

ANNOTATION REPORT



INFERIOR COLLICULUS (IC)

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Introduction

This report contains a gene expression summary of the inferior colliculus (IC), derived from the <u>Allen Brain Atlas</u> (ABA) in-situ hybridization (ISH) mouse data set. The structure's location and morphological characteristics in the mouse brain are described using the Nissl data found in the <u>Allen Reference Atlas</u>. Using an established algorithm, the expression values of the IC were compared to the values of the macro/parent-structure, in this case the midbrain, for the purpose of extracting regionally specific gene expression data. The highest ranking ratios were then manually curated and verified. The 50 Select Genes (at the time of report completion) were compiled for expression characterization. The experimental data for each gene may be accessed via the links provided; complementary sagittal data may also be accessed using the <u>ABA</u>. Correlation between gene expression in the IC and the rest of the brain, across all genes in the coronal dataset (~4300 genes), were derived computationally and are presented below. A gene ontology table (derived from DAVID Bioinformatics Resources 2007) is also included, highlighting possible functions of these 50 Select Genes.

To read more about how the 50 Select Genes list is derived, please refer the *Fine Structure Annotation white paper*.

Allen Reference Atlas Coronal Levels: 93-112 Allen Reference Atlas Sagittal Levels: 10-21 Shown below is a plate from the Allen Reference Atlas, depicting the inferior colliculus (level 103):



Description of Structure:

LOCATION and STRUCTURAL ANATOMY:

The hierarchical relationship within the brain is depicted below in the structure <u>legend</u>. The Allen Reference Atlas (based on Nisslstained sections scanned at 10X) was the primary resource for the following descriptions.

For additional information please refer to the <u>Allen Reference Atlas white paper</u>. <u>BrainInfo</u> houses a search engine that allows searches for structure name aliases.

The inferior colliculus (IC) is an ovoid sub-structure of the (sensory-related) midbrain that can be divided into external, central, and dorsal nuclei (ICe, ICc and ICd). In the coronal plane the ICe presents as the most rostral subdivision of the IC, appearing at the lateral border of the midbrain near the caudal pole of the cerebrum. The superior colliculus (SC) borders the IC dorsally, and the lateral lemniscus (a cell sparse fiber tract) forms a distinct ventral border. In the rostral half of the IC, a distinct lateral wall is formed by the brachium of the inferior colliculus (bic), at the lateral edge of the midbrain. Proceeding caudally in coronal cross-sections, the ICe increases in size until the the cerebellar flocculi appear and the caudal pole of the cortex disappears. Around this point, the central and dorsal subdivisions appear, and the IC replaces the SC as the most dorsal part of the midbrain. The subdivisions are difficult to discriminate, although the commissure of the inferior colliculus (cic) may aide in marking a border beneath ICd. The periaqueductal gray (PAG) separates the medial portion of the IC from the cerebral aqueduct throughout the rostrocaudal extent of the IC, except at the most posterior aspect where the IC adjoins the cerebellum.

From the sagittal perspective, the IC is observed as the lower of two small bumps (colliculus means "hill") that form the dorsal ridge of the midbrain. The SC can be seen anterior, and the cerebellum posterior, to the IC. The three subdivisions described above are not delineated in the sagittal plane in the Allen Reference Atlas.

The cells within the IC are primarily small, rounded cells with a scattered population of larger, darker staining cells. In NissI-stained sections, the three subdivisions of the IC can best be identified by contrasts in cellular density. The ICd contains the densest concentration of cells, whereas the ICe shows the lowest density, and the cell density of the ICc appears intermediate.

The appearance and location of the IC can be appreciated on the following two pages. Nisslstained sections and Allen Reference Atlas plates reveal the cytoarchitecture and extent of the IC, and its location in relation to surrounding structures.





Atlas and Nissl: Sagittal: **Rostral** Caudal a C de Atlas-Sagital-46-B Atlas-Sagital-48 Position: 3800 6208 - - -Reference Atlas PIN PINZ CUL4,5 (IV,V) CUL 4,5 (IV,V) POST IC IC IC SC MB NB Sto Level 16 Level 21 Level 10 A. 34 20 52 J.C. 51 Nissl . 70 - A

In-Situ Hybridization Expression Patterns of 50 Select Genes:

The ISH data presented below presents the anatomical and cytoarchitectural characteristics of the IC in the context of actual gene expression. In addition to presenting molecularly defined borders, ISH gene expression patterns also aid in phenotyping cell populations that otherwise can not be differentiated on purely morphological grounds. The 50 genes in this section were selected based on a mathematical algorithm to identify gene expression patterns that allow selective identification of the IC. The gene expression patterns were then verified manually. As such, these genes do not represent the only genes found in this structure, genes specific to this structure, or genes expressing at the highest level within this structure.

The ISH protocol is described in the <u>Data Production Processes white paper</u>. To read about heat map conversion, refer to the <u>Informatics Data Processing white paper</u>. The expression data subsequently presented can be further explored, in coronal and sagittal planes, at <u>brain-map.org</u>.

This survey of the 50 Select Genes showed several possible expression patterns in the IC. One recurring pattern was widespread expression across the entire IC. Another common, but less frequent pattern, revealed the borders of the ICc in contrast to the ICe and ICd. Several genes showed scattered expression within the IC. A few expression patterns suggested cell subpopulations within nuclei, but none displayed regional localization. The boundaries between the IC and nearby structures were clearly defined compared to the NissI-stained sections, and the borders within the IC were obvious but rarely stark.

Cellular density expression key		Cellular intensity expression key		
None	No expression	No color	Very low intensity	
Sparse	Very few cells expressing	Blue	Low intensity	
Scattered	Less than 10% of cells expressing in scattered pattern	Green	Medium intensity	
Medium	10-80% of cells expressing	Yellow	High intensity	
High	Greater than 80% of cells expressing	Red	Very high intensity	

To view heat map at <u>brain-map.org</u>, right click on an ISH image and select "Show Expression Analysis."

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ISH DATA The Allen Institute ISH images below were selected to highlight various expression patterns of the IC.

ISH D30017J20Rik

Coronal:

A widespread expression pattern can be seen below.



Heat map D30017J20Rik

Coronal:

A medium-high density and medium intensity expression pattern can be seen in the entire IC in this heat map image.



ISH <u>Ngef</u>

Coronal:

The external and dorsal nuclei of the IC are shown in the Ngef expression profile below.

Heat map Ngef

Coronal:

Ngef shows very high density and intensity expression in the ICd and ICe, but the density drops to scattered in the ICc.





ISH Ndst4

Coronal:

Ndst shows an expression pattern predominantly in the ICd.



Heat map Ndst4

Coronal:

The high density and intensity expression pattern can be seen in the dorsal subdivision of the IC.



Inferior colliculus, dorsal

ISH <u>Ndst4</u> Sagittal:

This view shows a caudal enrichment of expression.



Heat map

<u>Ndst4</u> Sagittal:

The high density and intensity expression pattern shown below.



50 SELECT GENES:

This gene list was generated by manual curation of an <u>algorithmically</u> derived list that compared gene expression values of IC to those of the midbrain. Categories of expression are subjectively grouped by relative expression characteristics. Curation of 50 Select Genes List: June 2007

Widespread Expression Pattern						
Number	Gene Symbol	Gene Name	Expression Pattern			
		sema domain, immunoglobulin domain (Ig),				
1	<u>Sema7a</u>	and GPI membrane anchor, (semaphorin) 7A	High density and intensity			
	A she she d		High density, medium intensity and medium			
2	Adarb1		density very high intensity			
3	D330017J20Rik	RIKEN CDNA D33001/J20 gene	Medium-high, density high intensity			
4	Rasgrp2	RAS, guanyl releasing protein 2	Medium-high, density medium intensity			
5	2310045A20RIK	RIKEN CDNA 2310045A20 gene	Medium density, medium-high intensity			
6	Canl	CAP, adenyiate cyclase-associated protein, 2	Madium density and intensity			
0	<u>Capz</u>	(yeasi)	Medium density and intensity			
/		unc-5 nonolog D (C. elegans)	Medium density and intensity			
0	<u>Ryss</u> Emb	ambigin	Medium density and intensity			
9		transforming growth factor, boto 1	Medium density and intensity			
10		transforming growth factor, beta i	Sectored medium density medium high			
11			intensity			
	D93004010124111K	KIKEN CDNA D930040M24 gene	Scattered medium density medium low in-			
12	Adamts15	ADAMTS-like 5	tensity			
13	Cckbr	cholecystokinin B receptor	Scattered-medium density low intensity			
14	Meaf11	multiple EGE-like-domains 11	Scattered density, very high intensity			
	Mogrin	ST3 beta-galactoside alpha-2 3-				
15	St3gal1	sialvitransferase 1	Scattered density, high intensity			
16	Col24a1	procollagen, type XXIV, alpha 1	Scattered density, high intensity			
		potassium voltage-gated channel, subfamily	,			
17	Kcnh7	H (eag-related), member 7	Scattered density, medium-high intensity			
		potassium voltage-gated channel, subfamily				
18	Kcnq4	Q, member 4	Scattered density, medium intensity			
19	Sox14	SRY-box containing gene 14	Scattered density, medium intensity			
20	Hs6st3	heparan sulfate 6-O-sulfotransferase 3	Scattered density, medium intensity			
21	Stard13	serologically defined colon cancer antigen 13	Scattered density, medium intensity			
		guanine nucleotide binding protein, alpha				
22	<u>Gnal</u>	stimulating, olfactory type	Scattered density, medium-low intensity			
			High density, medium intensity widespread;			
		calcium channel, voltage-dependent, gamma	Scattered density, very high intensity			
23	Cacng5	subunit 5	external nucleus			
		signal induced proliferation approximated 4 like	Medium density, low intensity widespread;			
24	Sipo111		Scattered density, medium intensity external			
24	Sipatri		Medium density low intensity widespread:			
			Scattered density, nedium intensity external			
25	00433254	1 00433254	nucleus			
	200100201		Medium density very high intensity wide-			
			spread: high density, very high intensity dor-			
26	Nrgn	neurogranin	sal nucleus			
			Medium density, high intensity widespread;			
27	Zfp365	zinc finger protein 365	High density high intensity central nucleus			
			Medium intensity and density widespread;			
			Medium-high density, medium intensity cen-			
28	Npy1r	neuropeptide Y receptor Y1	tral nucleus			
			Medium density, and intensity widespread;			
	1		High density, very high intensity external and			
29	Arpp21	cyclic AMP-regulated phosphoprotein, 21	dorsal nuclei			





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PTK2 protein tyrosine kinase 2 beta

RIKEN cDNA C630035N08 gene

RIKEN cDNA 2010300C02 gene

discs, large (Drosophila) homolog-

calcium channel, voltage-dependent,

G protein-coupled receptor 26

associated protein 2

neuropeptide Y

gamma subunit 3

cadherin 9

34 Ptk2b

35Cdh9

38 Dlgap2

39 Gpr26

40 Npv

41 Cacng3

36 C630035N08Rik

372010300C02Rik

Medium high, density and intensity external

Sparse density, medium-high intensity external nucleus; Medium density, high intensity

Medium high density and intensity external

Medium density and intensity external nucleus; High density and intensity dorsal nu-

High density, medium intensity external nu-

Medium density and intensity external and

cleus; Medium density and intensity dorsal nu-

Scattered density, very high intensity external

Scattered density, medium intensity external

and dorsal nuclei

dorsal nucleus

cleus

cleus

dorsal nuclei

and dorsal nuclei

and dorsal nuclei

and dorsal nuclei



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Correlated Expression:

The ABA coronal set contains the majority of genes of known scientific interest, as well as genes exhibiting marked or unique expression patterns. A correlation analysis of all available ABA coronal experiments (4376) was performed by comparing an expression value of the inferior colliculus (IC) to expression values in other regions of the brain. Following <u>image analysis</u>, the data values for each experiment were mapped to a 3-D reference brain at 200µm³ voxel resolution. Then, each voxel was assigned a single expression value based on the product of density and intensity of expression. Values from all 4376 experiments were computed, and the likelihood of co-expression between any two voxels or regions are reported as a Pearson's correlation coefficient.

For the purposes of determining correlated expression between the IC and other brain regions, expression values from all voxels within the IC were aggregated to form a single expression value. Two types of comparisons were then made. First, the aggregate expression values of the IC and those of other anatomically defined regions (~200 structures) were compared within the 3-D reference brain (structure vs. structure; table below). Second, a color map was then generated to display the correlation between the IC and each of the ~53,000 voxels of the reference volume (structure vs. voxel; correlation map on the following page).

STRUCTURE vs. STRUCTURE

The expression value of the IC was compared to expression values for all other defined atlas regions. Degree of correlation is displayed as a comparative fraction, with self-correlation = 1.000. Correlation between the IC and macro/parent-structures are presented, as well as correlation between the IC and the 25 highest ranking substructures. (The most highly correlated macro/parentstructures do not always contain the 25 top most correlated substructures). Columns match the Allen Reference Atlas palette.

Macro structure: Rostral-> Caudal	
CULE 1 25 3 5 4 1 5 3 5 6 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	
CL (Jalianin) CL (Jalianin) CL (Colos) CL (Colos)	Correlation
Inferior colliculus (IC)	1
Cuneiform nucleus (CUN)	0.958987
Superior colliculus, motor related (SCm)	0.958562
Superior colliculus, sensory related (SCs)	0.954491
Pretectal region (PRT)	0.944544
Nucleus of the lateral lemniscus (NLL)	0.941754
Anterior pretectal nucleus (APN)	0.940519
Periaqueductal gray (PAG)	0.939428
Parabrachial nucleus (PB)	0.939052
Nucleus of the optic tract (NOT)	0.938709
Supratrigeminal nucleus (SUT)	0.936548
Nucleus sagulum (SAG)	0.934455
Zona incerta (ZI)	0.934142
Hypothalamic lateral zone (LZ)	0.933689
Nucleus of the posterior commissure (NPC)	0.92957
Pedunculopontine nucleus (PPN)	0.928766
Nucleus incertus (IN)	0.927944
Pallidum, ventral region (PALv)	0.926593
Spinal nucleus of the trigeminal, caudal part (SPVC)	0.925037
Substantia inominata (SI)	0.924862
Midbrain reticular nucleus, retrorubral area (RR)	0.924343
Nucleus of the brachium of the inferior colliculus (NB)	0.923411
Principal sensory nucleus of the trigeminal (PSV)	0.922655
Midbrain reticular nucleus, magnocellular part, general (MRNmg)	0.92201
Superior central nucleus raphe (CS)	0.920854

STRUCTURE vs. VOXEL:

Correlation between the IC and all other 200um³ voxels in the brain. Degree of correlation assessed for each voxel is provided visually (lower value = the correlation value of the 25th ranked substructure reported on the previous page) using the "jet" color scale at rostrocaudal levels throughout the brain.



Gene Ontology (GO) Analysis:

GO TABLE: Below is an ontological analysis of the 50 Select Genes, using DAVID Bioinformatics Resources.

The functional terms that follow were returned using these constraints:

Category	Definition	Constraints	
P-value	Probability that the term is over-represented in this 50 Select Genes list relative to the mouse genome	when $p \le 0.05$	
Gene Count	The minimum number of genes that must fall into an onto- logical category to be considered a group	5 genes per term group	
GO Level	The level of functional specificity for GO functional cate- gories: Molecular Function (mf), Biological Process (bp) and Cellular Components (cc)	Level GO_All	
# of DAVID IDs	Number of unique DAVID gene IDs from user's input list	48 DAVID gene IDs/ 50 input genes	

Date of table completion: May 2007

GO Category	GO Term	Gene Count	% of Genes	p-value
GOTERM_MF_ALL	enzyme regulator activity	7	14.58%	0.001010798
GOTERM_MF_ALL	ion channel activity	5	10.42%	0.004760622
GOTERM_MF_ALL	alpha-type channel activity	5	10.42%	0.006424599
GOTERM_MF_ALL	transferase activity	10	20.83%	0.007153476
GOTERM_MF_ALL	channel or pore class transporter activity	5	10.42%	0.007705041
GOTERM_BP_ALL	cell communication	16	33.33%	0.008990189
GOTERM_CC_ALL	membrane	23	47.92%	0.012167082
GOTERM_CC_ALL	extracellular region	12	25.00%	0.015213919
GOTERM_BP_ALL	ion transport	6	12.50%	0.017880262
GOTERM_MF_ALL	cation transporter activity	5	10.42%	0.023974104
GOTERM_MF_ALL	protein kinase activity	5	10.42%	0.03079688
GOTERM_MF_ALL	kinase activity	6	12.50%	0.033947586
GOTERM_BP_ALL	signal transduction	14	29.17%	0.035284478
GOTERM_BP_ALL	protein amino acid phosphorylation	5	10.42%	0.039168191
GOTERM_CC_ALL	integral to membrane	18	37.50%	0.042444174
GOTERM_CC_ALL	intrinsic to membrane	18	37.50%	0.043195564
GOTERM_MF_ALL	signal transducer activity	13	27.08%	0.046880431
GOTERM_CC_ALL	extracellular space	10	20.83%	0.047689354
GOTERM_MF_ALL	phosphotransferase activity, alco- hol group as acceptor	5	10.42%	0.049044705
GOTERM_BP_ALL	intracellular signaling cascade	6	12.50%	0.049398881

Glynn Dennis Jr., Brad T. Sherman, Douglas A. Hosack, Jun Yang, Michael W. Baseler, H. Clifford Lane, Richard A. Lempicki. "DAVID: Database for Annotation, Visualization, and Integrated Discovery." *Genome Biology.* 2003 **4**(5): P3.

Inferior Colliculus (IC) Summary:

Anatomy

- In the coronal plane, the rostral aspect of the IC can be found at the lateral portion of the midbrain. Caudally, the IC occupies the dorsal-most aspect of the midbrain.
- In the sagittal plane, the IC presents between the cerebellum and the superior colliculus.
- The small cells within the IC exhibit a medium packing density in general, but there are also a population of larger, dark staining, multipolar cells scattered across the IC.
- The IC contains 3 subdivisions: the dorsal (ICd), central (ICc), and external (ICe).
- Without close inspection, the subdivisions of the IC are difficult to discern in NissI-stained sections; slight variations in cell density help in discriminating between the 3 subdivisions, with ICd appearing to have the highest, and ICe the lowest, density of cells.

Expression Patterns of the 50 Select Genes

- The most common pattern observed was widespread expression across the IC, yet this was seen across a range of cell densities.
- The borders between the 3 subdivisions could be clearly delineated by multiple genes.
- The border between the IC and the SC or PAG could be sharply defined by gene expression, and other borders tended to agree with the borders delineated by the reference atlas, with the rostral extent not exhibiting stark boundaries.

Expression Correlation with IC

- Midbrain and pons correlated most highly with the IC.
- Cerebellum and hippocampus were the least correlated regions.
- Of the top ranking 25 sub-structures highly correlated with the IC, most reside in the midbrain and pons, while the zona incerta and substantia inominata provide interesting exceptions.

Please send comments or questions by email to <u>!Annotation@alleninstitute.org</u>. To further explore the gene expression data and analytical tools referred to in this report, please access the genome-wide data set at <u>brain-map.org</u>.

Other Tools:

NEUROBLAST:

Many of the 50 genes listed in this report can be used to explore the NeuroBlast tool. This unique mining tool works seamlessly from within brain-map.org to produce a list of genes that share similar expression patterns to any gene in the coronal data set. Search for and select any gene, then select one of several brain regions from the NeuroBlast drop-tab to explore a ranked list of similarly expressed genes for that region.

To learn more about this function, please refer to the <u>NeuroBlast white paper</u>.

BRAIN EXPLORER:

To compare gene expression levels across anatomical structures in 3-D detail, download the <u>Brain Explorer</u> desktop application. This program is used to view gene expression in 3-D view (coronal, sagittal, horizontal and everywhere in between) across all brain structures and allows for simultaneous viewing of multiple expression profiles.

The NeuroBlast spatial homology function and an anatomic search tool are also available from within Brain Explorer to allow the user to search for and visualize genes with similar expression patterns.