & Sharp, J. J. (1997) *Nat. Genet.* **16,** 19–27.
7. Threadgill, D. W., Yee, D., Matin, A., Nadeau, J. H. & Magnuson, T. (1997) *Mamm. Genome* **8,** 390–393.
8. Ferraro, T. N., Golden, G. T., Smith, G. G., St. Jean, P., Schork, N. J., Mulholland, N., Ballas, C., Schill, J., Buono, R. J. & Berrettini, W. H. (1999) *J. Neurosci.* **19,** 6733–6739.
9. Hogan, B., Beddington, R., Costantini, F. & Lacy, E. (1994) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
10. Kubo, Y., Nakagawa, Y., Kakimi, K., Matsui, H., Higo, K., Wang, L., Kobayashi, H., Hirama, T. & Ishimoto, A. (1994) *J. Gen. Virol.* **75,** 881–888.
11. Muller, A. J., Chatterjee, S., Teresky, A. & Levine, A. J. (1998) *Mamm. Genome* **9,** 773–774.
12. Karschin, C., Dissmann, E., Stuhmer, W. & Karschin, A. (1996) *J. Neurosci.* **16,** 3559–3570.
13. Pellegrini-Giampietro, D. E., Peruginelli, F., Meli, E., Cozzi, A., Albani-Torregrossa, S., Pellicciari, R. & Moroni, F. (1999) *Neuropharmacology* **38,** 1607–1619.
14. Inglis, J. D., Lee, M., Davidson, D. R. & Hill, R. E. (1991) *Gene* **106,** 213–220.
15. Chiang, C. S., Hong, J. H., Stalder, A., Sun, J. R., Withers, H. R. & McBride, W. H. (1997) *Int. J. Radiat. Biol.* **72,** 45–53.
16. Pombo, C. M., Tsujita, T., Kyriakis, J. M., Bonventre, J. V. & Force, T. (1997) *J. Biol. Chem.* **272,** 29372–29379.
17. Ferraro, T. N., Golden, G. T., Smith, G. G., Schork, N. J., St. Jean, P., Ballas, C., Choi, H. & Berrettini, W. H. (1997) *Mamm. Genome* **8,** 200–208.
18. Demarest, K., McCaughran, J., Jr., Mahjubi, E., Cipp, L. & Hitzemann, R. (1999) *J. Neurosci.* **19,** 549–561.
19. Crabbe, J. C., Phillips, T. J., Buck, K. J., Cunningham, C. L. & Belknap, J. K. (1999) *Trends Neurosci.* **22,** 173–179.
20. Mayeda, A. R. & Hofstetter, J. R. (1999) *Behav. Genet.* **29,** 171–176.
21. Caldarone, B., Saavedra, C., Tartaglia, K., Wehner, J. M., Dudek, B. C. & Flaherty, L. (1997) *Nat. Genet.* **17,** 335–337.
22. Wehner, J. M., Radcliffe, R. A., Rosmann, S. T., Christensen, S. C., Rasmussen, D. L., Fulker, D. W. & Wiles, M. (1997) *Nat. Genet.* **17,** 331–334.
23. Flint, J., Corley, R., DeFries, J. C., Fulker, D. W., Gray, J. A., Miller, S. & Collins, A. C. (1995) *Science* **269,** 1432–1435.
24. Buck, K., Metten, P., Belknap, J. & Crabbe, J. (1999) *Mamm. Genome* **10,** 431–437.
25. Kubo, Y., Baldwin, T., Jan, Y. & Jan, L. (1993) *Nature (London)* **362,** 127–133.
26. Ouafik, L. H., Stoffers, D. A., Campbell, T. A., Johnson, R. C., Bloomquist, B. T., Mains, R. E. & Eipper, B. A. (1992) *Mol. Endocrinol.* **6,** 1571–1584.
27. Markel, P. D., Bennett, B., Beeson, M., Gordon, L. & Johnson, T. E. (1997) *Genome Res.* **7,** 92–99.
28. Duncan, C. C. & Erwin, V. G. (1992) *Alcohol* **9,** 23–29.
29. Bachmanov, A. A., Tordoff, M. G. & Beauchamp, G. K. (1996) *Alcohol Clin. Exp. Res.* **20,** 201–206.
30. Bachmanov, A. A., Reed, D. R., Ninomiya, Y., Inoue, M., Tordoff, M. G., Price, R. A. & Beauchamp, G. K. (1997) *Mamm. Genome* **8,** 545–548.
31. Tarantino, L. M., McClearn, G. E., Rodriguez, L. A. & Plomin, R. (1998) *Alcohol Clin. Exp. Res.* **22,** 1099–1105.
32. Blizard, D. A., Kotlus, B. & Frank, M. E. (1999) *Chem. Senses* **24,** 373–385.
33. Martin, B., Clement, Y., Venault, P. & Chapouthier, G. (1995) *J. Hered.* **86,** 274–279.
34. Gerlai, R. (1996) *Trends Neurosci.* **19,** 177–181.