

MOLECULAR HETEROGENEITY ALONG THE DORSAL–VENTRAL AXIS OF THE MURINE HIPPOCAMPAL CA1 FIELD: A MICROARRAY ANALYSIS OF GENE EXPRESSION

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Abstract—There has been increasing interest in functional heterogeneity along the septotemporal, dorsal–ventral (D–V) axis of the hippocampus. Although anatomical connectivity and lesion studies point to discrete roles for these subregions, the contribution of differential gene expression across this axis has not been systematically studied. Here we present findings from an Affymetrix microarray screen aimed at identifying genes in the CA1 region of the adult murine hippocampus that show significant differential expression along the D–V axis. Our results indicate that the vast majority of monitored genes (>90%) had tissue expression levels that differed by less than 20% between regions, while less than 0.1% of genes had expression levels that varied more than three-fold by sub-region. Only 23 probes showed a CA1 dorsoventral signal intensity ratio greater than three: 18 enriched dorsally and five enriched ventrally. Probes with the greatest difference in expression levels represent a range of genes with known functions in patterning and signaling, as well as genes without known function. Selective screening with digoxigenin-labeled *in situ* hybridization confirms the existence of CA1 sub-regionalized expression, with some genes exhibiting a graded expression pattern across the D–V axis, and others restricted to a discrete region. Our findings demonstrate that there are gene expression differences across the D–V axis of the adult murine hippocampus within traditionally recognized cytoarchitecturally defined boundaries. Combined with the previously recognized differences in connectivity and results from lesion studies, our data further confirm the existence of functional heterogeneity along the D–V axis. © 2005 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: BCIP/NBT, bromo-chloro-indolyl phosphate/nitroblue tetrazolium; CA1, hippocampal subregion CA1; CA2, hippocampal subregion CA2; CA3, hippocampal subregion CA3; cDNA, complementary DNA; cRNA, complementary RNA; DG, dentate gyrus; DIG, digoxigenin; D–V, dorsal–ventral; LPH, lactase-phlorizin hydrolase; SOCS-2, suppressor of cytokine signaling 2; VIP, vasoactive intestinal polypeptide.

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Traditionally, functional studies of the hippocampus have focused on its likely role in memory formation (Eichenbaum, 1997; Squire et al., 2004). However, there are significant data that point to a role for the hippocampus in regulating anxiety as well (Gray and McNaughton, 2000). More recently, lesion studies in rats have suggested that these two functions may be distributed across the dorsal–ventral (D–V) axis (Bannerman et al., 2004). Specifically, lesions of the dorsal hippocampus consistently result in impaired spatial learning without affecting anxiety-related measures, while ventral lesions affect anxiety-related measures without affecting spatial navigation (Bannerman et al., 1999, 2002; Kjelstrup et al., 2002; McHugh et al., 2004). Results of these studies point to a functional dissociation along the D–V axis of the hippocampus in rodents. Emerging imaging data in primates point to differential responses along the analogous anterior–posterior axis of the hippocampus, suggesting that such functional dissociation extends beyond the rodent model (Colombo et al., 1998; Strange and Dolan, 1999, 2001).

The functional dissociation seen in lesion studies of the hippocampus correlates with, and was foreshadowed by, observed differences in afferent and efferent connections along the septotemporal axis (Amaral and Witter, 1989). Despite this, traditional studies of hippocampal physiology have emphasized the intrinsic tri-synaptic circuit (dentate gyrus (DG)→CA3→CA1) that exists in the transverse plane and repeats itself in the longitudinal plane. Its highly organized, repetitive nature makes this circuit easily accessible, but lends itself to a unitary view of the hippocampus (Anderson et al., 1971). Dorsal and ventral regions receive distinct, separable inputs and send their projections to distinct targets. For example, visual, auditory and sensory association cortices project primarily to dorsal hippocampus via the lateral entorhinal cortex, while olfactory input is distributed evenly across the D–V axis (Kosel et al., 1981; Witter et al., 1989; Burwell and Amaral, 1998). Regarding efferent connections, the ventral hippocampus projects directly to the prefrontal cortex, the amygdala, and the shell of the nucleus accumbens, while the dorsal hippocampus does not (Pitkanen et al., 2000). In addition, hippocampal projections to the hypothalamus via the septum are topographically mapped, such that the dorsal CA1 and subiculum project to dorsal lateral septum and then to the mammillary body, while ventral CA1 and subiculum project

primarily to rostral and ventral lateral septum (Risold and Swanson, 1996). The septum in turn projects to hypothalamic medial zone nuclei involved in the expression of social behavior (Risold and Swanson, 1996). In summary, the dorsal hippocampus receives highly processed sensory information from neocortical areas consistent with a role in spatial learning/context acquisition while the ventral hippocampus receives significantly more inputs from regions that could impart affective valence to stimuli. Physiological studies citing higher densities and higher spatial selectivity of place cells in the dorsal versus ventral hippocampus are consistent with this interpretation (Jung et al., 1994). Combined, the evidence from both anatomical, behavioral and physiological studies now strongly argues for functional heterogeneity across the dorso-ventral axis.

In contrast to the anatomical and functional differences along the D–V axis, the possibility of molecular differentiation along this axis has not been well studied. There are recognized cytoarchitectural and connectivity differences in the classically defined structures of CA1, CA2, CA3 and the DG (Fig. 1C, D) (Ramón y Cajal, 1953), and molecular distinctions among these regions have been recently described as well, demonstrating good correlation between cytoarchitecturally defined boundaries and the boundaries of gene expression as confirmed by *in situ* hybridization (Zhao et al., 2001; Lein et al., 2004). However, there have been no systematic analyses of differences in gene expression within sub-regions of the hippocampus. Here we describe a DNA microarray study looking at gene expression differences in the CA1 region of the hippocampus across the D–V axis.

EXPERIMENTAL PROCEDURES

Animals and tissue

All procedures were approved by the Institutional Animal Care and Use Committee, and conform to the National Institutes of Health guidelines on the ethical use of animals. Eight-to-10-week-old 129SVEV male mice were obtained from Taconic (Germantown, NY, USA). For micro-dissection and DNA microarray analysis, brains were extracted and immediately placed on ice. Extracted brains were sliced coronally on a Series 1000 Vibratome (Intracel, Shepreth, UK) in 400 μm -thick sections and placed in RNA*later* (Sigma, St. Louis, MO, USA). Primary and secondary visual cortex (cortex overlying dorsal CA1 in the coronal plane), as well as dorsal and ventral CA1 areas, was dissected out as shown in Fig. 1. The tissue was hand dissected under a microscope using a fine surgical scalpel (part RS-6272; Roboz, Gaithersburg, MD, USA). Sub-dissected samples were pooled to yield sufficient RNA to be labeled per array experiment. For each region, material from six to seven mice was pooled. Three independent pools were processed per brain area (total=20 mice) on three separate arrays. For *in situ* hybridization, brains from 10-to-12-week-old mice were fresh-frozen on dry ice and stored at -80°C until sectioning. Every effort was made to minimize the number of mice used and their suffering.

RNA preparation and microarray hybridization

Total RNA was isolated from each pool of tissue using Trizol (Invitrogen, Carlsbad, CA, USA). RNA purity and integrity were assessed by optical densitometry, gel electrophoresis, and subsequent array parameters. Microarray samples were prepared

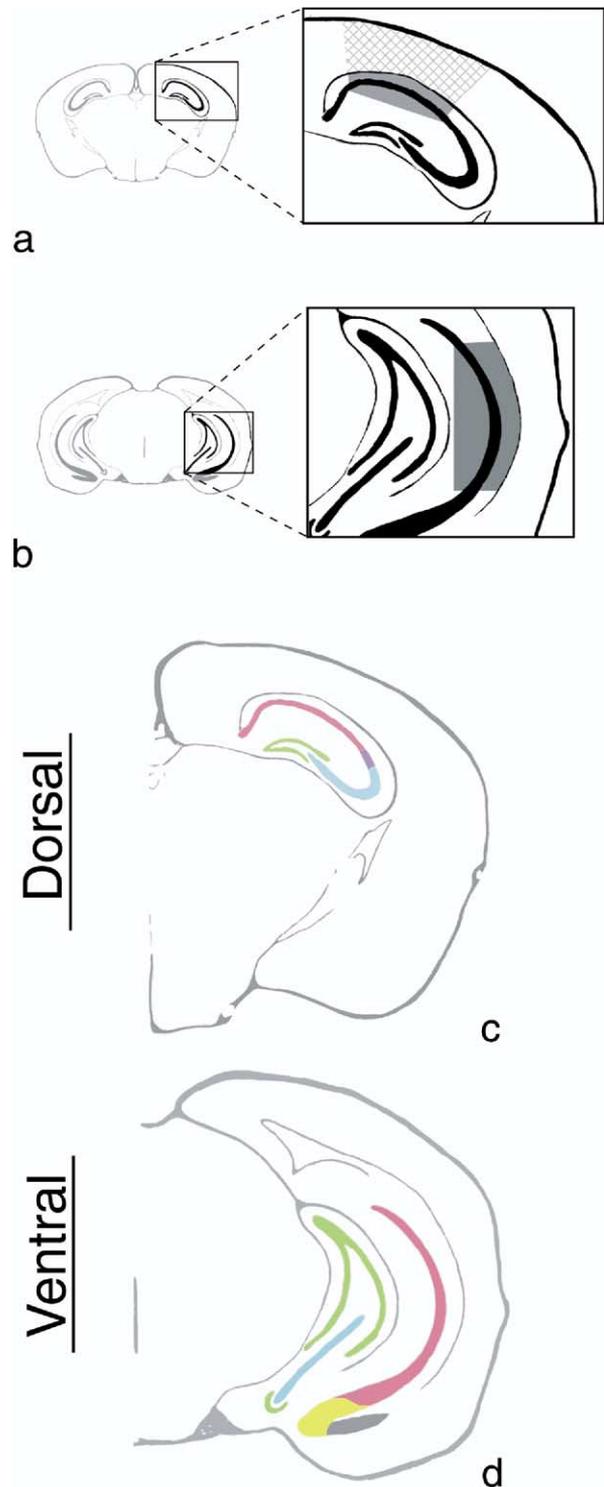


Fig. 1. Schematic of tissue microdissection. (a, b) Illustration of the coronal plane of section used to obtain dorsal and ventral CA1 samples. The shaded area represents the tissue that was isolated from CA1 for dorsal (a) and ventral (b) samples. (a) The crosshatched area shows the region that was used for the cortex sample. (c, d) Depicts the traditional hippocampal boundaries at these levels. Pink (CA1), purple (CA2), blue (CA3), green (DG), yellow (subiculum).

according to the Affymetrix protocol (<http://www.affymetrix.com/support/>). In brief, 6 μg of total RNA was reverse-transcribed and converted into double-stranded complementary DNA (cDNA). A biotinylated complementary RNA (cRNA) was then transcribed *in vitro* using an RNA polymerase T7 promoter site, which had been introduced during the reverse-transcription of RNA into cDNA. After fragmentation into pieces of 50–200 bases long, 15 μg of labeled cRNA sample was hybridized onto oligonucleotide MOE430A microarrays (Affymetrix, Palo Alto, CA, USA), using standard protocols with the Affymetrix microarray oven and fluidics station. A total of 22,690 annotated genes and well-characterized expressed sequence tag (EST) clusters is represented on MOE430A arrays. Quality control parameters on all nine arrays (three cortex, three dorsal CA1, three ventral CA1) were as follow: noise (RawQ) less than 2.26, background signal less than 34, consistent number of genes called as “present” across arrays, consistent scale factors, actin and GAPDH 5’/3’ signal ratios less than 1.66, and consistent detection of BioB and BioC hybridization spiked controls. Details of this procedure and microarray quality control parameters are described elsewhere (Galfalvy et al., 2003). For data analysis, probe set signal intensities were extracted with the robust multi-array average (RMA) algorithm (Irizarry et al., 2003). This algorithm is a part of the R package *affy* and can be downloaded from the Bioconductor project website (<http://www.bioconductor.org>).

Microarray data analysis

Data were imported into a Microsoft Excel spreadsheet where signal averages and standard deviations were calculated for each gene across the triplicate arrays. As these studies were exploratory and sample number precluded systematic statistical analysis, ratios of signal expression were calculated to seek genes with enrichment in expression levels in dorsal and ventral hippocampus, relative to the cortical samples. Results for selected genes were confirmed by *in situ* hybridization.

Digoxigenin (DIG)-labeled *in situ* hybridization

Using published reference sequences, we designed primers corresponding to the candidate genes and amplified 600–800 base-pair DNA fragments from mouse brain cDNA. Amplified fragments were cloned into the pCRII-TOPO TA vector (Invitrogen), and sequence-confirmed plasmids were linearized and used as templates to generate sense and anti-sense DIG-labeled riboprobes. The exceptions were the probe template for the gene *Wolframin*, which had been sub-cloned in to the pBluescript SK vector (Stratagene, La Jolla, CA, USA). Riboprobes were transcribed using the DIG RNA Labeling Kit (Roche, Mannheim, Germany) in accordance with kit instructions, using T7, Sp6, or T3 polymerases. *In situ* hybridization was performed as previously described (Schaeren-Wiemers and Gerfin-Moser, 1993), with hybridization temperature increased as needed to ensure low background. For cryo-sectioning, brains were mounted on optimal cutting temperature medium (TissueTek; Sakura Finetek, Torrance, CA, USA). Fourteen-micron sections were cut on a Leica CM3050 S cryostat (Bannockburn, IL, USA) and mounted on Superfrost slides (Fisher, Fairlawn, NJ, USA). Two sections containing dorsal CA1 and two containing ventral CA1 were mounted on each slide to ensure similarity in processing, with dorsal CA1 defined as Bregma -1.70 mm to -2.30 mm and ventral CA1 containing sections as Bregma -3.16 mm to -3.50 mm. DIG was detected with Anti-DIG AP Fab Fragments (Roche), and visualized with BCIP/NBT solution (Roche). As a reference, slides containing immediately adjacent sections were Nissl stained according to standard protocols.

Alkaline-phosphatase signals were allowed to develop for a period of six hours up to three days, depending on signal intensity and background. BCIP/NBT solution was changed every 24 h as needed. Slides were coverslipped with DAKO Glycergel mounting media (Carpentaria, CA, USA), and imaged using a Retiga EXi

imaging system (QImaging, Burnaby, Canada) and a Zeiss Axiovert 200 (Oberkochen, Germany) fitted with a 2.5 \times and a 10 \times objective. Images were assembled, using Adobe Photoshop. Final images were contrast enhanced and color matched for easier viewing.

RESULTS

Array data

Our aim was to identify genes that were differentially expressed along the dorsoventral axis of the CA1 region of the hippocampus enriched in the hippocampus relative to underlying cortex. An overview of the data reveals that of the 22,626 probes on the array, 6764 (29.9%) were “absent” (i.e. did not have expression above background levels). A number of genes showed enrichment in the cortical samples over the CA1 regions, with 233 genes showing greater than two-fold enrichment in the cortical sample over dorsal CA1 and 209 genes showing greater than two-fold enrichment in cortical sample over ventral CA1. Genes that were more than two-fold enriched in hippocampus numbered 158 in dorsal hippocampus and 139 in ventral hippocampus.

These numbers are consistent with other studies reporting regionally enriched signals on microarrays between brain regions (Zirlinger et al., 2001).

Within CA1, we identified 41 genes with greater than two-fold enrichment in the dorsal CA1 samples relative to the ventral CA1 samples and 67 genes with two-fold enrichment in ventral relative to dorsal CA1 samples (Fig. 2). Results showed remarkable consistency between arrays (Supplemental Fig. 1). Genes with highest “fold enrichment” across the D–V axis are shown in Table 1. Express-

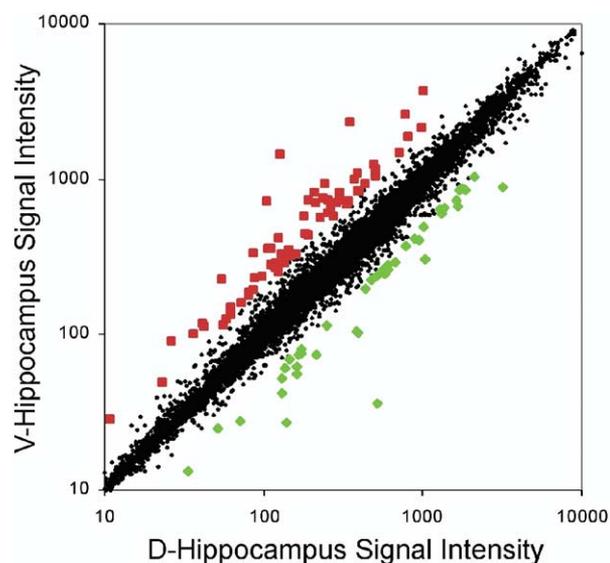


Fig. 2. Normalized dorsal CA1 versus ventral CA1 cDNA microarray results on log-log plots. Genes that show a greater than two-fold enrichment in the dorsal CA1 sample are shown in green (41 total). Genes that show a greater than two-fold enrichment in the ventral CA1 sample are shown in red (67 total). The remaining 22582 probe sets are shown in black. Each dot represents the average signal derived from three separate samples.

Table 1. Genes enriched in dorsal and ventral CA1

Accession number	Description	Raw signal		Fold enrichment	
		Dorsal CA1	Ventral CA1	Dorsal/ventral	Dorsal/cortex
Dorsally enriched					
C78577	EST weakly similar to Klotho lactose-phlorizin hydrolase ^a (LPH)	521±43	36±2.9	14.45	16.98
BG141874	Transthyretin ^{b,c}	140±100	27±4.4	5.14	-6.80
NM_013467	Aldehyde dehydrogenase family 1, subfamily 1A ^d (Wagner, et al. 2002)	396±12	102±31	3.87	1.47
NM_011716	Wolframin ^a	3217±521	888±133	3.62	6.99
BB075797	EphA7: Ephrin receptor A7 ^a	382±82	106±5.4	3.60	6.79
X79084	EphA7	1043±54	303±36	3.44	5.94
BB292785	EphA3: Ephrin receptor A3	130±25	42±5.9	3.06	4.10
BC026153	EphA7	160±46	56±8.1	2.89	4.20
BQ175355	Klotho ^p (TsujiKawa, et al. 2003)	215±50	75±8.5	2.87	4.11
BB831146	CCAAT/enhancer binding protein (C/EBP)	161±20	62±2.6	2.60	4.19
AV152953	Transthyretin ^b	33±22	13±1.3	2.54	-16.9
BC013499	Hypothetical protein MGC19163	71±5	28±1.6	2.53	2.24
Ventrally enriched					
NM_007833	Decorin ^a	129±18	1446±136	11.24	7.17
BC024515	Hypothetical protein LOC22564 (Gastrin-releasing peptide) ^e (Shumyatsky, et al. 2002)	105±26	723±74	6.86	2.79
AV218841	Neuromatin ^a	351±23	2335±451	6.66	6.02
NM_007706	Suppressor of cytokine signaling 2 (SOCS-2) ^f (Polizzotto, et al. 2000)	55±8	226±60	4.12	1.14
NM_030699	Netrin G-1 (Laminet-1) ^g (Yin, et al. 2002)	86±20	337±41	3.91	1.68
NM_138649	Brain&Kidney protein ^a	209±2.3	811±47	3.89	4.35
AW0448713	EST's	243±15	939±118	3.87	3.84
NM_011594	Tissue inhibitor of metalloproteinase 2	194±10	727±5	3.75	1.79
CR1601	Tissue inhibitor of metalloproteinase 2	1007±47	3638±146	3.61	1.77
BB244736	Expressed sequence AI527257	27±5	90±27	3.36	1.37
AF144628	Slit homolog 2 (<i>Drosophila</i>)	125±7.2	417±68	3.34	1.29
NM_026214	RIKEN cDNA 2210017A09 gene	107±19	357±101	3.32	2.87
BE653037	Coatomer protein complex, subunit γ2	213±37	703±171	3.30	-2.35
BF168458	Tissue inhibitor of metalloproteinase 2	789±34	2562±215	3.25	1.79
BB31657A	Coatomer protein complex, subunit γ2	182±56	576±120	3.16	-2.15
NM_009049	Regulated endocrine-specific protein 18	113±24	354±60	3.14	-1.13
BB426294	RIKEN cDNA 2810454P21 gene	239±26	750±68	3.13	2.04

^a Confirmed here by DIG-labeled *in situ* hybridization.

^b Expressed in choroid plexus—probable dissection contamination.

^c Confirmed by GENSAT databank.

^d Expressed in meningeal channels around the hippocampus.

^e Confirmed by literature.

^f Expressed in CA3—probable dissection contamination.

^g Expressed in thalamus and hippocampal interneurons.

sion levels for all 22,626 probe sets can be found in Supplemental Table 1.

Validation of microarray results

Other studies have demonstrated the usefulness and reliability of microarrays in identifying genes that are expressed in a region-specific manner (Lein et al., 2004). However, microarray results need to be validated by other means, both to confirm their accuracy, and to provide expression resolution at the cellular level within a given sub-region. We used our array data as a starting point for identifying genes differentially expressed in CA1 across the D–V axis. We sorted the array data looking for the highest dorsal to ventral and ventral to dorsal ratios and performed *in situ* hybridization on the top candidate genes for which expression data were not published. Good cor-

respondence was seen between genechip results and *in situ* data (see below).

A number of the genes with high fold enrichment have been described in the literature, as indicated in Table 1. One known expression pattern confirmed our array findings, while others suggested some contamination in our microdissection. Specifically, gastrin-releasing peptide appears to be expressed in the ventral CA1 region, but is absent in CA1 dorsally (Shumyatsky et al., 2002), in agreement with the D–V signal enrichment we found of 6.86. However, two genes with apparent dorsal enrichment, transthyretin and klotho, actually revealed the higher presence of choroid plexus RNA material in our dorsal versus ventral samples, reflecting the difficulty of removing this tissue during the dissection of the dorsal samples. Additionally, the gene SOCS-2, which showed ventral enrich-

ment in our screen, is expressed exclusively in the CA3 region within the hippocampus (Polizzotto et al., 2000), suggesting possible contamination of ventral CA1 with adjacent CA3 tissue. Lastly, aldehyde dehydrogenase family 1, subfamily 1a is expressed in the meningeal channels around the hippocampus (Wagner et al., 2002).

Validation by *in situ* hybridization

We sought to confirm differences in expression across the D–V axis for the top three genes in each group that did not have published expression data. All showed significant expression differences between dorsal and ventral CA1 areas and were significantly enriched in hippocampus versus cortex. Specifically, LPH, Wolframin, and the EphA7 were enriched in dorsal CA1 (Fig. 3), whereas Decorin, Brain&Kidney protein, and Neuronatin were enriched in ventral CA1 (Fig. 4). These patterns are discussed in detail below.

While the genes that we verified by *in situ* primarily showed differences attributable to pyramidal neurons in CA1, other cell types demonstrating mRNA expression across the D–V axis of the hippocampus were also repre-

sented in the array data. For instance, vasoactive intestinal polypeptide (VIP)-positive interneurons are found more frequently in ventral hippocampus than in dorsal hippocampus (Jinno and Kosaka, 2003). Despite being only a small percentage of the total cell type within the dissected tissue, this difference was detected by our microarray as a 1.78-fold enrichment of VIP signal in the ventral hippocampus. This suggests the existence of additional molecular heterogeneity along the D–V axis beyond genes verified here.

Dorsally enriched in CA1

Wolframin (Fig. 3a and g). This gene is highly restricted to CA1 within the hippocampus and is expressed in a graded fashion, with strong expression dorsally diminishing completely in the ventral third of CA1 (Fig. 5c). Additional, weaker, expression outside of the hippocampus is seen in the piriform cortex and the amygdala (Fig. 3 and data not shown). Mutations in this gene in humans are responsible for Wolfram syndrome, an autosomal recessive disease characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness. In addition, people

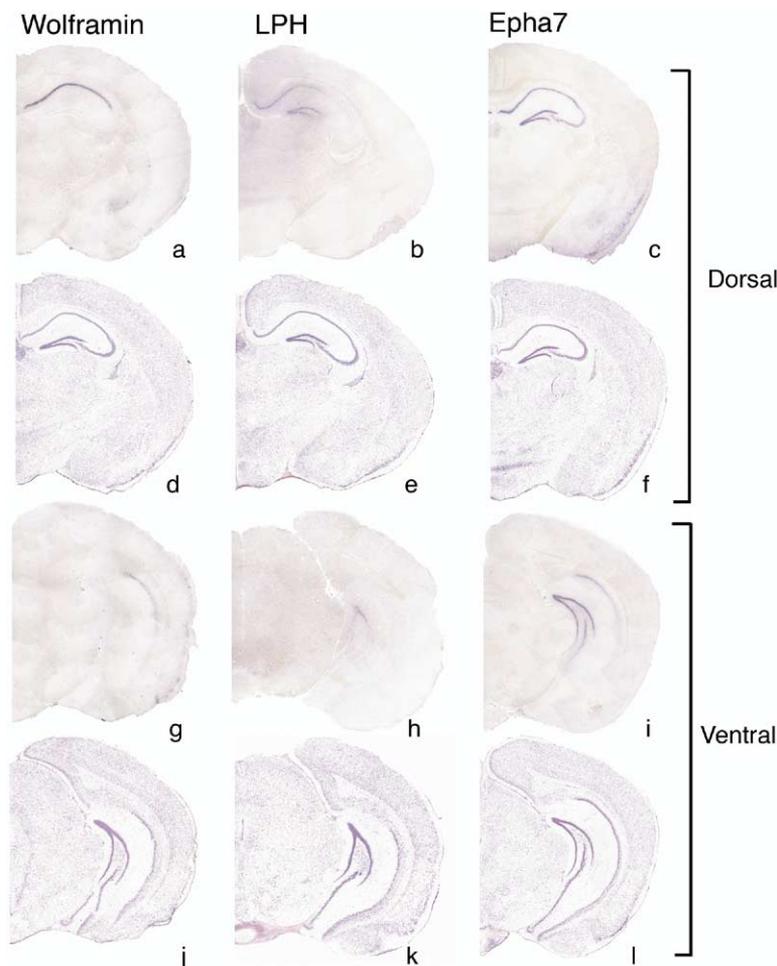


Fig. 3. Expression patterns of select dorsally enriched genes by *in situ* hybridization. (a–c) Shows DIG-labeled *in situ* hybridization for the genes LPH, Wolframin and EphA7 respectively in the dorsal hippocampus. (d–f) Adjacent Nissl sections for reference. (g–i) Show *in situ* hybridization for LPH, Wolframin and EphA7 at more ventral levels. (j–l) The adjacent Nissl sections for reference.

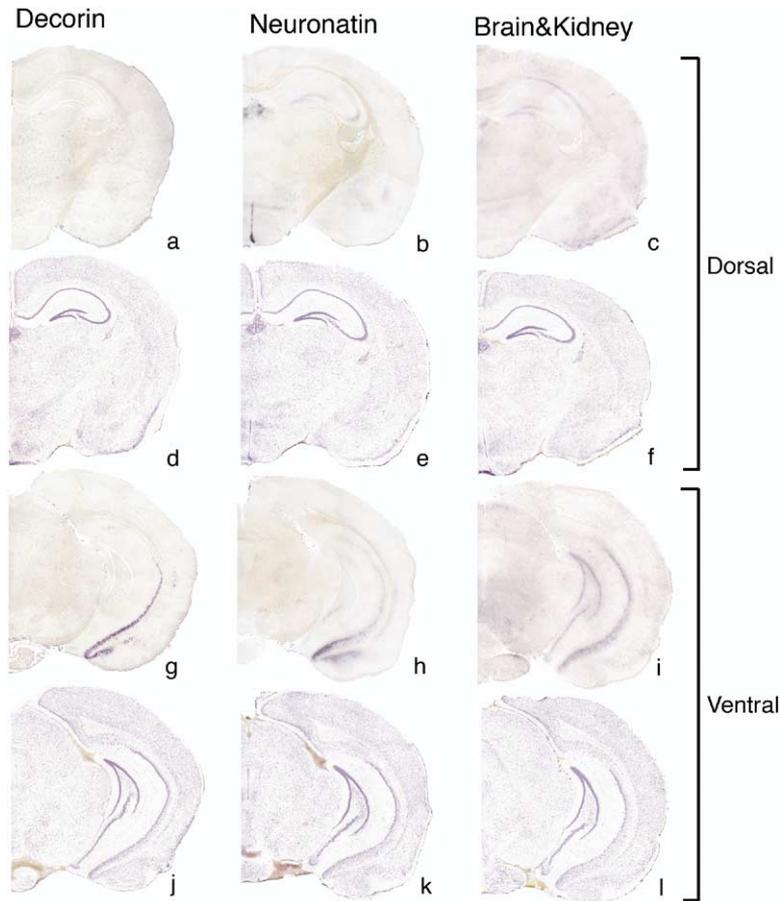


Fig. 4. Expression patterns of select ventrally enriched genes by *in situ* hybridization. (a–c) DIG-labeled *in situ* hybridizations for the genes Decorin, Neuronatin, and Brain&Kidney protein in sections containing dorsal hippocampus. (d–f) Adjacent Nissl sections for reference. (g–i) Show *in situ* hybridization for Decorin, Neuronatin and Brain&Kidney protein in sections containing ventral hippocampus. (j–l) Adjacent Nissl sections for reference.

who carry the gene but do not have the disorder have significantly increased risk for psychiatric hospitalization (Swift et al., 1998). More recently, polymorphisms in this gene have been associated with increased risk of depression (Koido et al., 2005).

LPH (Fig. 3b, h). Robust expression is seen dorsally in CA1 and DG; ventrally it is not expressed in CA1, or CA3, and gradually diminishes in the DG, and is indeed absent in the most ventral cells of the DG in the ventral slice (Fig. 6c). While the specific function for LPH in the brain has not been identified, LPH purified from the monkey gut has been shown to hydrolyze both lactose and cerebroside, suggesting that LPH may be modifying cerebroside in the hippocampus (Ramaswamy and Radhakrishnan, 1975).

EphA7 (Fig. 3c and i). Strong expression is seen dorsally in CA1, CA2, CA3, and the DG. The expression in all regions appears graded, with the diminishing expression in more ventral areas (Figs. 5a, 6a). Outside the hippocampus, it is expressed in the piriform cortex. Eph's are extensively involved in regionalization and topographic mapping of axonal projections during development and are often found in graded expression patterns (Flanagan and

Vanderhaeghen, 1998). Eph expression in the adult brain occurs primarily at sites that undergo ongoing remodeling/patterning (Yamaguchi and Pasquale, 2004), raising the possibility that presence of EphA7 in a graded pattern in adulthood may contribute to the maintenance of regional differences.

Ventrally Enriched in CA1

Decorin (Fig. 4a and g). Dorsally it is not detected in CA1, whereas strong expression is seen in ventral CA1 and subiculum. Outside of the hippocampus, weaker expression is seen in the endopiriform nucleus as well as the entorhinal cortex. Decorin is a small proteoglycan that is known to bind and inhibit the action of transforming growth factor- β , which in turn may help regulate neurotrophin signaling (Sometani et al., 2001).

Neuronatin (Fig. 4b and h). Dorsally it is not detected in CA1, although weak expression is seen in both the DG and CA3 regions. Ventrally it shows scattered expression in CA1, expression in CA3, and in the subiculum. Weak expression outside the hippocampus is seen in the amygdala, the habenular nucleus, regions of the hypothalamus, as well as the periventricular medial and lateral magnocel-

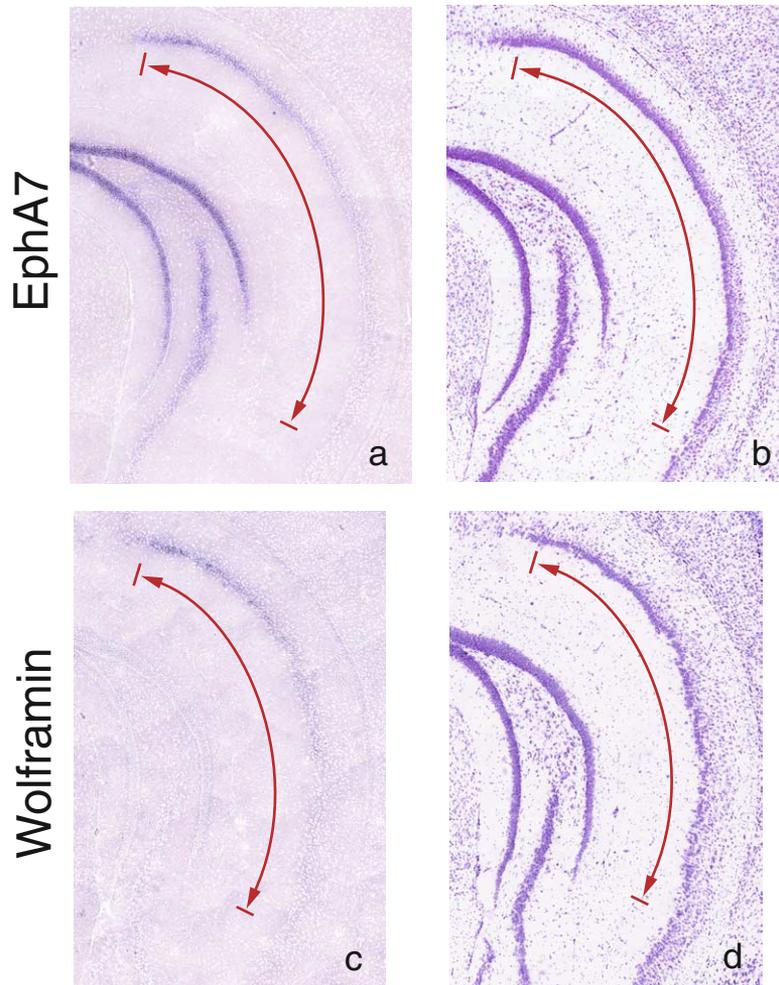


Fig. 5. (a) EphA7 *in situ* at the dorsal ventral boundaries of expression. (b) Adjacent Nissl section. (c) Wolframin *in situ* at the dorsal ventral boundaries of expression. (d) Adjacent Nissl section. Note expression in the dorsal but not ventralmost region of CA1 for both genes (double-headed arrows).

lular areas (Fig. 4 and data not shown). Neuronatin is a highly imprinted gene with currently unknown function (John et al., 2001).

Brain&Kidney protein (Fig. 4c and i). It is very weakly expressed in the most dorsal regions of CA1 (data not shown) and the adjacent CA2 region. Ventral regions of CA1 show uniform expression as does the ventral subiculum. In CA3 it is expressed only ventrally while in the DG expression is seen across the D–V axis. The function of Brain&Kidney protein is unknown, although it appears to be localized to the trans-Golgi network (Fukuda and Miko-shiba, 2001).

In addition to gene expression differences within one cytoarchitecturally-defined region, some genes also show differential patterns of D–V regulation throughout the major tripartite hippocampal sub-regions (CA1, CA3, and DG). Among the genes that we identified and confirmed, several patterns are apparent. First, EphA7 is expressed in a graded pattern in all three major regions, CA1, CA3, DG (Fig. 3c, 3i and Figs. 5a, 6a). One gene, Brain&Kidney protein, shows co-varying expression across two major

subregions and static expression in the third. Another, Neuronatin, also co-varies in a graded fashion across two out of three major sub-regions in which it is expressed; however, in this case the regions are regulated in opposite directions, with expression in dorsal but not ventral DG and expression in ventral but not dorsal CA1. Although these genes are but a small sample of those that showed enriched D–V ratios across CA1, these patterns likely exist for other genes as well. Interestingly, all three genes that showed enriched expression in ventral CA1 also showed expression in the ventral subiculum, raising the possibility that these two structures share a cellular population or a common origin.

DISCUSSION

Numerous groups have successfully used microarrays to define molecular markers of previously recognized anatomical structures, for example in sub-regions of the amygdala (Zirlinger et al., 2001) and the hippocampus (Lein et al., 2004). In general, those studies have concluded that gene expression patterns respect regional boundaries that

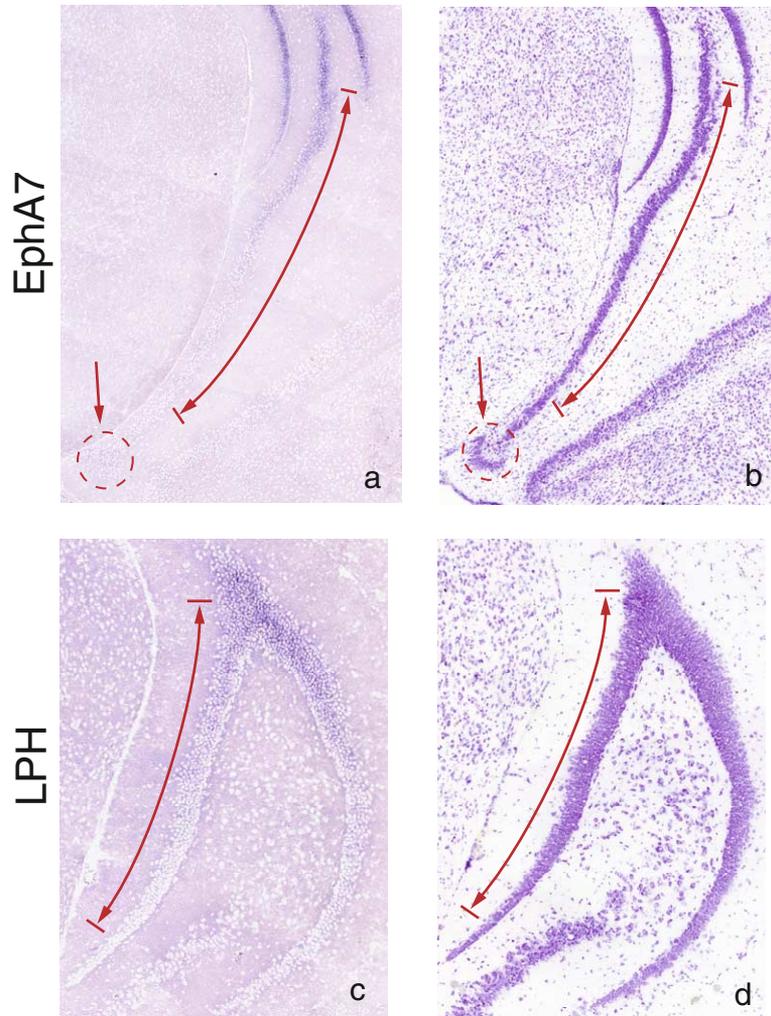


Fig. 6. (a) EphA7 *in situ* showing fading CA3 expression (double-headed arrow) and loss of expression in the ventralmost dentate (single arrow). (b) Adjacent Nissl section. (c) LPH *in situ* showing fading signal in the ventral DG (double-headed arrow). (d) Adjacent Nissl section.

have previously been established cytoarchitecturally. Here we present data from a screen for genes that are differentially expressed within one cytoarchitecturally-defined structure, CA1, across its longitudinal (dorsal-to-ventral) axis.

We have identified and confirmed at least six genes with differential expression across the D–V axis of CA1 in the adult mouse. In addition, our screen produced genes that showed differential regulation across the D–V axis in sub-regions of the hippocampus other than CA1. From these results we can conclude that: 1) differential gene regulation occurs within traditionally defined sub-regions across the D–V axis, and 2) expression across this axis of the hippocampus can be dependently or independently regulated among sub-regions, i.e. the hippocampus does not necessarily vary dorsally-to-ventrally as a concerted unit. These findings not only contradict the archetype of sub-region homogeneity within the hippocampus but also suggest the possibility that more subtle functional heterogeneity exists beyond the D–V distinction defined by anatomical connectivity and gross lesion studies. The identification of differential regulation of genes across the

longitudinal axis among subregions may elucidate yet another layer of complexity in the septotemporal differentiation of the hippocampus.

Identification of these differentially expressed genes not only provides molecular markers for new putative neuronal subpopulations, but also provides a molecular handle with which to study new populations of neurons. The functions of genes described here may themselves contribute to neurochemical and/or cellular properties, which in turn may help to define new subclasses of neurons. Moreover, taking advantage of transgenic technology using the promoters for these genes offers the possibility of mapping specific functions to these subpopulations, increasing our understanding of the relationships between connectivity and function in the brain.

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APPENDIX

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: [10.1016/j.neuroscience.2005.08.082](https://doi.org/10.1016/j.neuroscience.2005.08.082).

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