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## Research Report

# Patterned expression of ion channel genes in mouse dorsal raphe nucleus determined with the Allen Mouse Brain Atlas

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## ARTICLE INFO

## Article history:

Accepted 28 March 2012

Available online 4 April 2012

## Keywords:

Serotonin

Depression

Ion channel

Dorsal raphe

## ABSTRACT

The dorsal raphe nucleus (DR) is the major source of serotonin (5-hydroxytryptamine, 5-HT) in the forebrain and dysfunction of this midbrain structure is implicated in affective disorders. The DR is composed of several types of 5-HT and non-5-HT neurons and their excitable-membrane properties are heterogeneous and overlapping. In order to understand how these properties may be generated, we examined the mRNA expression patterns of voltage- and ligand-gated ion channels in the DR using the Allen Mouse Brain Atlas. Since DR cytoarchitecture is organized with respect to the midline, we sought to identify genes that were expressed in a pattern with respect to the midline, either enriched or depleted, rather than those that were homogeneously expressed throughout the DR. Less than 10% of the screened genes for voltage-gated ion channels showed patterned expression within the DR. Identified genes included voltage-gated sodium channel beta subunits, potassium channels, P/Q-, N-type calcium channels, as well as the alpha2/delta-1 calcium channel. Several voltage-gated chloride channels were also identified, although these may function within intracellular compartments. Of the ligand-gated ion channels examined, 20% showed patterned expression. These consisted primarily of glutamate and GABA-A receptor subunits. The identified genes likely contribute to unique excitable properties of different groups of neurons in the DR and may include novel pharmacologic targets for affective disorders.

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## 1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) neurotransmission modulates a variety of behavioral states such as mood, motivation and arousal. Reflecting its widespread behavioral influence, 5-HT producing neurons of the dorsal raphe nucleus (DR) widely innervate the brain (Peyron et al., 1998; Steinbusch et al., 1981). 5-HT neurons represent about a third

of the neurons within the DR and are located primarily in clusters on the midline (Descarries et al., 1982; Jacobs and Azmitia, 1992). When neurons in the DR were initially characterized electrophysiologically, unique properties of putative 5-HT neurons were identified. In vivo extracellular recordings of rat midline DR identified characteristic pacemaker firing and biphasic extracellular waveforms (Aghajanian et al., 1968). Later work described a broad action

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potential, large slow afterhyperpolarization (AHP) potential, slow interspike depolarization, and high input resistance (Vandermaelen and Aghajanian, 1983).

Subsequently, electrophysiological recordings coupled with post-hoc immunohistochemical identification of 5-HT neurons revealed that 5-HT neurons themselves exhibit a range of characteristics, overlapping with those of non-5-HT neurons (Calizo et al., 2011; Kirby et al., 2003; Li et al., 2001; Marinelli et al., 2004). 5-HT neurons tended to have a broader action potential and greater input resistance than non-5-HT neurons, but these characteristics do not definitively identify 5-HT neurons. Moreover, features of the AHP are highly variable in DR neurons. Evidence suggests that fast-firing neurons in the DR are largely GABAergic (Allers and Sharp, 2003), however there may be diversity within GABAergic neuron populations as well.

In order to identify genes that could contribute to the membrane properties of DR neurons we sought to identify voltage- and ligand-gated ion channel genes that had a patterned distribution in the DR, rather than those that were present ubiquitously. The DR is a midline structure, with pronounced medio-lateral organization (Fig. 1). Therefore we screened to identify genes that exhibited patterns of expression with respect to the midline. Identification of the ion channels that influence the active membrane properties of various DR cell populations could enable future pharmacologic interventions for affective disorders. Additionally, mapping the topography of enriched ion channels provides future targets for genetic or pharmacological manipulation and should accelerate unraveling the electrochemical underpinnings of DR neuronal function.

## 2. Results

All of the identified genes are summarized in Table 1.

### 2.1. Voltage gated ion channels

Of the 139 voltage gated ion channels listed by IUPHAR, 21 genes were not available on the Allen Mouse Brain Atlas. Of the remaining 118 genes, 51 (42%) had some expression visible in the DR but were ubiquitous throughout the DR and did not exhibit a pattern. Only 12 (10%) exhibited a patterned expression with respect to the midline. An additional 5 ion channels were identified using the NeuroBLAST tool to have selective expression patterns.

### 2.2. Voltage gated sodium channels (Nav)

9 sodium channel alpha subunits were searched, of these subunits 2 were not available, and among the remainder none exhibited a notable midline or paramedial expression pattern. However, one sodium channel beta subunit, Scn3b, was identified via a NeuroBLAST search, and subsequently we examined Scn1–4b. Of these SCN2b was enriched on the midline (Fig. 2) and Scn4b was enriched paramedially, just ventral to the DR.

**Table 1 – Identified genes and their midline enrichment score (midline expression intensity–paramedial expression intensity). Positive numbers reflect genes enriched on the midline, and negative numbers reflect genes enriched paramedially.**

<i>Sodium channels</i>		<i>Calcium channels</i>		<i>Glutamate receptors</i>	
Scn2b	1.5	Cacna1a	2	Gria3	–1
Scn3b	2.5	Cacna1b	2	Gria4	1
Scn4b	–2	Cacna1h	0.5	Grik1	–1
		Cacna2d1	2.5	Grik2	1
		Cacnag3	2	Grik5	2
<i>Potassium channels</i>		<i>Chloride channels</i>		<i>GABA receptors</i>	
Kcna1	–2	Cacnag5	1	Gabra1	–1
Kcna2	–1			Gabrb1	1
Kcna4	2	Ttyh3	2	Gabrb2	–1
Kcnc1	–1	Clcn3	2	Gabrg2	1
Kcnh8	1	Clcn5	1	Gabrg3	1
Kcnj6	1	Clcn6	0.5	Gabrq	–1
Kcnk1	–1				
Kcnk9	1	<i>Other</i>			
Kcnq3	1	Chrna6	1		
		Chrb2	0.5		
		Gla2	2		
		Glr2	–1		

### 2.3. Voltage-gated potassium channels (Kv) channels

40 listed on the IUPHAR database were searched, of these 6 were not available. From the remainder, Kcna4 (Kv1.4, shaker family related) was present in a restricted area within the DR at the base of the aqueduct (Fig. 3A). In addition, Kcnq3 (Kv7.3, an M-channel) appeared modestly enriched on the midline in coronal series (Fig. 3B). In the sagittal series for this gene, the sections through the precise midline were not available, and the distribution was not confirmed (Fig. 3B1). Several additional genes had subtle expression patterns with respect to the midline and these included Kcnh8 (Kv12.1) or on the midline and Kcna1 (Kv1.1), kcna2 (Kv1.2), kcnc1 (Kv3.1) in paramedial areas (data not shown).

### 2.4. Two-pore-domain potassium channels (K<sub>2P</sub>)

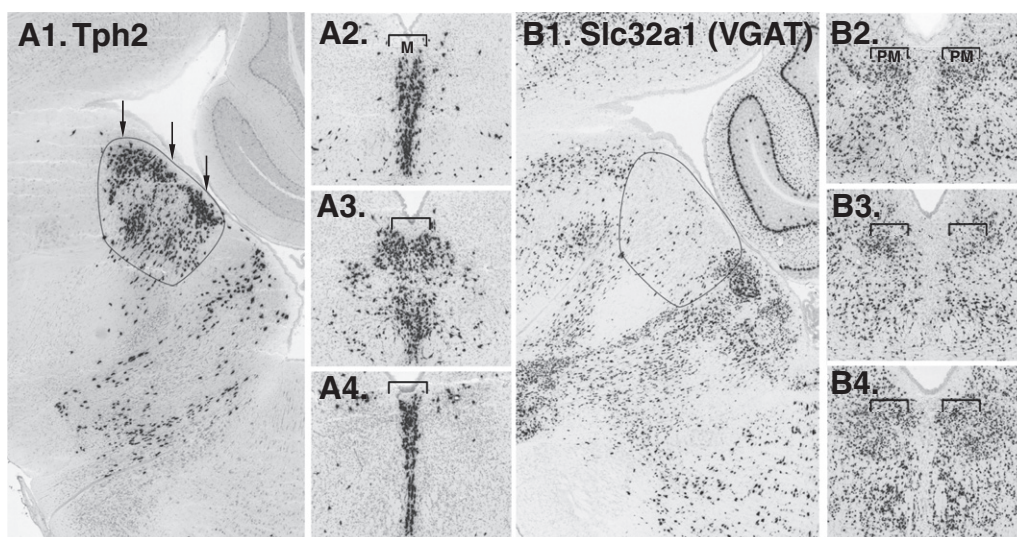
15 were searched, and 2 were not available. Kcnk9 (K<sub>2P</sub> 9.1) was enriched on the midline (Fig. 3C). This channel is also known as TASK3. In addition, Kcnk1 (K<sub>2P</sub> 1.1) had a subtle paramedial expression pattern (not shown).

### 2.5. Inwardly rectifying potassium channels (Kir)

15 were examined, and 2 of these were not available. One gene, Kcnj6 (Kir 3.2), also known as GIRK2 (G protein-coupled inwardly rectifying potassium channel 2), had modest enriched on the midline (Fig. 3D).

### 2.6. Voltage gated calcium channels (Cav)

10 were examined and all were available. 3 were identified as enriched along the midline: Cacna1a (calcium channel, voltage-dependent, P/Q-type, alpha1A; Cav2.1; Fig. 4A); Cacna1b (calcium channel, voltage dependent, N-type, alpha1B; Cav2.2; Fig. 4B) and with a more subtle expression pattern, Cacna1h (calcium



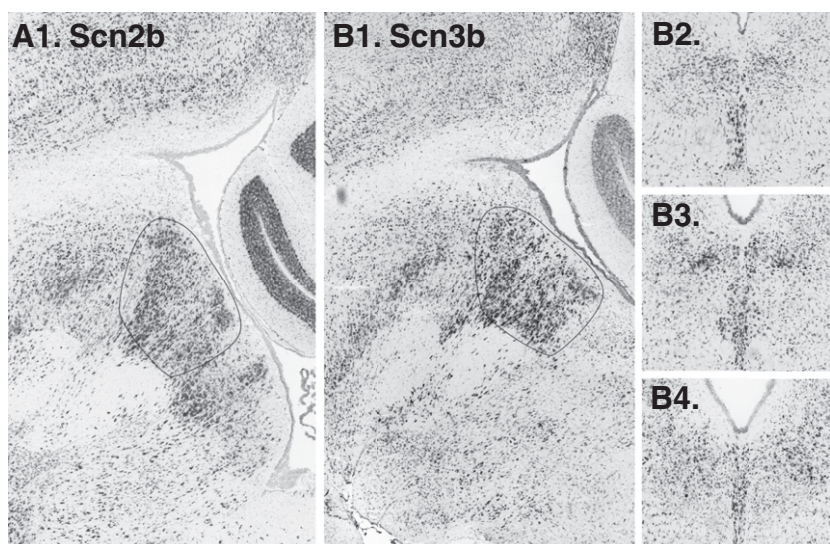
**Fig. 1 – Reciprocal midline and paramedial expression of two major cell types in the DR provided the basis for screening for genes expressed in medio-lateral patterns. A1. Expression of tryptophan hydroxylase 2 (Tph2), a marker for 5-HT neurons, is visible at the base of the aqueduct (arrows); Tph2 cells are outlined. The outline from this image is transferred to several other sagittal sections in subsequent figures as a reference for the distribution of 5-HT cells. Coronal sections at rostral (A2.), middle (A3.), and caudal (A4.) levels: note Tph2 expressing cells are enriched on the midline (bracketed area ‘M’), with less expression paramedially. B1–4. GABAergic neurons in the DR defined by the expression of the Vesicular GABA Transporter, Slc32a1 or VGAT. In sagittal section (B1.) note the low density of expression in the circled area where Tph2-expressing cells would lie. In coronal sections at rostral (B2.), middle (B3.), and caudal (B4.) levels, VGAT expressing neurons are more abundant paramedially (bracketed area, ‘PM’) than on the midline.**

channel, voltage-dependent, T-type; Cav3.2). Neuroblast also identified Cacna2d1 (Calcium channel voltage-dependent alpha2/delta-1 subunit; Fig. 4C). Cacna2d1 expression seemed to extend in a broader area than TPH2 expression, extending caudally on the midline (Fig. 4C). Cacng3, voltage-dependent calcium channel gamma 3 subunit, which is also known as a type 1 transmembrane AMPA regulatory protein (TARP) was also identified using Neuroblast (Fig. 4D). In both coronal and sagittal image series Cacng3 had the highest expression levels in the

rostral pole of the DR (Fig. 4D). Subsequently Cacng1–8 genes were examined, and Cacng5 was identified with a midline expression pattern in a ventral subset of cells (Fig. 4E).

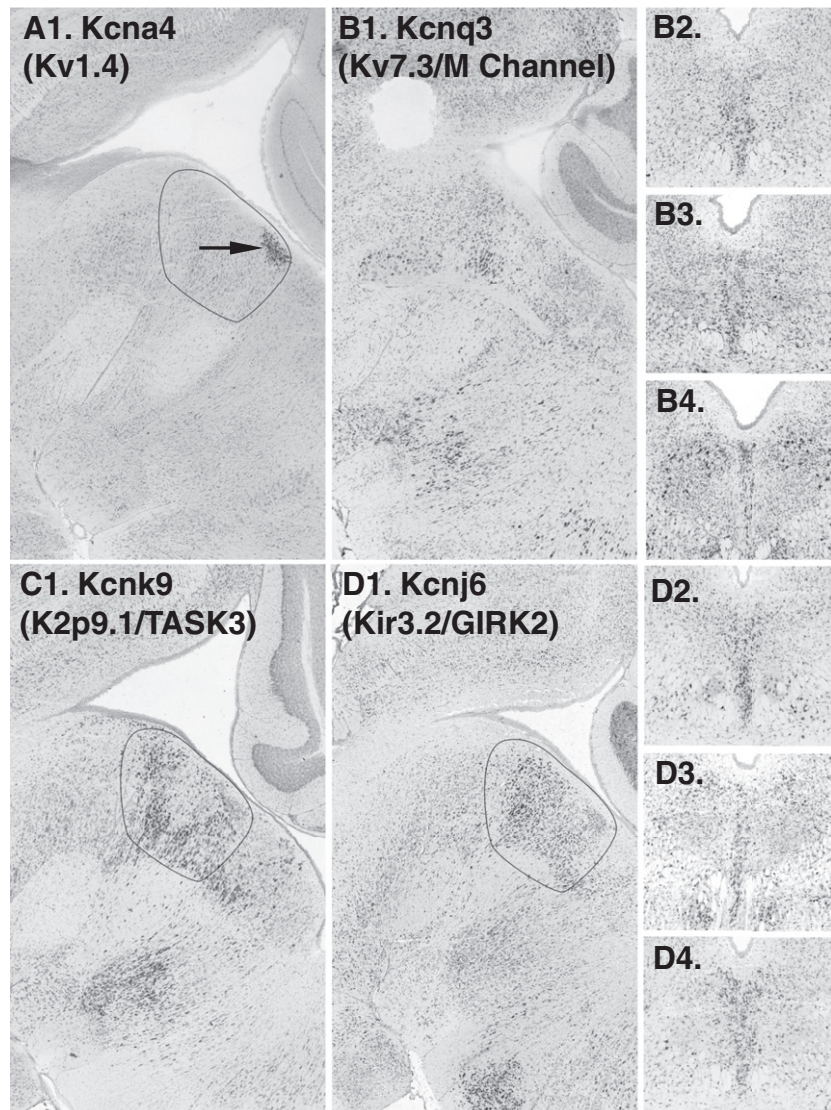
## 2.7. Chloride Channels

No Chloride Channels (Clc) were listed on the IUPHAR database. However, 2 were identified using NeuroBLAST. Ttyh3 (Twenty-like homolog 3) and Clcn5 (Chloride channel 5) (Fig. 5). Ttyh3 was



**Fig. 2 – Voltage-dependent sodium channel beta subunits Scn2b (A1. in a sagittal section, not available in coronal series) and Scn3b (B1. sagittal, B2–4. coronal) both appear enriched on the midline.**





**Fig. 3 – Voltage dependent potassium (Kv), inward-rectifying potassium (Kir), and two-pore potassium channels ( $K_{2P}$ ) with midline expression patterns. A1. In the sagittal plane Kcna4, (not available in coronal series), appears expressed in a cluster of cells on the midline (arrow). B1–4. Sagittal and coronal sections showing modest enrichment of Kcnq3 on the midline. C1. Kcnk9 is enriched on the midline of the DR. D1–4. Kcnj6 in sagittal and coronal series.**

distinctive in the rostral component of the DR whereas Clcn5 predominated ventrally (Fig. 5). Subsequently we searched additional voltage-gated chloride channels including members of the Ttyh, Clca, Clcn and Clcnk families and found Clcn3 and Clcn6 enriched on the midline (Fig. 5).

### 2.8. Remaining voltage-gated ion channels

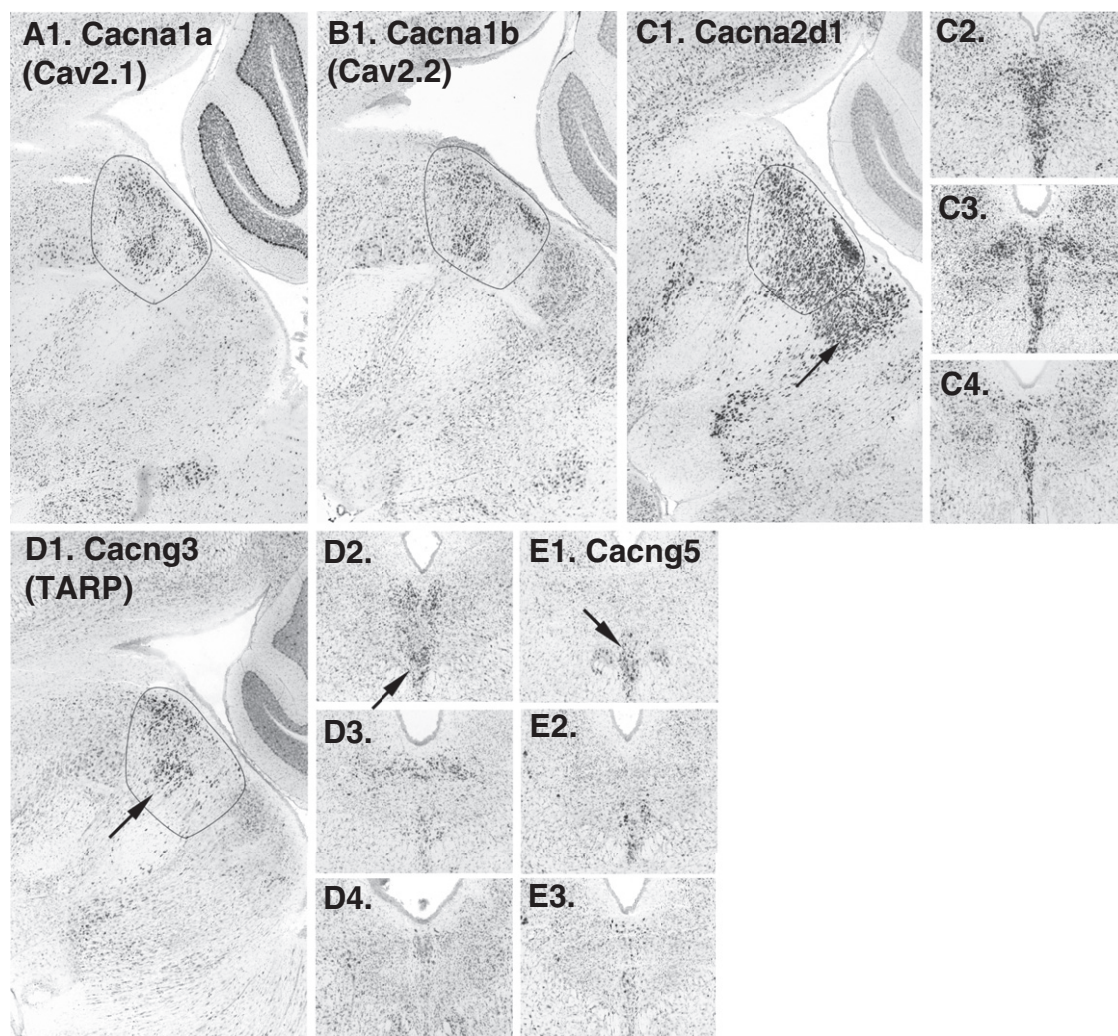
Several groups of voltage-gated ion channels had no members with a detected in a midline or paramedial expression pattern. These included Calcium-Activated Potassium channels (8 searched, 1 not available); CatSper and Two-Pore Calcium Channels (6 searched, 1 not available); Cyclic Nucleotide Regulated Channels (10 searched, 2 not available); and Transient Receptor Potential Channels (28 searched; 4 not available).

### 2.9. Ligand-gated ion channels

Of the 67 ligand-gated ion channels listed by IUPHAR, 3 were not available. Of the remaining 64, 18 (28%) appeared ubiquitously expressed in the DR. Another 14 (22%) showed patterned expressions. No additional genes were identified using NeuroBLAST searches.

### 2.10. Glutamate receptor subunits

18 were searched, all available. Three showed enrichment on the midline: Gria4 (glutamate receptor, ionotropic AMPA 4), Grik2 (glutamate receptor, ionotropic, kainate 2) and Grik5 (glutamate receptor, ionotropic, kainite 5) (Fig. 6). Of these channels the Gria4 expression pattern was most pronounced. Two glutamate



**Fig. 4 – Calcium channels.** A1. *Cacna1a* and B1. *Cacna1b* in sagittal sections are enriched in the area of TPH2 expression (circled). *Cacna2d1* in sagittal (C1.) and coronal series (C2–4.) series: expressing cells can be seen extending caudal to the DR (arrow). D1–4. In both sagittal and coronal series, *Cacng3* appears preferentially expressed in the rostral pole of the DR on the midline (arrows in D1. and D2.). E1–3. *Cacng5* appears enriched in ventrally located cells, particularly at rostral levels (arrow in E1.).

receptor subunits appeared more abundant paramedially than on the midline: *Gria3* and *Grik1* (Fig. 7).

### 2.11. GABA-A receptor subunits

19 were searched, and 1 was not available. Among the remainder, 3 subunits appeared in a midline pattern, *Garb1* (GABA-A receptor, beta 1) and *Gabrg2* (GABA-A receptor, gamma 2), *Gabrg3* (GABA-A receptor, gamma 3) (Fig. 8). Of these *Garb1* and *Gabrg2* were enriched on the midline throughout the rostro-caudal axis of the DR, whereas *Gabrg3* was enriched in the ventral DR at mid-rostro-caudal levels. Three other subunits, *Gabra1* (GABA-A receptor alpha1), *Gabrb2* (GABA-A receptor, beta-2) and *Gabrq* (GABA-A receptor, theta) appeared enriched in a paramedial distribution (Fig. 9).

### 2.12. Other ligand gated ion channels

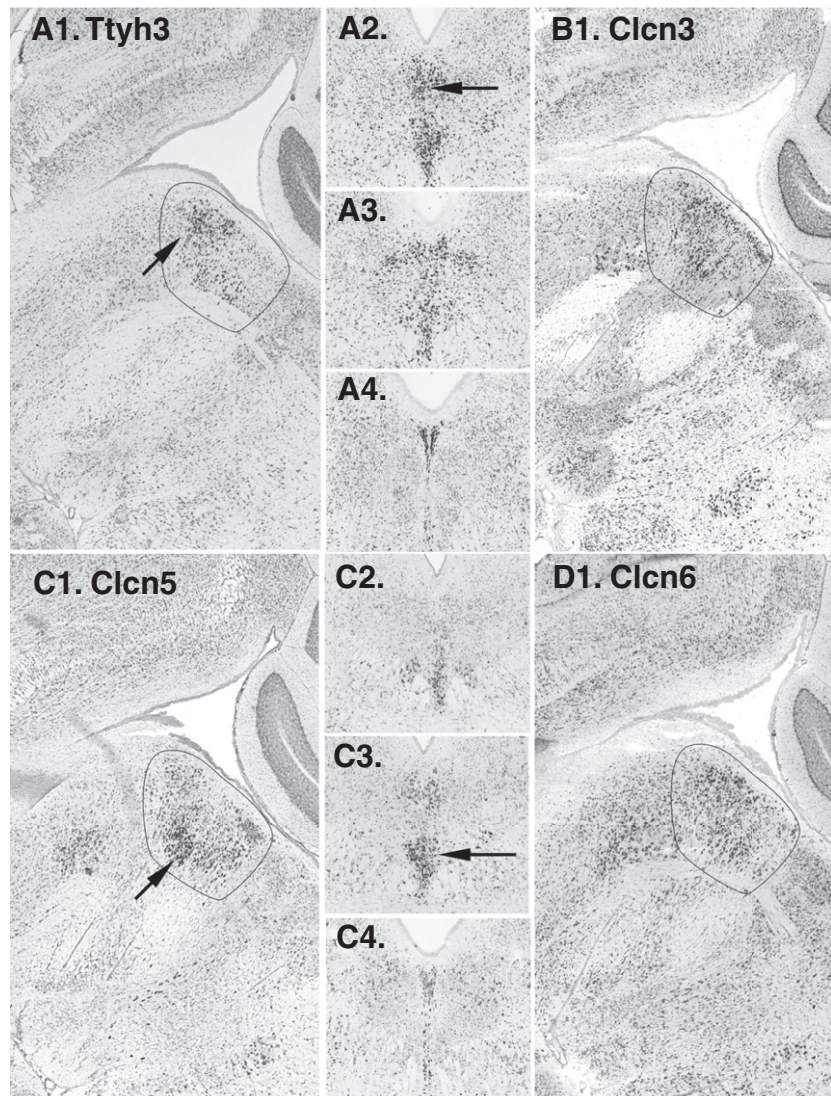
Additional receptor subunits searched include 5-HT<sub>3</sub> (2 searched), glycine subunits (5 searched), nicotinic acetylcholine receptor

subunits (16 searched, 1 was not available) and P2X (7 searched, 1 was not available). Of these, *Gla2* was present on the midline, *Glrh* paramedially, *Chrna6* appeared expressed in cells contiguous with the A10 dorsal-caudal cell group and *Chrb2* was modestly enriched on the midline (not shown).

## 3. Discussion

In this study we analyzed gene expression of voltage- and ligand-gated ion channels in the DR and identified several genes with medio-lateral expression patterns. Selective expression of particular genes may underlie the unique electrophysiological characteristics of cell populations within the DR. Few of the identified genes appeared to precisely recapitulate the pattern of either 5-HT or GABAergic neurons in the DR (Fig. 1), consistent with the observations that 5-HT and non-5-HT neurons have overlapping excitable properties. Some genes exhibited dorsal-ventral or rostral-caudal gradients of expression within the DR, suggesting association with





**Fig. 5 – Voltage-dependent chloride channels. A1–4. Ttyh3 expression domain includes rostral and dorsal components of the DR, seen in both sagittal and coronal series (arrows). B1. Clcn3 in sagittal section is visible on the midline. C1–4. Clcn5 appears predominantly expressed in the ventral component of the DR on the midline (arrows). D1. Clcn6 in sagittal section is enriched on the midline.**

functional subgroups of 5-HT and/or non-5-HT neurons. Although there are important species differences, some of the identified channels could be relevant to human disorders associated with 5-HT dysfunction.

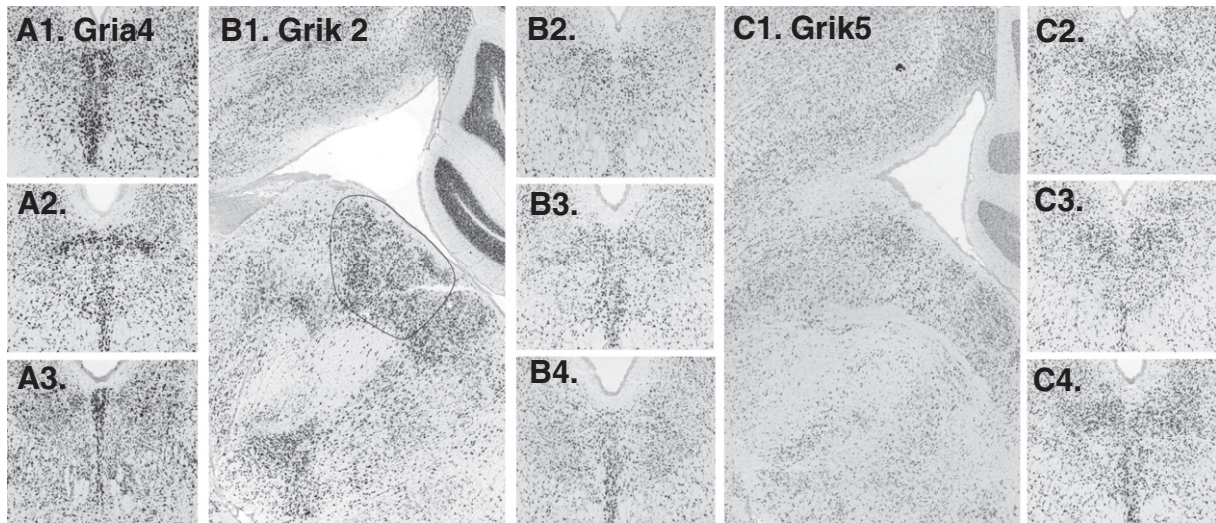
### 3.1. Methodological considerations

The Allen Brain Atlas is high-throughput and therefore has the potential to produce both false negative and false positive findings, based upon the known characteristics of the database (Lein et al., 2007). In addition, the method of colorimetric detection of in situ hybridization signal also has limited quantitative validity and indeed, it is not unusual for different series of in situ hybridization results on the Allen Mouse Brain Atlas to have different signal intensity. Relative expression levels that generate patterns however were more

consistent, and often replicated in both coronal and sagittal series, as shown in this report. However, there is the potential that genes with patterned expression were not detected, in particular when genes were only available in sagittal series where medio-lateral patterns may be harder to detect. Likewise, some genes may be regulated at a post-transcriptional level, therefore some of the detected genes may not be present as functional protein. Furthermore, it deserves emphasis that while we focused on genes with patterned expression, there are many additional voltage- and ligand-gated channel genes are expressed in the DR more ubiquitously.

Supporting the validity of the approach we used, we were able to identify two genes that have already been established to have high expression levels in the DR, and coincidentally within 5-HT neurons. These include *Kcnj6* also known as





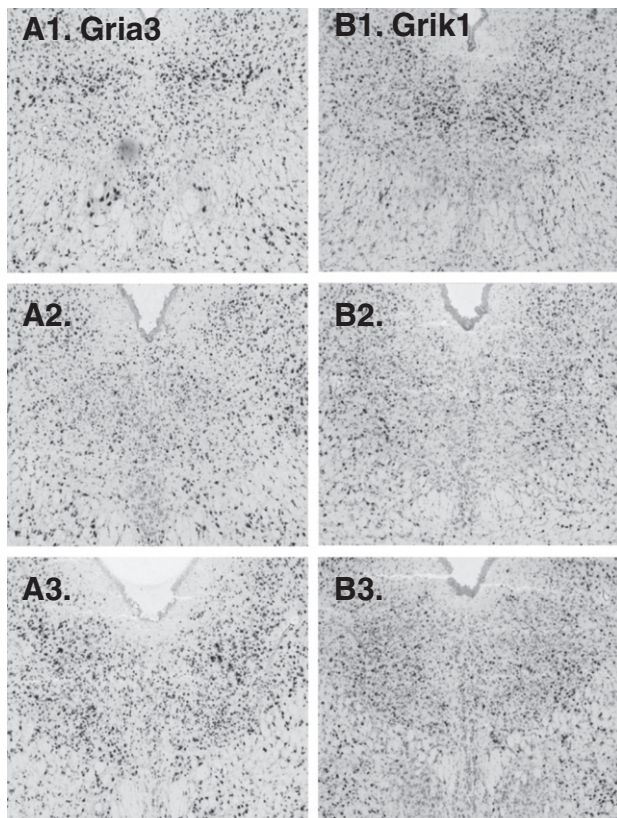
**Fig. 6 – Glutamate receptor subunits with notable expression on the midline include A1–3. Gria4 (not available in sagittal series); B1–4. Grik2 and C1–4. Grik5.**

‘weaver gene’, Kir 3.2 or GIRK2. Expressed in DR 5-HT neurons, this channel is implicated in coupling to 5-HT<sub>1A</sub> receptors (Costa et al., 2005; Penington et al., 1993; Saenz del Burgo et al., 2008). The second gene previously identified as highly expressed in 5-HT neurons is Kcnk9 (*K<sub>2P</sub>9.1*) or TASK-3 a “leak” channel that influences the resting membrane potential (Berg et al., 2004; Lesage and Lazdunski, 2000; Marinc et al.,

2010). TASK3 contributes to sleep behavior and response to anesthetics (Linden et al., 2007; Pang et al., 2009). TASK3 channels account for the pH sensitivity of 5-HT neurons, are modified by Gq second messenger signaling and appear to be located somato-dendritically on 5-HT neurons (Marinc et al., 2010; Veale et al., 2007; Washburn et al., 2002).

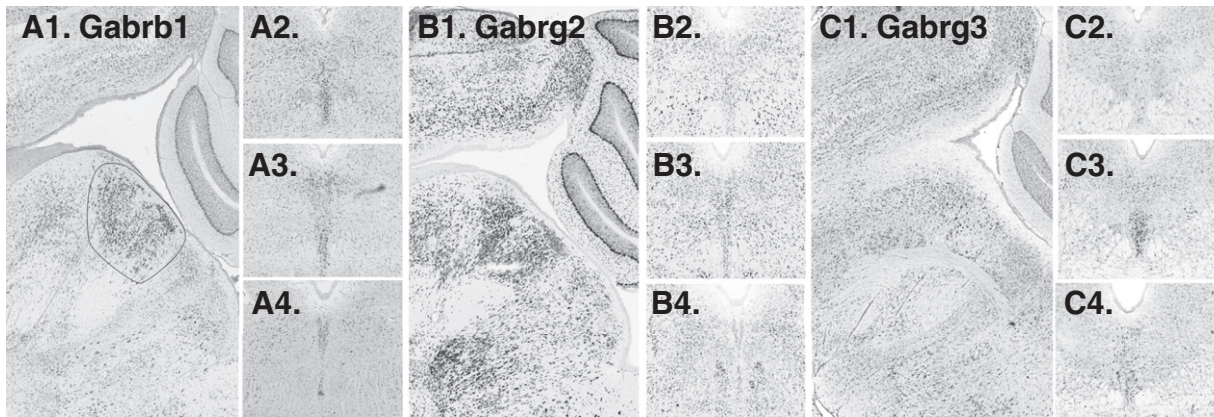
We found that sodium channel beta subunits Scn2b and more so, Scn3b, are expressed on the midline of the DR. Beta subunits encoded by Scn2b and Scn3b function to regulate Nav alpha subunits, influencing the activation and inactivation kinetics of sodium channels. Scn3b, when expressed with the alpha subunit Nav1.3, promotes slower channel inactivation, which would increase the duration of the action potential (Cusdin et al., 2010). This finding raises the possibility that expression of these subunits may contribute to the broad action potential associated primarily, but not exclusively, with 5-HT neurons (Calizo et al., 2011; Kirby et al., 2003; Li et al., 2001; Marinelli et al., 2004).

Despite the fact that voltage-gated potassium channels accounted for the most numerous type of channels screened, few had pronounced patterns of expression. Kcnq3, or Kv7.3, a member of the KQT-like subfamily, was enhanced on the midline although the intensity of the pattern was not very robust. Kcnq3 is thought to function as a heteromer in conjunction with either Kcnq2, Kcnq4 or Kcnq5 to mediate M-currents, which are voltage dependent, slowly activating and deactivating potassium currents (Wang et al., 1998; Yang et al., 1998). Indeed, Kcnq4 or Kv7.4 has been reported in 5-HT neurons in the rat DR, although little expression is detectable in the Allen Mouse Brain Atlas (Hansen et al., 2008; Kharkovets et al., 2000). The late phase of the afterhyperpolarization (AHP) is mediated in part by a calcium dependent potassium conductance (Crunelli et al., 1983; Pan et al., 1994) and in the hippocampus, Kcnq3 channels contribute to the calcium activated medium and slow AHP (Tzingounis and Nicoll, 2008). Therefore, this channel could contribute to some features of the AHP in DR neurons. However, there is considerable variability within the AHP in both 5-HT and



**Fig. 7 – Glutamate receptors showing notable paramedial expression patterns include: A1–3. Gria3 and B1–3. Grik1.**

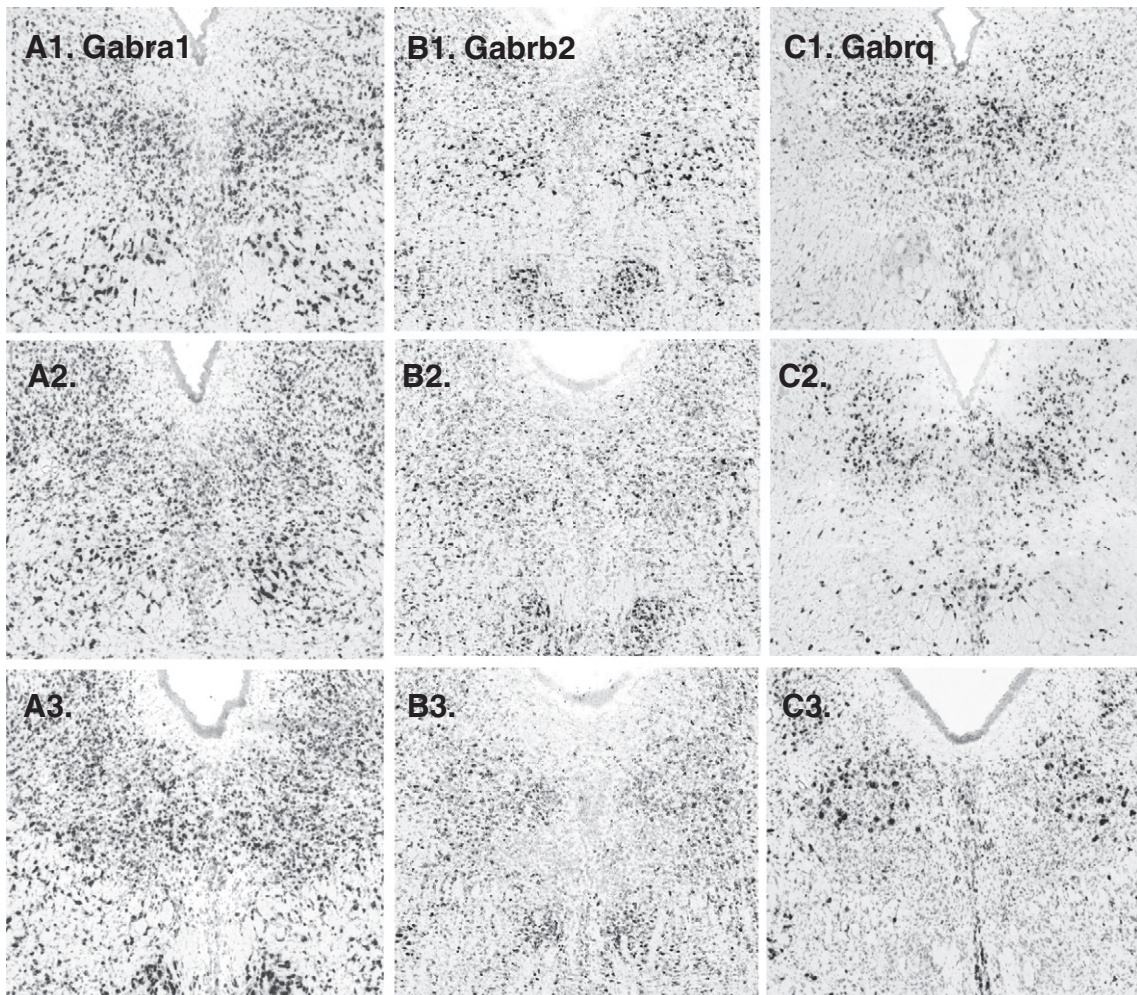




**Fig. 8** – GABA receptor subunits with expression on the midline. A1. sagittal and A2–A4. cononal series of Gabrb1. B1–B4. Gabrg2 is expressed along the midline. C1–C4. Gabrg3 appears enriched, particularly in the ventral part of the DR, in mid-rostro-caudal location (C3. arrow).

non-5-HT neurons in the DRN (Kirby et al., 2003; Li et al., 2001; Marinelli et al., 2004), and this is consistent with our finding that the majority of potassium channels that could contribute to these characteristics did not have robustly patterned expression.

We found Kv1.4 expressed in a subgroup of cells within the DR, perhaps corresponding to the B6 cluster of neurons. However, immunohistochemical localization of Kv1.4 does not confirm this distribution (Lujan et al., 2003) thus this finding awaits further evaluation.



**Fig. 9** – GABA receptor subunits with paramedial expression patterns. A1–3. Gabra1, B1–3. Gabrb2, and C1–3. Gabrgq.



A few calcium channels exhibited interesting patterns of expression in the DR. One of them is *Cacna1a*, which is a P/Q type calcium channel, important for presynaptic neurotransmitter release (Catterall, 1999). The *Rolling* mouse Nagoya has a mutation in this gene and these mice have age related alterations in markers of 5-HT neuron function and behavioral abnormalities in tests for anxiety and depression-like behavior, suggesting that this gene may be important for 5-HT neuron function (Takahashi et al., 2009). Mice lacking *Cacna1b* have altered activity, arousal and are hyperaggressive (Beuckmann et al., 2003; Kim et al., 2009). These mutants also display increased firing of 5-HT neurons, suggesting this channel participates in controlling the excitability of 5-HT neurons.

*Cacna2d1*, the L-type voltage-dependent calcium channel  $\alpha 2/\delta$ -1 subunit, was expressed in a subset of DR cells. The  $\alpha 2/\delta$ -1 subunit possesses a stereoselective, high-affinity binding site for gabapentin and pregabalin, which are primarily used to treat chronic pain (Dooley et al., 2007; Gee et al., 1996; Marais et al., 2001). Gabapentin has been associated with increased suicidality, however pregabalin may have positive effects for anxiety (Pande et al., 2003). While  $\alpha 2/\delta$ -1 is expressed in many brain areas (Taylor and Garrido, 2008) the expression pattern we found in the DR raises the possibility that the DR, and perhaps 5-HT, may contribute to the physiological effects of gabapentin and pregabalin.

*Cacng3* and *Cacng5*, members of the TARP family, were found enriched in different subregions of the DR. *Cacng3* was located rostral and dorsal in the nucleus, whereas *Cacng5* was distinguished ventrally. Related to Stargazin, TARP proteins selectively bind to AMPA receptor subunits and are implicated in both gating and trafficking of AMPA receptors (reviewed by: Jackson and Nicoll, 2011; Kato et al., 2010; Payne, 2008). TARPs are enriched in postsynaptic densities and may change biophysical channel properties by stabilizing specific conformations of AMPA receptors. Therefore *Cacng3* and *Cacng5* expression may confer differential AMPA receptor function within the expressed cells. *Cacng5* is reported to be a susceptibility locus for schizophrenia and bipolar disorder (Curtis et al., 2011), and function within the DR could be relevant to this observation.

The role of voltage-dependent chloride channels in neurons is poorly understood, however several were identified with a patterned distribution in the DR. *Tyh3*, is a homolog of the drosophila gene *tweety*, located in the *flightless* locus. *Tyh3* encodes a large conductance calcium-activated chloride current (Suzuki, 2006), whose functional importance may be interesting to pursue since this gene had a particularly clear expression pattern. *Clcn3*, *Clcn5* and *Clcn6* are associated with acidifying intracellular compartments, including synaptic vesicles and endosome–lysosome compartments (Riazanski et al., 2011; Smith and Lippiat, 2010). Loss of all three chloride channels is associated with a neurodegenerative disorder, neuronal ceroid lipofuscinosis (NCL), indicating their importance to neuron function (Poet et al., 2006; Pontikis et al., 2004; Pressey et al., 2010; von Schantz et al., 2008).

Glutamate neurotransmission is of particular interest in the DR due to the potential use of glutamate receptor antagonists for treatment-resistant depression (Machado-Vieira et al., 2009; Pittenger et al., 2007; Yilmaz et al., 2002; Zarate et al., 2004). Among AMPA receptor subunits, *Gria4* was

the most abundantly expressed in the midline region showing a clear pattern of expression that overlaps with the TPH2 pattern, suggesting an association with 5-HT neurons. Interestingly, *Gria4*-KO mice show increased anxiety-like behavior in response to light (Sagata et al., 2010). *Gria3* showed a paramedial pattern of expression and polymorphism of this gene has been identified as a genetic risk factor for loss of libido in citalopram-treated patients (Perlis et al., 2009) as well as with suicidal ideation emerging during citalopram treatment of major depression, together with *Grik2* (Laje et al., 2007).

Two kainate receptor subunits appear particularly enriched on the midline in the DR, and this could contribute to some of the behavioral effects associated with these receptors. Specifically, polymorphisms of *Grik2* have been associated with obsessive–compulsive disorder and sexual dysfunction in citalopram-treated patients (Perlis et al., 2009; Sampaio et al., 2010). *Grik5*, which contributes to slowly-deactivating currents, has been associated with bipolar disorder (Barberis et al., 2008; Yosifova et al., 2011). *Grik1*, which has paramedial expression, is expressed in GABAergic interneurons in the hippocampus (Segerstrale et al., 2010) and the *Grik1*-KO mice have increased anxiety-related behaviors (Wu et al., 2007). Taken together these observations would suggest the possibility that *Grik1* could be selectively associated with GABAergic neurons.

GABA-A subunits that displayed enriched midline expression include *Gabrb1*, *Gabrg2*, and *Gabrg3* genes while those associated with lateral expressions are *Gabra1*, *Gabrb2*, and *Gabrq* genes. Some of the midline-enriched genes have been reported to be associated with drug dependence and mood disorders. For instance, possible genetic linkage in humans has been reported between *Gabrb1* gene and alcohol dependence as well as bipolar disorder (Craddock et al., 2008; Kertes et al., 2011; Parsian and Zhang, 1999). In mice, *Gabrg2* receptor deficits result in anxiety-like, depressive-like, and anhedonia-like behavior, which are ameliorated by antidepressants (Shen et al., 2010). Genes that are enriched laterally have been implicated in mediating actions of anesthetics or alcohols. A few studies showed that point mutations of *Gabra1* and *Gabrb2* reduce currents potentiated by isoflurane or alcohols (Borghese et al., 2006; McCracken et al., 2010; Nishikawa et al., 2002).

Overall, ligand-gated ion channels, primarily glutamate and GABA receptors, were twice as likely as the voltage-gated ion channels to show patterned expression in the DR. This finding suggests that DR neurons in particular vary in how they receive and integrate synaptic glutamatergic and GABAergic neurotransmission. This conclusion would be consistent with electrophysiological evidence suggesting differences in the regulation of glutamatergic and GABAergic innervation of 5-HT and non-5-HT neurons in the DR (Kirby et al., 2007).

In this study we identified several voltage- and ligand-gated ion channels that have patterned expression through the DR, and therefore may contribute to the heterogeneity of membrane properties of neurons recorded in this nucleus. Additional studies are required to determine how these genes distribute to 5-HT and non-5-HT cell populations in the DR, and the functional importance of each gene. However, the current results have the capacity to drive hypotheses in this regard.

#### 4. Experimental procedures

The source of data for this project was the Allen Mouse Brain Atlas (ABA) (<http://mouse.brain-map.org>), a genome-wide database of high-throughput in-situ hybridization data for over 20,000 genes (Lein et al., 2007). Two approaches were used to identify genes with expression patterns that could be selective for different groups of neurons within the DR. The first entailed a manual examination of a list of ion channel genes provided by the International Union of Basic and Clinical Pharmacology (IUPHAR) (<http://www.iuphar-db.org/>) (Sharman et al., 2010). IUPHAR's list comprised 142 voltage-gated channels and 72 ligand-gated channels. We previously reported a preliminary analysis of glutamate receptor genes (Soiza-Reilly and Commons, 2011), but include this group into the current report to complete the data set. The second approach was to identify genes using the Allen Brain Atlas' NeuroBLAST tool.

For the IUPHAR database search, individual genes were searched. If the gene had a coronal series, this was examined. If there were two or more coronal series, the one with greater signal strength was examined. Sections were chosen from the center of the rostro-caudal extent of the DR, between 3450 and 3850  $\mu\text{m}$  ABA coordinates. On the selected section, gene expression was scored in two zones: one on the midline, and the other paramedial to the midline. The location of these zones were defined by reference sections for the serotonin biosynthetic enzyme tryptophan hydroxylase 2 (TPH2) and the Vesicular GABA Transporter (Slc6a1, VGAT; Fig. 1), which exhibit reciprocal patterns with respect to the midline: TPH2 is enriched on the midline, whereas VGAT is present in a paramedial distribution. In each of these two zones, expression level was evaluated by visual inspection of the ABA generated heatmap; ranging from absent (black, 0), sparse green (low, 1) to green/yellow (medium, 2) to orange/red (high, 3) with 0.5 increments used for intermediate values. Once one series of sections was evaluated, a second series was evaluated to confirm the observations. The pattern of expression was then defined as the midline score subtracted from the paramedial score. For genes that were only available in a sagittal series, the section closest to the midline was evaluated, and genes were given one score for expression intensity within the area where 5-HT cells are located, based on TPH2 expression in an analogous section. Subsequently, more lateral sections were inspected to determine if there was a patterned expression. All the genes identified in these screens were subsequently re-evaluated by a second observer to reduce the incidence of false-positive identification. Some genes in the IUPHAR list were either not available on the ABA or were present only in sagittal series that did not include a section close enough to the midline to capture the location of 5-HT cells. The frequency of these events is noted in results as the number of genes 'not available' for each family of ion channels.

For the NeuroBLAST search, we selected coronal and sagittal series of TPH2 gene expression and designated the area 'midbrain'. The output list was then examined using NIH's DAVID gene identification tool (<http://david.abcc.ncifcrf.gov/>) to identify ion channels. Image series of these genes were subsequently evaluated to eliminate false-positives. If

we found a gene using NeuroBLAST search, we subsequently searched other members of the same class or gene family.

To generate figures, all the images were downloaded from the ABA website (Allen Mouse Brain Atlas [Internet]. Seattle (WA): Allen Institute for Brain Science. ©2009. Available from: <http://mouse.brain-map.org>) and assembled using Adobe Photoshop. Images were adjusted for contrast and brightness using 'image-adjust-brightness-contrast' or 'image-adjust-curves' functions.

#### Acknowledgments

This work was supported by a grant from NIH, DA-021801, and the Sara Page Mayo Endowment for Pediatric Pain Research. We appreciate the comments of Drs. Lynn Kirby and Chris Vaughan on draft versions of the manuscript.

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