Cellular/Molecular

A Transcriptome Database for Astrocytes, Neurons, and Oligodendrocytes: A New Resource for Understanding Brain Development and Function

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Understanding the cell–cell interactions that control CNS development and function has long been limited by the lack of methods to cleanly separate neural cell types. Here we describe methods for the prospective isolation and purification of astrocytes, neurons, and oligodendrocytes from developing and mature mouse forebrain. We used FACS (fluorescent-activated cell sorting) to isolate astrocytes from transgenic mice that express enhanced green fluorescent protein (EGFP) under the control of an S100β promoter. Using Affymetrix GeneChip Arrays, we then created a transcriptome database of the expression levels of >20,000 genes by gene profiling these three main CNS neural cell types at various postnatal ages between postnatal day 1 (P1) and P30. This database provides a detailed global characterization and comparison of the genes expressed by acutely isolated astrocytes, neurons, and oligodendrocytes. We found that Aldh1L1 is a highly specific antigenic marker for astrocytes with a substantially broader pattern of astrocyte expression than the traditional astrocyte marker GFAP. Astrocytes were enriched in specific metabolic and lipid synthetic pathways, as well as the draper/Megf10 and Mertk/integrin $\alpha_v\beta_5$ phagocytic pathways suggesting that astrocytes are professional phagocytes. Our findings call into question the concept of a "glial" cell class as the gene profiles of astrocytes and oligodendrocytes are as dissimilar to each other as they are to neurons. This transcriptome database of acutely isolated purified astrocytes, neurons, and oligodendrocytes provides a resource to the neuroscience community by providing improved cell-type-specific markers and for better understanding of neural development, function, and disease.

Key words: **astrocyte; neuron; oligodendrocyte; GeneChip; Aldh1L1; culture; gene profiling; microarray; transcriptome; phagocytosis; astroglia; Megf10; Mertk; Draper; Mfge8**

Introduction

(, 1980; , 2004).

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 0270-6474/08/280264-15$15.00/0

Materials and Methods

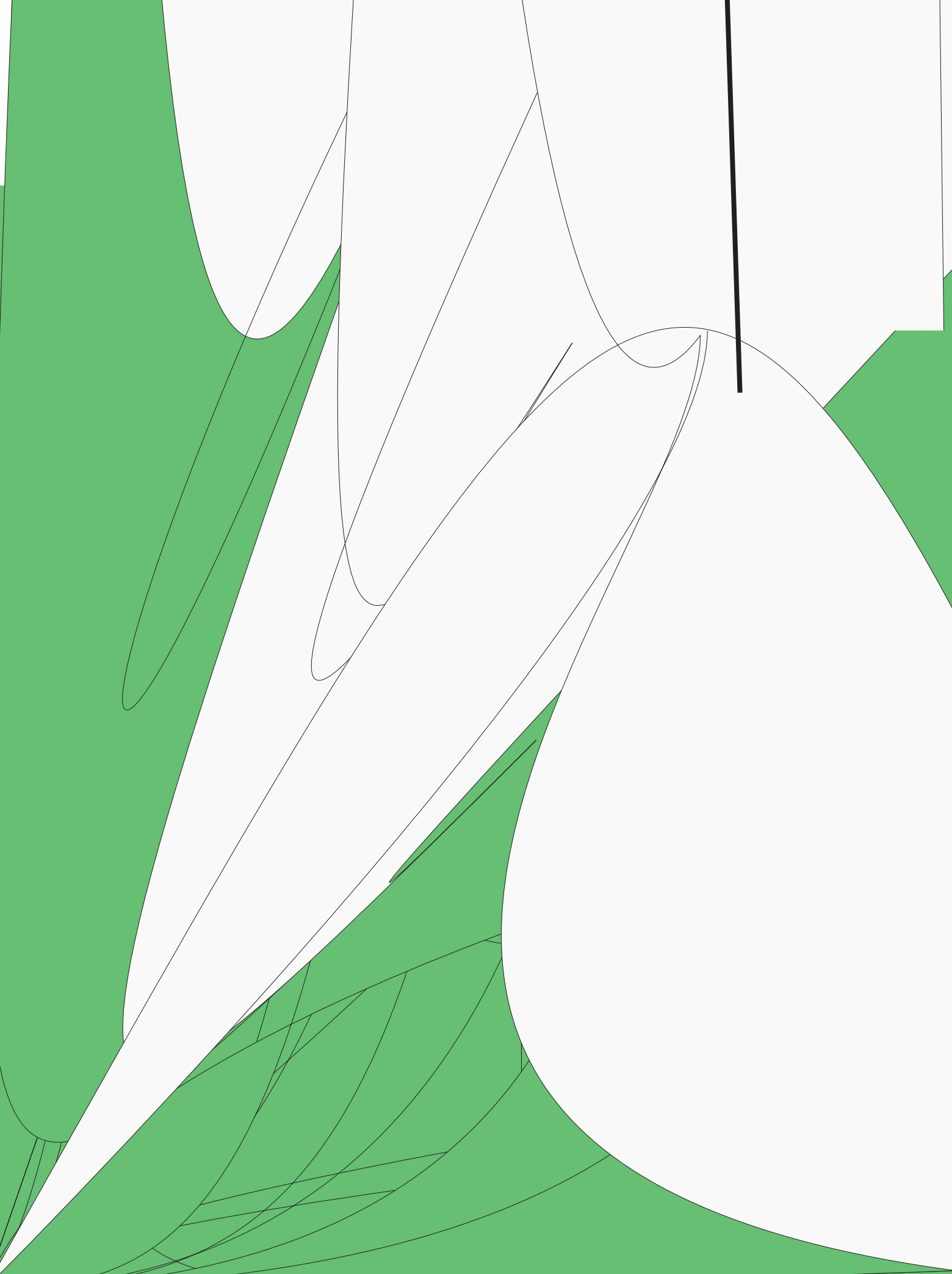
FACS purification of astrocytes.

FACS purification of neurons.

Panning purification of oligodendrocyte lineage cells.

Data normalization and analysis.

Immunohistochemistry.



Gene expression profiling of CNS cell types

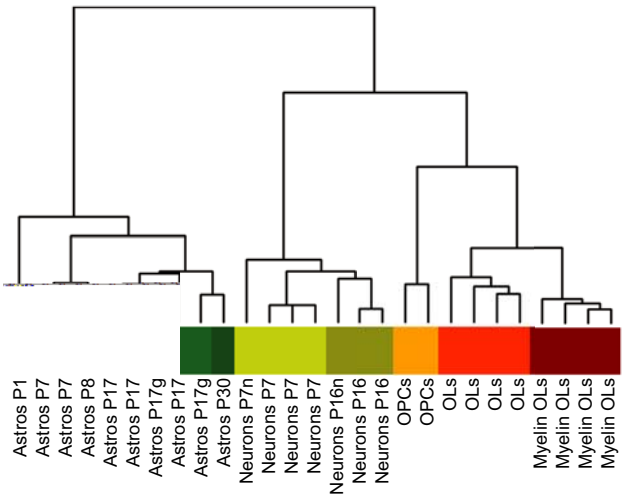


Figure 2. Dendrogram and sample clustering of purified CNS cell types. Hierarchical clustering of highly purified CNS cell type samples from different developmental stages reveals three distinct clusters representing astrocytes, neurons, and oligodendrocytes. The similarity of gene expression between different samples is represented by the vertical distances on each branch of the dendrogram. Biological replicates show the highest degree of correlation within samples, represented by short vertical distances. Within each cell population, gene expression is more highly correlated between maturing and mature samples (Astros P7, Astros P17, OLs, Myelin OLs) than between immature and maturing samples (Astros P1, Astros P7, OPCs, OLs). Color bar and sample labels describe each individual sample type (green, astrocytes; yellow, neurons; orange–red, OL lineage cells; P, postnatal day, represented by different color shades; g, cerebral cortical gray matter astrocytes; n, neuron samples depleted of residual endothelial cells).

Identification and validation of neural cell-type-specific genes

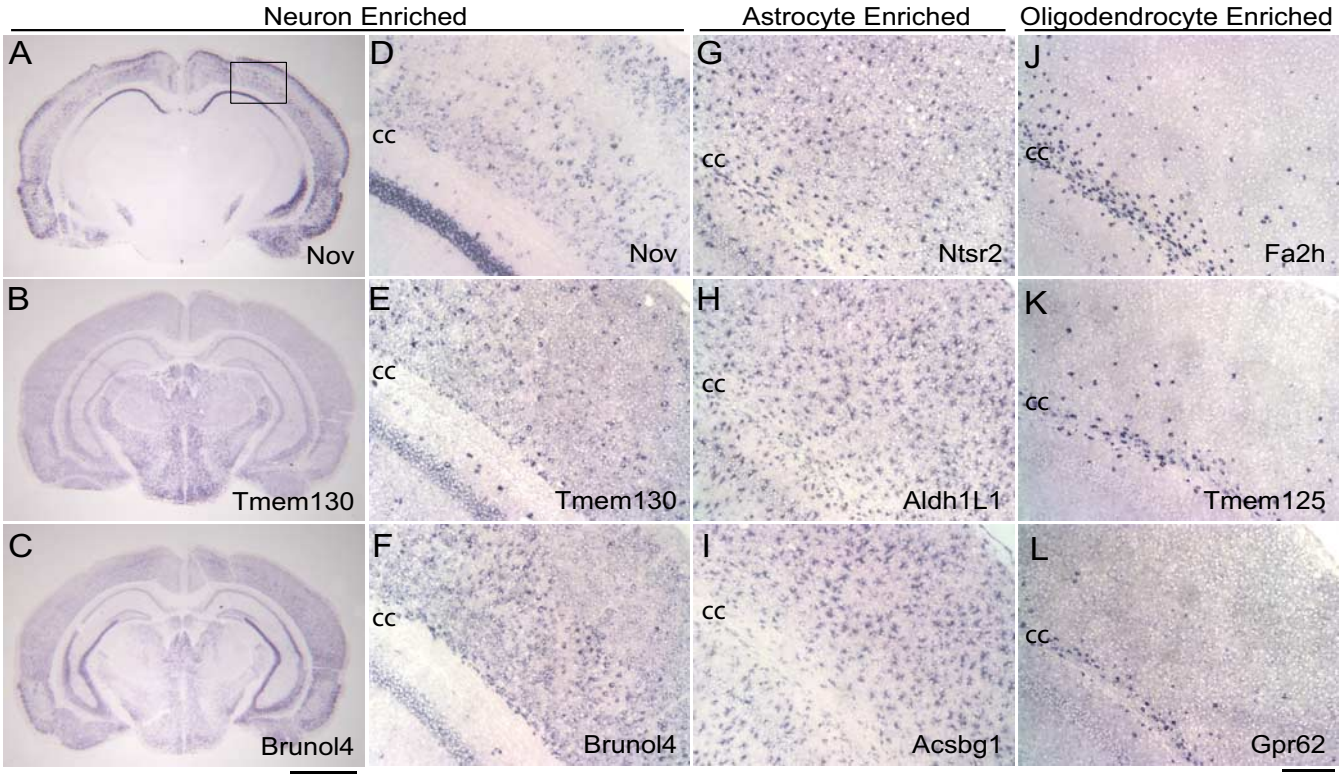


Figure 4. Validation of gene expression data by *in situ* hybridizations. ***A–C***, Coronal brain sections showing ISH for genes identified by array data as having specific neuronal expression: ***A***, Nov; ***B***, Tmem130; ***C***, Brunol4. ***D–L***, Higher-magnification images corresponding to outlined region in ***A*** showing hippocampus, corpus callosum (cc), and the overlying cortex. ***D–F***, Genes identified by array data as having specific neuronal expression, displaying expression in the hippocampus and cortex: ***D***, Nov; ***E***, Tmem130; ***F***, Brunol4. ***G–I***, Genes identified by array data as having astrocyte-enriched expression, showing fibrous, positive cells throughout the white and gray matter: ***G***, Ntsr2; ***H***, Aldh1L1; ***I***, Acsbg1. ***J–L***, ISH for genes identified by array data as having specific OL expression, showing white matter expression in the corpus callosum and the occasional positive cell in the overlying cortex: ***J***, Fa2h; ***K***, Tmem125/6330530A05Rik; ***L***, Gpr62. All ISH performed on P17 mouse brains. Scale bars: ***A–C***, 2 mm; ***D–L***, 200 μm.

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44 , 78

69

3′-

4 6 (191 , 178 (93%) 3′- , 141 (79%) >1.5- , 106 (60%) >4- 30 (17%) >20- ( ) 23 (13%)

80%

Aldh1L1 is a new astrocyte-specific marker

( ) 1 1 ( ) 10- (, 1991; , 2007). (.4*H*), (, 1997). (.5*A–C*). 1 1, 1 1 ( ) ( ) 7,

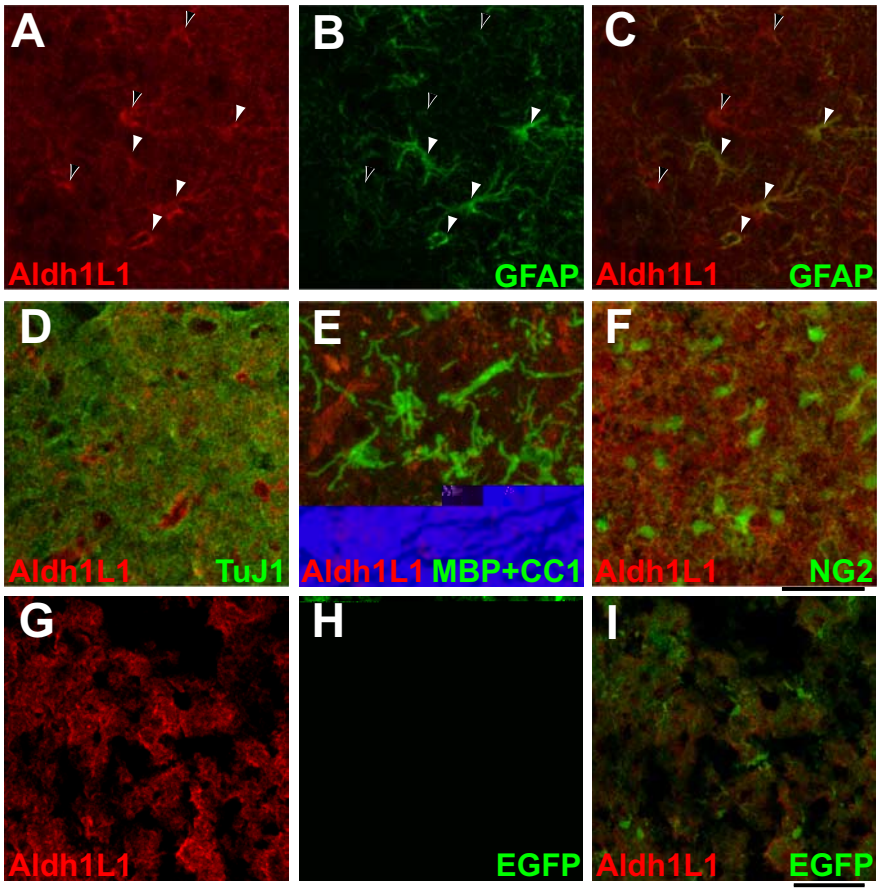

Figure 5. Aldh1L1 is a specific pan-astrocyte marker. ***A–F***, Immunohistochemical staining of P15 rat cortex shows Aldh1L1 is a cell-type-specific pan-astrocyte marker. ***A–C***, Costaining of rat cortex for Aldh1L1 (red) and GFAP (green) staining. ***A***, Aldh1L1 labels both the cell bodies and extensive processes of astrocytes in the cortex. ***B***, GFAP labels the astrocyte intermediate filament cytoskeleton but not the finer processes that Aldh1L1 is capable of labeling. ***C***, GFAP labels a subset of astrocytes: cells labeled by GFAP are also Aldh1L1 positive (white arrowheads), whereas Aldh1L1 labels many astrocytes not labeled by GFAP (black arrowheads). ***D–F***, Aldh1L1 does not label neurons (***D***, Tuj1), OLs (***E***, MBP + CC1), or OPCs (***F***, NG2). ***G–I***, The merge (***I***) of Aldh1L1 immunostaining (***G***) and strong BAC Aldh1L1-EGFP fluorescence (***H***) seen in the Aldh1L1-EGFP transgenic mouse cortex shows that all cells expressing the EGFP transgene also express the endogenous Aldh1L1 protein. Scale bars: ***A–F***, 40 μm; ***G–I***, 60 μm.

Analysis of canonical pathways enriched in the main CNS cell types

Table 1. Ingenuity pathway analysis identified cell-type-enriched pathways

A: Astrocyte enriched signaling pathways

	P-value	Signaling Pathway
1	1.82E-05	Notch Signaling
2	5.01E-05	Xenobiotic Metabolism Signaling
3	1.32E-04	Wnt/β-catenin Signaling
4	2.34E-04	Cell Cycle: G1/S Checkpoint Regulation
5	6.76E-04	TGF-β Signaling
6	7.24E-04	Axonal Guidance Signaling
7	8.91E-04	Ephrin Receptor Signaling
8	2.63E-03	Integrin Signaling
9	3.80E-03	Sonic Hedgehog Signaling
10	9.55E-03	PTEN Signaling
11	1.02E-02	p38 MAPK Signaling
12	1.05E-02	PI3K/AKT Signaling
13	1.07E-02	Circadian Rhythm Signaling
14	1.17E-02	Apoptosis Signaling
15	1.26E-02	PPAR Signaling
16	2.00E-02	Actin Cytoskeleton Signaling
17	2.69E-02	Toll-like Receptor Signaling
18	3.09E-02	ERK/MAPK Signaling
19	3.80E-02	Antigen Presentation Pathway
20	4.79E-02	JAK/Stat Signaling

B: Oligodendrocyte enriched signaling pathways

	P-value	Signaling Pathway
1	3.98E-11	Axonal Guidance Signaling
2	5.25E-10	Integrin Signaling
3	7.76E-09	Ephrin Receptor Signaling
4	2.45E-07	ERK/MAPK Signaling
5	6.03E-06	PI3K/AKT Signaling
6	7.08E-06	Neuregulin Signaling
7	2.63E-05	Actin Cytoskeleton Signaling
8	1.82E-04	SAPK/JNK Signaling
9	4.57E-04	PTEN Signaling
10	5.25E-04	Estrogen Receptor Signaling
11	5.25E-04	B Cell Receptor Signaling
12	8.91E-04	PPAR Signaling
13	1.17E-03	PDGF Signaling
14	1.20E-03	Protein Ubiquitination Pathway
15	1.48E-03	Leukocyte Extravasation Signaling
16	1.51E-03	Apoptosis Signaling
17	1.86E-03	IGF-1 Signaling
18	1.91E-03	Natural Killer Cell Signaling
19	2.14E-03	VEGF Signaling
20	2.95E-03	JAK/Stat Signaling
21	3.02E-03	TGF-β Signaling
22	3.39E-03	Insulin Receptor Signaling
23	3.80E-03	Fc Epsilon RI Signaling
24	6.03E-03	NF-kapaB Signaling
25	8.13E-03	Huntington's Disease Signaling
26	9.77E-03	Neurotrophin/TRK Signaling
27	1.10E-02	Cell Cycle: G2/M DNA Damage Checkpoint
28	1.12E-02	GM-CSF Signaling
29	1.58E-02	Xenobiotic Metabolism Signaling
30	2.04E-02	IL-6 Signaling
31	2.14E-02	Wnt/β-catenin Signaling
32	2.88E-02	IL-2 Signaling
33	3.31E-02	Notch Signaling
34	3.80E-02	Hypoxia Signaling in the CV System
35	3.98E-02	Cardiac β-adrenergic Signaling
36	4.17E-02	EGF Signaling
37	4.68E-02	G-Protein Coupled Receptor Signaling
38	4.90E-02	T Cell Receptor Signaling

C: Neuron enriched signaling pathways

	P-value	Signaling Pathway
1	7.94E-26	Calcium Signaling
2	1.58E-15	Axonal Guidance Signaling
3	5.01E-15	Synaptic Long Term Depression
4	3.16E-13	Synaptic Long Term Potentiation
5	2.51E-11	Glutamate Receptor Signaling
6	1.74E-10	cAMP-mediated Signaling
7	5.62E-09	Huntington's Disease Signaling
8	3.16E-07	Chemokine Signaling
9	3.98E-07	G-Protein Coupled Receptor Signaling
10	4.68E-07	Ephrin Receptor Signaling
11	5.50E-07	Cardiac β-adrenergic Signaling
12	3.16E-06	Nitric Oxide Signaling in the CV System
13	2.57E-05	B Cell Receptor Signaling
14	7.76E-05	GABA Receptor Signaling
15	2.69E-04	Amyloid Processing
16	3.31E-04	ERK/MAPK Signaling
17	5.01E-04	Amyotrophic Lateral Sclerosis Signaling
18	9.55E-04	Dopamine Receptor Signaling
19	1.91E-03	Actin Cytoskeleton Signaling
20	6.17E-03	Integrin Signaling
21	1.12E-02	Neuregulin Signaling
22	1.20E-02	GM-CSF Signaling
23	1.23E-02	SAPK/JNK Signaling
24	1.23E-02	IGF-1 Signaling
25	1.38E-02	Protein Ubiquitination Pathway
26	1.78E-02	Phototransduction Pathway
27	1.91E-02	T Cell Receptor Signaling
28	2.69E-02	Xenobiotic Metabolism Signaling
29	3.24E-02	Parkinson's Signaling

D: Astrocyte enriched metabolic pathways

	P-value	Metabolic Pathway
1	1.58E-11	Valine, Leucine and Isoleucine Degradation
2	7.94E-07	Propanoate Metabolism
3	1.38E-04	Fatty Acid Metabolism
4	8.91E-04	Citrate Cycle
5	1.26E-03	Butanoate Metabolism
6	2.45E-03	Pyruvate Metabolism
7	2.95E-03	Alanine Metabolism
8	7.08E-03	Lysine Degradation
9	1.70E-02	Nitrogen Metabolism
10	1.74E-02	N-Glycan Degradation
11	1.82E-02	Glycolysis/Gluconeogenesis
12	4.27E-02	Glycine, Serine and Threonine Metabolism

E: Oligodendrocyte enriched metabolic pathways

	P-value	Metabolic Pathway
1	6.76E-03	Inositol Phosphate Metabolism
2	2.09E-02	Lysine Degradation
3	2.88E-02	Nicotinate and Nicotinamide Metabolism

F: Neuron enriched metabolic pathways

	P-value	Metabolic Pathway
1	2.29E-04	Inositol Phosphate Metabolism
2	1.91E-02	Nicotinate and Nicotinamide Metabolism
3	2.40E-02	Aminosugars Metabolism

Canonical signaling pathways (A–C) and metabolic pathways (D–F) statistically enriched in astrocytes, OLs, and neurons at $p < 0.05$ (5.00E-02). Genes enriched in astrocytes, OLs, and neurons (supplemental Tables S4–S6, available at www.jneurosci.org as supplemental material) were compared to Ingenuity's database of 72 canonical signaling pathways and 80 metabolic pathways. Pathways enriched at $p < 0.001$ (1.00E-03) are considered highly significant.

Analysis of genes expressed by astrocytes

Table 2. Genes in defined phagocytosis and engulfment pathways

Probe set ID	Astrocytes (P7)	Astrocytes (P17)	Neurons (P16)	Myelin oligos	Gene symbol	Gene title
1451086_s_at	12835	17817	9937	19272	Rac1 (ced-10)	RAS-related C3 botulinum substrate 1
1421840_at	13229	11313	443	239	Abca1 (ced-7)	ATP-binding cassette, subfamily A (ABC1), member 1
1453771_at	840	770	105	69	Gulp1 (ced-6)	GULP, engulfment adaptor PTB domain containing 1
1429841_at	4746	8268	149	2340	Megf10 (ced-1)	Multiple EGF-like-domains 10
1448655_at	4671	5922	1693	86	Lrp1 (ced-1)	Low-density lipoprotein receptor-related protein 1
1448248_at	5831	6609	4578	5183	Crk (ced-2)	v-crk sarcoma virus CT10 oncogene homolog (avian)
1452220_at	4040	3898	175	1574	Dock1 (ced-5)	Dedicator of cyto-kinesis 1
1456098_a_at	1232	4115	1553	801	Elmo2 (ced-12)	Engulfment and cell motility 2, ced-12 homolog (*C. elegans*)
1422869_at	1948	7370	59	89	Mertk	c-mer proto-oncogene tyrosine kinase
1423586_at	2145	2598	52	43	Axl	AXL receptor tyrosine kinase
1417399_at	503	1991	1485	268	Gas6	Growth arrest specific 6
1452784_at	9018	10595	1757	4162	Itgav	Integrin α V
1417533_a_at	8569	6783	193	231	Itgb5	Integrin β 5
1420911_a_at	25011	31089	617	754	Mfge8	Milk fat globule-EGF factor 8 protein
1417876_at	36	50	11	36	Fcgr1	Fc receptor, IgG, high-affinity I
1435477_s_at	48	21	32	54	Fcgr2b	Fc receptor, IgG, low-affinity IIb
1448620_at	24	33	34	72	Fcgr3	Fc receptor, IgG, low-affinity III
1418340_at	21	55	22	80	Fcer1g	Fc receptor, IgE, high-affinity I, gamma polypeptide
1450678_at	55	33	79	79	Cd11/Itgb2	Integrin β 2
Reference cell-type-enriched genes						
1440142_s_at	9742	13827	31	77	Gfap	Glial fibrillary acidic protein
1448768_at	7	14	6	24964	Mog	Myelin oligodendrocyte glycoprotein
1433884_at	326	339	18197	239	Syt1	Synaptotagmin I

Molecular components of phagocytosis and engulfment pathways are enriched and expressed at high levels in astrocytes. See Results and Discussion for a complete description of the different pathways.

(., 1999).

1, 1 2, 1/2/3, 3 (, 2004) x - 11,). (1) 2 3 (., 2002; ., 2005). 2 (2), -β ( ), /β- x, (1A). (., 2001), 2). x ( ) *C. elegans* *Drosophila* (., 2004; ., 2006). -10 (1) -1 (10, *Drosophila*), -7 (1), -6 (1), -1 1 (0.91), (., 1993). -2 ( ), -5 (1), -12 ( ) -7/ -6/ -1 x (2). α β5 x (., 2006), 8 6, 8 x

Analysis of genes expressed by oligodendrocytes

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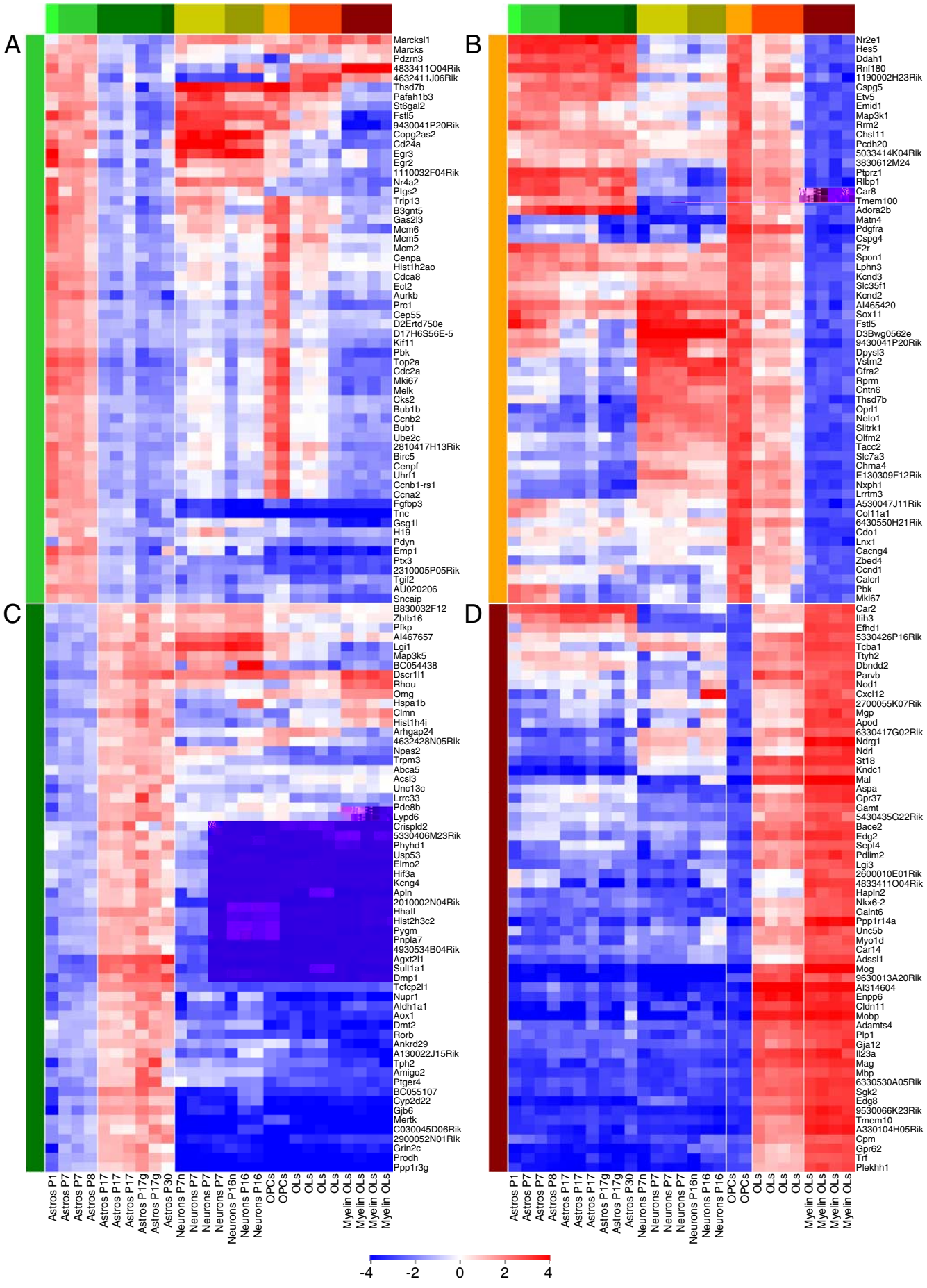
Analysis of genes expressed by neurons

Gene expression changes during astrocyte and oligodendrocyte development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Rrm2
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Comparison of *in vivo* astrocytes with *in vitro* cultured astroglia

Discussion

A database of transcriptional profiles for CNS neural cell types

Figure 6. Genes upregulated and downregulated during astrocyte and oligodendrocyte development. The top 60 genes most downregulated during astrocyte development (***A***, light green bar) and OL development (***B***, orange bar), and the top 60 genes most upregulated during astrocyte development (***C***, dark green bar) and OL development (***D***, red bar). The genes are plotted on a heat map to illustrate gene expression patterns in all CNS cell types at different developmental stages. The individual gene expression level for each cell type is normalized to the age averaged astrocyte expression (***A***, ***C***) and the age averaged OL expression (***B***, ***D***). The normalized values are plotted on a $\log_2$ color scale, with blue representing low expression and red representing high expression. The fold enrichment can be estimated from the $\log_2$ color bar scale. For example, the change from light blue (−1) to medium red (2) represents an eightfold difference in expression level. Note that, although few genes strongly downregulated during development (***A***, ***B***) are expressed in a cell-type-specific pattern, the majority of genes strongly upregulated during development (***C***, ***D***) are expressed in a cell-type-specific pattern.

Identification of new cell-type-specific markers

The astrocyte transcriptome provides many new clues to astrocyte development and function

Several evolutionarily conserved phagocytic pathways are highly enriched in astrocytes

Drosophila (2003; 2006). *C. Elegans* *in vivo*. -1/ / 10 (1/ 91), -7, -6/ 1 -2/ -5/ 1, -12/ / , α β5 (2002; 2003; 2006; 2007), *in vivo*. 8, *in vivo*. 11/ 18 / β2 *in vivo* (1995). *Drosophila*, 10), 1, 1 (2007). *in vivo* (2004). (1978). 10 *in vitro*, 2003), 8 (2007).

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(2005) 45:207 221.

(2006) -6 *Drosophila* 50:855 867.

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101:8384 8389.

(1992) 70:31 46.

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