

#### **ANNOTATION REPORT**

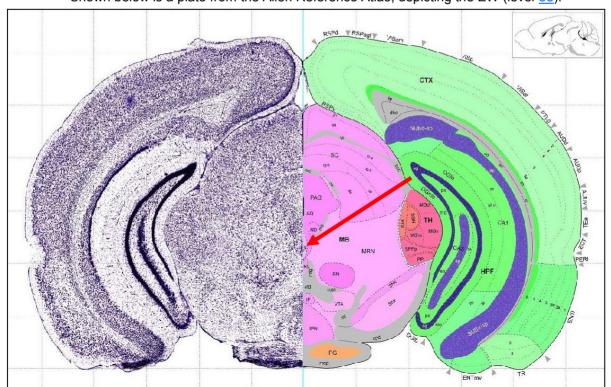
# **EDINGER-WESTPHAL NUCLEUS (EW)**

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### Introduction

ALLEN BRAIN ATLAS MOUSE BRAIN

This report contains a summary of expression patterns for genes that are enriched in the Edinger-Westphal nucleus (EW) of the midbrain. All data are derived from the <u>Allen Brain Atlas</u> (ABA) in situ hybridization mouse project. 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 Edinger-Westphal nucleus were compared to the values of its larger parent structure, in this case the midbrain, for the purpose of extracting regionally selective gene expression data. The highest ranking genes were manually curated and verified. 50 genes were then selected and compiled for expression analysis. The experimental data for each gene may be accessed via the links provided; additional data in the sagittal plane may also be accessed using the <u>ABA</u>. Correlations between gene expression in the Edinger-Westphal nucleus and the rest of the brain, across all genes in the coronal dataset (~4300 genes), were derived computationally. A gene ontology table (derived from DAVID Bioinformatics Resources 2007) is also included, highlighting possible functions of the 50 genes selected for this report.



Allen Reference Atlas Coronal Levels: 83-92 Allen Reference Atlas Sagittal Levels: 20-21 Shown below is a plate from the Allen Reference Atlas, depicting the EW (level 88):

# **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.

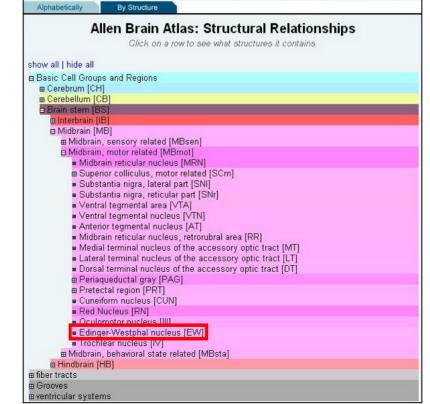
For additional information please refer to the <u>Allen Reference Atlas white paper</u>. <u>BrainInfo</u> houses a search engine that allows searches for structure name aliases.

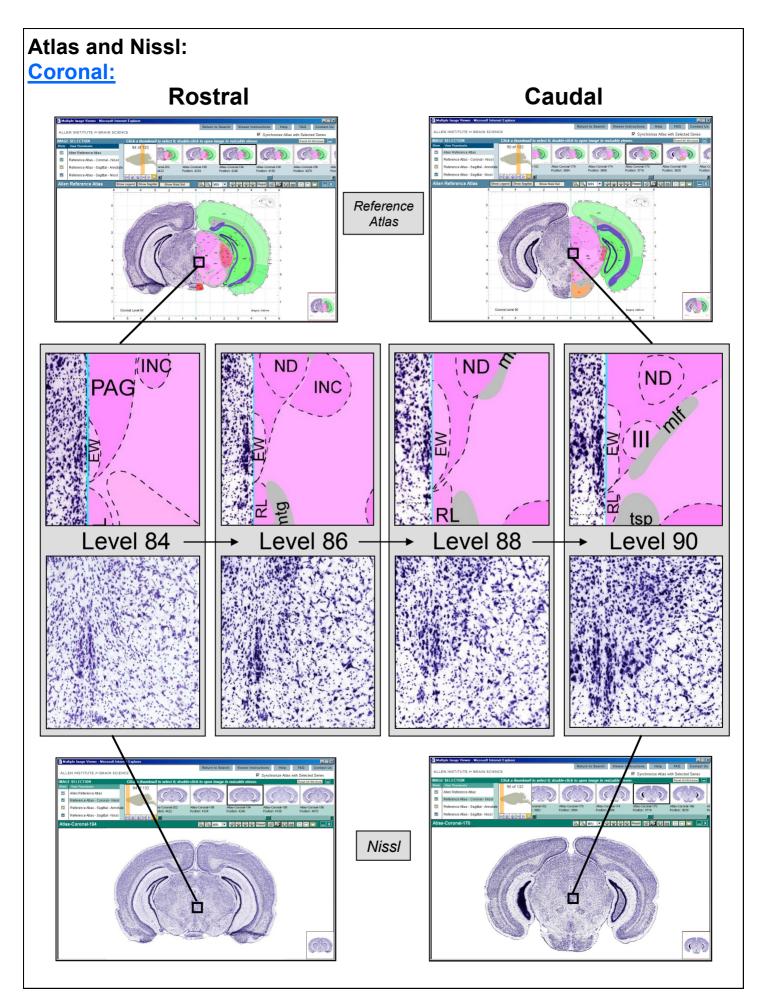
The Edinger-Westphal nucleus (EW) is located at the ventro-medial border of the periaqueductal gray matter (PAG) of the midbrain. It is the accessory parasympathetic nucleus of the oculomotor nerve, and consists of mid-sized, preganglionic neurons that are darkly stained and visible in both the coronal and sagittal Nissl sections.

In the coronal plane, its long, thin appearance and its proximity to the midline make it easy to locate. At its rostral edge, the cells of the EW are separated from the large neurons of the nucleus of Darkschewitsch, which lies dorsal and lateral to the EW, by a thin portion of the PAG. The medial longitudinal fascicle (mlf) and the mammillotegmental tract also serve to separate the EW from the surrounding neurons of the midbrain reticular nucleus (MRN), which are large but scattered. More caudally, the oculomotor nucleus (III) lies lateral to, and abuts, the EW; both lie medial to the mlf. These two nuclei can easily be differentiated from one another by the larger neurons found in III, and the more columnar arrangement of neurons in the EW.

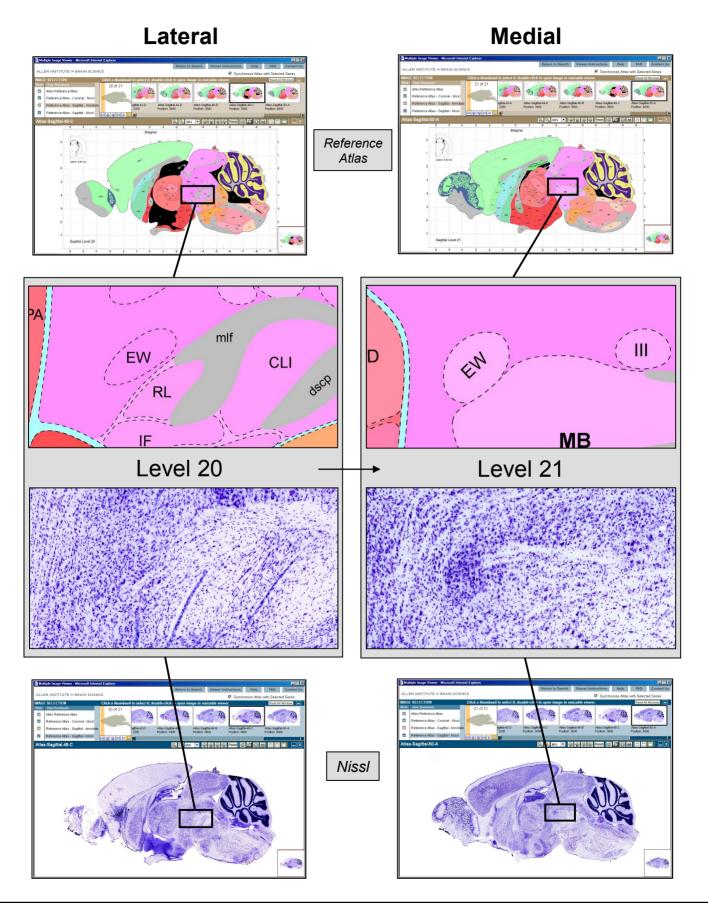
In the sagittal plane, the EW is somewhat more difficult to distinguish from surrounding structures than in the coronal plane. At the midline of the brain, it appears as a circular structure along the floor of the periaqueductal gray, located between the thalamus and the oculomotor nucleus. At its lateral aspect, it sits adjacent to the mlf, while at its medial aspect, it can be differentiated from the surrounding periaqueductal grey matter due to the striated appearance of the PAG. In this view, the oculomotor nucleus lies caudal to the EW, while the nucleus of Darkschewitsch lies dorsal and caudal.

The appearance and location of the Edinger-Westphal nucleus can be appreciated on the following two pages. Nissl-stained sections and Allen Reference Atlas (ARA) plates reveal the cytoarchitecture and extent of the Edinger-Westphal nucleus, and its location in relation to surrounding structures.





# Atlas and Nissl: Sagittal:



# In Situ Hybridization Expression Patterns of 50 Select Genes:

The in situ hybridization (ISH) data below presents the Edinger-Westphal nucleus' anatomical and cytoarchitectural characteristics 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 Edinger -Westphal nucleus. 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.

Please refer to our protocol 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 presented can be further explored, in coronal and sagittal planes, at <u>brain-map.org</u>.

Identification of the Edinger-Westphal nucleus in ISH labeled coronal sections is straightforward due to the unique appearance of the nucleus itself. In the coronal plane, the cells appear densely packed in thin, nearly adjacent columns, two to three cells wide, on either side of the midline. In the sagittal plane, they form a cohesive structure seen only in the most medial sections.

The 50 selected genes presented in this report show a consistently uniform expression pattern, with expression in all or nearly all cells within the nucleus. Expression gradients are not observed, nor are distinct sub-divisions or subnuclei. However, some genes are also expressed in the surrounding PAG and midbrain reticular nucleus. The expression patterns suggest that the EW extends farther caudally than is portrayed in the ARA sagittal view.

Cellular density expression key		Cellular int	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		

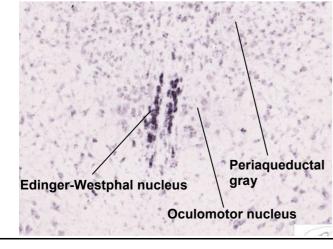
To view heat map at brain-map.org, right click on the ISH image and select "Show Expression Analysis."

ISH DATA The images below were selected to highlight various expression patterns of the Edinger-Westphal nucleus.

## ISH <u>Nenf</u>

## Coronal:

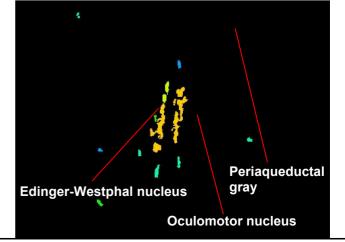
*Nenf* expression shows the characteristic expression pattern for the EW, highlighting the columnar arrangement of cells.



Heat map <u>Nenf</u> Coronal:

#### Coronal:

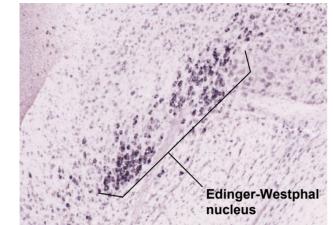
The heat map image shows high density and high intensity expression of *Nenf*.



#### ISH <u>Nenf</u> Sagitta

## Sagittal:

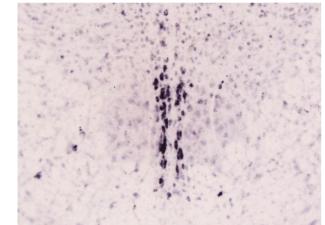
A sagittal view of *Nenf* suggests that the EW continues farther caudally than is portrayed in the sagittal views of the ARA.



#### ISH Spint2 Corona

Coronal:

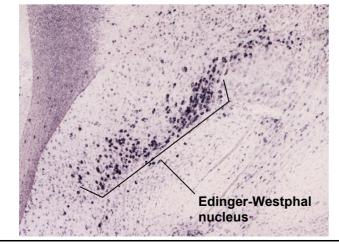
Spint2 expression clearly differentiates EW from the neighboring oculomotor nucleus.



## ISH <u>Spint2</u>

## Sagittal:

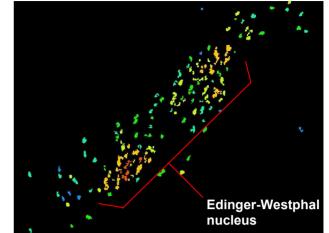
*Spint2* expression also suggests that the EW extends farther caudally than is portrayed in the ARA.



## Heat map <mark>Nenf</mark>

## Sagittal:

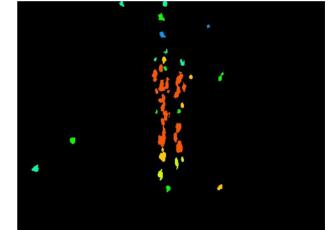
Again, the high density and high intensity of *Nenf* expression is evident in the heat map image.



## Heat map Spint2

## Coronal:

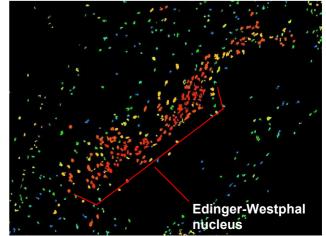
The columnar arrangement of the cells within the EW is clearly visualized in the heat map image.



# Heat map Spint2

## Sagittal:

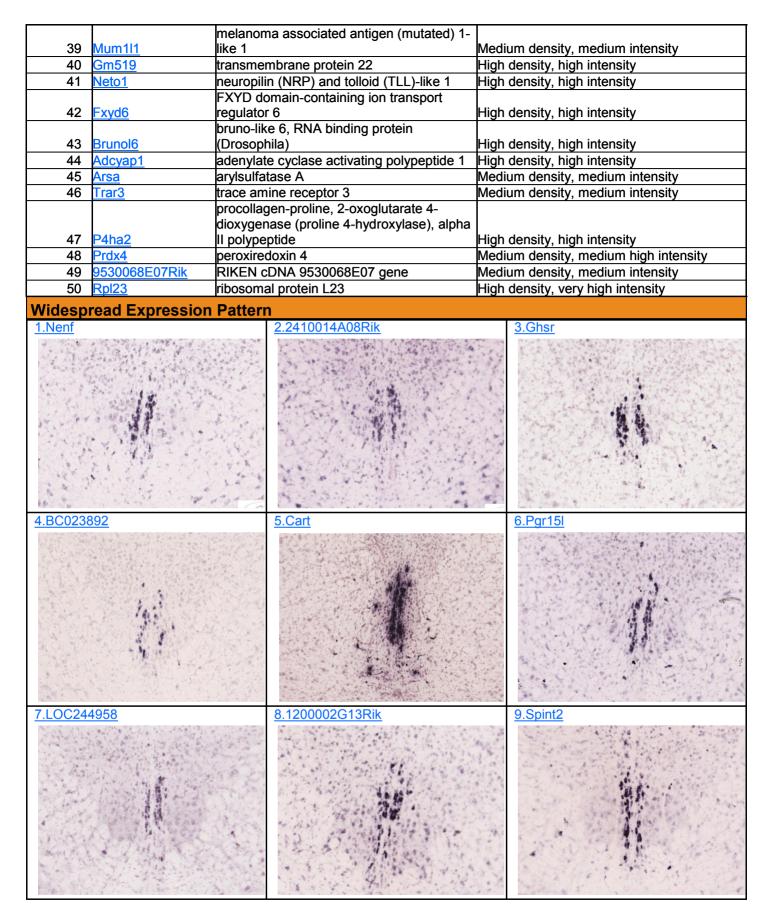
Although expressed in cells outside of the EW, the intensity of *Spint2* expression is greatest in the EW.

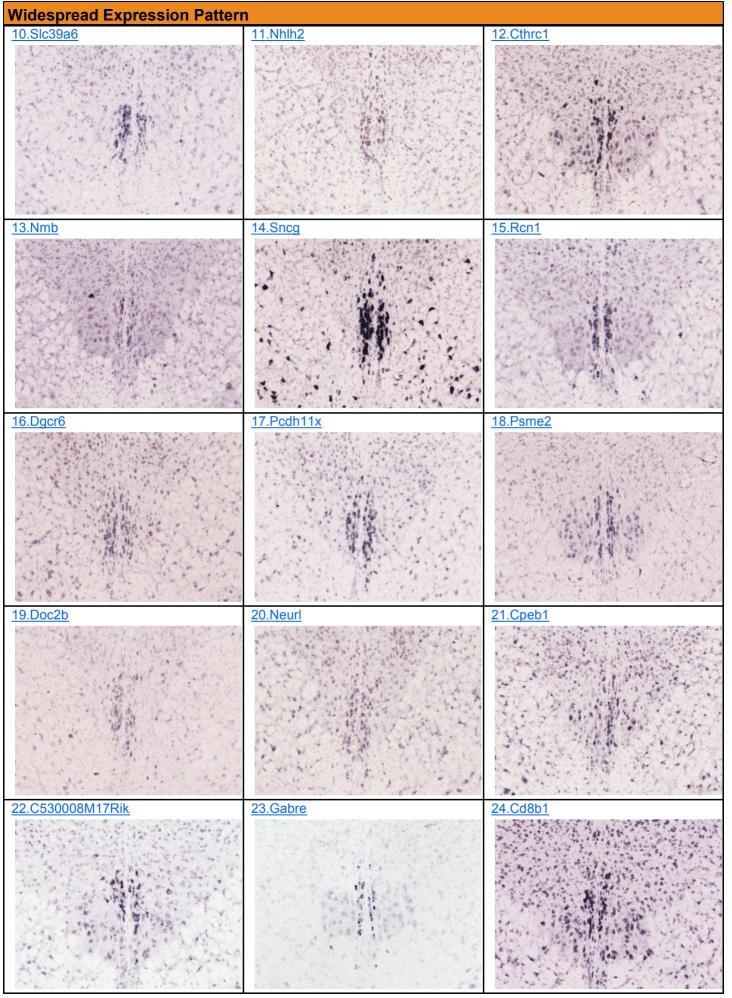


### **50 SELECT GENES:**

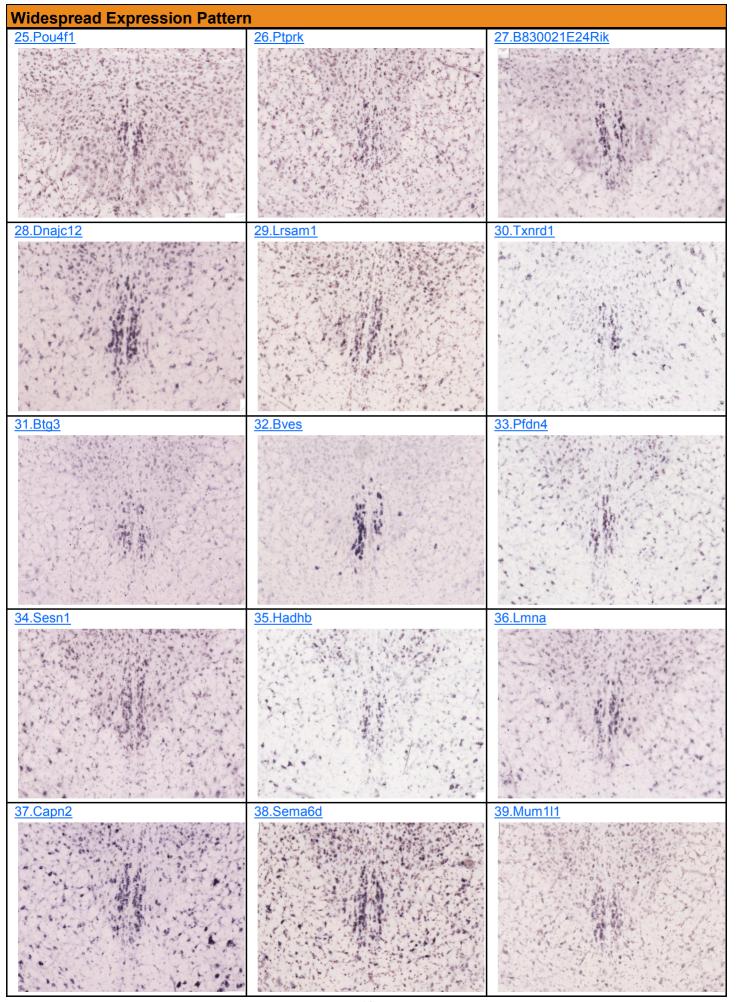
This gene list was generated by manual curation of an <u>algorithmically</u> derived list that compared gene expression values of EW to those of the midbrain. Categories of expression are subjectively grouped by relative expression characteristics. Curation of 50 Select Genes List: July, 2008

Widesp	Widespread Expression Pattern					
Number	Gene Symbol	Gene Name	Expression Pattern			
1	Nenf	neuron derived neurotrophic factor	High density, high intensity			
2	2410014A08Rik	RIKEN cDNA 2410014A08 gene	High density, medium-high intensity			
3	Ghsr	growth hormone secretagogue receptor	High density, high intensity			
4	BC023892	cDNA sequence BC023892	Medium density, medium high intensity			
		cocaine and amphetamine regulated tran-				
5	Cart	script	High density, very high intensity			
6	<u>Pgr15l</u>	G protein-coupled receptor 15-like	High density, high intensity			
7	LOC244958	similar to Chromosome 6 open reading frame 117	High density, medium-high intensity			
		transmembrane emp24 domain containing				
	1200002G13Rik	3	High density, high intensity			
9	Spint2	serine protease inhibitor, Kunitz type 2	High density, high intensity			
	<u>Slc39a6</u>	solute carrier family 39 (metal ion trans- porter), member 6	High density, medium-high intensity			
	Nhlh2	nescient helix loop helix 2	Medium density, medium-low intensity			
12	Cthrc1	collagen triple helix repeat containing 1	Medium density, high intensity			
13	<u>Nmb</u>	neuromedin B	Medium density, medium-low intensity			
	<u>Sncg</u>	synuclein, gamma	High density, high intensity			
	Rcn1	reticulocalbin 1	High density, high intensity			
16	Dgcr6	DiGeorge syndrome critical region gene 6	High density, medium intensity			
17	Pcdh11x	protocadherin 11 X-linked	High density, high intensity			
		proteasome (prosome, macropain) 28 sub-				
18	Psme2	unit, beta	High density, medium-high intensity			
19	Doc2b	double C2, beta	Medium density, medium intensity			
20	Neurl	neuralized-like homolog (Drosophila)	High density, medium intensity			
		cytoplasmic polyadenylation element bind-				
21	Cpeb1	ing protein 1	Medium density, high intensity			
22	C530008M17Rik	RIKEN cDNA C530008M17 gene	Medium density, high intensity			
		gamma-aminobutyric acid (GABA-A) recep-				
	<u>Gabre</u>	tor, subunit epsilon	Medium density, medium-low intensity			
	<u>Cd8b1</u>	CD8 antigen, beta chain 1	High density, high intensity			
25	Pou4f1		Medium density, medium intensity			
		protein tyrosine phosphatase, receptor				
	Ptprk	type, K	Medium density, medium intensity			
27	<u>B830021E24Rik</u>	SID1 transmembrane family, member 1	Medium density, medium-high intensity			
		DnaJ (Hsp40) homolog, subfamily C, mem-				
28	Dnajc12	ber 12	High density, high intensity			
		leucine rich repeat and sterile alpha motif				
	Lrsam1	containing 1	Medium density, medium-high intensity			
30	Txnrd1	thioredoxin reductase 1	Medium density, medium intensity			
	Btg3	B-cell translocation gene 3	Medium density, medium intensity			
	<u>Bves</u>	blood vessel epicardial substance	Medium density, high intensity			
	Pfdn4	prefoldin 4	Medium density, medium-high intensity			
34	Sesn1	sestrin 1	High density, medium intensity			
		hydroxyacyl-Coenzyme A dehydrogenase/3				
		-ketoacyl-Coenzyme A thiolase/enoyl-				
		Coenzyme A hydratase (trifunctional pro-				
	Hadhb	tein), beta subunit	Medium density, medium intensity			
	Lmna	lamin A	Medium density, medium-high intensity			
37	Capn2	calpain 2	High density, high intensity			
		sema domain, transmembrane domain				
		(TM), and cytoplasmic domain,				
38	Sema6d	(semaphorin) 6D	High density, high intensity			
		7				

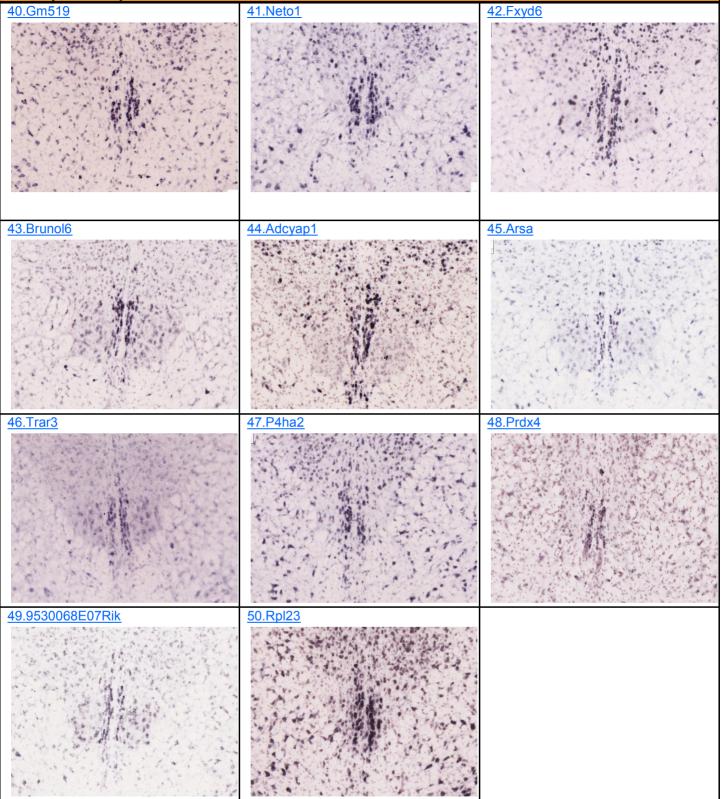




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## Widespread Expression Pattern



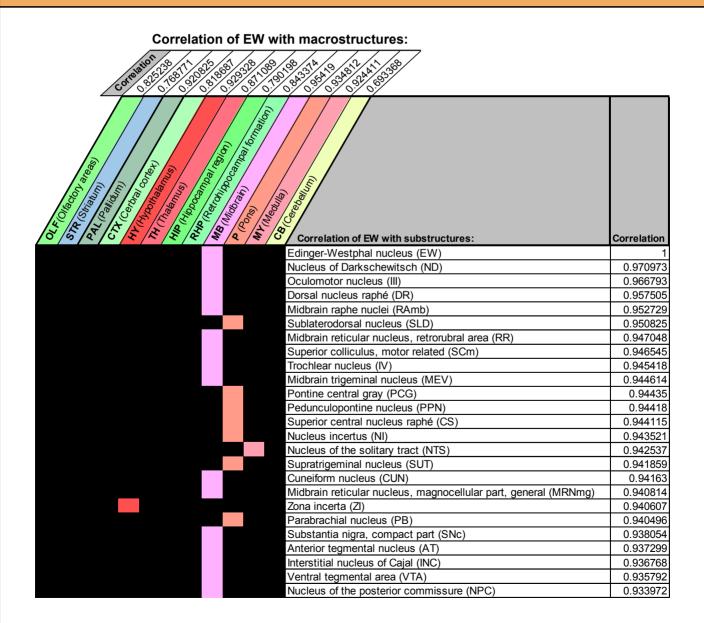
# **Correlated Expression:**

The ABA coronal set contains many genes of known scientific interest as well as genes exhibiting combinatorial or unique expression patterns. A correlation analysis of all available ABA coronal experiments (4376) was performed by comparing the expression value of the Edinger-Westphal nucleus 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  $(200\mu m)^3$  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 Edinger-Westphal nucleus and other brain regions, expression values from all voxels within the Edinger-Westphal nucleus were aggregated to form a single expression value. Two types of comparisons were then made. First, the aggregate expression values of the Edinger-Westphal nucleus 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 Edinger-Westphal nucleus and each of the ~53,000 voxels of the reference volume (structure vs. voxel; correlation map on the following page).

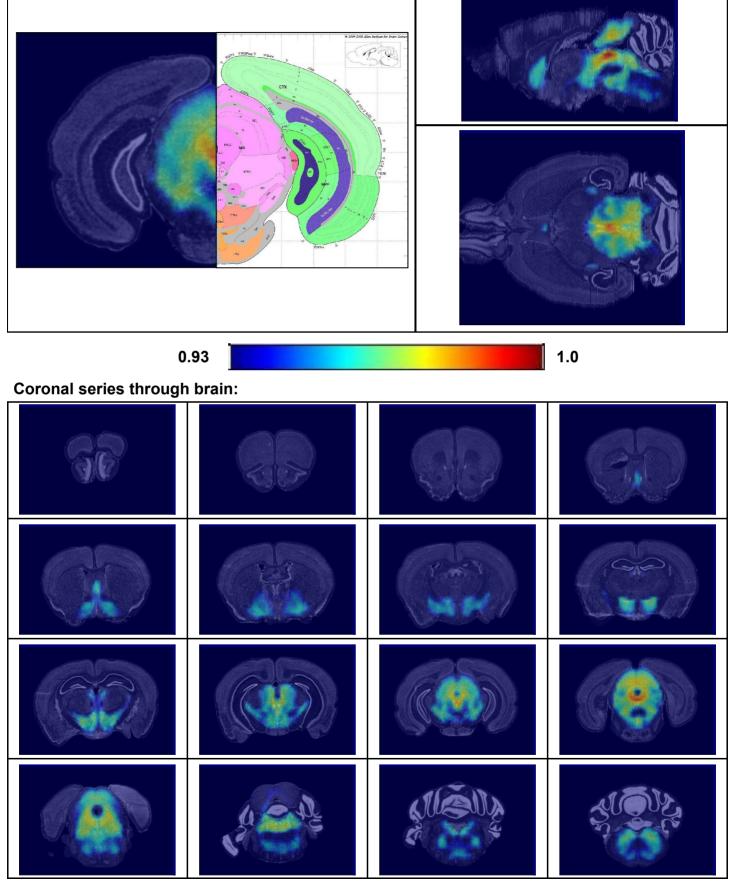
### **STRUCTURE vs. STRUCTURE**

The expression value of the Edinger-Westphal nucleus 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 Edinger-Westphal nucleus and macro/parent-structures are presented, as well as correlation between the Edinger-Westphal nucleus and the 25 highest ranking substructures. The most highly correlated macro/parent-structures don't always contain the 25 top most correlated substructures. Columns match the Allen Reference Atlas palette.



### STRUCTURE vs. VOXEL:

Correlation between the Edinger-Westphal nucleus and all other  $(200 \mu m)^3$  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 rostro-caudal levels throughout the brain.



# Gene Ontology (GO) Analysis:

## GO TABLE:

Below is an ontological analysis of the 50 Select Genes, using <u>DAVID</u> 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	46 gene IDs/ 50 input genes	

## Date of table completion: July, 2008

GO Category	GO Term	Gene Count	% of Genes	p-value
GOTERM_CC_ALL	extracellular region part	13	28.26%	0.00634
GOTERM_BP_ALL	localization	13	28.26%	0.029029
GOTERM_CC_ALL	extracellular region	14	30.43%	0.004237
GOTERM_CC_ALL	extracellular space	12	26.09%	0.011178

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.

# Edinger-Westphal nucleus Summary:

## Anatomy

- The Edinger-Westphal nucleus (EW), located along the ventral border of the periaqueductal grey, is a thin, elongated structure consisting of mid-sized, parasympathetic preganglionic neurons.
- It is closely apposed to the nucleus Darkschewitsch, the oculomotor nucleus, and the rostral linear nucleus of the raphé.

# **Expression Patterns of the 50 Select Genes**

- In this gene set, the dominant expression pattern seen is widespread expression throughout the nucleus.
- No gradients or subdivisions are observed for these fifty genes.
- Although most of the genes presented here make identification of the EW easy due to a lack of expression in structures in the immediate vicinity (for example, *Nenf* and *Ghsr*), some label the surrounding PAG, MRN, and III as well, such as *Fxyd6*, *Rpl3*, and *P4ha2*.
- Given the gene expression patterns presented here, it is probable that the EW extends farther caudally than is portrayed in the sagittal view of the ARA, although it is consistent with the coronal view.

# Correlation

- Macrostructure correlation identifies the midbrain and pons as the most highly correlated structures.
- The most highly correlated structures are the adjacent nucleus of Darkschewitsch, the oculomotor nucleus, and the raphé nucleus, portions of which sit just ventral to the EW.
- The cerebellum shows the least correlation with the EW.
- The structure to voxel correlation and heat map reveals additional correlations with portions of the medial septum (MS), substantia inominata (SI), lateral habenula (LH), zona incerta (ZI) and the posterior hypothalamic nucleus (PH).

We encourage you to reply with any 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 our genome-wide data set at <u>brain-map.org</u>.

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# 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.