Recent advances in our understanding of the organization of dorsal horn neuron populations and their contribution to cutaneous mechanical allodynia

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Electronic Supplementary Material:

Number of Figures: 4 tables

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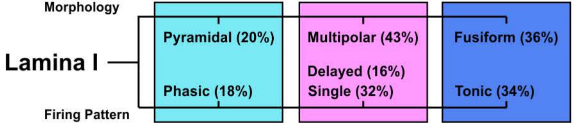


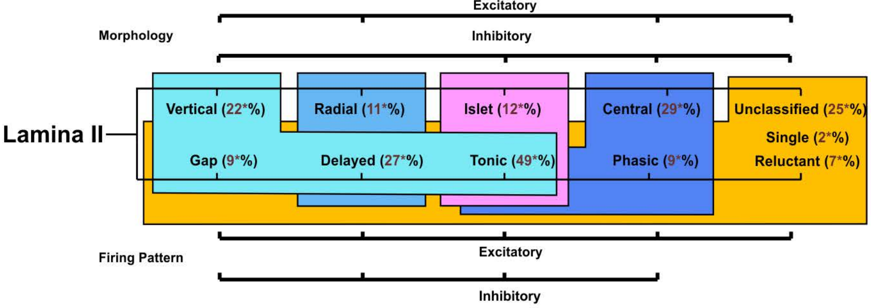
Projection Neurons

% of lamina	5%-11%
Expression of other markers	NK1R 80%-90% MOR 86% DOR 56% SP 16% SST2A 9/10-15% DYN 1% GRP 0% GRPR 0%

Excitatory — Inhibitory

Marker	NK1R	NKB	MOR1	PKCγ	SOM	CCK	DYN	GABA	SST2A	GLY	GAL	NPY	NNOS
Expression	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
% of lamina	19-45%	11%	11%	8%	0-7%	7%	17%	25/31-36%	13-17%	12%	7%	6%	4%
Expression of other markers	PKCγ 22% NKB 3% SST2A 0-2% SOM 0% GABA 0% GRP 0% GRPR 0%	GAD 0%		GABA 0% GLY 0% NNOS 0%	SST2A 0% NK1R 0%		GABA 47% GAL 47% NNOS 0% PKCγ 0%	GLY 33-47% NK1R 0%	GABA 100%-100% GLY 83% NK1R 0-1% SOM 0%	GABA100% PKCγ 0%	GABA 100% SST2A 100% DYN 96% NPY 0% NNOS 0%	GABA 100%-100% GLY 6% GAL 0%	GABA 100% PKCγ 0% GAL 0% DYN 0%

ESM_1 Populations of dorsal horn neurons in lamina I for which quantitative expression within laminae is available. Lamina I neurons can be sorted based on their morphology and firing patterns (upper schematic). Neurons that are pyramidal have phasing firing (blue square), those that are multipolar have delayed or single firing (pink square), and the last class of fusiform neurons are mostly tonic (dark blue square). Percentages represent the proportion of neurons within the lamina that belong to each population. Lamina I neurons can also be organized based on their molecular profile. Using such criteria, populations of neurons that are mostly excitatory are displayed in light blue and those that are mostly inhibitory neurons are displayed in light pink. A population that is equally composed of excitatory and inhibitory neurons is represented in grey. Projection neurons of lamina I that send axons to supraspinal areas are represented in green. Range of values correspond to the minimum and maximum percentage of neurons that express a marker reported for each population. Values obtained in mice are shown in black and those obtained in rats are displayed in brown. References used in this table can be find in ESM_4



Excitatory

Marker	SOM	CR	PKCγ	MOR1	SP	GRP	NKB	CCK	NTS
Expression	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
% of lamina	37*%-45%	39%	19*%-21%	20*%	14-20%	11-14%	10*%-12%	5-7*%	7%
Morphology	V, R, C	V, R, I, C	R 55% C 26% V 7% I 5%		R	C 84% V 3%			
Firing pattern	D 45% S 17% P 32%	D 62% S 24% T 5% P 5% R 5%	T 46% P 16% G 16% S 21%		D 79% T 8% S 5% P 3% G 3% R 2%	P 49-94% S 0-33% T 6-8% R 0-6% D 0-3%			
Expression of other markers	VGLUT2 94% CR 53% CB 46% PKCγ 35%/45% tVGLUT3 28% NKB 21% GRP 15% NTS 10% GAD67 2% GABA 0%	SOM 67% PKCγ 22% NKB 21% GRP 19% PAX2 15-17% VGLUT3 7-45% NTS 0% NECAB1 45%	NECAB2 95-89% SOM 74% NECAB1 0-55% CR 50% CCK 47% NTS 31% NKB 29-31% VGLUT3 25% GAD67 20-24*% GABA 8*% GRP 5-11% SP 4% NPYY1R 0% PV 0% NNOS 0%	PKCγ 5% GABA 5%	CB 44% SOM 57% PAX2 12-19% GRP 0-11% CCK 3% PKCγ 2%	VGLUT2 100% LMX1B 74-86% TLX3 70-79% SOM 59% CR 55% CB 21-29% PKCγ 3-17% SP 0-12% NPFF 0-6% NTS 1% NKB 0% CCK 0% VGAT 0% PAX2 0%	SOM 31-91% TLX3 85% CR 84% CB 84% PKCγ 20*-76% NTS 0-6% NK1R 5% CCK 2% PAX2 2% MOR1 1% GRP 0% NPFF 0% GAD 0*%	VGLUT2 93% PKCγ 47% SP 21-29% NTS 1% NKB 1% GRP 0% PAX2 0%	PKCγ 76-98% CB 75% SOM 73% NKB 0-13% TRH 4% GRP 2% CR 0% GABA 0*%

Marker	NPFF	NK1R	DOR	GRPR	NPYY1R	CB	TRH	NECAB1	NECAB2
Expression	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
% of lamina	5%	5*-6*%							
Morphology									
Firing pattern			D D 35% S 25% G 20% T 15% P 5%	D 56-58% T 21-23% P 15% G 0-7% S 0-5%					
Expression of other markers	SOM 85% SP 5% CCK 1% NTS 1% GRP 0-33% NKB 0% PAX2 0%	GABA 0% SST2A 0-0% GRPR 0%	VGLUT2 85% SOM 62% CB 50% CR 27% VGAT 25% NNOS 12% PAX2 8% PKCγ 7%	VGLUT2 63-81% LMX1B 62% PAX2 27% VGAT 19% NK1R 0%	CALB 36% CR 18% PAX2 2% PKCγ 0% PV 0*%	NTS 13% SOM 11% SP 10% NECAB1 48%	PKCγ 91% CCK 85% NTS 18%	VGLUT2 72% CB 38% CR 33% PKCγ 0-12% SST2A 2-11% PV 5%	VGLUT2 90% GAD67 0-0%

Inhibitory

Marker	GABA	PAX2	NNOS	SST2A	DYN	GLY	NPY	PRP	GAL	PV	BHLHB5
Expression	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Development
% of lamina	24*%-46*%	25%	18*%	14-14%	4*-14%	12*%	5*-8%	3/4*-7%	3%		
Morphology	I 43% C 14% V 11%				V		I 0%	C 100%		C-I 82%	C
Firing pattern	T 83% P 13% G 4% D 4%				T, P		T 84% P 8% S 7%	T 72-93 P 13% S 7%		T 71% P 29%	
Expression of other markers	PAX2 100*% GLY 37*-43*% SOM 0% NTS 0*% NK1R 0%	GABA 100% CR 27% GRP 0% CCK 0% NPFF 0%	SST2A 57% GABA 33*% PKCγ 0-32*% PV 0% GAL 0% DYN 0%	GABA 99-100% GLY 83% NECAB1 15-54% GAL 26-44% NNOS 30-36% PRP 29% NECAB2 7% NK1R 0-1% PV 0%	GAD67 86% GABA 69-79*% PAX2 67-73% SST2A 72% GAL 70*% GLYT2 28% VGLUT2 12% NPY 7-9% PV 0*% NNOS 0%	GABA 99*-100%	GABA 100*% PAX2 100% SST2A 15-24% DYN 13% GLY 6*% NNOS 4% BHLHB5 2% GAL 0%	SST2A 100% GABA 80% NNOS 70% GAL 28% NPY 0% PV 0%	GABA 100% SST2A 97-100% BHLHB5 95% DYN 92*% PRP 41% PV 0% NNOS 0% NPY 0%	GABA 70-75% NECAB1 31% NPY 4% SST2A 0-1% PKCγ 0% GAL 0% DYN 0*% NNOS 0% NPYYR1 0*%	LMX1B 30% PAX2 70% GAL 78%

ESM_2 Populations of dorsal horn neurons in lamina II for which quantitative expression within laminae is available. Lamina II neurons can be sorted based on their morphology and firing patterns (upper schematic). Vertical cells have gap, delayed or tonic firing (blue square), radial cells have delayed firing (medium blue square), islet cells are invariable tonic (pink square) and central cells have either tonic or phasic firing (dark blue square). A last class of lamina II neurons for which morphology does not fit usual categories can display all firing pattern that exist in this lamina. Percentages represent the proportion of neurons within the lamina that belong to each population. Lamina II neurons can also be organized based on their molecular profile. Using such criteria, populations of neurons that are mostly excitatory are displayed in light blue and those that are mostly inhibitory neurons are displayed in light pink. Range of values correspond to the minimum and maximum percentage of neurons that express a marker reported for each population. Values obtained in mice are shown in black and those obtained in rats are displayed in brown. Data that were obtained exclusively in lamina II are shown with an asterisk. Other data include analysis of both lamina I and lamina II. References used in this table can be find in ESM_4

Lamina III

Morphology: Multipolar, Pyramidal, Flattened, Antenna

Firing Pattern: Tonic (43*%), Phasic (25*%), Delayed (21*%), Single (10*%), Gap (2*%), Reluctant

Excitatory

Marker	VGLUT3	SOM	CCK	PKCγ	NK1R	CBLN2	IGFBP5	HTR6	NTS	NKB	SP	DYN
Expression	Development	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
% of lamina	32%	16-17%	3*-15%	6-14%	6-11%	11*%	11*%	10*%	8%	4-4%	4%	1-4%
Firing pattern	P 17-58% T 8-54% D 0-66% S 7-9%		P 67*% T 33*%	P 29*% D 56*% T 14*%		P48*% S 25*% T 21*%	T 76*% R 22*%	R 76*% T 10*% S 10*%				
Expression of other markers	VGLUT2 96% PKCγ 16-42% SOM 38% CR 10% NK1R 1%	PKCγ 50% NKB 25% GLYT2 5% NTS 3%	VGLUT2 92-98*% SP 13-21% NEUD4 4*% PKCγ 1*% GRP 1% NKB 1% NTS 1% CBLN2 1*% HTR6 1*% VGAT 1*% IGFPB5 0*% PV 0*% PAX2 0%	VGLUT2 97*% NEUD4 91*% SOM 56% CCK 4*-47% NTS 45 NKB 13% GABA 8% IGFPB5 3*% CBLN2 2*% HTR6 2*% VGAT 1*% SP 0% GLY 0% PV 0*%	PKCγ 37% GABA 10%	VGLUT2 81*% VGAT 5*% CCK* 2% HTR6* 8% PKCγ* 3% PV 0*%	VGLUT2 73*% VGAT 15*% CCK 2*% HTR6 4*% PKCγ 3*%	VGLUT2 88*% VGAT 6*% CCK 4*% PKCγ 3*% NEUD4 2*% CBLN2 1*% IGFBP5 1*%	PKCγ 75-79% NKB 0-34% SOM 10% TRH 4% GABA 0%	SOM 55% PKCγ 46% NTS 0-30% CCK 2% GRP 0% GAD67 0%	CCK 10-40% PAX2 21% GRP 0% PKCγ 0%	GABA 0% PV 0% GAL 0%

Marker	NEUD4	LMX1B	RORα	MAFA	MAFB	CMAF	LBX1	TRH	NECAB1	CB	CR	GRP
Expression	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
% of lamina	2*%											
Firing pattern	T 100*%											
Expression of other markers	VGLUT2 85*% PKCγ 18*% HTR6 5*% CCK 4*% VGAT 3*%	PAX2 0% GBX1 0% GAD67 0%	VGLUT2 92% LMX1B 86-92% CCK 60% CMAF 49% MAFA 48% PKCγ 22% GAD67 3% GBX1 3% PAX2 1%	LMX1B 93% GAD67 0% GBX1 0%	LMX1B 62% PAX2 29% GBX1 19% GAD67 13%	LMX1B 62% PAX2 29% GBX1 19% GAD67 18%	LMX1B 79% GBX1 21% PAX2 18% GAD67 11%	PKCγ 91% CCK 85% NTS 18%	CB 25% PV 16% CR 8%	NECAB1 23%	NECAB1 18%	NKB 0% SP 0%

Inhibitory

Marker	PAX2	GABA	GLY	PV	NPY	KCNIP2	SST2A	CDH3	GAL	RET	GBX1	GAD67
Expression	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Development	Adult	Adult
% of lamina	40%	30-53%	20-36%	13*%	4-9%	9*%	6%	5*%	2%			
Firing pattern				T 100*%	T 84% P 8% S 7%	D 100*%		G 46*% T 38*% P 8*% D 7*%		T 64-80% D 8-26% P 3-4% S 3-8%		
Expression of other markers	GABA 100% GAD67 82% GBX1 81% LMX1B 0% CCK 0%	PAX2 100% GLY 64-89% NTS 0% DYN 0%	GABA 96-98% PKCγ 0%	GABA 70-75% VGAT 63*% VGLUT2 35*% CDH3 35*% NECAB1 17% IGFBP5 2*% NPY 2% RORβ 1*% NEUD4 1*% HTR6 1*% CBLN2 0*% KCNIP2 0*% CCK 0*% PKCγ 0*% GAL 0% SST2A 0% NNOS 0-0% DYN 0%	GABA 100% PAX2 100% NNOS 1-6% SST2A 4% GAL 0%	VGAT 92*% VGLUT2 7*% CDH3 1*% RORβ 1*% PV 0*%	GABA 50-92% PV 0%	VGAT 79*% VGLUT2 8*% RORβ 3*% KCNIP2 2*%	GABA 100% NNOS 83% SST2A 10% GLY 1% DYN 0% PV 0% NPY 0%	VGAT 91-93% GLYT2 51% GAD67 47% PV 24-31% VGLUT2 11%	PAX2 87-95% GAD67 77% LMX1B 0% MAFA 0%	PAX2 82% GBX1 77% RORβ 32% CMAF 18% MAFB 13% LBX1 11% LMX1B 0% MAFA 0% NKB 0%

Mixte

Marker	RORβ	NNOS
Expression	Adult	Adult
% of lamina	8*%	5%
Firing pattern	D 70*% T 29*%	
Expression of other markers	VGAT 80*% PAX2 54-58% GBX1 51% LMX1B 48% VGLUT2 11*-43% GAD67 32% NNOS 21% GAD65 20% DYN 11% GAL 9% PV 6% NPY 5% CDH3 1*% KCNIP2 0*%	PKCγ 53% GABA 50% SST2A 29% PV 0% NPY 0%

Lamina IV-VI

Marker	ZIC2	ATOH1	SATB1/2	TCFAP2B
Expression	Development	Development	Development	Development
% of lamina				
Firing pattern				
Expression of other markers	LMX1B 64% RORα 28% SATB1/2 16% TCFAP2B 15% PAX2 4%	VGLUT2 82%	TLX3 52% PAX2 22%	GAD65 67% GAD67 40% VGLUT2 26%

Projection Neurons

% of lamina	2%
Expression of other markers	NK1R 33-44%

ESM_3 Populations of dorsal horn neurons in lamina III for which quantitative expression within laminae is available. Lamina III neurons can be sorted based on their morphology and firing patterns but no correlation has been established between these two criteria (upper schematic). Lamina III cells can have multipolar, pyramidal, flattened or antenna-type morphologies. Their firing pattern can be tonic, phasic, delayed, single, gap or reluctant. Percentages represent the proportion of neurons within the lamina that belong to each population. Lamina II neurons can also be organized based on their molecular profile. Using such criteria, populations of neurons that are mostly excitatory are displayed in light blue and those that are mostly inhibitory neurons are displayed in light pink. Populations that are equally composed of excitatory and inhibitory neurons are represented in grey. Other populations of the deep dorsal horn are displayed in yellow. Projection neurons of lamina III that send axons to supraspinal areas are represented in green. Range of values correspond to the minimum and maximum percentage of neurons that express a marker reported for each population. Values obtained in mice are shown in black and those obtained in rats are displayed in brown. Data marked by an asterisk include neurons from lamina II_i to lamina III. Other data were obtained from analysis of lamina III exclusively. References used in this table can be find in ESM_4

Marker	References
ATOH1	(Yuengert et al. 2015)
BHLHB5	(Kardon et al. 2014; Ross et al. 2010)
CB	(Yoshida et al. 1990)
CBLN2	(Abraira et al. 2017)
CCK	(Abraira et al. 2017; Gutierrez-Mecinas et al. 2019b; Xu et al. 2008)
CDH3	(Abraira et al. 2017)
CMAF	(Del Barrio et al. 2013)
CR	(Cheng et al. 2017; Gutierrez-Mecinas et al. 2019c; Peirs et al. 2015; Smith et al. 2015; Smith et al. 2016; Zhang et al. 2014)
DYN	(Boyle et al. 2017; Duan et al. 2014; Huang et al. 2018; Sardella et al. 2011a; Xu et al. 2008)
DOR	(Wang et al. 2018)
GABA	(Abraira et al. 2017; Larsson 2017; Polgar et al. 2013a; Polgar et al. 2003; Polgar et al. 2013b; Todd and Sullivan 1990; Yasaka et al. 2010)
GAD67	(Del Barrio et al. 2013)
GAL	(Iwagaki et al. 2013; Kardon et al. 2014; Polgar et al. 2013b; Sardella et al. 2011a; Simmons et al. 1995; Tiong et al. 2011)
GBX1	(Del Barrio et al. 2013)
GLY	(Abraira et al. 2017; Polgar et al. 2013a; Polgar et al. 2003; Todd and Sullivan 1990)
GRP	(Albisetti et al. 2019; Dickie et al. 2018; Gutierrez-Mecinas et al. 2016; Gutierrez-Mecinas et al. 2014; Pagani et al. 2019; Solorzano et al. 2015)
GRPR	(Aresh et al. 2017; Bardoni et al. 2019)
HTR6	(Abraira et al. 2017)
KCNIP2	(Abraira et al. 2017)
LBX1	(Gross et al. 2002)
IGFBP5	(Abraira et al. 2017)
LMX1B	(Del Barrio et al. 2013; Gross et al. 2002; Qiu et al. 2009)
MAFA	(Del Barrio et al. 2013)
MAFB	(Del Barrio et al. 2013)
MOR1	(Kemp et al. 1996; Polgar et al. 1999; Spike et al. 2002)
NECAB1	(Zhang et al. 2016; Zhang et al. 2014)
NECAB2	(Zhang et al. 2016; Zhang et al. 2014)
NEUD4	(Abraira et al. 2017)
NK1R	(Bardoni et al. 2019; Duan et al. 2014; Littlewood et al. 1995; Polgar et al. 2013a; Polgar et al. 1999; Polgar et al. 2006; Todd et al. 1998)
NKB	(Gutierrez-Mecinas et al. 2016; Mar et al. 2012; Polgar et al. 2006)
NNOS	(Huang et al. 2018; Iwagaki et al. 2013; Martin et al. 1999; Polgar et al. 2013a; Polgar et al. 2013b; Sardella et al. 2011b; Tiong et al. 2011)
NPFF	(Gutierrez-Mecinas et al. 2019a)
NPY	(Boyle et al. 2017; Iwagaki et al. 2013; Kardon et al. 2014; Polgar et al. 2013b; Polgar et al. 2011; Rowan et al. 1993; Tiong et al. 2011)
NPYY1R	(Nelson et al. 2019)
NTS	(Gutierrez-Mecinas et al. 2019b; Gutierrez-Mecinas et al. 2016; Polgar et al. 1999; Proudlock et al. 1993; Todd et al. 1992)
PAX2	(Del Barrio et al. 2013; Gross et al. 2002; Gutierrez-Mecinas et al. 2017; Larsson 2017)
PKCγ	(Abraira et al. 2017; Alba-Delgado et al. 2015; Dickie et al. 2018; Gutierrez-Mecinas et al. 2019b; Gutierrez-Mecinas et al. 2019c; Gutierrez-Mecinas et al. 2016; Martin et al. 1999; Peirs et al. 2014; Peirs et al. 2015; Polgar et al. 1999; Sardella et al. 2011a; Sardella et al. 2011b; Solorzano et al. 2015; Zhang et al. 2016)
PRP	(Hantman et al. 2004; Iwagaki et al. 2013)
PV	(Abraira et al. 2017; Boyle et al. 2019; Boyle et al. 2017; Hughes et al. 2012; Laing et al. 1994; Martin et al. 1999; Polgar et al. 2013b; Tiong et al. 2011; Yoshida et al. 1990)
RET	(Cui et al. 2016)
RORα	(Bourane et al. 2015; Del Barrio et al. 2013; Paixão et al. 2019)
RORβ	(Abraira et al. 2017; Del Barrio et al. 2013; Koch et al. 2017)
SATB1/2	(Levine et al. 2014)
SP	(Dickie et al. 2018; Gutierrez-Mecinas et al. 2017; Gutierrez-Mecinas et al. 2019b; Gutierrez-Mecinas et al. 2019c; Xu et al. 2008)
SOM	(Cheng et al. 2017; Duan et al. 2014; Gutierrez-Mecinas et al. 2016; Proudlock et al. 1993)
SST2A	(Cameron et al. 2015; Iwagaki et al. 2013; Polgar et al. 2013a; Todd et al. 1998; Zhang et al. 2016)
TCFAP2B	(Levine et al. 2014)
TRH	(Gutierrez-Mecinas et al. 2019b)
VGLUT2	(Abraira et al. 2017; Gutierrez-Mecinas et al. 2019b; Gutierrez-Mecinas et al. 2019c; Wang et al. 2018)
VGLUT3	(Cheng et al. 2017; Peirs et al. 2015)
ZIC2	(Paixão et al. 2019)
Projection neurons	(Abraira et al. 2017; Bardoni et al. 2019; Bice and Beal 1997; Cameron et al. 2015; Gamboa-Esteves et al. 2004; Grudt and Perl 2002; Gutierrez-Mecinas et al. 2019a; Marshall et al. 1996; Polgar et al. 1999; Solorzano et al. 2015; Spike et al. 2003; Todd et al. 2000; Wang et al. 2018; Yuengert et al. 2015)

ESM_4 References used in ESM_1-3 for each dorsal horn population for which quantitative expression within laminae is available. Studies performed in mice are shown in black and those performed in rats are displayed in brown. ATOH1, Atonal Basic helix-loop-helix Transcription Factor 1; BHLHB5, Basic helix-loop-helix Domain Containing, Class B, 5; CB, Calbindin, CBLN2, Cerebellin-2; CCK, Cholecystokinin; CDH3, Cadherin-3; CMAF, Proto-Oncogene C-Maf, CR, Calretinin; DYN, Dynorphin; DOR, Opioid receptor δ; GABA, γ-aminobutyric acid; GAD67, Glutamic acid decarboxylase 67; GAL, Galanin; GBX1, Gastrulation Brain Homeobox 1; GLY, Glycine; GRP, Gastrin releasing peptide; GRPR, Gastrin-releasing peptide receptor; HTR6, Serotonin Receptor 6; KCNIP2, Kv Channel Interacting protein-2; LBX1, Ladybird homeobox 1; IGFBP5, Insulin-like Growth Factor Binding Protein 5; LMXB, LIM homeobox transcription factor 1 beta; MAFA, V-maf musculoaponeurotic fibrosarcoma oncogene homolog A; MAFB, V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; MOR1, Opioid receptor μ 1; NECAB1, Neuronal calcium-binding protein 1; NECAB2, Neuronal calcium-binding protein 2; NEUD4, Neurogenic Differentiation Factor-4; NK1R, Neurokinin 1 receptor; NKB, Neurokinin B; NNOS, Neuronal nitric oxide synthase; NPFF, Neuropeptide FF; NPY, Neuropeptide Y; NPYY1R, Neuropeptide Y Y1 receptor; NTS, Neurotensin; PAX2, Paired box gene 2; PKCγ, Gamma isoform of protein kinase C; PRP, Prion promoter; PV, Parvalbumin; RET, Receptor tyrosine kinase; RORα, ROR α nuclear orphan receptor; RORβ, ROR β nuclear orphan receptor; SATB1/2, Special AT-rich sequence-binding proteins 1 and 2; SP, Substance P; SOM, Somatostatin; SST2A, Somatostatin receptor subtype 2A; TCFAP2B, Transcription factor AP-2 beta; TRH, Thyrotropin Releasing Hormone; VGLUT2, Vesicular glutamate transporter 2; VGLUT3, Vesicular glutamate transporter 3; ZIC2, Zic Family Member 2

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