

TECHNICAL WHITE PAPER: MOUSE DIVERSITY STUDY

Overview

While there is a significant literature on changes in the brain mediated by strain or sex that presumably contribute to behavioral differences, the influence of genetic background or sex on gene expression in the brain has not been investigated on a systematic, brain-wide basis.

The Allen Institute Mouse Diversity Study characterizes gene expression across genetic backgrounds and sex, expanding beyond the adult male *C57BL/6J* reference brain comprising the Allen Mouse Brain Atlas to include seven strains of male mice and female *C57BL/6J* mice. Gene expression was detected using colorimetric RNA *in situ* hybridization (ISH) that provides cellular level anatomic resolution. Data are publicly accessible via a Web application that presents searchable image series organized by gene, strain, or sex. This freely accessible online resource allows for simultaneous navigation and comparison of different mouse brains at high resolution, providing users with a dataset to probe for possible differences in gene regulation.

For both the strain and sex components of the project, a panel of 49 well known “drug target” genes that are expressed in the adult and developing brain and encompass sites of action for FDA-approved drugs were assayed (Table 1). These include genes encoding proteins involved in the synthesis, transport and signaling of neurotransmitters, G protein-coupled receptors and ion channels, as well as other intracellular signaling molecules. In addition to holding clinical relevance, the diversity of genes selected allows for the identification of possible trends that might emerge by gene class or family between strains or sex.

ISH assays were performed for these “drug target” genes in the sagittal plane (24 sections/gene) using a 200 μ m section interval across one hemisphere of the brain. This sampling strategy generates two or more sections for each of 200 brain areas, allowing for detailed anatomical comparison of labeling density, intensity, and distribution of expressing cells. Nissl stained sections were generated from all brains at 200 μ m intervals in order to provide detailed cytoarchitectural comparisons between the strains. Biological replicates were generated for 20 of the drug target genes across the strains.

For each component of the project, an additional 12 genes were selected to augment the research, as described below. For these experiments, coronal sections (60 sections/gene) were obtained at 200 μ m intervals, providing additional sampling, as both hemispheres of the brain are included.

Protocols used for data generation were similar to those used for the Allen Mouse Brain Atlas^{1,2}. Experiments were organized by gene into work packets in order to provide a high level of experimental control. Tissue collection and processing was uniform across groups, and ISH conditions were matched and performed simultaneously across all comparison groups whenever possible.

Strain

For the study, three closely related inbred laboratory strains [C57BL/6J, 129S1/SvImJ, DBA/2J], three wild-derived inbred *Mus*. sub-species [*Domesticus* (WSB/EiJ), *Castaneus* (CAST/EiJ), and *Musculus* (PWD/PhJ)], and one wild-derived inbred species *Mus*. *Spretus* (SPRET/EiJ) were used. The representative sub-species and species were selected based on genome sequence availability.

In addition to the drug target panel, 12 additional genes were examined: 6 genes (*Calb1*, *Grin2b*, *Zfhx1b*, *Cap1*, *Nnat*, *Cacna2d1*) that were predicted to show strain differences from other studies³, and 6 genes (*A930038C07RIK*, *Wfs2*, *Rorb*, *Etv1*, *Rprm*, *Inpp4b*) selected for enrichment in each of the primary lamina of the neocortex¹.

Data from the WSB and PWD strains were generated together, separate from the other strains, except for the biological replicates for 20 of the 49 drug targets where all 7 groups were concurrently processed.

Sex

In this study, gene expression was assayed in female C57BL/6J mice at three stages of estrus and in adult C57BL/6J male mice. Stages of estrus were estimated cytologically (modified PAP smear) after at least 2 consistent, regular cycles.

In addition to the drug target genes, 12 additional genes were assayed. These genes were chosen based on known sexual dimorphisms in other species and/or unstudied across the brain of the mouse (*Avpr1a*, *Cacna1g*, *Cckbr*, *Eif2s3x*, *Gabre*, *Gal*, *Nos1*, *Htr2c*, *Ncoa1*, *Pgr*, *Npy1r*, *Gabrd*).

Table 1. Drug target genes

Gene	Class	Family	Indication / Symptom	Drug Name	Brand Name / Aliases	Notes
Abcc1	Transporter	Abc trans	Transplant rejection, Psoriasis	Cyclosporine	Gengraf, Neoral, Restasis, Sandimmune	Immunosuppressant
Ache	Catalytic	Cholinergic	Dementia (Alzheimer's associated)	Donepezil	Aricept	Cholinesterase inhibitor
Adra2a	GPCR	Adrenergic	Hypertension	Clonidine	Catapres	Alpha agonist
Adrb1	GPCR	Adrenergic	Hypertension	Clonidine	Catapres	Alpha agonist
Agtr1a	GPCR	Angiotensin	Hypertension	Losartan	Cozaar	Angiotensin II receptor antagonist
AR	Nuc Receptor	Hormone	Prostate Cancer	Flutamide	Eulexin	Luteinizing hormone-releasing antagonist
Bche	Catalytic	Cholinergic	Dementia	Rivastigmine	Exelon	Cholinesterase inhibitor; Alzheimer's, Parkinson's
Cacna1b	Ion Channel	Ion Channel	Angina	Amlodipine	Norvasc	Calcium channel blockers
Chrm1	GPCR	Cholinergic	Muscle spasms, Motility, Shortness of b	Atropine	Isopto Atropine, Lomotil, Sal-Tropine	Muscarinic acetylcholine receptor inhibitor
Chnb2	Ion Channel	Cholinergic	Nicotine addition, Hypertension	Mecamylamine	Inversine	Ganglionic blocker
Cnr1	GPCR	Cannabinoid	Obesity, Nicotine addiction	Rimonabant	removed from market	Cannabinoid receptor CB1 inverse antagonist
Comt	Catalytic	Dopamine	Parkinson's Disease	Entacapone	Comtan	COMT inhibitor
Cyp19a1	Catalytic	Hormone	Post-menopausal breast cancer	Anastrozole	Arimidex	Aromatase inhibitor
Drd1a	GPCR	Dopamine	Schizophrenia	Clozapine	Clozaril	Atypical anti-psychotic
Drd2	GPCR	Dopamine	Dementia	Olanzapine	Zyprexa	Atypical anti-psychotic: schizophrenia, depression, mania
Drd4	GPCR	Dopamine	Dementia	Olanzapine	Zyprexa	Atypical anti-psychotic: schizophrenia, depression, mania
Ednrb	GPCR	Endothelin	Hypertension	Bosentan	Tracleer	Endothelin receptor antagonist: lung-related
Egfr	TK Receptor	EGF	Lung Cancer	Gefitinib	Iressa	EGFR tyrosine kinase inhibitor
Erb2	TK Receptor	Oncogene	Breast Cancer	Trastuzumab	Herceptin	HER2/neu receptor antagonist
Esr1	Nuc Receptor	Hormone	Breast Cancer	Tamoxifen	Nolvadex, Soltamox	Antiestrogen
Esr2	Nuc Receptor	Hormone	Prostate Cancer	Bicalutamide	Casodex	Nonsteroidal antiandrogen
Faah	Catalytic	Hydrolase	Anesthesia	Propofol	Diprivan	Faah inhibitor
Gabra2	Ion Channel	GABA	Anxiety, Seizures, Muscle tension	Diazepam	Valium, Diastat	Benzodiazepine
Gabra3	Ion Channel	GABA	Anxiety, Seizures, Muscle tension	Diazepam	Valium, Diastat	Benzodiazepine
Gabrb1	GPCR	GABA	Muscle spasms, Alcohol withdrawal	Baclofen	Lioresal	GABA receptor antagonist
Gabrb2	GPCR	GABA	Muscle spasms, Alcohol withdrawal	Baclofen	Lioresal	GABA receptor antagonist
Gabrb3	GPCR	GABA	Muscle spasms, Alcohol withdrawal	Baclofen	Lioresal	GABA receptor antagonist
Gnrh	Peptide	Hormone	Prostate Cancer, Endometriosis	Leuprolide	Lupron, Eligard, Viadur	GNRH, SHRH antagonist
Gsk3a	Cell signaling	Kinase	Bipolar disorder	Lithium	Eskalith	Antimanic agent
Gsk3b	Cell signaling	Kinase	Bipolar disorder	Lithium	Eskalith	Antimanic agent
Hrh1	GPCR	Histamine	Allergies, Parkinson's (early)	Diphenhydramine	Benadryl, AllerMax, Cmproz	Antihistamine
Htr1a	GPCR	Serotonin	Anxiety	Buspirone	Buspar	Serotonin receptor antagonist
Htr1b	GPCR	Serotonin	Migraines	Sumatriptan	Imitrex	Selective serotonin receptor antagonist
Htr2a	GPCR	Serotonin	Schizophrenia	Olanzapine	Zyprexa	Atypical anti-psychotic
Inpp1	Cell signaling	Phosphatase	Bipolar disorder	Lithium	Eskalith	Antimanic agent
Maob	Catalytic	Dopamine	Parkinson's	Selegiline	Eldepryl	MAOB inhibitors
Oprk1	GPCR	Opioid	Pain	Propoxyphene	Darvon, Darvon-N	Narcotic pain reliever
Oprm1	GPCR	Opioid	Pain	Morphine	Morphine	Narcotic pain reliever
Pdgfra	TK Receptor	PDGF	Leukemia	Imatinib	Gleevec	Protein-tyrosine kinase inhibitor
Pdgfrb	TK Receptor	PDGF	Leukemia	Imatinib	Gleevec	Protein-tyrosine kinase inhibitor
Ppp3ca	Cell signaling	Phosphatase	Transplant rejection, Arthritis	Ciclosporin	Gengraf, Neoral, Restasis, Sandimmune	Immunosuppressant
Ptgs2	Catalytic	Prostaglandin	Pain	Prostaglandin	Many	Eicosanoid
Scn10a	Ion Channel	Ion Channel	Anesthesia	Tetracaine	Amethocaine, Pontocaine	Ryanodine receptor antagonist
Slc6a1	Transporter	GABA	Epilepsy	Tiagabine	Gabitril	Anticonvulsant
Slc6a2	Transporter	Adrenergic	Depression, OCD	Bupropion	Wellbutrin, Zyban	Antidepressant
Slc6a4	Transporter	Serotonin	Depression	Fluoxetine	Prozac, Sarafem	Serotonin reuptake inhibitor
Srd5a1	Catalytic	Hormone	BPH, Male pattern hair loss	Finasteride	Proscar, Propecia	5-alpha reductase inhibitor
Th	Catalytic	Dopamine	Hypertension (pheochromocytoma)	Alpha-methyltyrosine	Alpha-methyltyrosine	Th inhibition
Top1	Catalytic	DNA enzyme	Colorectal Cancer	Irinotecan	Camptosar	Topoisomerase I inhibitor

References

- Lein et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. *Nature* 445: 168-76.
- Data Production Processes*. <http://mouse.brain-map.org/pdf/ABADataProductionProcesses.pdf>
- Rob Williams and Daniel Ciobanu selected 6 genes, from hippocampal array data, that are associated with strong evidence of genetic variation. Additional criteria for selection included patterned expression in other areas of the brain. Hippocampus Consortium M430v2; June06; (http://www.genenetwork.org/dbdoc/HC_M2_0606_P.html).