THE EF-HAND CA^{2+}-BINDING PROTEIN SUPER-FAMILY: A GENOME-WIDE ANALYSIS OF GENE EXPRESSION PATTERNS IN THE ADULT MOUSE BRAIN

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Abstract—In mice, 249 putative members of the superfamily of EF-hand domain Ca^{2+}-binding proteins, manifesting great diversity in structure, cellular localization and functions have been identified. Three members in particular, namely, calbindin-D28K, calretinin and parvalbumin, are widely used as markers for specific neuronal subpopulations in different regions of the brain. The aim of the present study was to compile a comprehensive atlas of the gene-expression profiles of the entire EF-hand gene superfamily in the murine brain. This was achieved by a meticulous examination of the *in-situ* hybridization images in the Allen Brain Atlas database. Topographically, our analysis focused on the olfactory bulb, cerebral cortex (barrel cortex in the primary somatosensory area), basal ganglia, hippocampus, amygdala, thalamus, hypothalamus, cerebellum, midbrain, pons and medulla, and on clearly identifiable sub-structures within each of these areas. The expression profiles of four family-members, namely hippocalcin-like 4, neurocalcin-δ, plastin 3 and tescalcin, that have not been hitherto reported, at either the mRNA (*in-situ*-hybridization) or the protein (immunohistochemical) levels, are now presented for the first time. The fruit of our analysis is a document in which the gene-expression profiles of all members of the EF-hand family genes are compared, and in which future possible neuronal markers for specific cells/brain areas are identified. The assembled information could afford functional clues to investigators, conducive to further experimental pursuit.

Key words: Allen Brain Atlas, hippocalcin-like 4, neurocalcin-δ, plastin 3, tescalcin, neuronal markers.

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Abbreviations: ABA, Allen Brain Atlas; AMY, amygdala; AON, anterior olfactory nucleus; APN, anterior pretectal nucleus; ARH, arcuate hypothalamic nucleus; AV, anteroventral nucleus of the thalamus; BG, basal ganglia; BLA, basolateral nucleus of the amygdala; BMA, basomedial nucleus of the amygdala; CA1/2/3, Ammon's horn regions 1/2/3; *Calb1*, Calb-D28K-encoding gene; *Calb2*, CalR-encoding gene; Calb-D28K, calbindin protein; CalR, calretinin protein; CBX, cerebellum; CEA, central nucleus of the amygdala; CLA, claustrum; CM, central medial nucleus of the thalamus; COA, cortical nucleus of the amygdala; CP, caudoputamen; CS, superior central raphé nucleus; CSm, superior-central raphé, nucleus, medial part; CTX, cerebral cortex; CUN, cuneiform nucleus; DCN, deep cerebellar nuclei; DG, dentate gyrus; DG sg, dentate gyrus, granule cell layer; DL, dorsolateral quadrant of the PAG; DMH, dorsomedial hypothalamic nucleus; DMX, dorsal motor nucleus of the Vagus nerve; DR, dorsal raphé nucleus; DTN, dorsal tegmental nucleus; EF-CaBP, EF-hand domain containing Ca^{2+}-binding protein; END, endopiriform nucleus; EW, Edinger–Westphal nucleus; Golgi c., golgi cells; GPE, globus pallidus, external segment; GPI, globus pallidus, internal segment; gl, glomerula layer of the olfactory bulb; gr, granular layer of the olfactory bulb; Gr, granular layer of the cerebellum; *Hpcal4*/Hpcal4, respectively hippocalcin-like 4 gene/protein; HPF, hippocampus formation; HY, hypothalamus; IC, inferior colliculus; III, oculomotor nucleus; IO, inferior olivary complex; ISH, *in situ* hybridization; IV, trochlear nucleus; LAV, lateral vestibular nucleus; LC, locus coeruleus; LD, latero-dorsal nucleus of the thalamus; LDT, latero-dorsal tegmental nucleus; LGd, dorsal part of the geniculate complex; LH, lateral habenula; LHA, lateral hypothalamic area; LM, lateral mammillary nucleus; LP, lateral posterior nucleus of the thalamus; LRN, lateral reticular nucleus; LS, lateral septum; MARN, magnocellular reticular nucleus; MB, midbrain; ME, medial nucleus of the amygdala; MEV, midbrain trigeminal nucleus; MH, medial habenula; mi, mitral cells layer, olfactory bulb; MM, medial mammillary nucleus; Mo, molecular layer of the cerebellum; MS, medial septum; MV, medial vestibular nucleus; MY, medulla oblongata; *Ncald*/Ncald, repectively neurocalcin-δ gene/protein; nd, not detected; ND, nucleus of Darkschewitz; NDB, nucleus of the diagonal band; NLL, nucleus of the lateral lemniscus; NTB, nucleus of the trapezoid body; NTS, nucleus of the solitary tract; OB, olfactory bulb; opl, outer plexiform layer, olfactory bulb; P, pons; PA, posterior nucleus of the amygdala; PAG, periaqueductal gray; Parv, parvalbumin-protein; PBG, parabigeminal nucleus; PBl, parabrachial nucleus, lateral division; PCG, pontine central gray; Pch, plexus choroideus; PCN, paracentral nucleus; pCTX, prefrontal cortex; PF, parafascicular nucleus; PFA, paraformaldehyde; PG, pontine gray; PGRN, paragigantocellular nucleus; PH, posterior hypothalamic nucleus; *Pls3*, plastin3-encoding gene; PMd, dorsal premammillary nucleus; PRN, pontine reticular nucleus; PRP, nucleus prepositus; PSV, principal sensory nucleus of the trigeminal; PT, parataenial nucleus; Pu, Purkinje cells layer; PV1, PV1 nucleus; PV2, PV2 nucleus of the PAG; *Pvalb*, Parv-encoding gene; PVH, paraventricular hypothalamic nucleus; PVT, paraventricular nucleus of the thalamus; RE, reunions nucleus; RH, rhomboid nucleus; RM, nucleus raphé magnus; RMS, rostral migratory stream; RN, red nucleus; RT, reticular nucleus of the thalamus; S, striatum; SC, superior colliculus; SCH, suprachiasmatic nucleus; sg, granule cell layer, dentate gyrus; SI, substantia innominata; SLD, sublatero-dorsal nucleus; SNc, substantia nigra, compact part; so, oriens layer of the hippocampus; sp, pyramidal layer of the hippocampus; spa, sparse distribution of stained elements; SPIV, spinal nucleus of the trigeminus, interpolar part; SPV, spinal nucleus of the trigeminal; STN, subthalamic nucleus; SUMI, supramammillary nucleus, lateral part; TBS, tris-buffered saline; *Tesc*/Tesc, respectively tescalcin gene/protein; TH, thalamus; TM, tuberomammillary nucleus; TRN, tegmental reticular nucleus; ubi, ubiquitary distribution of stained elements; V, motor nucleus of trigeminal nerve; VAL, ventral anterior nucleus of the thalamus; VI, abducens nucleus; VII, facial motor nucleus; VI, lateral ventricle; VM, ventral medial nucleus of the thalamus; VMH, ventro-medial hypothalamic nucleus; VPL, ventral postero-lateral nucleus of the thalamus; VPM, ventral postero-medial nucleus of the thalamus; VTA, ventral tegmental area; VTN, ventral tegmental nucleus; Wm, white matter; XII, hypoglossal nucleus; ZI, zona incerta.

INTRODUCTION

Following the advent of the modern genome-sequencing techniques, a number of studies were undertaken in which the assimilated know-how was utilized to gain insights into gene functions. The fruits of these investigations are numerous published web databases, which furnish information appertaining to, for example, nomenclature, gene/protein sequences, protein/gene interactions, expression patterns, biological activity, integrated biological systems and disease-associations in humans. One such large-scale genomic database – the Allen Brain Atlas (ABA) database (http://www.brain-map.org/) – yields information of inestimable value on the expression patterns of genes in the adult murine brain (Lein et al., 2007). These data have served as the basis for more restricted analyses, which have focused, for example, on the gene-expression profiling of particular neuro-anatomical structures, gene families and non-coding RNAs (Sunkin and Hohman, 2007; Mercer et al., 2008; Olszewski et al., 2008; Thompson et al., 2008; Dahlin et al., 2009; Dong et al., 2009; Tebbenkamp and Borchelt, 2010; Girard et al., 2011, 2014; Ko et al., 2013).

During the past three decades, three members of the superfamily of EF-hand Ca^{2+}-binding proteins (EF-CaBPs), namely, calbindin-D28K (Calb-D28K) (*Calb1* gene), calretinin (CalR) (*Calb2* gene) and parvalbumin (Parv) (*Pvalb* gene) have been widely used as specific and robust markers for discrete, often GABAergic, neuronal populations (Celio, 1990; Baimbridge et al., 1992; Andressen et al., 1993; Bastianelli, 2003; Schwaller, 2009, 2010). A classical example is the subpopulation of fast-spiking GABAergic, Parv-positive interneurons existing in several different brain regions including the neocortex, striatum, hippocampus and cerebellum (Hu et al., 2014). More recently, secretagogin (Scgn), a member of the family of hexa-EF-hand proteins has been recruited as a neuro-morphological marker and used to further characterize novel neuronal populations (Alpar et al., 2012; Kosaka and Kosaka, 2013; Gati et al., 2014; Romanov et al., 2015). An interesting outcome of these studies is that Ca^{2+}-binding proteins do not necessarily delineate a uniform neuronal subpopulation. For example, Calb-D28K is expressed at low levels in pyramidal neurons of cortical layers 2–3 and CA1-pyramidal cells and at high levels in scattered interneurons (Celio, 1990). CalR is expressed not only in mature layer-2 GABAergic interneurons but also in interneurons of the adult striatum (Ernst et al., 2014). Although neurons expressing Parv also have different morphologies and distributions, as exemplified by the interneurons of the cerebral cortex and in the hippocampus (DeFelipe et al., 2013), and by the long-axon neurons such as the Purkinje cells of the cerebellum and the oculomotor motoneurons of the midbrain (Solbach and Celio, 1991), they share in common a high firing rate (Kawaguchi et al., 1987). Moreover, while the developmental patterns of Calb-D28K-, CalR- and Parv-expressing neurons in the neocortex are fairly consistent between species, in other parts of the brain and among mammalian orders, neurons expressing any of these EF-CaBPs exhibit great difference in the morphology and distribution (Hof et al., 1999). In the basal forebrain for example, immunoreactivity for Calb-D28k reveals the cholinergic cells of the basal forebrain only in primates, not in rodents (Celio and Norman, 1985). Furthermore, CalR and Calb-D28K are considered to be useful markers for corticogenesis in humans since they are expressed at very early embryonic stages [(Carnegie stage (CS) 17 (Gonzalez-Gomes and Meyer, 2014)], namely during the phases that precede the generation and the migration of the various interneuronal subtypes. Proteins including CalR and other EF-CaBPs have also proved to be useful in delineating the various stages of adult hippocampal neurogenesis (Brandt et al., 2003; Kempermann et al., 2004). During this process, an active interchange in the expression of the EF-CaBP takes place: CalR is expressed only transiently in newly formed (glutamatergic) granule cells; in the more mature ones it is replaced by Calb-D28K (Brandt et al., 2003). The molecular basis for this switch remains an enigma; we know only that in adult CalR-knockout mice the absence of this protein leads to a permanent impairment of neurogenesis (Todkar et al., 2012). The latter finding also points to an important distinction: the expression of a given EF-CaBP in a well-defined neuronal subpopulation permits an investigation not only of the role of a particular protein in this group of cells, using for instance transgenic or knockout animals, but it also allows studying the role of the neuronal population itself, by targeting it genetically – irrespective of the role the EF-CaBP may play in this population. Studies of this type include the use of transgenic mice expressing Cre-recombinase under the control of the *Pvalb*-promoter (Hippenmeyer et al., 2005). By means of optogenetic experiments, in which the selective expression of the hyperpolarizing halorhodopsin in Parv-positive nerve cells is triggered, a suppression of γ-oscillations *in vivo* was demonstrated, whereas a channelorhodopsin-mediated activations of this neuronal subpopulation leads to emergent γ-frequency rhythmicity (Sohal et al., 2009). This finding is of some functional relevance, in so far as alterations in either the function, the number or the mRNA/protein-expression level of Parv-immunoreactive neurons correlates not only with experience-dependent plasticity (Donato et al., 2013), but also with the manifestation of neurological disturbances including schizophrenia, bipolar disorder and autism (Lewis et al., 2005; Torrey et al., 2005; Gogolla et al., 2009). In addition, chronic stress down-regulates significantly the expression of hippocampal Parv (Hu et al., 2010; Filipovic et al., 2013).

The four summarized proteins (Calb-D28K, CalR, Parv, Scgn) represent but a minute contingent of the richly endowed superfamily to which they belong, which, in eukaryotic organisms, is the most abundant category of Ca^{2+}-binding proteins. Hence, it would be of interest to identify other potential neuronal markers within this family. The conserved EF-hand domain consists of two alpha helices, which are joined by a Ca^{2+}-binding loop. The canonical sequence is comprised of about 30 residues, and the EF-hand motifs commonly occur in adjacent pairs (Kawasaki et al., 1998; Lewit-Bentley and Rety, 2000). By 1990, 160 members of the EF-CaBP

superfamily were identified. Following the publication of mammalian genomes, the number rose to over 200, and even this estimate was deemed to be a conservative one (Persechini et al., 1989; Moncrief et al., 1990; Haiech et al., 2004). EF-CaBPs play a central role in all aspects of Ca^{2+} signaling, which include the control of Ca^{2+} gating, modulation of the amplitude and duration of Ca^{2+} signals and the transduction of Ca^{2+} signals into biochemical responses. The involvement of the EF-CaBPs in such a broad array of functions is rendered possible by the great diversity that they manifest in structure, cellular localization and molecular activity (Schwaller, 2009, 2010).

It was the aim of the present study to map in the murine brain the expression profiles of all members of the EF-CaBP superfamily at the gene level by an exhaustive analysis of the *in-situ*-hybridization data in the ABA. Our analysis has revealed potential new markers for specific neurons, as well as for certain brain nuclei, areas and layers, and possibly also for specific functional systems.

EXPERIMENTAL PROCEDURES

Bioinformatics

For listing the genes of the EF-hand super-family, we searched the Uniprot database (http://www.uniprot.org/), selecting the term ''EF-hand'' as query, as well as the NCBI Gene database (http://www.ncbi.nlm.nih.gov/gene/), selecting the terms ''EF-hand'' and ''Mus musculus'' as queries. For analyzing gene expression patterns in the mouse brain, we consulted the ABA database (http://mouse.brain-map.org/), and examined available *in situ* hybridization (ISH) images for each of the EF-hand genes (see Table 2). The number of ISH-series available in the ABA for the detection of the expression of a given gene varies from 1 (sagittal series for 61 genes), 2 (sagittal + coronal series for 60 genes), 3 or more (sagittal + coronal for 14 genes). (Table 2, 2nd column: ISH Nr.). The schematic drawings in Figs. 1 and 2 were adapted from Dong (2008) and the ISH images presented in Figs. 3–5 were taken from the ABA.

Animals

Adult mice (C57Bl6), males and females, aged 8–12 weeks, were used in this study for brain ISH and immunostaining. Mice were housed in an animal facility approved by the Veterinary Office of the Canton of Fribourg (Switzerland), and according to the present Swiss law and the European Communities Council Directive of November 1986 (86/609/EC). Animals were housed in groups of 3–5 individuals on a 12-h/12-h light/dark cycle (light onset at 7 a.m.) with free access to food and water. The study was approved by the Veterinary Office of the Canton of Fribourg (Switzerland).

In situ hybridization

Total RNA from mouse brain (Zyagen, San Diego, USA) was reverse transcribed using oligodT as primer, and the reaction mixture was used as template for PCR reactions using primers specific for each of the genes tested (*Pvalb*, *Ncald*, *Pls3*, *Eps15* and *Sparcl1*), the sequence of which is described in the ABA database. Forward and reverse primers were flanked by T3 and SP6 RNA polymerase core promoter sequence, respectively (Girard et al., 2011). Digoxygenin-labeled antisense and sense RNA probes were prepared from each specific PCR template by *in vitro* transcription with either SP6 or T3 RNA-polymerases and DIG-labeled UTP (Roche Applied Science, Switzerland). Twelve-to-fourteen micrometer coronal brain cryosections were prepared on Superfrost gold slides (Medite, Nunningen, Switzerland), and stored at −70 °C until use. *In situ* hybridization was performed essentially as previously described (Girard et al., 2011), after fixation of the brain sections in 4% paraformaldehyde in PBS.

Immunostaining and antibodies

Immunostaining experiments were performed on 8-to-12-week-old mouse brains, under standard conditions. Animals were anesthetized with pentobarbital (100 mg/kg), perfused through the left ventricle with ice-cold 0.9% NaCl, followed by fixation with 4% paraformaldehyde (PFA). Brains were excised, post-fixed overnight at 4 °C in PFA, then immersed in 20% sucrose solution in 0.1 M Tris buffer pH 7.3 (TBS) until cryo-sectioning. Brains were cryo-sectioned into 30- or 40-μm coronal sections collected and maintained until use in TBS containing 0.02% sodium azide. Immunostaining experiments were performed following standard protocols. Free-floating sections were incubated 18–24 h at 4 °C with primary antibodies diluted in TBS containing 0.1% Triton-X100.

The following primary antibodies were used: anti-hippocalcin-like 4 and anti-tescalcin (rabbit, 1/750, this study), anti- neurocalcin-δ (rabbit, 1/500 to 1/750, Enzo Life Sci., Lausen, Switzerland), anti-parvalbumin (mouse PV235 1/2'000, guinea pig PV690 1/1500 Swant, Marly, Switzerland), anti-calbindin (mouse CB300, 1/2'000, Swant, Marly, Switzerland), anti-calretinin (mouse CR6B3, 1/2'000, Swant, Marly, Switzerland). Secondary antibodies employed were Alexa 488-conjugated donkey anti-rabbit (Luboscience, Luzern, Switzerland), Cy3-conjugated donkey anti-mouse, Cy5-conjugated donkey anti-guinea pig, Cy2- and Cy3-conjugated streptavidin (Jackson Immunoresearch Laboratory, Rheinfelden, Switzerland), biotinylated anti rabbit (Vector Laboratories, Servion, Switzerland).

The image analysis was performed with a Zeiss Axiophot fluorescence microscope, a Hamamatsu Nanozoomer and a Leica TCS SP5 confocal laser microscope. Image post-processing and contrast adjustments were performed with Adobe Photoshop and Nanozoomer slide processing software.

Polyclonal antibodies to Hpcal4 and Tesc were obtained the following way. In a larger and ongoing project aiming to obtain reliable antibodies against all EF-CaBPs, the selected cDNA sequences (for each of the EF-CABP-encoding genes) were analyzed for both CG content and the absence of BamHI and SalI restriction sites. For each gene, cDNA was then

Table 1. The EF-hand super-family in the mouse genome. For each EF-hand family gene, the abbreviated usual name, the complete name, and the function of the encoded protein, according to Gene Ontology are given. The function in the CNS, when described (in italics), is mentioned. Genes in bold show a detectable gene expression in the brain, according to the ABA data; (n.e.d): no expression detected in brain; (n.d): no data available in the ABA database

Gene	Complete name (*alternative name*)	Gene ontology/*CNS function*(*s*)
Ankrd5 (n.e.d)	Ankyrin repeat domain 5 (=*Ankef1*)	Unknown
Actn1	Actinin, alpha 1	Actin filament binding/assembly; *neurite extension*
Actn2 (n.e.d)	Actinin, alpha 2	Actin filament binding/assembly
Actn3	Actinin, alpha 3	Actin filament binding/assembly
Actn4	Actinin, alpha 4	Actin filament binding/assembly
Aif1 (n.e.d)	Allograft inflammatory factor 1 (=*Iba1*)	Actin filament binding/assembly
Aif1l (n.e.d)	Allograft inflammatory factor 1-like	Actin filament binding/assembly
Cabp1	Caldendrin, Ca^{2+}-binding protein 1	Modulator of Ca^{2+} channel activity; *Ca^{2+} sensor; fine tuning of CaV.1/2*
Cabp2 (n.e.d)	Ca^{2+}-binding protein 2	Modulator of Ca^{2+} channel activity
Cabp4	Ca^{2+}-binding protein 4	Modulator of Ca^{2+} channel activity
Cabp5	Ca^{2+}-binding protein 5	Modulator of Ca^{2+} channel activity
Cabp7	Ca^{2+}-binding protein 7	Modulator of Ca^{2+} channel activity
Calb1	Calbindin D-28 K	*Ca^{2+} sensor/buffer activity*
Calb2	Calretinin	*Ca^{2+} sensor/buffer activity*
Calm1	Calmodulin 1	Ca^{2+} sensor/Ca^{2+} signaling
Calm2	Calmodulin 2	Ca^{2+} sensor/Ca^{2+} signaling
Calm3	Calmodulin 3	Ca^{2+} sensor/Ca^{2+} signaling
Calm4 (n.e.d)	Calmodulin 4	Ca^{2+} sensor/Ca^{2+} signaling
Calm5 (n.e.d)	Calmodulin 5	Ca^{2+} sensor/Ca^{2+} signaling
Calml3 (n.e.d)	Calmodulin-like 3	Ca^{2+} sensor/Ca^{2+} signaling
Calml4 (n.e.d)	Calmodulin-like 4	Ca^{2+} sensor/Ca^{2+} signaling
Caln1 (n.e.d)	Calneuron 1	Ca^{2+} buffer/sensor activity
Calu	Calumenin	Unknown
Capn1	Calpain 1	Ca^{2+}-dependent cysteine-type endopeptidase activity; *neural stem cell renewal|differentiation*
Capn2	Calpain 2	Ca^{2+}-dependent cysteine-type endopeptidase activity; *modulator of gliogenesis*
Capn3	Calpain 3	Ca^{2+}-dependent cysteine-type endopeptidase activity
Capn8	Calpain 8 (=*Capn10*)	Ca^{2+}-dependent cysteine-type endopeptidase activity
Capn9 (n.e.d)	Calpain 9	Ca^{2+}-dependent cysteine-type endopeptidase activity
Capn11 (n.d)	Calpain 11	Ca^{2+}-dependent cysteine-type endopeptidase activity
Capn13 (n.e.d)	Calpain 13	Ca^{2+}-dependent cysteine-type endopeptidase activity
Capns1	Calpain, small subunit 1	Ca^{2+}-dependent cysteine-type endopeptidase activity; *synaptic plasticity; traumatic brain injury*
Capns2 (n.d)	Calpain, small subunit 2	Ca^{2+}-dependent cysteine-type endopeptidase activity
Caps2 (n.e.d)	Calcyphosphine 2	Secretory granule exocytosis
Capsl (n.e.d)	Calcyphosine-like	Secretory granule exocytosis
Cbara1	Ca^{2+}-binding atopy-related autoantigen 1 (=*Micu1, mitochondrial Ca^{2+} uptake 1*)	Mitochondrial Ca^{2+} homeostasis
Cbl	Casitas B-lineage lymphoma	Ubiquitin ligase activity
Cblb	Casitas B-lineage lymphoma b	Ubiquitin ligase activity
Cblc (n.e.d)	Casitas B-lineage lymphoma c	Ubiquitin ligase activity
Ccdc48 (n.e.d)	Coiled-coil domain containing 48 (=*Efcc1, EF-hand and coiled-coil domain containing 1*)	Unknown
Cetn1 (n.e.d)	Centrin 1	Microtubule dynamics
Cetn2	Centrin 2	Microtubule dynamics
Cetn3	Centrin 3	Microtubule dynamics
Cetn4 (n.e.d)	Centrin 4	Microtubule dynamics
Cgref1	Cell growth regulator with EF hand domain 1	Unknown
Chp1 (n.d)	Calcineurin-like EF-hand protein 1	Regulation of pH
Chp2 (n.e.d)	Calcineurin-like EF-hand protein 2	Regulation of pH
Cib1 (n.e.d)	Ca^{2+}- and integrin-binding 1 (=*calmyrin*)	Pleiotropous effects
Cib2	Ca^{2+}- and integrin-binding family member 2 (=*Calmyrin 2*)	Unknown
Cib3 (n.e.d)	Ca^{2+}- and integrin-binding family member 3	Unknown
Cib4 (n.d)	Ca^{2+}- and integrin-binding family member 4	Unknown
Crnn (n.d)	Cornulin	Unknown
Dgka	Diacylglycerol kinase, alpha	Diacylglycerol kinase activity
Dgkb	Diacylglycerol kinase, beta	Diacylglycerol kinase activity

(*continued on next page*)

Table 1 (*continued*)

Gene	Complete name (*alternative name*)	Gene ontology/*CNS function*(*s*)
Dgkg	Diacylglycerol kinase, gamma	Diacylglycerol kinase activity
Dmd (n.e.d)	Dystrophin	Muscle cell structure/homeostasis
Drp2	Dystrophin-related protein 2	Muscle cell structure/homeostasis
Dst (n.e.d)	Dystonin	Cytoplasmic microtubule organization/axonogenesis
Dtna	Dystrobrevin alpha	Muscle homeostasis
Dtnb	Dystrobrevin beta	Muscle homeostasis
Duox1 (n.e.d)	Dual oxidase 1	Oxidoreductase activity
Duox2 (n.d)	Dual oxidase 2	Oxidoreductase activity
Dytn (n.e.d)	Dystrotelin	Muscle homeostasis
Efcab1	EF hand Ca^{2+}-binding domain 1	Unknown
Efcab2 (n.e.d)	EF-hand Ca^{2+}-binding domain 2	Unknown
Efcab3 (n.e.d)	EF-hand Ca^{2+}-binding domain 3	Unknown
Efcab4a	EF-hand Ca^{2+}-binding domain 4A	*Ca^{2+} sensor activity*
Efcab4b (n.e.d)	EF-hand Ca^{2+}-binding domain 4B	Unknown
Efcab5 (n.e.d)	EF-hand Ca^{2+}-binding domain 5	Unknown
Efcab6 (n.d)	EF-hand Ca^{2+}-binding domain 6	Unknown
Efcab7 (n.d)	EF-hand Ca-binding domain 7	Unknown
Efcab8 (n.d)	EF-hand Ca^{2+}-binding domain 8	Unknown
Efcab9 (n.e.d)	EF-hand Ca^{2+}-binding domain 9	Unknown
Efcab10 (n.e.d)	EF-hand Ca^{2+}-binding domain 10	Unknown
Efcab11 (n.e.d)	EF-hand Ca^{2+}-binding domain 11 (=*Egfem1, EGF-like and EMI domain containing 1*)	Unknown
Efcab12	EF-hand Ca^{2+}-binding domain 12 (=BC060267)	Unknown
Efcab14 (n.d)	EF hand Ca^{2+}-binding domain 14	Unknown
Efha1 (n.e.d)	EF hand domain family A1 (=*Micu2, mitochondrial Ca^{2+} uptake 2*)	Mitochondrial Ca^{2+} homeostasis
Efha2	EF-hand domain family, member A2 (=Micu3, mitochondrial Ca^{2+} uptake 3)	Mitochondrial Ca^{2+} homeostasis
Efhb	EF hand domain family, member B	Unknown
Efhc1 (n.e.d)	EF-hand domain (C-terminal) containing 1	Unknown
Efhc2 (n.e.d)	EF-hand domain (C-terminal) containing 2	Unknown
Efhd1 (n.e.d)	EF hand domain containing 1	Unknown
Efhd2	EF hand domain containing 2 (=*Swiprosin-1*)	B cell receptor signaling, kinesin-mediated transport in neurites
Ehd1	EH-domain containing 1	Endocytosis; *axonal targeting*
Ehd2	EH-domain containing 2	Endocytosis
Ehd3	EH-domain containing 3	Endocytosis
Ehd4 (n.e.d)	EH-domain containing 4	Endocytosis
Eps15	Epidermal growth factor receptor pathway substrate 15	Endocytosis
Eps15l1	Epidermal growth factor receptor pathway substrate 15-like 1	Endocytosis
Fkbp7 (n.e.d)	FK506-binding protein 7	Peptidyl-prolyl cis–trans isomerase activity
Fkbp9	FK506-binding protein 9	Peptidyl-prolyl cis–trans isomerase activity
Fkbp10	FK506-binding protein 10	Peptidyl-prolyl cis–trans isomerase activity
Flg (n.d)	Filaggrin	Cytoskeleton organization
Flg2 (n.d)	Filaggrin family member 2	Unknown
Fstl1	Follistatin-like 1	BMP antagonist
Fstl4	Follistatin-like 4 (=*Spig1*)	Unknown; *negative regulator of BDNF maturation*
Fstl5	Follistatin-like 5	Unknown
Gca	Grancalcin	Leukocyte adhesion
Gpd2	Glycerol phosphate dehydrogenase 2, mitochondrial	Glycerol-3-phosphate dehydrogenase activity
Guca1a (n.e.d)	Guanylate cyclase activator 1a	Ca^{2+}-sensitive guanylate cyclase activator activity, Ca^{2+} sensor activity
Guca1b	Guanylate cyclase activator 1B	Ca^{2+}-sensitive guanylate cyclase activator activity, Ca^{2+} sensor activity
Hpca	Hippocalcin	*Ca^{2+} sensor activity*
Hpcal1	Hippocalcin-like 1 (=*Vilip3*)	*Ca^{2+} sensor activity*
Hpcal4	Hippocalcin-like 4 (=*Vilip2*)	*Ca^{2+} sensor activity*
Hrnr (n.e.d)	Hornerin	Unknown
Itsn1	Intersectin 1 (SH3 domain protein 1A)	Guanyl-nucleotide exchange factor activity; *synaptic vesicle recycling*
Itsn2	Intersectin 2	Guanyl-nucleotide exchange factor activity; *synaptic vesicle recycling*

Table 1 (*continued*)

Gene	Complete name (*alternative name*)	Gene ontology/*CNS function*(*s*)
Kcnip1	kV channel-interacting protein 1 (=*Kchip1*)	*Ca^{2+} sensor activity; modulation of Kv4 activity/control of neuronal excitability*
Kcnip2	kV channel-interacting protein 2 (=*Kchip2*)	*Ca^{2+} sensor activity; modulation of Kv4 activity/control of neuronal excitability*
Kcnip3	kV channel-interacting protein 3 (=*Kchip3, DREAM, calsenilin*)	Transcription factor (repressor)
Kcnip4	kV channel-interacting protein 4 (=*Kchip4*)	*Ca^{2+} sensor activity; modulation of Kv4 activity/control of neuronal excitability*
Lcp1	Lymphocyte cytosolic protein 1	Actin filament bundle assembly
Letm1	Leucine zipper-EF-hand containing transmembrane protein 1	Mitochondrial Ca^{2+} antiporter
Letm2 (n.e.d)	Leucine zipper-EF-hand containing transmembrane protein 2	Mitochondrial Ca^{2+} antiporter
Lpcat1 (n.e.d)	Lysophosphatidylcholine acyltransferase 1	1-Acylglycerophosphocholine O-acyltransferase activity
Lpcat2	Lysophosphatidylcholine acyltransferase 2	1-Acylglycerophosphocholine O-acyltransferase activity
Lpcat2b (n.e.d)	Lysophosphatidylcholine acyltransferase 2B	Lysophosphatidylcholine acyltransferase activity
Macf1	Microtubule-actin crosslinking factor 1	Cytoskeletal organization; *neuronal migration during development*
Mcc	Mutated in colorectal cancers	Wnt receptor signaling pathway
Mcfd2	Multiple coagulation factor deficiency 2	Unknown; *survival factor for neural stem cells*
Myl1	Myosin, light polypeptide 1	Motor activity
Myl2 (n.e.d)	Myosin, light polypeptide 2	Motor activity
Myl3 (*unexploitable data*)	Myosin, light polypeptide 3	Motor activity
Myl4	Myosin, light polypeptide 4	Motor activity
Myl6	Myosin, light polypeptide 6	Motor activity
Myl6b (n.e.d)	Myosin, light polypeptide 6B	Motor activity
Myl7 (n.e.d)	Myosin, light polypeptide 7	Motor activity
Myl9 (n.e.d)	Myosin, light polypeptide 9,	Motor activity
Myl12a (n.e.d)	Myosin, light chain 12A, regulatory, non-sarcomeric (=2900073G15Rik)	Motor activity
Myl12b (n.e.d)	Myosin light chain, regulatory B	Motor activity
Mylc2pl (n.d)	Myosin light chain 2, precursor lymphocyte-specific	Motor activity
Mylpf	Myosin light chain, phosphorylatable, fast skeletal muscle	Motor activity
Ncald	Neurocalcin delta	*Ca^{2+} sensor activity*
Ncs1	Neuronal Ca^{2+} sensor1 (=*frequenin homolog*)	*Ca^{2+} sensor activity; modulation of synaptic plasticity/ neuronal secretion*
Necab1	N-terminal EF-hand Ca^{2+}-binding protein 1	Unknown
Necab2	N-terminal EF-hand Ca^{2+}-binding protein 2	Unknown
Necab3	N-terminal EF-hand Ca^{2+}-binding protein 3	Unknown
Nin	Ninein	Microtubule dynamics
Ninl (n.d)	Ninein-like	Microtubule dynamics
Nkd1	Naked cuticle 1 homolog	Wnt receptor signaling pathway
Nkd2	Naked cuticle 2 homolog	Wnt receptor signaling pathway
Nucb1	Nucleobindin 1	Ca^{2+} storage regulation in Golgi
Nucb2	Nucleobindin 2 (=*Nesfatin*)	Three different peptides (neuropeptide/DNA binding/Ca^{2+} binding)
Ocm (n.e.d)	Oncomodulin	Ca^{2+} buffer activity
P4htm	Prolyl 4-hydroxylase, transmembrane	Oxidoreductase activity
Pdcd6	Programed cell death 6 (=*Alg2*)	Apoptosis
Pef1	Penta-EF hand domain containing 1	Unknown
Pkd2	Polycystic kidney disease 2	Ca^{2+} channel activity
Pkd2l1 (n.e.d)	Polycystic kidney disease 2-like 1	Cation channel activity
Plcd1 (n.e.d)	Phospholipase C, delta 1	Phosphoinositide phospholipase C activity
Plcd4 (n.d)	phospholipase C, delta 4	Phosphoinositide phospholipase C activity
Plcg1	Phospholipase C, gamma 1	Phosphoinositide phospholipase C activity
Plch1	Phospholipase C, eta 1	Phosphoinositide phospholipase C activity; *Ca^{2+} sensor activity*
Plch2	Phospholipase C, eta 2	Phosphoinositide phospholipase C activity
Plcz1 (n.e.d)	Phospholipase C, zeta 1	Phosphoinositide phospholipase C activity
Pls1 (n.e.d)	Plastin 1 (I-isoform)	Actin filament binding/assembly
Pls3	Plastin 3 (T-isoform)	Actin filament binding/assembly

(*continued on next page*)

Table 1 (*continued*)

Gene	Complete name (*alternative name*)	Gene ontology/*CNS function*(*s*)
Ppef1 (n.e.d)	Protein phosphatase with EF hand Ca^{2+}-binding domain 1	Protein serine/threonine phosphatase activity
Ppef2 (n.e.d)	Protein phosphatase, EF hand Ca^{2+}-binding domain 2	Protein serine/threonine phosphatase activity
Ppp2r3a (n.e.d)	Protein phosphatase 2, regulatory subunit B'', alpha	Protein phosphatase activity
Ppp2r3c	Protein phosphatase 2, regulatory subunit B'', gamma	Protein phosphatase activity
Ppp2r3d	Protein phosphatase 2, regulatory subunit B'', delta	Protein phosphatase activity
Ppp3r1	Protein phosphatase 3, regulatory subunit B, alpha isoform (=*calcineurin B, type I*)	Protein phosphatase activity; *regulation of synaptic transmission/plasticity*
Ppp3r2 (n.e.d)	Protein phosphatase 3, regulatory subunit B, alpha isoform (=*calcineurin B, type II*)	Protein phosphatase activity
Prkcsh	Protein kinase C substrate 80 K-H	*Ca^{2+} sensor activity*
Pvalb	Parvalbumin	*Ca^{2+} sensor/buffer activity*
Rab11fip3	RAB11 family-interacting protein 3	Cell signaling
Rab11fip4	RAB11 family-interacting protein 4	Cell signaling
Rasef (n.e.d)	RAS and EF hand domain containing	Cell signaling
Rasgrp1	RAS guanyl-releasing protein 1	Guanyl-nucleotide exchange factor activity
Rasgrp2	RAS, guanyl-releasing protein 2	Guanyl-nucleotide exchange factor activity
Rasgrp3 (n.e.d)	RAS, guanyl-releasing protein 3	Guanyl-nucleotide exchange factor activity
Rcn1	Reticulocalbin 1	Unknown
Rcn2	Reticulocalbin 2	Unknown
Rcn3 (n.e.d)	Reticulocalbin 3	Unknown
Rcvrn	Recovering	*Ca^{2+} buffer activity in phototransduction; Ca^{2+} sensor activity*
Reps1	RalBP1-associated Eps domain containing protein	Cell signaling
Reps2	RalBP1-associated Eps domain containing protein 2	Cell signaling
Rhbdl3	Rhomboid, veinlet-like 3	Peptidase activity
Rhot1	Ras homolog gene family, member T1 (=*Miro1*)	GTPase activity; *Ca^{2+} sensor activity /mitochondrial trafficking in neurons*
Rhot2	Ras homolog gene family, member T2 (=*Miro2*)	GTPase activity
Rptn	Repetin	Unknown
Ryr1	Ryanodine receptor 1	*ryanodine-sensitive Ca^{2+}-release channel activity*
Ryr2	Ryanodine receptor 2, cardiac	*ryanodine-sensitive Ca^{2+}-release channel activity*
S100a1 (n.e.d)	S100 Ca^{2+}-binding protein A1	Ca^{2+} buffer activity
S100a3 (n.e.d)	S100 Ca^{2+}-binding protein A3	Ca^{2+} buffer activity
S100a4 (n.e.d)	S100 Ca^{2+}-binding protein A4	Ca^{2+} buffer activity
S100a5 (n.e.d)	S100 Ca^{2+}-binding protein A5	Ca^{2+} buffer activity
S100a6 (n.e.d)	S100 Ca^{2+}-binding protein A6 (=*calcyclin*)	Ca^{2+} buffer activity
S100a7a (n.e.d)	100 Ca^{2+}-binding protein A7A	Ca^{2+} buffer activity
S100a8 (n.e.d)	S100 Ca^{2+}-binding protein A8 (=*calgranulin A*)	Ca^{2+} buffer activity
S100a9	S100 Ca^{2+}-binding protein A9 (=*calgranulin B*)	Ca^{2+} buffer activity; *neuro-inflammatory process*
S100a10	S100 Ca^{2+}-binding protein A10 (=*calpactin*)	Ca^{2+} buffer activity; *serotonergic signaling*
S100a11	S100 Ca^{2+}-binding protein A11 (=*calgizzarin*)	Ca^{2+} buffer activity
S100a13 (n.e.d)	S100 Ca^{2+}-binding protein A13	Ca^{2+} buffer activity
S100a14 (n.e.d)	S100 Ca^{2+}-binding protein A14	Ca^{2+} buffer activity
S100a16	S100 Ca^{2+}-binding protein A16	Ca^{2+} buffer activity
S100b	S100 protein, beta polypeptide, neural	*Ca^{2+} buffer activity; biological marker of brain damage*
S100g (n.e.d)	S100 Ca^{2+}-binding protein G	Ca^{2+} buffer activity
S100z (n.d)	S100 Ca^{2+}-binding protein, zeta	Ca^{2+} buffer activity
Scgn	Secretagogin	Ca^{2+} sensor/buffer activity
Sdf4	Stromal cell-derived factor 4	Unknown
Slc25a12	Solute carrier family 25 (mitochondrial glutamate/aspartate carrier), member 12 (=*Aralar*)	Transporter activity
Slc25a13 (n.e.d)	Solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 13	Transporter activity
Slc25a23	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 23	Transporter activity
Slc25a24 (n.e.d)	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 24	Transporter activity
Slc25a25 (n.e.d)	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	Transporter activity
Smoc1	SPARC related modular Ca^{2+}-binding 1	Extracellular matrix organization
Smoc2	SPARC related modular Ca^{2+}-binding 2	Extracellular matrix organization
Sntn (n.e.d)	Sentan, cilia apical structure protein	Microtubule dynamics

Table 1 (*continued*)

Gene	Complete name (*alternative name*)	Gene ontology/*CNS function*(*s*)
Sparc	secreted acidic cysteine rich glycoprotein (=*osteonectin*)	Extracellular matrix organization; *astrocyte-regulated synaptogenesis*
Sparcl1	SPARC-like 1 (=*hevin*)	Extracellular matrix organization
Spata21 (n.e.d)	Spermatogenesis-associated 21	Unknown
Spna1 (n.e.d)	Spectrin alpha 1	Cytoskeletal organization
Spna2	Spectrin alpha 2	Cytoskeletal organization
Spock1	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan 1	Extracellular matrix organization
Spock2	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2	Extracellular matrix organization
Spock3	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan 3	Extracellular matrix organization
Sri	Sorcin	Intracellular Ca^{2+} transport; *modulator of ryanodine-sensitive* Ca^{2+} *-release channel*
Stim1	Stromal interaction molecule 1	Ca^{2+} *sensor activity;* Ca^{2+} *signaling/storage/release*
Stim2	Stromal interaction molecule 2	Ca^{2+} *sensor activity*
Synrg (n.e.d)	Synergin, gamma	Unknown
Tchh (n.d)	Trichohyalin	Cytoskeletal organization
Tchhl1	Trichohyalin-like 1 (=*S100a17*)	Unknown
Tesc	Tescalcin (=*calcineurin-like protein 3*)	Unknown
Tescl (n.d)	Tescalcin-like	Unknown
Tnnc1	Troponin C, cardiac/slow skeletal	Actin filament binding/assembly
Tnnc2 (n.e.d)	Troponin C2, fast	Actin filament binding/assembly
Usp32	Ubiquitin-specific peptidase 32	Peptidase activity
Utrn	Utrophin	Muscle homeostasis
Vsnl1	Visinin-like 1 (=*Vilip-1*)	Ca^{2+} *sensor activity; regulator of receptors* (*P2X, glycine, nicotinic acteylcholine*)
Zzef1	Zinc finger, ZZ-type with EF hand domain 1	Unknown
1500003O03Rik	RIKEN cDNA 1500003O03 gene (=*calcineurin-like protein 1*)	Unknown
1700008P20Rik (n.e.d)	RIKEN cDNA 1700008P20 gene	Unknown
1700023F06Rik (n.e.d)	RIKEN cDNA 1700023F06 gene	Unknown
1700109H08Rik (n.e.d)	RIKEN cDNA 1700109H08 gene	Unknown
2010110P09Rik (n.e.d)	RIKEN cDNA 2010110P09 gene (=*calcineurin B homologous protein 2*)	Unknown
4732418C07Rik	RIKEN cDNA 4732418C07 gene	Unknown
4930443G12Rik (n.e.d)	RIKEN cDNA 4930443G12 gene	Unknown
9130204L05Rik (n.e.d)	RIKEN cDNA 9130204L05 gene	Unknown
Gm1254 (n.e.d)	Predicted gene 12854 (=*S100A11-like*)	Unknown
Gm2446 (n.d)	Predicted gene 2446	Unknown
Gm5849 (n.d)	Predicted gene 5849	Unknown
Gm5958 (n.d)	Predicted gene 5958	Unknown
Gm9229 (n.d)	Predicted gene 9229	Unknown
Gm20056 (n.d)	Predicted gene 20056	Unknown
LOC101056100 (n.d)	Centrin-1-like	Unknown
LOC100861614 (n.d)	Hippocalcin-like protein 1-like	Unknown
LOC101055809 (n.d)	Ca^{2+} and integrin-binding family member 4-like	Unknown

synthesized *in vitro* (Eurofins MWGOperon, Ebersberg, Germany), with the addition of a 6xHis C-terminal tail and BamH1/Sal1 restriction sequences at the extremities, and further cloned into a variety of standard vectors (pCR2.1, pUC57, pEX-A, pSK+). All plasmids generated at Eurofins were digested with BamHI and SalI and each excised insert independently ligated into the expression plasmid pGEX-4P-1. The recombinant plasmids were double checked by restriction analysis and sequencing, and transformed into the *Escherichia coli* strain JM109 for protein expression under control of an IPTG inducible promoter. Proteins expressed in

distinct brain areas: main olfactory bulb (OB), cerebral cortex (CTX), Basal ganglia (BG), hippocampal formation (HPF), amygdala (AMY), thalamus (TH), hypothalamus (HY), midbrain (MB), pons (P), cerebellum (CBX), medulla (MY). +: corresponds to a measurable expression; *nd*: no expression detected. The ISH signal was considered as positive when observed in several sections in a given structure. For *S100a11* the OB area is missing in the ABA database. Pu identifies the Purkinje cell layer of the cerebellum. In this layer the main neuron is the Purkinje cell, but Golgi-epithelial cells (Bergmann-glia), Golgi-interneurons and Lugaro cells can occur in the same plane. Therefore, Pu does not always automatically imply that the expressing cell is the Purkinje neuron. ''Railroad-track'' describes two parallel continuous lines of stained cells in the Purkinje-cells layer and at the boundary between granular layer and white matter (Wm). Puffs indicate an accumulation of signal at the apex of cerebellar circumvolutions. Mol: molecular layer; Pu: Purkinje-cells layer; Gr: granular layer. In addition, some sites (i.e. identifiable nuclei) of stronger or specific expression are listed in brackets (see the list of abbreviations). The term ''*scattered cells*'' is used when numerous cells expressing the gene of interest are scattered throughout the structure under consideration (in this situation, nuclei are identifiable). The term ''*sparse cells*'' is used when only few cells in the area considered express the gene of interest, and when no nuclei are identifiable. For 22 genes, expression and localization studies have been performed by *in-situ* hybridization or immunohistochemistry (numbered in column 1). [1](Kim et al., 2014), [2](Vasiljevic et al., 2012) [3](Konig et al., 2003), [4](Blazejczyk et al., 2009), [5](Dixon et al., 1997), [6](Bohm et al., 2008), [7](Yang et al., 2009), [8](Masuda et al., 2014), [9](Saitoh et al., 1993), [10](Xiong et al., 2004), [11](Bhat et al., 1994), [12](Zhang et al., 2014), [13](Sugita et al., 2002), [14](Kanemaru et al., 2010), [15](Meyer, 2014), [16](Kuwajima et al., 1992), [17](Sah et al., 1993), [18](Sturchler et al., 2006), [19](Vives et al., 2003), [20](Mulder et al., 2010), [21](Skibinska-Kijek et al., 2009), [22](Saitoh et al., 1994)

Gene name	ISH Nr	Marker	Telencephalon					Diencephalon		Mesencephalon	Metencephalon			Myelencephalon
			OB	CTX	BG	HPF	AMY	Thalamus (TH)	Hypothalamus (HY)	Midbrain (MB)	Pons (P)	Cerebellum (CBX)	DCN	Medulla oblongata (MY)
Actn1	1		+	+	+	+	+	Scattered cells	Scattered cells	Scattered cells	Scattered cells	Gr	*nd*	+ (Pch + +)
Actn3	1		+	+	+	+	*nd*	Scattered cells	Scattered cells	Scattered cells	+	Pu	+	+
Actn4	1		+	+	*nd*	+	+	*nd*	*nd*	*nd*	+ (PG)	Pu	*nd*	*nd*
Cabp1[1]	1		+	+	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Mol, Pu, Gr	+	Scattered cells
Cabp4	1		+	*nd*	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu, Gr (railroad track)	+	Scattered cells
Cabp5	1		+	*nd*	+	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu, Gr	*nd*	*nd*
Cabp7	1		*nd*	*nd*	+	+	*nd*	*nd*	+ (LM, ZI)	+ (III, *ND*, APN, RN)	+ (PG, TRN)	Pu, Gr	+ +	+ (VI, VII, PRP, XII, PGRN)
Calb1	20	v	+	+	+	+	+	+ (VM, RE, MH, PF)	+ (VMH, DMH, PMd)	+ (VTA, EW, DR)	+ (NTB)	Pu	+	+ (MARN, IO, SPV, NTS)
Calb2	17	v	+	+	*nd*	+	+	+ (PVT, RH, RE and more)	Scattered cells	+ (SC, VTA, PAG: DL and VL + +)	+ (PG, TRN and more)	Gr	*nd*	+ (IO and more)
Calm1	3	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous (III, DR)	Ubiquitous	Pu, Gr	+ +	Ubiquitous
Calm2	2	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous (III, DR)	Ubiquitous	Pu, Gr	+	Ubiquitous
Calm3	1		+	+	*nd*	+	+	Sparse cells (weak)	Sparse cells (weak)	+ (RN, MEV, III)	+ (PG, TRN)	Few Pu and Gr	+	+
Calu[2]	1		+	*nd*	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu	+	Sparse cells (weak)
Capn1	1		*nd*	*nd*	*nd*	+	*nd*	*nd*	*nd*	III, IV, TRN	+ (PG, TRN, V)	*nd*	(+)	+ VII, LRN, XII, NTS and scattered cells
Capn2	3		+	+	+	+	(+)	+ (RT, AD and scattered cells)	Sparse cells (weak)	+ (RN, EW, MEV, *ND*; III, IV, V)	+ (PG, TRN, NTB, V)	Pu	+	+ VI, VII, XII and scattered cells
Capn3[3]	1		+	+	*nd*	+	*nd*	+	*nd*	Sparse cells (weak)	Sparse cells (weak)	Pu, Gr	(+)	+
Capn 8 (10)	2		+	+	+	+	(+)	+	Sparse cells (weak)	Sparse cells (weak)	Sparse cells (weak)	Pu	(+)	Sparse cells (weak)

Capns1	2	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous (III, V, DR)	Ubiquitous	Pu, Gr	+ +	Ubiquitous
Cbara1	2		+	+	+	+	+	+	Scattered cells	+ (RN, EW, DR, PAG, MEV, III)	+ (PG, V, TRN)	Pu, Gr	+	Ubiquitous strong
Cbl	1		*nd*	*nd*	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Cblb	1		*nd*	+	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Gr	*nd*	*nd*
Cetn2	2		+	+	+	+	+	Sparse cells (weak)	Sparse cells (weak)	*nd*	Sparse cells (weak)	Pu	+	Sparse cells (weak)
Cetn3	2		*nd*	*nd*	*nd*	+	+	*nd*	*nd*	*nd*	*nd*	Pu	*nd*	*nd*
Cgref1	1		*nd*	*nd*	+	+	+	+ (AD, PVT)	Scattered cells	Scattered cells	+ (PCG)	Gr	(+)	+ (VII)
Cib2[4]	1		+	+	+	+	*nd*	+ (AD, PVT and scattered cells)	+	+	+	*nd*	*nd*	+ (LC)
Dgka	4		*nd*	*nd*	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Dgkb	3		+	+	+	+	+	+ (RT, MH)	+ (MM, SUMI, TM and scattered cells)	Scattered cells, DR	+ (LC)	Pu (rare cells in Gr)	(+)	Scattered cells
Dgkg	3		+	+	+	+	+	AD, RT	+	*nd*	+ (PG, TRN)	Pu	*nd*	*nd*
Drp2[5]	1		+	+	+	+	+	+ (weak)	+ (weak)	+ (weak)	+ (weak)	Pu, Gr (railroad track)	+	+ (weak)
Dtna[6]	2		+	+	+	+	+	+ (weak)	+ (weak)	+ (DR strong and scattered cells)	+ (TRN and scattered cells)	Pu, Gr	+	+ (VII and scattered cells)
Dtnb	2	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu (Rare sp in Gr)	+	Ubiquitous
Efcab1	1		*nd*	*nd*	*nd*	+	(+)	*nd*	Sparse cells (weak)	*nd*	*nd*	*nd*	*nd*	*nd*
Efcab4a	1		+	+	*nd*	+	*nd*	*nd*	*nd*	*nd*	+	Pu (rare cells in Gr)	*nd*	+
Efha2	1		*nd*	+	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu	(+)	Sparse cells (weak)
Efhb	1		+	+	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu	*nd*	*nd*
Emd2	1		+	+	+	+	+	+	+	+ (RN and scattered cells)	+ (PG, TRN and scattered cells)	Pu (rare cells in Gr)	+	+ (LRN and scattered cells)
Ehd1	1		*nd*	+	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu, Gr	+	*nd*
Ehd2	3		+	+	+	+	*nd*	Sparse cells (weak)	Sparse cells (weak)	Sparse cells (weak)	Sparse cells (weak)	Pu, Gr	+	Sparse cells (weak)
Ehd3	2		+	+	*nd*	+	+	+ (AD, VAL, LD, PF)	Sparse cells (weak)	+ (MEV, ND, RN, III)	+, LC	Pu, Gr	+ +	+ (VII, XII, LRN)
Eps15	2		+	+	+	+	+	Ubiquitous	+	Ubiquitous (DR)	Ubiquitous (LC, PG, TRN, strong)	Mol, Pu, Gr	+	Ubiquitous
Eps15l1	2		+	+	+	+	+	Scattered cells	Scattered cells	Scattered cells	Scattered cells	Pu, Gr	+	Scattered cells
Fkbp9	2		+	+	+	+	*nd*	Sparse cells	Sparse cells	*nd*	Sparse cells	Pu, Gr	+	Sparse cells
Fkbp10	2		+	+	*nd*	+	*nd*	*nd*	Sparse cells	*nd*	Sparse cells	Pu, Gr	+	Scattered cells
Fstl1[7]	2		+	+	+	+	+	+ (AD, PVT and scattered	Sparse cells	+ (RN, III, DR, MEV, IV, V, VTN)	+ (PG, NLL, V, TRN, NTB)	Gr	+ + +	+ (VI, VII, XII, IO, PGRN, LRN)

(*continued on next page*)

Gene name	ISH Nr	Marker	Telencephalon					Diencephalon		Mesencephalon	Metencephalon			Myelencephalon
			OB	CTX	BG	HPF	AMY	Thalamus (TH)	Hypothalamus (HY)	Midbrain (MB)	Pons (P)	Cerebellum (CBX)	DCN	Medulla oblongata (MY)
								cells)						
Fstl4	2		+	+	*nd*	+	+	+ (VAL, VPM/VPL, VM, weak)	*nd*	+ (III, IV weak)	*nd*	Pu	*nd*	*nd*
Fstl5[8]	2		+	*nd*	*nd*	+	+	*nd*	*nd*	PV2	+ (LC)	*nd*	*nd*	*nd*
Gca	2		*nd*	+	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Gpd2	2		+	+	*nd*	+	+	*nd*	+ (VMH)	Sparse cells, DR. LC	Sparse cells	Gr	*nd*	Scattered cells
Guca1b	1		+	+	+	+	+	Sparse cells	Scattered cells	*nd*	Scattered cells	Gr	+	Scattered cells
Hpca[9]	2		+	+	+	+	+	+ (RT and Sparse cells)	Ubiquitous	Ubiquitous	+ (LC and scattered cells)	Mol, Pu, Gr	(+)	+ (IO and scattered cells)
Hpcal1	2		+	*nd*	+	+	+	+ (RT and Sparse cells)	Scattered cells	Scattered cells, DR, LC (rostrocaudal changing pattern)	Sparse cells	Pu	*nd*	+ (IO, DMX and scattered cells)
Hpcal4	2	v	+	+	+	+	+	+ (PVT, RE)	+ (VMH)	+ (DR, VTA)	+ (LC)	Gr	*nd*	+ (NTS)
Itsn1	2	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr	+	Ubiquitous
Itsn2	2		+	*nd*	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	Sparse cells
Kcnip1[10]	2	V	+	+	(+)	+	+	+ (RT, MH, part LP, PT)	*nd*	Sparse cells, MEV	Sparse cells	Pu	*nd*	Sparse cells
Kcnip2[10]	2		+	+	+	+	+	Clustered scattered cells	Scattered cells	+ (IC, weak)	*nd*	*nd*	*nd*	+ (IO, weak)
Kcnip3[10]	2		+	+	+	+	+	Clustered scattered cells	Sparse cells	Sparse cells, DR, LC	+ (PG, LC)	Mol, Pu, Gr	(+)	Sparse cells
Kcnip4[10]	2		+	+	+	+	*nd*	Clustered scattered cells	Sparse cells	Scattered cells, DTN	+ (LC and Sparse cells)	Pu	(+)	+ (PRP, DMX)
Lcp1	1		+	+	+	+	(+)	Clustered Sparse cells	Sparse cells	Sparse cells	Scattered cells (V, strong)	Pu, Gr	+	+ (VII, AMB, XII and scattered cells)
Letm1	2		+	+	+	+	+	Clustered Sparse cells	Sparse cells	Sparse cells	Scattered cells	Pu, Gr	+	Scattered cells
Lpcat2	2		+	+	+	+	+	*nd*	*nd*	*nd*	Sparse cells	Pu	+	Sparse cells
Macf1	1	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr	+	Ubiquitous
Mcc[11]	1		*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	Pu, Gr	*nd*	*nd*
Mcfd2	1		+	+	+	+	+	Clustered sparse cells	Scattered cells	Sparse cells	Sparse cells	Pu, Gr	+	Sparse cells
Myl1	1		+	+	*nd*	+	+	*nd*	*nd*	*nd*	*nd*	Gr	+	*nd*
Myl4	2	V	*nd*	+	(+)	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	PGRN, VII, DMX
Myl6	1	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr	+	Ubiquitous
Mylpf	1		+	*nd*	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu, Gr	*nd*	*nd*
Ncald	1		+	+	+	+	+	+ (PVT,	Scattered cells	+ (DR strong,	+ (LC and scattered	Gr	*nd*	Scattered cells

							MH, AD and scattered cells)		PBG, LC, scattered cells)	cells)			
Ncs1	1		+	+	+	+	+	Clustered scattered cells	Sparse cells	+ (RN, III and scattered cells)	Scattered cells	Mol, Pu. Gr	+	Scattered cells
Necab1[12]	2	V	+	+	+	+	+	+ (PVT, RT, RE, MV, LP)	Sparse cells	Sparse cells	Sparse cells	Pu, Gr	(+)	Scattered cells
Necab2[13]	2	V	+	+	+	+	+	+ (PVT, MH, LH and scattered cells)	Sparse cells	+ (DR strong and scattered cells)	+ (PG, TRN, PSV, PBl, SPIV)	Pu	*nd*	+ (IO and scattered cells)
Necab3	3		+	+	*nd*	+	+	+ (MH, LH and scattered cells	*nd*	+ (RN, MEV and scattered cells)	+ (V and scattered cells)	Pu, Gr	+	+ (VII and scattered cells)
Nin	2		+	+	+	+	+	Ubiquitous	+ (VMH)	Sparse cells, III	+ (PG, TRN, V and sparse cells)	Pu, Gr	(+)	Scattered cells
Nkd1	2		+	*nd*	*nd*	+	*nd*	+ (PVT, AD/AV, PF, CM, PCN, RE)	+ (SCH)	Sparse cells	*nd*	Pu, Gr	(+)	Sparse cells
Nkd2	2	V	*nd*	*nd*	*nd*	+	*nd*	*nd*	VMH sc, PH, ARH, PVT (faint)	SC scattered cells	*nd*	*nd*	*nd*	*nd*
Nucb1	2		+	+	+	+	+	*nd*	Sparse cells	Sparse cells, LC	+ (V and scattered cells)	Pu	+	+ (VII and scattered cells)
Nucb2	2		+	+	+	+	+	+ (PF and sparse cells)	+ (LHA, PVH and sparse cells)	+ (EW and sparse cells, LC, MEV	+ (LC and sparse cells)	*nd*	*nd*	Sparse cells
P4htm	1		+	+	+	+	+	Sparse cells	Scattered cells	Sparse cells	Scattered cells	Pu, Gr	+	Scattered cells
Pdcd6	1		+	+	+	+	+	Sparse cells	*nd*	+ (RN)	+ (V, PRN, PG, TRN)	Pu	+	+ (VII)
Per1	1		+	+	+	+	*nd*	Sparse cells, PF, AD	Scattered cells	Sparse cells	+ (V and sparse cells)	Pu	+	NTS, DMX, XII and scattered cells)
Pkd2	1		*nd*	*nd*	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu	*nd*	*nd*
Plcg1	1		+	+	+	+	+	Clustered sparse cells, AD	Scattered cells	Sparse cells	Scattered cells	Pu, Gr	+	Scattered cells
Plch1	1		*nd*	*nd*	*nd*	*nd*	*nd*	+ (RT, LH, weak)	+ (STN, weak)	Sparse cells, CUN	*nd*	*nd*	*nd*	Sparse cells
Plch2[14]	1		+	+	+	+	+	+ (PVT, LH and scattered cells)	+ (SCH)	Sparse cells	Sparse cells	Gr	+	Scattered cells
Plcz1	2		+	+	*nd*	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	+	*nd*
Pls3	2		*nd*	+	*nd*	+	+	+ (AD, LD, PF, MH, VPN)	Sparse cells	+ (RN, III, MEV and sparse cells)	+ (PG, TRN, V and sparse cells)	Pu	*nd*	+ (VII, IO, XII, DMX and scattered cells)
Ppp2r3c	1		+	+	+	+	+	Sparse cells	*nd*	Sparse cells	Scattered cells	Pu, Gr	+	Scattered cells
Ppp2r3d	1		+	+	+	+	+	Scattered	Scattered cells	Scattered cells	Scattered cells	Pu, Gr	+	Scattered cells

(*continued on next page*)

Gene name	ISH Nr	Marker	Telencephalon					Diencephalon		Mesencephalon	Metencephalon			Myelencephalon
			OB	CTX	BG	HPF	AMY	Thalamus (TH)	Hypothalamus (HY)	Midbrain (MB)	Pons (P)	Cerebellum (CBX)	DCN	Medulla oblongata (MY)
								cells (weak)	(weak)	(weak)	(weak)			(weak)
Ppp3r1	3	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous, DR, LC	Ubiquitous	Pu, Gr	+	Ubiquitous
Prkcsh	3		+	+	+	+	+	Scattered cells (weak)	Scattered cells (weak)	Scattered cells (weak), DR, LC	Scattered cells (weak)	Pu, Gr	+	Scattered cells (weak)
Pvalb	22		+	+	+	+	+	+ (RT and sparse cells)	+ (PV1, STN, MM and sparse cells)	+ (RN, III, MEV ND and more)	+ (PG, NLL, TRN and more)	Mol, Pu	+	+ (PRP, VII, LRN and more)
Rab11fip3	1	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr	+	Ubiquitous
Rab11fip4	2	Ub	+	+	(+)	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr	(+)	Ubiquitous
Rasgrp1[15]	2	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr	+	Ubiquitous
Rasgrp2	2		+	+	+	*nd*	*nd*	Sparse cells	Sparse cells	Sparse cells	Sparse cells	*nd*	*nd*	Sparse cells
Rcn1	2		+	+	+	*nd*	+	+ (PVT, MH)	+ (LHA and Scattered cells)	Sparse cells, DR, EW	*nd*	*nd*	*nd*	Sparse cells
Rcn2	2	Ub	+	+	+	+	+	+ (MH, AD, PVT, PF)	Ubiquitous	Ubiquitous (DR strong)	Ubiquitous (LC strong)	Pu, Gr	+	Ubiquitous
Rcvrn	1		+	+	+	+	*nd*	*nd*	*nd*	*nd*	*nd*	Pu, Gr (railroad track)	(+)	*nd*
Reps1	1		+	+	+	+	+	Scattered cells	Scattered cells	Scattered cells	Scattered cells	Pu, Gr	+	Scattered cells
Reps2	2		+	+	+	+	+	Ubiquitous	Scattered cells	Ubiquitous, III, DK	Ubiquitous	Mol, Pu, Gr (sp)	+ +	Ubiquitous
Rhbdl3	1		+	+	+	+	+	Scattered cells	Scattered cells	Scattered cells	Scattered cells	Pu, Gr (railroad track, puffs)		Scattered cells
Rhot1	1		+	+	+	+	+	*nd*	*nd*	Sparse cells	Sparse cells	Pu, Gr	(+)	Sparse cells
Rhot2	2		+	+	+	+	+	Scattered cells	Scattered cells	Scattered cells	Scattered cells	Pu, Gr	+	Scattered cells
Ryr1[16]	2		+	+	+	+	+	*nd*	*nd*	*nd*	*nd*	Pu	+	Scattered cells
Ryr2[17]	1	Ub	+	+	+	+	+	+ (RT, LH)	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr (railroad track	+	Ubiquitous
S100a9	1		+	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
S100a10	2		+	+	+ sp, SI, NDB, Cla	+ MS, LS	+	+ (AD)	+ (PVH strong)	+ (RN, III, MEV, DR, SNc & VTA strong), CSm, V)	+ (TRN, CS, V, LDT, SLD, LC strong, VII, MARN, LAV, IO)	Pu, rare cells in Gr	(+)	+ (VII, RM, AMB -strong-, XII, DMX, NTS, DMV)
S100a11	1		**?**	+	*nd*	+	+	Sparse cells	Sparse cells	Sparse cells	Sparse cells	Pu	+	Sparse cells
S100a16[18]	1		+	+	+	+	(+)	Ubiquitous (weak) glia ?	Ubiquitous (weak) glia	Ubiquitous (weak)	Ubiquitous (weak)	Pu, Gr, Wm	+	Ubiquitous (weak)
S100b[19]			+	+	+	+	+	Scattered cells	Scattered cells	Ubiquitous (RN, ME, III strong)	Ubiquitous (V strong)	Mol, Pu, Gr, Wm	+	Ubiquitous (VII, AMB, XII strong)
Scgn[20]	1		+	+	*nd*	+	(+)	*nd*	*nd*	*nd*	*nd*	Pu	*nd*	DMX, LC (?), sparse cells
Sdf4	1		+	+	+	+	+	Sparse cells	Scattered cells	Scattered cells	Ubiquitous (weak)	Pu, Gr	+	Ubiquitous (weak)
Slc25a12	1	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous (strong)	Pu, Gr	+ +	Ubiquitous

														(strong)
Slc25a23	1	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr	+	Ubiquitous
Slc25a25	2		+	+	+	+	(+)	+ (AD and Sparse cells)	Sparse cells	+ (RN, MEV, III, IV and more) patchy	+ (PG, TRN, V and more)	Pu	*nd*	+ (VI, VII, XII and more)
Smoc1	2		+	*nd*	*nd*	*nd*	+	+ (PVT and AD weak, MH strong)	*nd*	*nd*	+ (LC weak)	*nd*	*nd*	*nd*
Smoc2	2		*nd*	*nd*	*nd*	+	+	*nd*	*nd*	*nd*	Nd	*nd*	*nd*	*nd*
Sparc	4	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Mol, Pu, Gr, pia mater	+	Ubiquitous
Sparcl1	2	ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Mol (basket cells?), Pu, Gr	++	Ubiquitous
Spna2	1	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Pu, Gr	+	Ubiquitous
Spock1	2		+	+	+	+	+	Ubiquitous (PVT, PT, RE, AD, RT)	Scattered cells	Scattered cells	Ubiquitous (PG, TRN, V, PSV strong)	Mol, Pu, Gr (Golgi cells?)	+	Ubiquitous (VII, XII, AMB, PGRN strong)
Spock2	2	Ub	+	+	+	+	(+)	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Mol, Pu, Gr	+	Ubiquitous
Spock3	2		+	+	+	+	(+)	+ (PVT, RE, LD, RT) Ubiquitous	Scattered cells	Scattered cells (DR strong, III)	+ (V, CSm, LC)	Mol, Pu, Gr	+	+ (VI, VII, AMB, XII)
Sri	2		+	+	+	+	+	Sparse cells	Sparse cells	Sparse cells	Sparse cells	Pu, Gr (railroad track)	+	Sparse cells
Stim1[21]	1	Ub	+	+	+	+	+	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous (V strong)	Pu	+	Ubiquitous (VII strong)
Stim2	2		+	+	+	+	+	*nd*	*nd*	Sparse cells (weak)	Sparse cells (weak)	Pu, Gr	+	Sparse cells (VII)
Tesc	2		+	+	+	+	+	+ (MH, PVT, AD)	Sparse cells	+ (RN, MEV, SC, III)	+ (PG, V, TRN, NLL)	Gr	+	+ (VI, VII, LAV, XII, AMB)
Tnnc1	2	V	*nd*	+ CLA	Nd	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Usp32	1		+	+	(+)	+	(+)	Sparse cells (weak)	Sparse cells (weak)	Sparse cells (weak)	Sparse cells	Pu	*nd*	Sparse cells
Utrn	2		+	+	+	*nd*	(+)	*nd*	*nd*	+ (MEV)	+ (PG, V, TRN)	Pu, Gr	(+)	+ (VII, MV, XI, PGRN)
Vsnl[22]	3		+	+	+	+	(+)	+ (AD, LD and sparse cells)	+ (ZI, STN and scattered cells)	Ubiquitous	+ (PG, TRN and scattered cells)	Gr	+	Ubiquitous
Zzef1	1		*nd*	+	Nd	+	*nd*	*nd*	*nd*	*nd*	Sparse cells (weak)	Pu	(+)	Sparse cells (weak)
1500003O03Rik	1		+	+	+	+	+	Scattered cells	Sparse cells	Scattered cells	Scattered cells (V strong)	Pu, Gr	+	Scattered cells (VII, LRN, AMB strong)
4732418C07Rik	1		+	+	+	+	+	Scattered cells	Scattered cells	Scattered cells	Scattered cells	Pu, Gr, Wm	+	Scattered cells

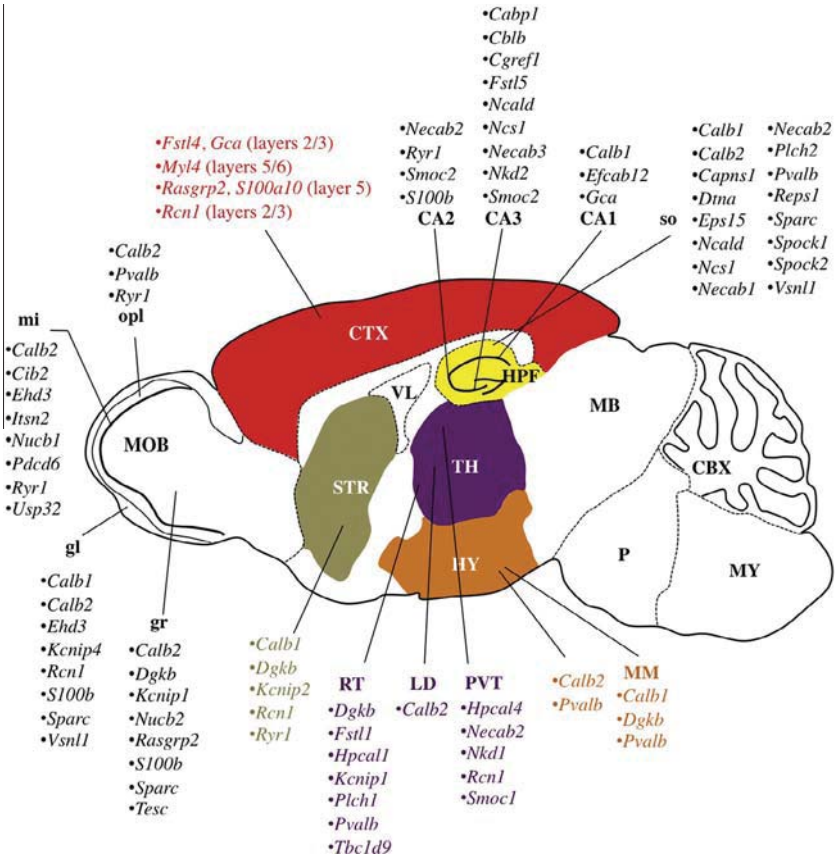

Fig. 1. Schematic brain representation showing specific expression of EF-hand genes in discrete areas, Part 1. A schematic view of a brain sagittal section, highlighting the different areas analyzed in this study. For each area, the genes which are preferentially expressed in this region are listed. For details, see Tables 2–7. *Abbreviations:* MOB: main olfactory bulb; CTX: cerebral cortex; HPF: hippocampal formation; so: stratum oriens; STR: striatum; HY: hypothalamus; TH: thalamus; VL: lateral ventricle; MB: midbrain; P: pons; MY: medulla; CBX: cerebellum. All other abbreviations are detailed in the list of abbreviations. Drawing is adapted from Dong (2008).

E. coli were affinity purified through their GST-tag, which was later removed by thrombin cleavage. Purified proteins were checked by gel electrophoresis and used for immunizing rabbits (Eurogentec, Seraing, Belgium). The specificity of the obtained antibodies was tested by immunoblotting. The diluted, straight antisera were used for immunohistochemistry.

Immunoblotting

Extracts of mouse brains were subjected to 12% sodium dodecyl sulfate (SDS)-gel-electrophoresis (50-µg protein loaded per lane) followed by transfer on nitrocellulose paper and immunoblot using the antisera against Hpcal44 and Tesc diluted 1:2000. The bound antibody was revealed with an HRP-labeled secondary antibody and the reaction amplified by chemiluminescence.

RESULTS

Identification of genes of the EF-hand super-family in the mouse genome

Previous studies on the proteins of the EF-hand super-family of Ca^{2+}-binding proteins have revealed an extreme diversity in their structure, functional activities and cellular localization (extracellular, membrane-bound, cytosolic, cytoskeleton-associated, in Golgi compartments, in other vesicles, in the nucleus and more) (Kawasaki et al., 1998; Persechini et al., 1989; Moncrief et al., 1990; Lewit-Bentley and Rety, 2000; Haiech et al., 2004; Schwaller, 2009, 2010; Yanez et al., 2012). Through extensive examination of the Uniprot and NCBI Gene databases (see Experimental procedures, Section ''Bioinformatics''), we found that this family comprises in mouse at least 249 putative members. According to Gene Ontology terms, we have classified these proteins into several categories: Ca^{2+} sensors/buffers, enzymes, cytoskeleton organization/dynamics (actin filament binding/assembly, microtubule dynamics, motor proteins, muscle cell structure/homeostasis), transporters, extracellular matrix organization/dynamics, ion channels (Ca^{2+}, K^{+}), modulators of ion channels, guanyl-nucleotide exchange factors and more (alphabetical list in Table 1).

Gene expression pattern atlas of the EF-hand family genes in the mouse brain

For each gene encoding an EF-hand domain containing protein, the ABA database of ISH data was consulted, and the different images available (mostly sagittal, but

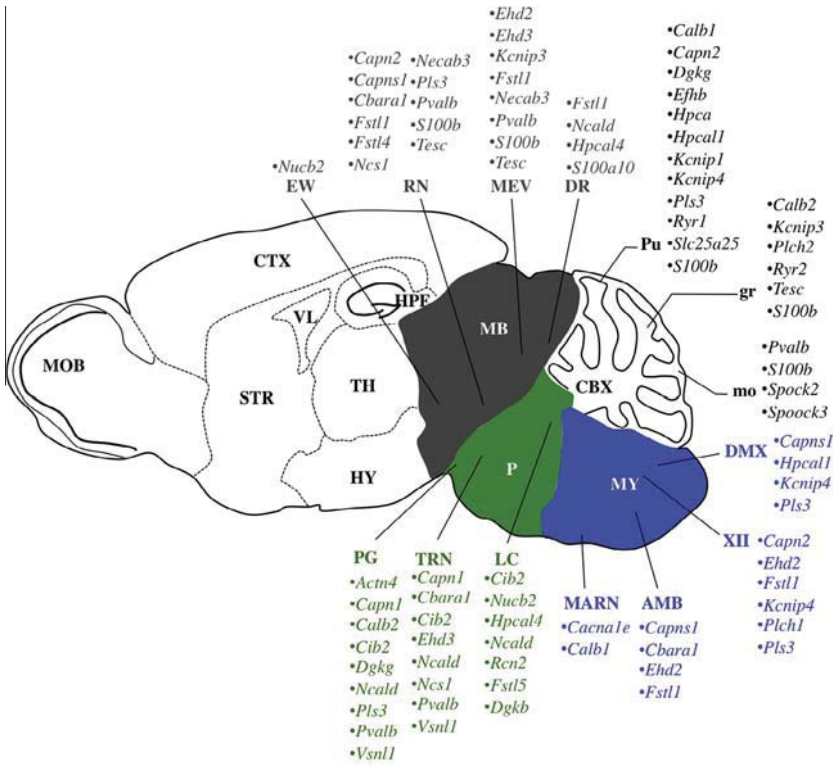

Fig. 2. Schematic brain representation showing specific expression of EF-hand genes in discrete areas, Part 2. See legend to Fig. 1.

also coronal, see Table 2, column 2) were analyzed in detail focusing on different brain areas: main olfactory bulb (OB), accessory olfactory nucleus (AON), cerebral cortex (CTX), basal ganglia (BG), hippocampus (HPF), amygdala (AMY), thalamus (TH), hypothalamus (HY), midbrain (MB), cerebellum (CBX), pons (P), medulla (MY), and in clearly identifiable substructures within each areas. Of the 249 genes forming the EF-hand super-family, ABA data were available for 222 genes, among which 135 showed a detectable expression pattern in the brain. For 87 genes (“n.e.d” in Table 1), no discernible signals were observed with respect to brain expression. For the remaining 27 genes (“n.d” in Table 1), no expression data were available in the ABA. The results for the 135 genes clearly expressed in the brain are presented in Table 2, and schematized in Figs. 1 and 2. Tables 3–7 summarize expression details in the OB/AON, the cerebral cortex (barrel field of the primary somatosensory area), the basal ganglia, the hippocampal formation and the amygdala, respectively. Figs. 3–5 show examples of genes expressed in the cerebral cortex, the hippocampus and the cerebellum, respectively. Most of the EF-CaBP-encoding genes are expressed concomitantly in different brain areas; nevertheless some genes show detectable expression in unique, or at least a limited number of brain structures (Table 2). These include *Cabp1* (weak expression in CTX and PIR, in HPF CA3 sp, and few sparse cells in MY); *Cblb* (weak expression in all layers of CTX, strong expression in dentate gyrus, granule cell layer (DG sg) and CA3 sp); *Cbl* (weak expression in DG sg and CA1-3 sp); *Cetn3* (sparse cells in CA1-3 sp and DG sg); *Dgka* (sparse cells in CA3 sp and DG sg); *Efcab1* (weak expression in CTX and HPF); *Efcab12* (weak expression in CBX and HPF); *Ehd1* (sparse cells in CA1-3 sp and strong expression in DG sg); *Gca* (CTX all layers, CA1 sp); *Mcc* (CBX Pu); *Myl4* (CTX layers 5/6, lateral amygdalar nucleus); *Nkd3* (CA3 sp); *Pkd2* (sparse cells in CA1-3 sp and DG sg); *Plch1* (weak expression in TH and MY; *S100a9* (weak expression in OB); *Tnnc1* (expressed in different CTX layers – 2/3, 4, 6 – depending on the area).

Expression patterns of the EF-hand family genes in the olfactory bulb

Most of the EF-hand family genes appear to be expressed in the main olfactory bulb: 111 out of the 135 genes expressed in brain are present with detectable expression levels in the different layers (Table 3). Most of these genes are expressed in more than one layer, and very few genes occur specifically in only one layer in the OB. These include *Calb1*, *Calu*, *Kcnip4*, *Rcn1* and *Vsnl1* for the glomerular layer; *Pvalb* for the outer plexiform layer; *Calm3*, *Cib2* and *Nucb1* for the mitral layer; *Fstl1*, *Kcnip1*, *Nucb2, Rasgrp2* and *Tesc* for the granular layer. Two genes encoding proteins with Ca^{2+}-buffering activity (S100A10 and S100A16) highlight the rostral migratory stream, which conveys newborn neurons to the olfactory bulb; in addition *Cib2* marks the accessory olfactory bulb specifically.

Expression patterns of the EF-hand family genes in the cerebral cortex

112 out of the 135 genes are expressed in the cerebral cortex. By examining expression in the barrel field of the

primary somatosensory area, we notice that several EF-CaBP-encoding genes have a rather ubiquitous distribution, but nevertheless some genes are expressed in specific layers (Figs. 1 and 3, and Table 4). These include for example *Cabp1* (weak expression in sparse cells in layers 2 and 5a), *Fstl4*, *Gca*, *Efcab4a* or *Rcn1* (weak expression in sparse cells in layers 2 and 3), *Myl4* (weak to moderate expression in layers 5 and 6), *S100a10* (weak expression in layer 4), *Tnnc1* (expressed in different CTX layers [2/3, 4, 6], depending on the area).

Expression pattern of the EF-hand family genes in the basal ganglia

Compared to the striatum, the globus pallidus expresses less often EF-hand genes. The ventral pallidum is highlighted specifically by *Myl4*. *Ehd3*, *Reps2* and *Vsnl1* are expressed only in the external globus pallidus (GPE), whereas *Dgkb* and *S100A11* expressing cells are concentrated in the internal globus pallidus (GPI) (Table 5). In a number of cases, the gene expression-pattern replicated the subdivision of the striatum in striosome-matrix compartments.

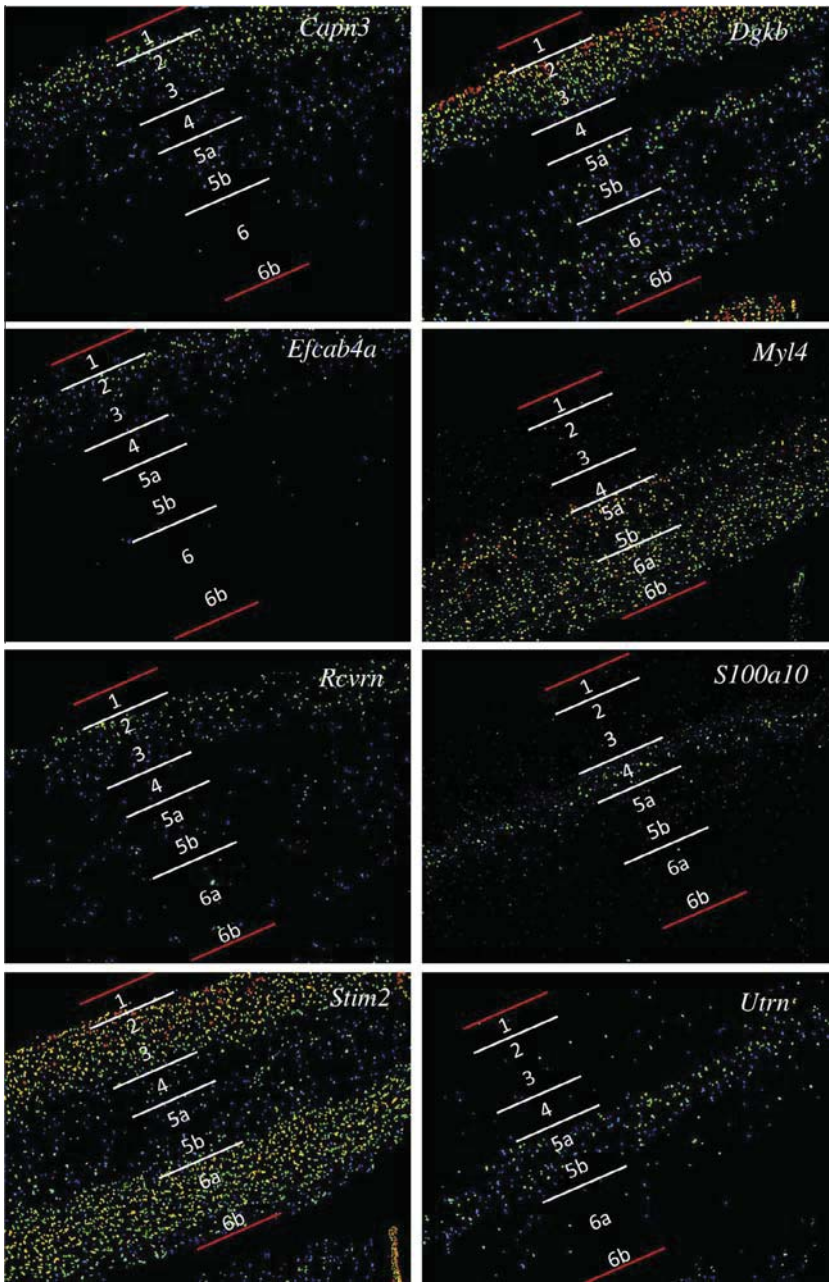

Fig. 3. Gene expression patterns in the cerebral cortex. ISH images for eight genes, listed in alphabetical order, and presenting a characteristic expression pattern in the cerebral cortex (barrel field of the primary somatosensory area), were taken from the ABA database. All images correspond to sagittal sections. Gene expression levels range from weak (blue/green), moderate (yellow) to strong (red). The different layers are highlighted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

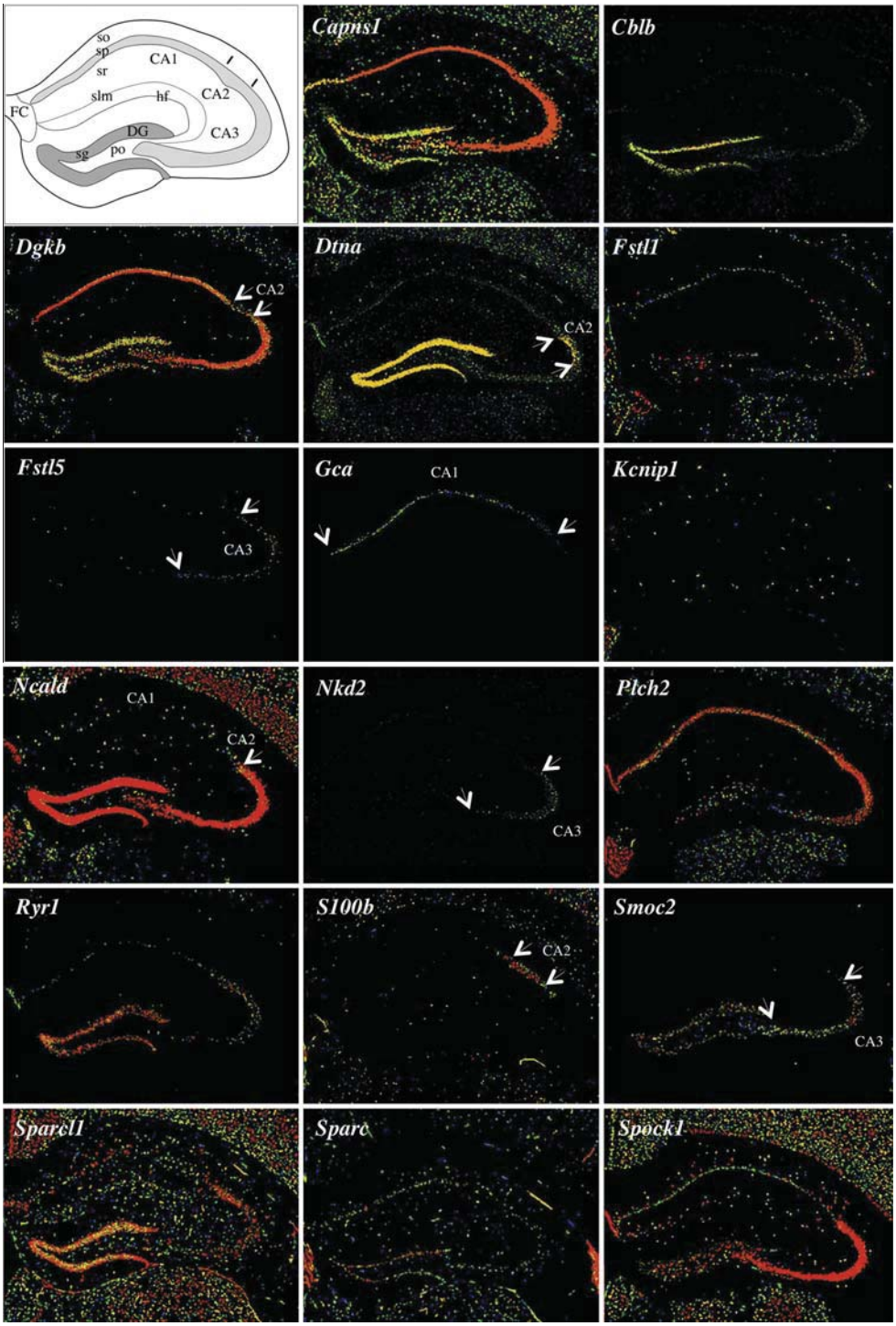

Fig. 4. Gene expression patterns in the hippocampus. The upper left panel is a schematic, coronal, view of the hippocampal formation. *Abbreviations:* CA1/2/3: hippocampal fields; so: stratum oriens; sp: stratum pyramidale; sr: stratum radiatum; slm: stratum lacosum moleculare; DG: dentate gyrus; sg: granule cell layer; po: polymorph layer; hf: hippocampal fissure. ISH images for 17 genes, listed in alphabetical order, and presenting a characteristic expression pattern in the hippocampus, were taken from the ABA database. All are coronal sections. Gene expression levels range from weak (blue/green), moderate (yellow) to strong (red). Notice that stratum oriens interneurons are visible for *Ncald*, *Capns1* or *Spock1*. Some are clearly negative in the granular layer of the DG (*Fstl5, Gca, Nkd2*, *S100b*). Arrows highlight the CA1, CA2 or CA3 boundaries. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Expression patterns in the hippocampus

The three ''classical'' EF-hand proteins Calb-D28K, CalR and Parv have been extensively used as markers to define discrete populations of neurons in the hippocampus, in conjunction with immunostaining for neurotransmitters/neuropeptides (see for example Jinno and Kosaka, 2002). Examining the ABA database, most of the EF-hand genes appear to be expressed in the hippocampus: 126 out of the 135 expressed in the brain,

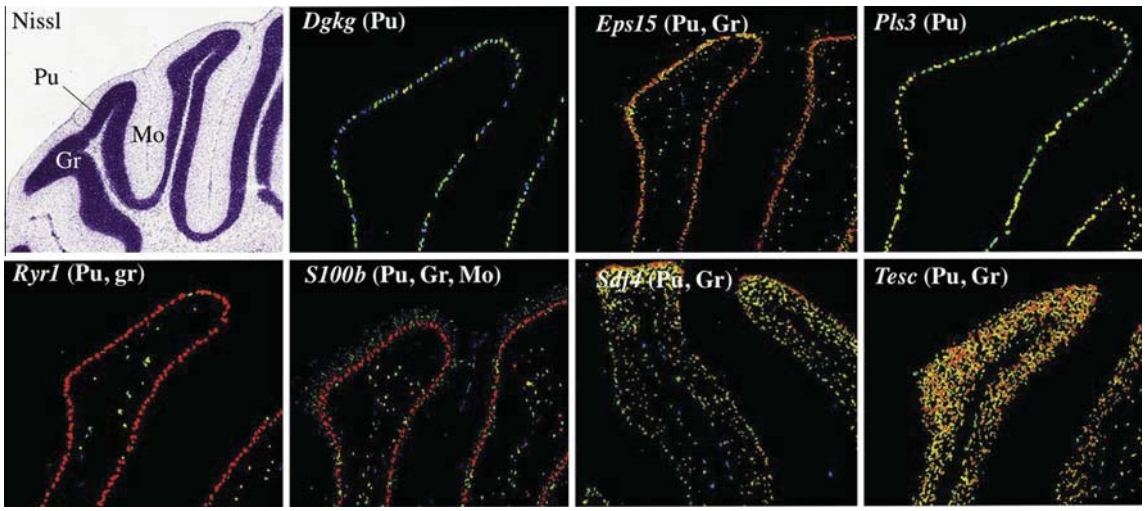

Fig. 5. Gene expression patterns in the cerebellum. The upper left panel shows the Nissl staining of a coronal section, highlighting the Purkinke- (Pu), the molecular- (Mo) and the granular (Gr) layers. The other seven pictures, taken from the ABA database, show the ISH images for seven genes, listed in alphabetical order, presenting a characteristic expression pattern. All are coronal sections, except Sdf4 (sagittal). Gene expression levels range from weak (blue/green), moderate (yellow) to strong (red). Some genes are strongly expressed in the Pu layer (*Dgkg, Eps15, Pls3, Ryr1*), with little or no staining in other layers. Inversely, some are expressed in the Gr layer (*Sdf4, Tesc*), with eventually few cells stained in the Pu layer. Only few genes (see list Table 2) show expression in the Mo layer (an example is given here with *S100b*). Note the presence of a ''railroad track'' of parallel cells expressing the *Sdf4*-gene. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

present a detectable HPF expression. Most of them are distributed ubiquitously according to the known subdivisions (CA1/2/3 (Ammon's horn regions 1/2/3), DG (dentate gyrus)), but some reveal an interesting parcellation (Table 6 and Fig. 4). Indeed, few genes are expressed in unique areas in the CA fields, and thus could serve as specific markers: CA1 (*Calb1*, *Efcab12*, *Gca*); CA2 (*Necab2*, *S100b*); CA3 (*Cabp1*, *Cblb*, *Cgref1*, *Dgka*, *Fstl5*, *Ncald*, *Necab3*, *Nkd2, Smoc2*). Most of these genes show detectable expression in the DG at different levels (only eight are clearly negative in DG: *Cabp1, Cgref1, Efha2, Fstl5, Gca, Mylpf, Nkd2, Tnnc2*), and we found none that was expressed only in DG and not in the CA fields.

An additional focus was given to possible expression in nerve cells of the stratum oriens, which are exclusively short axon cells (interneurons). A significant proportion of Parv-immunoreactive neurons are found in the stratum oriens of the CA1 and CA3 regions, and a few are located in the radiatum layer (Celio, 1990). As listed in Table 6 , 51 EF-CaBP-encoding genes show expression in the stratum oriens, and thus could partly be co-expressed with *Pvalb*. Most of these are ubiquitously expressed genes and therefore of minor anatomical relevance as specific markers, but 17 (*Calb1, Calb2, Capns1, Dtna, Eps15, Ncald, Ncs1, Necab1, Necab2, Plch2, Pvalb, Reps 1, Ryrt, Sparc, Spock1, Spock3, Vsnl1*) are selectively expressed in scattered neurons and could help subdivide the interneuronal population in additional subclasses. Particularly interesting in this context are *Sparc*, *Spock1* and *Spock3*, which are also expressed in the periglomerular cells of the OB, the RT and the molecular layer of the cerebellum.

Two experimental approaches, ISH and immunostaining, were performed in order to analyze, respectively at the gene and protein level, the co-expression in the hippocampus of *Pvalb*/Parv, with either *Ncald* (neurocalcin-δ), *Hpcal4* (hippocalcin-like4) or *Pls3* (plastin3). *Ncald* is mainly expressed in the granule layer of the DG, and in the pyramidal layer of the CA3 region. In addition, sparse cells are present in the hilus of the DG, in the stratum oriens, stratum radiatum and stratum lacunosum moleculare, and isolated cells can be found also in the pyramidal layer of the CA1 region (Fig. 4). Only a subset of *Pvalb* expressing cells also expressed *Ncald* in the CA1 region, both in the pyramidal layer of the hippocampus (sp) and stratum oriens. (Fig. 6C, D). *Pls3* is strongly expressed in the pyramidal layer of the CA1-2-3 regions (Fig. 6E, J). No clear expression is visible in the stratum oriens. In addition, isolated cells are present in the DG and in the hilus (Fig. 6H). Co-localization of *Pls3* with *Pvalb* mRNA is observed in the pyramidal layer of CA1/3 regions, in the stratum lacunosum moleculare and in the granular layer of the DG (Fig. 6H–K). For *Hpcal4*, we found no evidence for co-expression with *Pvalb* (not shown). Similar co-expression experiments by ISH were performed with *Eps15* and *Sparcl1*, and the results highlighted the co-expression of these two genes in virtually all *Pvalb* expressing neurons in the HPF (data not shown).

Immunostaining experiments using anti-Ncald, respectively -Hpcal4, combined with anti-Parv, have confirmed these results. Indeed, Ncald-immunoreactive neurons in the CA1 region (in stratum oriens and sp) represent a subset of Parv-immunoreactive ones. Nevertheless, some neuronal populations stained positive for Parv but negative for Ncald, while in others it was the other way round (Fig. 7A, B). The same was true for the CA3 region (not shown). For Hpcal4, we found no evidence for co-expression in Parv-positive neurons in the hippocampus in the CA sp and stratum oriens (Fig. 7C–E).

Table 3. EF-hand family genes expressed in the main olfactory bulb and accessory olfactory nucleus. List of the EF-hand genes showing a detectable expression in the main olfactory bulb (OB), and the accessory olfactory nucleus (AON), according to the ISH images of the ABA database. + + corresponds to moderate/strong expression, and + to low expression. *Abbreviations:* AOB: accessory olfactory bulb; END: endopiriform nucleus; CLA: Claustrum; RMS: rostral migratory stream; gl: glomerular layer; gr: granular layer; mi: mitral layer; opl: outer plexiform layer

Gene	gl	opl	mi	gr	AON	Gene	gl	opl	mi	gr	AON
Actn1	+		+	+	+	*Necab1*	+			+	Sparse cells
Actn3	+		+	+	+	*Necab2*	+			+	Sparse cells
Actn4			+	+	+	*Necab3*			+ +	+ +	+
Cabp1			+		+	*Nin*	+	+	+ +	+ +	+
Cabp4	+		+	+	+	*Nkd1*	+			+	Sparse cells
Cabp5	+		+	+		*Nucb1*			+		
Calb1	+				Sparse cells	*Nucb2*				+	+
Calb2	+ +	+ +	+ +	+ +	+ +	*P4htm*			+	+	+
Calm1	+ +		+ +	+ +	+ +	*Pdcd6*			+		Sparse cells
Calm2	+ +		+ +	+ +	+ +	*Pef1*	+		+ +	+	
Calm3			+		+	*Plcg1*	+ +		+ +	+	+
Calu	+					*Plch2*	+	+	+ +	+ +	+ +
Capn2	+		+ +	+	Sparse cells	*Pls3*					+ + END, CLA
Capn3	+ +		+ +	+	Sparse cells	*Ppp2r3c*	+		+	+	Sparse cells
Capn10	+		+	+	Sparse cells	*Ppp2r3d*	+	+	+	+	+
Capns1	+ +	+	+ +	+	+ +	*Ppp3r1*	+ +	+	+ +	+ +	+ +
Cbara1	+ +	+	+ +	+	+ +	*Prkcsh*	+	+	+	+	+
Cetn2	+		+	+	+	*Pvalb*		+ +			Sparse cells
Cgref1					+ +	*Rab11fip3*	+ +	+ +	+ +	+ +	+ +
Cib2			+		AOB + + +	*Rab11fip4*	+ +	+ +	+ +	+ +	+ +
Dgkb			+	+ +	+ +	*Rasgrp1*	+ +	+	+ +	+	+
Dgkg	+		+ +	+	+ +	*Rasgrp2*				+	
Drp2	+		+	+ +	+ +	*Rcn1*	+				
Dtna	+	+	+ +	+	+ +	*Rcn2*	+ +	+	+ +	+	+
Dtnb	+		+		+	*Rcvrn*	+		+	+	+
Efcab4a	+		+			*Reps1*	+ +	+	+ +	+	+
Efhb	+		+	+		*Reps2*	+	+	+	+	+ +
Efhd2	+ +	+	+ +	+	+ +	*Rhbdl3*	+ +		+ +	+ +	
Ehd2	+		+	+	+	*Rhot1*	+		+	+	Sparse cells
Ehd3	+ +		+ +		+	*Rhot2*	+ +		+ +	+ +	+ +
Eps15	+	+	+ +	+	+	*Ryr1*	+ +	+	+ +		
Eps15l1	+		+	+	+	*Ryr2*	+ +	+	+ +	+ +	+ +
Fkbp9	+			+	Sparse cells	*S100a9*	+		+	+	
Fkbp10			+	+		*S100a10*			+	+	RMS
Fstl1				+	+ +	*S100a16*	+	+		+	Sparse cells, RMS
Fstl4		+	+			*S100b*	+			+	Sparse cells
Fstl5		+ +	+ +			*Scgn*	+ +		+ +	+ +	
Gpd2	+			+	+	*Sdf4*	+ +		+ +	+	Sparse cells
Guca1b	+ +	+	+	+		*Slc25a12*	+ +	+	+ +	+ +	+ +
Hpca	+			+	+ +	*Slc25a23*	+ +	+	+ +	+ +	+
Hpcal1	+			+	Sparse cells	*Slc25a25*	+		+	+	+
Hpcal4			+	+ +	+ +	*Smoc1*	+			+	
Itsn1	+		+	+ +	+	*Sparc*	+ +	+	+	+	Sparse cells
Itsn2			+	+		*Sparcl1*	+ +	+ +	+ +	+ +	+ +
Kcnip1				+	Sparse cells	*Spna2*	+ +	+	+ +	+	+ +
Kcnip2	+ +			+ +	+	*Spock1*	+ +	+	+ +	+	+ +
Kcnip3	+		+ +	+	+ +	*Spock2*	+ +	+	+ +	+	+ +
Kcnip4	+				Sparse cells	*Spock3*	+		+	+	Sparse cells
Lcp1	+ +		+ +	+ +	+	*Sri*	+		+	+	+
Letm1	+		+ +		Sparse cells	*Stim1*	+		+	+	+
Lpcat2	+			+	Sparse cells	*Stim2*	+		+	+	+
Macf1	+ +	+	+ +	+	+	*Tesc*				+	+
Mcfd2	+		+		+	*Usp32*	+		+ +		Sparse cells
Myl1	+		+	+		*Utrn*	+		+	+	+
Myl6	+ +	+	+ +	+	+ +	*Vsnl1*	+				+
Mylpf	+		+ +	+		*1500003O03Rik*	+		+ +	+	+
Ncald	+	+	+	+	+ +	*4732418C07Rik*	+		+ +	+	Sparse cells
Ncs1	+		+ +	+	+						

Table 4. EF-hand family genes expressed in the cerebral cortex. The table lists the expression of the 135 Ef-hand family genes in the six layers of the somatosensory isocortex, at the level of the barrel cortex. The analysis was based on both sagittal and coronal ISH images of the ABA. Legends: nd: not detected; spa.+/−: few isolated cells, weakly stained; spa.+, spa.++, spa.+++: sparse cells, weakly/moderately/strongly stained; ub.+, ub.++, ub.+++: ubiquitous weak/moderate/strong staining; SM: somatomotor cortex; SS: somatosensory cortex; VIS: visual cortex

Gene name	Layer 1	Layer 2	Layer 3	Layer 4	Layer 5a	Layer 5b	Layer 6
Actn1	nd	Ub.+++	Ub.++	Spa.+	Spa.+	Spa.+	Ub.++
Actn3	nd	Spa.+	Spa.+	Spa.+/−	Spa.+	Spa.+	Spa.+
Actn4	nd	Spa.+	Spa.+	Spa.+/−	Spa.+	Spa.+	Spa.+
Cabp1	nd	Spa.+	nd	nd	Spa.+	nd	nd
Calb1	Spa.+/−	Ub.+++	Ub.+++	Ub.+	Spa.++	Spa.++	Spa.++
Calb2	nd	Spa.+	Spa.+	Spa.+	Spa.+	Spa.+	Spa.+
Calm1	nd	Ub.++	Ub.++	Spa.+	Ub.++	Ub.++	Ub.++
Calm2	Spa.+	Ub.+++	Ub.+++	Ub.++	Ub.+++	Ub.+++	Ub.+++
Calm3	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Capn2	nd	Spa.+/−	Spa.+	Spa.+	Spa.+/−	Spa.++	Spa.+
Capn3	Spa.+	Ub.+	Ub.+	nd	Spa.+	Spa.+	nd
Capn8	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Capns1	nd	Spa.+	Spa.+	nd	Spa.++	Spa.++	Spa.+
Cbara1	nd	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+
Cblb	nd	Spa.+	Spa.+	Spa.+/−	Spa.+	Spa.+	nd
Cetn2	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Cib2	nd	Spa.+	Spa.+	nd	Spa.+/−	Spa.+/−	Spa.+/−
Dgkb	nd	Ub.++	Ub.+	nd	Spa.+	Spa.+	Spa.+
Dgkg	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Drp2	nd	Ub.++	Ub.++	Ub.++	Ub.++	Ub.++	Ub.+
Dtna	Spa.+	Ub.+	Ub.+	Spa.+	Ub.+	Ub.+	Ub.+
Dtnb	nd	Spa.+	Spa.+	nd	Ub.+	Ub.+	Ub.+
Efcab4a	nd	Spa.+	Spa.+	nd	nd	nd	nd
Efha2	nd	Spa.+/−	nd	nd	Spa.+/−	Spa.+/−	nd
Efhb	nd	Spa.+/−	Spa.+/−	nd	Spa.+/−	nd	nd
Efhd2	Spa.+	Ub.+++	Ub.++	Spa.+	Ub.++	Ub.++	Ub.++
Ehd1	nd	Spa.+/−	Spa.+/−	nd	Spa.+/−	Spa.+/−	Spa.+/−
Ehd2	Ub.++	Ub.+++	Ub.+++	Ub.++	Ub.++	Ub.++	Ub.++
Ehd3	Spa.+	nd	nd	nd	Spa.++	Spa.++	Spa.+/−
Eps15	Ub.++	Ub.++	Ub.++	Ub.++	Ub.++	Ub.++	Ub.++
Eps15l1	nd	nd	nd	nd	Spa.+/−	Spa.+/−	Spa.+/−
Fkbp9	nd	Ub.++	Ub.++	Spa.+	Ub.+	Ub.+	Ub.+
Fkbp10	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	nd
Fstl1	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Fstl4	nd	Spa.+	Spa.+	nd	nd	nd	nd
Gca	nd	Spa.+/−	Spa.+/−	nd	nd	nd	nd
Gpd2	nd	Ub.+	Ub.+	Spa.+	Ub.+	Ub.+	Ub.+
Guca1b	Spa.++	Ub.++	Ub.+	Spa.+	Ub.+	Ub.+	Ub.+
Hpca	Spa.+	Ub.++	Ub.++	Ub.+	Spa.++	Spa.++	Ub.++
Hpcal4	Spa.+	Ub.+++	Ub.++	Spa.+/−	Spa.++	Spa.+	Ub.+++
Itsn1	nd	Spa.+	Spa.+	Spa.+	Ub.+	Ub.+	Ub.+
Kcnip1	nd	Spa.+	Spa.+	nd	Spa.+/−	Spa.+/−	Spa.+/−
Kcnip2	nd	Ub.+	Ub.+	Ub.+	Spa.++	Spa.++	Ub.+
Kcnip3	nd	Ub.+	Ub.+	Ub.+	Ub.+++	Ub.+++	Ub.++
Kcnip4	nd	Spa.+/−	nd	nd	Spa.+/−	Spa.+/−	Spa.+/−
Lcp1	Spa.+	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Letm1	nd	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+
Lpcat2	nd	Spa.+/−	Spa.+/−	nd	nd	nd	Spa.+/−
Macf1	Spa.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+
Mcfd2	nd	Spa.+	Spa.+/−	nd	Spa.+	Spa.+	Spa.+
Myl1	Spa.+	Spa.+	Spa.+	Spa.+	Ub.+	Ub.+	Ub.+
Myl4	nd	nd	nd	nd	Spa.++	Spa.++	Ub.+
Myl6	nd	Ub.++	Ub.++	Ub.++	Ub.+++	Ub.+++	Ub.+++
Ncald	Spa.+++	Ub.+++	Ub.++	Spa.+	Ub.+++	Spa.+++	Ub.+++
Ncs1	Spa.+++	Ub.+	Ub.+	Spa.+	Ub.++	Ub.++	Ub.+
Necab1	Spa.+	Spa.+++	nd	nd	Spa.++	Spa.+	Spa.+
Necab2	Spa.+	Spa.+	nd	nd	nd	nd	Spa.+/−
Necab3	nd	Ub.+	Ub.+	nd	nd	nd	Ub.+
Nin	nd	Ub.+	Ub.+	Spa.+	Spa.+	Spa.+	Ub.+
Nucb1	nd	Spa.+	nd	nd	Spa.+	Spa.+	Spa.+/−

Table 4 (*continued*)

Gene name	Layer 1	Layer 2	Layer 3	Layer 4	Layer 5a	Layer 5b	Layer 6
Nucb2	nd	Ub.+	Spa.+/–	nd	Spa.+	Spa.+	Ub.+
P4htm	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Pdcd6	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	nd
Pef1	Spa.+	Ub.+	Ub.+	Spa.+	Spa.+	Spa.+	Spa.+
Plcg1	Spa.+	Spa.+	Spa.+	Spa.+	Spa.+	Spa.+	Spa.+
Plch2	Spa.+	Ub.+	Ub.+	Ub.+	Spa.+	Spa.+	Spa.+
Pls3	nd	Spa.+	nd	nd	Spa.+	Spa.+	Spa.+/–
Ppp2r3c	nd	Ub.+	Ub.+	Spa.+/–	Spa.+	Spa.+	Spa.+
Ppp2r3d	nd	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+
Ppp3r1	Spa.+	Ub.+++	Ub.+++	Ub.+	Ub.++	Ub.++	Ub.++
Prkcsh	nd	Ub.+	Ub.+	nd	Ub.+	Ub.+	Ub.+
Pvalb	nd	Spa.+++	Spa.+++	Spa.+++	Spa.+++	Spa.+++	Spa.+++
Rab11fip3	Spa.+	Ub.+++	Ub.+++	Ub.+++	Ub.+++	Ub.+++	Ub.+++
Rab11fip4	Spa.+	Ub.++	Ub.++	Spa.+	Ub.++	Ub.++	Ub.++
Rasgrp1	Spa.+	Ub.+++	Ub.+++	Ub.+	Ub.+	Ub.+	Ub.++
Rasgrp2	nd	nd	nd	nd	Spa.+	Spa.+	nd
Rcn1	nd	Spa.+	Spa.+	nd	nd	nd	nd
Rcn2	Spa.+	Spa.+	Spa.+	Spa.+/–	Ub.++	Ub.++	Spa.+
Rcvrn	nd	Spa.+	Spa.+	nd	Spa.+/–	Spa.+/–	nd
Reps1	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Reps2	Spa.++	Ub.++	Ub.++	Ub.++	Ub.++	Ub.++	Ub.++
Rhbdl3	nd	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+	Spa.+
Rhot1	Spa.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+
Rhot2	Spa.+	Ub.++	Ub.+	Ub.+	Ub.++	Ub.++	Ub.+
Ryr1	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Ryr2	Spa.++	Ub.+++	Ub.+++	Ub.++	Ub.++	Ub.++	Ub.++
S100a10	nd	nd	nd	Spa.+	nd	nd	nd
S100a11	nd	Spa.+/–	Spa.+/–	nd	Spa.+/–	nd	nd
S100a16	nd	nd	nd	nd	Spa.+	Spa.+	Spa.+
S100b	nd	nd	nd	Ub.++	Ub.++	Ub.++	Spa.++
Scgn	nd	Spa.+	Spa.+	nd	nd	nd	Spa.+/–
Sdf4	Spa.+	Ub.++	Ub.+	nd	Spa.+	Spa.+	Spa.+
Slc25a12	Spa.+	Ub.+	Ub.+	Spa.+	Ub.++	Ub.++	Ub.+
Slc25a23	Spa.+	Ub.+++	Ub.++	Ub.+	Ub.++	Ub.++	Ub.+
Slc25a25	nd	Spa.+	Spa.+	nd	Spa.+	Spa.+	Spa.+
Sparc	Spa.+	Spa.+	Spa.+	Spa.+	Spa.+	Spa.+	Spa.+
Sparcl1	Ub.+++	Ub.+++	Ub.+++	Spa.+++	Ub.+++	Ub.+++	Ub.+++
Spna2	Spa.+	Ub.+++	Ub.++	Ub.+	Ub.++	Ub.++	Ub.+
Spock1	Spa.+	Ub.++	Ub.++	Ub.++	Ub.++	Ub.++	Ub.+
Spock2	Ub.+++	Ub.+++	Ub.++	Ub.++	Ub.+++	Ub.+++	Ub.+++
Spock3	Spa.+	Spa.++	Spa.+	Sp+/–	Ub.++	Spa.+	Spa.+
Sri	nd	Spa.+	Spa.+	nd	nd	Spa.+	Spa.+
Stim1	Spa.+	Ub.+	Ub.+	Spa.+/–	Ub.+	Ub.+	Ub.++
Stim2	Spa.+/–	Ub.+++	Ub.++	Spa.+/–	Spa.+/–	Spa.+/–	Ub.++
Tesc	nd	Ub.+	nd	nd	Spa.+/–	nd	Spa.+
Tnnc1	nd	VIS(4), SS(2)	SM	Spa.+ VIS, SS (barrel), SM	nd	nd	Spa.+ VIS, SS, SM
Usp32	nd	Spa.+	nd	nd	Spa.+	Spa.+	Spa.+
Utrn	nd	Spa.+/–	nd	nd	Spa.+	Spa.+	Spa.+/–
Vsnl1	nd	Spa.+	Spa.+	nd	Ub.+	Ub.+	Ub.+
Zzef1	nd	nd	nd	nd	Spa.+/–	Spa.+/–	nd
1500003O03Rik	Spa.+	Spa.+	Spa.+	nd	Spa.++	Spa.+/–	Spa.+/–
4732418C07Rik	Spa.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+	Ub.+

Expression pattern of the EF-hand family genes in the amygdala

The most specific marker for an amygdalar subnucleus is *Myl4*, which only marks the lateral amygdala nucleus, part of basomedial amygdalar nucleus and endopriform nucleus. *Rcn1* is positive in the cortical amygdalar area, posterior part medial zone (layer 2), *Rhot2* in basolateral amygdalar nucleus; *Smoc1* in CoAPm layers 2 + 3 and posterior amygdalar nucleus and *Smoc2* in posterior amygdalar nucleus (Table 7).

Expression patterns of the EF-hand family genes in the cerebellum

Calb-D28K and CalR are useful markers for neuronal populations in the cerebellum, being specific for Purkinje

Table 5. EF-hand family genes expressed in the basal ganglia. The table lists the 135 genes of the EF-hand family expressed in three compartments of the basal ganglia, the striatum (S) (caudoputamen, CP), the Nucleus accumbens and the Globus pallidus. *Abbreviations:* GPE: Globus pallidus, external; GPI: Globus pallidus, internal: *nd*: no positive elements detected; mtx: probably neurons of the matrix compartment positive; msi: medium spiny interneurons; SI: substantia innominata; spa +/spa.++/spa.+++: sparse cells, weak/moderate/strong signal; (+) rare or few positive cells

Gene name	Striatum	Accumbens	Globus pallidus
Actn1	+ mspi	+++	–
Actn3	+	+	+
Cabp5	+ dorsal	*nd*	*nd*
Cabp7	(+)	+	(+)
Calb1	+ mspi	+	+
Calm1	+	+	+
Calm2	+	+	+
Capn2	spa +	*nd*	+
Capn3	(+)	*nd*	*nd*
Capn8	+	+	+
Capns1	spa +	+	+
Cbara1	+ msi	+	spa +
Cetn2	+	+	+
Cgref1	spa +	spa +	spa +
Cib2	+	(+)	+
Dgkb	+ mtx	+	GPI spa +
Dgkg	+ mtx	+	+
Drp2	+ mtx	+	+
Dtna	+	+	+
Dtnb	+ mtx	+	+
Efha2	*nd*	*nd*	+
Efhd2	spa +	spa +	+
Ehd1	*nd*	*nd*	+
Ehd2	+	*nd*	+
Ehd3	*nd*	*nd*	+ GPE
Eps15	+ mtx	+	+
Eps15l1	+	+	+
Fkbp9	+ mtx	+	+
Fstl1	spa +	spa +	+
Guca1b	+	+	+
Hpca	+ mtx	+	+
Hpcal1	spa +	spa +	*nd*
Hpcal4	+ mtx	+	*nd*
Itsn1	spa +	spa +	+
Itsn2	*nd*	*nd*	(+)
Kcnip1	(+) spa	(+) spa	(+) spa
Kcnip2	+	+	*nd*
Kcnip3	+ mtx	+	*nd*
Kcnip4	(+) spa	(+) spa	+
Lcp1	+	+	+
Letm1	+	+	+
Lpcat2	(+) spa	(+) spa	(+) spa
Macf1	+	+	+
Mcfd2	+	+	+
Myl4	*nd*	*nd*	Ventral pallidum
Myl6	+	+	+
Ncald	spa +++	+	spa +++
Ncs1	spa +	+	+
Necab1	+	+	*nd*
Necab2	+	+	(+)
Nin	+	+	(+)
Nucb1	*nd*	*nd*	+
Nucb2	+	+(>than CP)	*nd*
P4htm	spa +	spa +	spa +
Pdcd6	+	+	*nd*
Pef1	+	+	+
Plcg1	+	+	+
Plch2	+	+	(+)
Ppp2r3c	spa +	spa (+)	+
Ppp2r3d	+	+	+

Table 5 (*continued*)

Gene name	Striatum	Accumbens	Globus pallidus
Ppp3r1	+	+	*nd*
Prkcsh	+	+	+
Pvalb	spa + + +	spa + + +	spa + + +
Rab11fip3	+	+	+
Rab11fip4	(+)	(+)	(+)
Rasgrp1	+	+	(+)
Rasgrp2	+ +	+ +	*nd*
Rcn1	+ ant	+ (< than S)	sp + +
Rcn2	+	+	+
Rcvrn	+ ant	+ (> than S)	*nd*
Reps1	spa (+)	+ (> than S)	+
Reps2	+	+	+ GPE
Rhbdl3	+	+	(+)
Rhot1	spa +	*nd*	+
Rhot2	+	+	+
Ryr1	+ + +	+	*nd*
Ryr2	+	+	+ +
S100a10	spa + +	spa + +	spa + + (SI)
S100a11	*nd*	*nd*	+ GPI
S100a16	+	+	+
S100b	+	+	+
Sdf4	+	+	+
Slc25a12	+ mtx	+	+ + +
Slc25a23	+	+	+
Slc25a25	+ dorsal	+ (< than S)	*nd*
Sparc	+	+	+ + vessels and pia
Sparcl1	+	+	+ GPE > GPI
Spna2	+	+	+
Spock1	+ vm	+	+
Spock2	+	+	+ +
Spock3	+ + +	+	(+)
Sri	spa +	spa +	spa +
Stim1	+	+	+
Stim2	+	+	*nd*
Tesc	+ +	+ +	*nd*
Usp32	(+)	(+)	(+)
Utrn	+	+	*nd*
Vsnl1	*nd*	(+)	+ GPE
1500003O03Rik	+	+	+
4732418C07Rik	+	+	+

cells, and cells of the granular layers respectively, which are mostly granule cells. However also unipolar brush cells (Floris et al., 1994), the Golgi- and Lugaro cells, as well as the monodendritic cells (Braak and Braak, 1993; Dino et al., 1999) express CalR. Screening the ABA database with a special focus on the cerebellum, 100 genes presenting a detectable expression were found (Table 2, and Figs. 2 and 5). The following 19 genes are specific for the granular layer: *Actn1*, *Cabp5*, *Calb2*, *Efcab12*, *Ehd3*, *Fstl1*, *Gpd2*, *Guca1b*, *Itsn1*, *Kcnip3*, *Myl1*, *Ncs1*, *Necab3*, *Plch2*, *Pls3*, *Rbhdl3*, *Ryr2*, *Sri*, and *Vsnl1*. The following 32 genes are specific for the Purkinje layer: *Actn3*, *Calb1*, *Calu*, *Capn2*, *Capn10*, *Cetn2*, *Dgkb*, *Dgkg*, *Efha2*, *Efhb*, *Efhd2*, *Fstl4*, *Hpca*, *Hpcal1*, *Kcnip1*, *Kcnip4*, *Letm1*, *Mcc*, *Mcfd2*, *Nucb1*, *P4htm*, *Pdcd6*, *Pef1*, *Prkcsh*, *Rab11fip4*, *Ryr1*, *S100a10*, *S100a11*, *Scgn*, *Slc25a25*, *Stim1*, and *Zzef1*. In the molecular layer a restricted number of six genes are expressed: *Pvalb* (stellate and basket cells), *S100b* (probably Bergmann glia), *Sparc* (neuronal/glial cells), *Sparcl1*, *Spock2*, *Spock3* (Fig. 5). Expression in the Purkinje-cell layer (Pu) does not implicitly mean expression in the Purkinje-neuron themselves. The resolution of the ISH does not allow for differentiating between Purkinje-neurons and Golgi-epithelial cells, which give rise to the Bergmann glia. One such example is *Calu*, which is expressed in the Purkinje cell layer (Table 2), but found immunohistochemically in the Golgi-epithelial cells and Bergmann glia (Vasiljevic et al., 2012).

At least four genes (*Rhbdl3, Ryr2, Sdf4, Sri*) are expressed both in the Purkinje-cell layer and in a parallel layer of cells located at the boundary between the granular layer and white matter (Fig. 5). No indication of the presence of this row of cells is found in the reference book on the cerebellum (Palay and Chan-Palay, 1974), but in the classical studies by Golgi (1903) and Cajal (1955) single fusiform cells located in this boundary region are depicted.

Table 6. EF-hand family genes expressed in the hippocampus. The table lists the EF-hand genes showing a detectable expression in the hippocampal formation (Ammon's horn region CA1, 2, 3, dentate gyrus and the interneuron-rich Stratum oriens), according to the ISH-images of the ABA-database. An additional column indicates other interneuron-rich regions (cerebellum, olfactory bulb) expressing the same genes. *nd*: no expression detected; EPL: external plexiform layer; RT: reticular nucleus of the thalamus

Gene name	Expression level	Detailed expression in CA fields	Expression in dentate gyrus	Expression in stratum oriens	Expression in interneuron-rich areas comments
Actn1	Ubiquitous strong	Pyramidal cells CA1-2-3	Granular cells	*nd*	
Actn3	Ubiquitous weak to moderate	Pyramidal cells CA1-2-3, hylus	Granular cells	*nd*	
Actn4	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Cabp1	Patterned	String of cells in CA3 and hylus (weak), CA1-2: *nd*	*nd*	*nd*	
Cabp4	Ubiquitous weak to moderate	String of cells in CA1-2-3	String of cells in DG	*nd*	
Cabp5	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Cabp7	Patterned	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Calb1	Patterned	Pyramidal cells CA1, CA2-3: *nd*	Granular cells DG (strong)	+	Purkinje, periglomerular OB, sparse interneurons
Calb2	Patterned	Scattered neurons	Scattered neurons	+	Glomerular cells OB, periglomerular OB, EPL, sparse interneurons
Calm1	Ubiquitous strong	Pyramidal cells CA1-2-3, hylus	Granular cells, po	+	
Calm2	Ubiquitous strong	Pyramidal cells in CA1-2-3, hylus	Granular cells, po	+	
Calm3	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Calu	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Capn1	Ubiquitous weak	String of cells in CA1-2-3 and hylus	String of cells in DG	*nd*	
Capn2	Ubiquitous weak	String of cells in CA1-2-3 and hylus	String of cells in DG	*nd*	
Capn3	Ubiquitous weak	String of cells in CA1-2-3 and hylus	String of cells in DG	*nd*	
Capn10	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Capns1	Patterned	Pyramidal cells CA1-2-3 and hylus (strong),	Granular cells (moderate), po (strong)	+	Rare molecular layer CBX, RT faint, periglomerular OB, EPL, sparse interneurons
Cbara1	Ubiquitous moderate to strong	Pyramidal cells CA1-2-3 and hylus	Granular cells	*nd*	
Cbl	Ubiquitous weak	Pyramidal cells CA1-2-3	Granular cells	*nd*	
Cblb	Patterned	Sparse cells in CA3 (weak), CA1-2: *nd*	Granular cells (moderate)	*nd*	
Cetn2	Ubiquitous weak	String of cells in CA1-2-3 and hylus	String of cells in DG	+	
Cetn3	Ubiquitous weak	String of cells in CA1-2-3 and hylus	String of cells in DG	*nd*	
Cgref1	Patterned	Sparse cells in CA3 (weak), CA1-2: nd	*nd*	*nd*	
Cib2	Ubiquitous weak	Pyramidal cells CA1-2-3	Granular cells	*nd*	
Dgka	Patterned	Pyramidal cells CA3 (weak), CA1-2: *nd*	Granular cells (weak)	*nd*	
Dgkb	Patterned	Pyramidal cells CA1-3 (strong), CA2 (weak), and hylus	Granular cells (moderate)	*nd*	
Dgkg	Ubiquitous strong	Pyramidal cells in CA1-2-3, hylus (weak)	Granular cells	+	

Table 6 (*continued*)

Gene name	Expression level	Detailed expression in CA fields	Expression in dentate gyrus	Expression in stratum oriens	Expression in interneuron-rich areas comments
Drp2	Ubiquitous strong	Pyramidal cells CA1-2-3	Granular cells	*nd*	
Dtna	Patterned	Pyramidal cells CA1-2-3 (moderate)	Granular cells (strong)	+	Periglomerular OB, EPL
Dtnb	Ubiquitous strong	Pyramidal cells CA1-2-3	Granular cells	+	
Efcab1	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Efcab4a	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Efcab12	Patterned	String of cells in CA1 (weak), CA2-3: *nd*	String of cells in DG (weak)	*nd*	
Efha2	Patterned	String of cells in CA1-2-3 (weak)	*nd*	*nd*	
Efhb	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Efhd2	Ubiquitous weak	Scattered cells in CA1-2-3, hylus	Scattered cells in DG	+	
Ehd1	Patterned	Pyramidal cells CA1-2-3 (weak)	Granular cells (moderate)	*nd*	
Ehd2	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Ehd3	Patterned	Pyramidal cells in CA2 (strong), CA1-3 and hylus (weak)	Granular cells (strong)	*nd*	
Eps15	Patterned	Pyramidal cells CA1-2-3 (strong)	Granular cells (moderate)	+	Molecular layer CBX, RT, periglomerular OB, EPL, layer 2
Eps15l1	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Fkbp9	Ubiquitous weak	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Fkbp10	Ubiquitous weak	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Fstl1	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	+	
Fstl4	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Fstl5	Patterned	Scattered cells in CA3 and hylus, CA1-2: *nd*	*nd*	*nd*	
Gca	Patterned	Pyramidal cells CA1 (weak), CA2-3: *nd*	*nd*	*nd*	
Gpd2	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Guca1b	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	+	
Hpca	Ubiquitous strong	Pyramidal cells CA1-2-3 (strong), and hylus (weak)	Granular cells	*nd*	
Hpcal1	Ubiquitous weak	String of pyramidal cells	String of Granular cells	*nd*	
Hpcal4	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Itsn1	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	+	
Itsn2	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Kcnip1	Ubiquitous weak	Scattered interneurons in the whole hippocampus	Few sparse cells (weak)	+	
Kcnip2	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Kcnip3	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	*nd*	

(*continued on next page*)

Table 6 (*continued*)

Gene name	Expression level	Detailed expression in CA fields	Expression in dentate gyrus	Expression in stratum oriens	Expression in interneuron-rich areas comments
Kcnip4	Ubiquitous weak to moderate	Pyramidal cells CA1 (moderate), CA2-3 (weak)	Granular cells	*nd*	
Lcp1	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	+	
Letm1	Ubiquitous weak	Pyramidal cells in CA1-2-3, hylus	String of cells in DG	*nd*	
Lpcat2	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Macf1	Ubiquitous moderate	Pyramidal cells CA1-2-3 (moderate), hylus (weak)	Granular cells	+	
Mcfd2	Ubiquitous moderate	Pyramidal cells CA1-2-3 (moderate, hylus (weak)	Granular cells	*nd*	
Myl1	Ubiquitous weak	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Myl6	Ubiquitous strong	Pyramidal cells in CA1-2-3	Granular cells	+	
Mylpf	Patterned	String of cells in CA1-2-3 (weak)	*nd*	*nd*	
Ncald	Patterned	Pyramidal cells CA3 (strong), CA2 (low), CA1 isolated cells	Granular cells (strong)	+	RT, periglomerular OB, interneurons basal ganglia
Ncs1	Patterned	Pyramidal cells CA3 (strong), CA1-2: sparse cells	Granular cells (strong)	+	Hylus, molecular layer CBX, RT, periglomerular OB
Necab1	Patterned (strong in entorhinal cortex)	Few sparse cells	Few sparse cells	+	NRTH, periglomerular OB
Necab2	Patterned (strong in entorhinal cortex)	Pyramidal cells in CA2 (strong), sparse cells in CA3 (weak), CA1: *nd*	Sparse cells in DG	+	Periglomerular OB
Necab3	Patterned	Few pyramidal cells in CA3 (weak), CA1-2: *nd*	String of cells in DG	*nd*	
Nin	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Nkd1	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Nkd2	Patterned	Only pyramidal cells in CA3	*nd*	*nd*	
Nucb1	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Nucb2	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
P4htm	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Pdcd6	Ubiquitous weak to moderate	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Pef1	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Pkd2	Ubiquitous weak to moderate	String of cells in CA1-2-3	String of cells in DG	+	
Plcg1	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	+	
Plch2	Patterned	Pyramidal cells in CA1-2-3 (strong)	Sparse Granular cells, strong in po	+,	hylus DG, molecular layer CBX, RT
Pls3	Patterned	Pyramidal cells in CA1-2-3 (strong), hylus	Granular cells (moderate), po	*nd*	
Ppp2r3c	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Ppp2r3d	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Ppp3r1	Ubiquitous	Pyramidal cells in CA1-2-3	Granular cells	*nd*	

Table 6 (*continued*)

Gene name	Expression level	Detailed expression in CA fields	Expression in dentate gyrus	Expression in stratum oriens	Expression in interneuron-rich areas comments
	strong				
Prkcsh	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells, po	+	
Pvalb	Patterned	Few sparse cells	Few sparse cells	+	Molecular layer CBX, RT, periglomerular OB
Rab11fip3	Ubiquitous strong	Pyramidal cells in CA1-2-3	Granular cells	+	
Rab11fip4	Ubiquitous strong	Pyramidal cells in CA1-2-3	Granular cells	+	
Rasgrp1	Patterned	Pyramidal cells in CA1-2-3 (strong), hylus (weak)	Few sparse cells (weak)	*nd*	
Rcn2	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	+	
Rcvrn	Ubiquitous weak	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Reps1	Patterned	Pyramidal cells in CA1-2-3 (stronger in CA1), hylus cells (weak)	Granular cells (moderate)	+	RT, periglomerular OB
Reps2	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3	Granular cells	+	
Rhbdl3	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	+	
Rhot1	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Rhot2	Ubiquitous strong	Pyramidal cells in CA1-2-3	Granular cells	+	
Ryr1	Patterned	Pyramidal cells in CA2 (strong), string of cells in CA1-3 (weak)	Granular cells (strong)	+,	EPL, periglomerular OB
Ryr2	Ubiquitous strong	Pyramidal cells in CA1-2-3	Granular cells	+	
S100a10	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	+	
S100a11	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
S100a16	Ubiquitous weak	Scattered cells in CA1-2-3	Scattered cells in DG	+	
S100b	Patterned	Pyramidal cells in CA2 (moderate), CA1-3: *nd*	Scattered cells in DG	*nd*	
Scgn	Ubiquitous weak	String of cells in CA1-2-3	String of cells in DG	*nd*	
Sdf4	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	+	
Slc25a12	Ubiquitous strong	Pyramidal cells in CA1-2-3, hylus	Granular cells	+	
Slc25a23	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3	Granular cells	+	
Slc25a25	Ubiquitous moderate	Pyramidal cells in CA1-2-3	Granular cells	+	
Smoc2	Patterned	Pyramidal cells in CA3, CA1-2: *nd*	Granular cells	*nd*	
Sparc	Patterned	Scattered cells (weak) (glial cells)	Scattered cells in DG	+	Molecular layer CBX, RT, periglomerular OB. (Ependyma, pia, capillaries)
Sparcl1	Ubiquitous moderate to strong	Scattered cells (glial cells and neurons)	Granular cells (strong), po	+	
Spna2	Ubiquitous strong	Pyramidal cells in CA1-2-3	Granular cells	+	
Spock1	Patterned	Pyramidal cells in CA3	Scattered	+,	Golgi/Lugaro, RT, periglomerular

(*continued on next page*)

Table 6 (*continued*)

Gene name	Expression level	Detailed expression in CA fields	Expression in dentate gyrus	Expression in stratum oriens	Expression in interneuron-rich areas comments
		(strong), scattered cells in CA1-2 and hylus	granular cells, po (strong)		OB
Spock2	Ubiquitous strong	Pyramidal cells in CA1-2-3	Granular cells	+	
Spock3	Patterned	Pyramidal cells in CA3 (strong), string of cells CA1-2	Granular cells	+,	CBX, faint RT, periglomerular OB
Stim1	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3, hylus	Granular cells, po	+	
Stim2	Ubiquitous strong	Pyramidal cells in CA1-2-3	Granular cells, po	*nd*	
Tesc	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
Tnnc2	Ubiquitous weak	String of cells in the pyramidal cell layers	*nd*	*nd*	
Usp32	Ubiquitous weak to moderate	Pyramidal cells in CA1-2-3 (moderate), hylus cells (weak)	Sparse granular cells	*nd*	
Vsnl1	Patterned	Pyramidal cells CA1-3 (moderate), hylus cells (strong)	Sparse granular cells	+,	Periglomerular OB
Zzef1	Weak	Sparse cells in CA1-2-3	Sparse granular cells	*nd*	
1500003O03Rik	Ubiquitous moderate to strong	Pyramidal cells in CA1-2-3	Granular cells	*nd*	
4732418C07Rik	Ubiquitous weak to moderate	Pyramidal cells in CA1-2-3	Granular cells	+	

Three members of the EF-hand gene family (*Cabp1, Spock1* and *Spock3*) appear as specific markers of cerebellar interneurons in the publication by (Schilling and Oberdick, 2009).

Expression patterns of the of the EF-hand family genes in the mesencephalon and myelencephalon

In the periaqueductal gray (PAG), a few genes are highly expressed in the Edinger–Westphal nucleus (EW) (*Nucb2* and *Rcn1*), or the Nucleus of Darkschewitz (*Itsn1* and *Reps2*). Patchy, interesting new patterns of labeling seemingly not restricted to the classical functional columns of the PAG (Bandler and Shipley, 1994) were also seen for *Kcnip3*, *Nin* and *Slc25a25*.

Detailed expression pattern of the Neuronal Calcium Sensor family proteins neurocalcin-δ and hippocalcin-like 4

A particular attention was paid to two members of the NCS family, neurocalcin-δ (*Ncald*) and hippocalcin-like 4 (*Hpcal4*), which also include the following genes: *Ncs1*, *Vsnl1*, *Hpcal1*, *Hpca*, *Guca1a*, *Guca1b*, *Rcvrn*, and *Kcnip1* to *4*. The NCS family proteins bind Ca^{2+} with high affinities, undergo conformational changes and interact with and regulate other proteins, leading to changes in physiological functions including regulation of neurotransmitter release, regulation of cell-surface receptors and ion channels, control of gene transcription, cell growth and survival (Burgoyne, 2004; McCue et al., 2010). We performed immunostaining experiments for Ncald and Hpcal4, for which protein expression data in rodent brain are missing in the literature. In both cases, when comparing mRNA (ABA data/our ISH experiment) and protein expression patterns (our immunostaining experiment), we found a good correspondence.

Sites of Hpcal4 protein expression (Table 2, Fig. 8) include the main olfactory bulb (granular and mitral cells) (Fig. 8A, B), the anterior olfactory nucleus (AON), the piriform area, the striatum (caudoputamen (CP), nucleus accumbens, lateral septal nucleus), the cerebral cortex (Fig. 8C), the bed nuclei of the stria terminalis, the thalamus (strong expression in the paraventricular nucleus, sparsely located cells in the nucleus of reuniens and central medial nucleus) (Fig. 8C), the hypothalamus (weak expression in sparse cells in several hypothalamic nuclei), the hippocampus (strong expression in Ammon's horn regions CA1 lateral, CA3 pyramidal layer, and in granular cells in the dentate gyrus) (Figs. 7C and 8C, D), the midbrain (strong expression in ventral tegmental area (VTA) and dorsal raphé nucleus, and sparse cells in inferior colliculus and midbrain reticular nucleus) (Fig. 8G), the pons (strong in locus coeruleus (LC), superior central raphé nucleus (CS), parabranchial nucleus (PB), and sparse cells in pontine reticular nucleus (PRN)) (Fig. 8E), the medulla

Table 7. EF-hand family genes expressed in the Amygdala. List of genes expressed in the various amygdalar subdivisions: CEA: central amygdalar nucleus; MEA: medial amygdalar nucleus; LA: lateral nucleus; BMA: basomedial amygdalar nucleus; BLA: basolateral amygdalar nucleus; CO: Cortical amygdalar area; PA: posterior amygdalar nucleus. Indications: + + +: strong positive; + +: moderate positive; +: positive; (+): faint or not observed on all sections; nd: not detected; ubi: ubiquitous; sca: scattered cells; spa: sparse cells. *Other abbreviations:* BLAa: basolateral amygdalar nucleus, anterior part; BLAv: basolateral amygdalar nucleus, ventral part; BMAa: basomedial amygdalar nucleus, anterior part; BMAp: basomedial amygdalar nucleus, posterior part; CEAl: central amygdalar nucleus, lateral part; COAPm2: cortical amygdalar area, posterior part, medial zone; EPv: endopiriform nucleus ventral part; IA: intercalated amygdalar nucleus; NLOT: nucleus of the lateral olfactory tract

Gene name	CEA	MEA	LA	BMA	BLA	COA	PA
Actn1	+			+ +	+	+ +	
Actn4	(+)	+	+	+	(+)	+ +	
Calb1					+ spa		
Calb2							+ + +
Calm1	+	+	+	+	+	+ + +	
Calm2	+	+	+	+	+	+ + +	
Calm3				+	+	+ +	
Capn2	(+)	(+)	(+)	(+)	(+)	(+)	
Capn 8	(+)	(+)	(+)	(+)	(+)	(+)	
Capns1	+	+	+	+	+	+	
Cbara1						+	
Cetn2	+	+	+	+	+	+	
Cetn3						+	
Cgref1				+		+	
Dgkb	+	+	+	+	+	+	
Dgkg	+	+	+	+	+	+	
Drp2	+	+	+	+	+	+	
Dtna	+	+	+	+	+	+	
Dtnb	+	+	+	+	+	+	
Efcab1	(+)	(+)	(+)	(+)	(+)	(+)	
Efhd2	+	+	+	+	+ +	+	
Ehd3	+ +				+ + BMAp		
Eps15					+ + BMAp		
Eps15l1	+	+	+	+	+	+	
Fstl1					+		
Fstl4					+		
Fstl5					+		
Gpd2				+		+	
Guca1b	+	+	+	+	+	+	
Hpca	+	+	+	+	+	+	
Hpcal1				+		+	
Hpcal4	+	+	+	+	+	+	+
Itsn1				+	+	+	+
Kcnip1	sca.	sca.	sca.	sca.	sca.	sca.	spa.
Kcnip2	+	+	+	+	+	+	+
Kcnip3	+	+	+	+	+	+	+
Lcp1					(+)		
Letm1	+	+	+	+	+	+	+
Lpcat2				+		+	
Macf1	+	+	+	+	+	+	+
Mcfd2	+	+	+	+	+	+	+
Myl1	+	+	+	+	+	+	+
Myl4			+		+ BLAv, BMAp, EPv		
Myl6	+	+	+	+	+	+	+
Ncald	+	+	+	+	+ +	+	+
Ncs1	+	+	+	+	+	+	+
Necab1				+ + BMAa	+ BLAa		
Necab2						+ +	+ +
Necab3					+	+	
Nin	+	+	+	+	+	+	+
Nucb1					+		
Nucb2					+	+	
P4htm	+	+	+	+	+	+	+
Pdcd6					+		
Plcg1	+	+	+	+	+	+	+
Plch2				+	+		
Plcz1						+	

(*continued on next page*)

Table 7 (*continued*)

Gene name	CEA	MEA	LA	BMA	BLA	COA	PA
Pls3				+ BMAp	+ BLAa		+
Ppp2r3c	+	+	+	+	+	+	+
Ppp2r3d	+	+	+	+	+	+	+
Ppp3r1	+	+	+	+	+	+	+
Prkcsh	+	+	+	+	+	+	+
Pvalb	(+)	+	+	+	+	+	+
Rab11fip3	+	+	+	+	+	+	+
Rab11fip4				+			
Rasgrp1	+	+	+	+ +	+	+	+
Rcn1		+ +				+ + + COApm2	
Rcn2	+	+	+	+	+	+	+
Reps1				+	+	+	+
Reps2	(+)	(+)	(+)	+	+ +	+	+
Rhbdl3	+	+	+	+	+	+	+
Rhot1				+	+	(+)	(+)
Rhot2					+ BLAp		
Ryr1					+	(+)	
Ryr2	+	+	+	+	+	+	+
S100a10					+		
S100a11	+	+	+	+	+	+	+
S100a16	(+)	(+)	(+)	(+)	(+)	(+)	(+)
S100b	+ CEAl, IA			+ BMA			
Scgn	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Sdf4	+	+	+	+	+	+	+
Slc25a12	+	+	+	+	+	+	+
Slc25a23	+	+	+	+	+	+	+
Slc25a25	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Smoc1						+ COApm2/3, PAA	
Smoc2						+ PAA	
Sparc	+	+	+	+	+	+	+
Sparcl1	+	+	+	+	+	+	+
Spna2	+	+	+	+	+	+	+
Spock1	+ +	+		+ +	+ +	+ +	NLOT
Spock2	ubi	ubi	ubi	ubi	ubi	ubi	ubi
Spock3	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Sri	spa	spa	spa	spa	spa	spa	spa
Stim1	+	+	+	+	+	+	+
Stim2	+	+	+	+	+	+	+
Tesc	+	+	+	+	+	+	+
Usp32	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Utrn	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Vsnl1	(+)	(+)	(+)	(+)	(+)	(+)	(+)
1500003O03Rik	+	+	+	+	+	+	+
4732418C07Rik	+	+	+	+	+	+	+

(nucleus of the solitary tract) (Fig. 8F). A weak immunostaining staining is also observed in the cerebellum although ABA ISH images show no mRNA expression above the detection level (not shown).

Ncald protein expression is more widespread (Figs. 6, 7, 9 and 10), including the main olfactory bulb (strong in glomerula layer of the olfactory bulb (gl) and granular layer of the olfactory bulb (gr), sparse cells in mitral cells layer, olfactory bulb (mi) and outer plexiform layer, olfactory bulb (opl)) (Figs. 9A and 10), the AON (Fig. 9B), the cerebral cortex (Fig. 9C), the striatum (strong in lateral septal nucleus, septohippocampal nucleus, few isolated cells in nucleus accumbens, caudoputamen and medial septal nucleus) (Fig. 9C), the thalamus (widespread thalamic expression, stronger in medial habenula, paraventricular nucleus, antero-dorsal and antero-ventral nuclei of the thalamus) (Fig. 9D), the hypothalamus (ubiquitous), the hippocampus (mainly CA3 sp and DG sg, strong in the Fasciola cinerea) (Figs. 6A and 7A, 9E), the midbrain (sparse cells throughout, stronger in the dorsal raphé nucleus) (Fig. 9K), the pons (sparse cells throughout, stronger in the LC and tegmental reticular nucleus (TRN)) (Fig. 9L), the medulla (weak staining in sparse cells throughout, stronger in the nucleus of the solitary tract), the cerebellum (granular layer) (Fig. 9I). We detected no protein in the reticular thalamic nucleus, although the mRNA is present according to ABA. From co-immunostaining experiments in the MOB with Ncald and the three classical EF-CaBPs, we obtained the following data (Fig. 10): (1) In the outer plexiform layer: co-expression of Ncald in all GABAergic interneurons expressing Parv. In addition, Ncald also stains a significant population of Parv-negative cells in the opl.

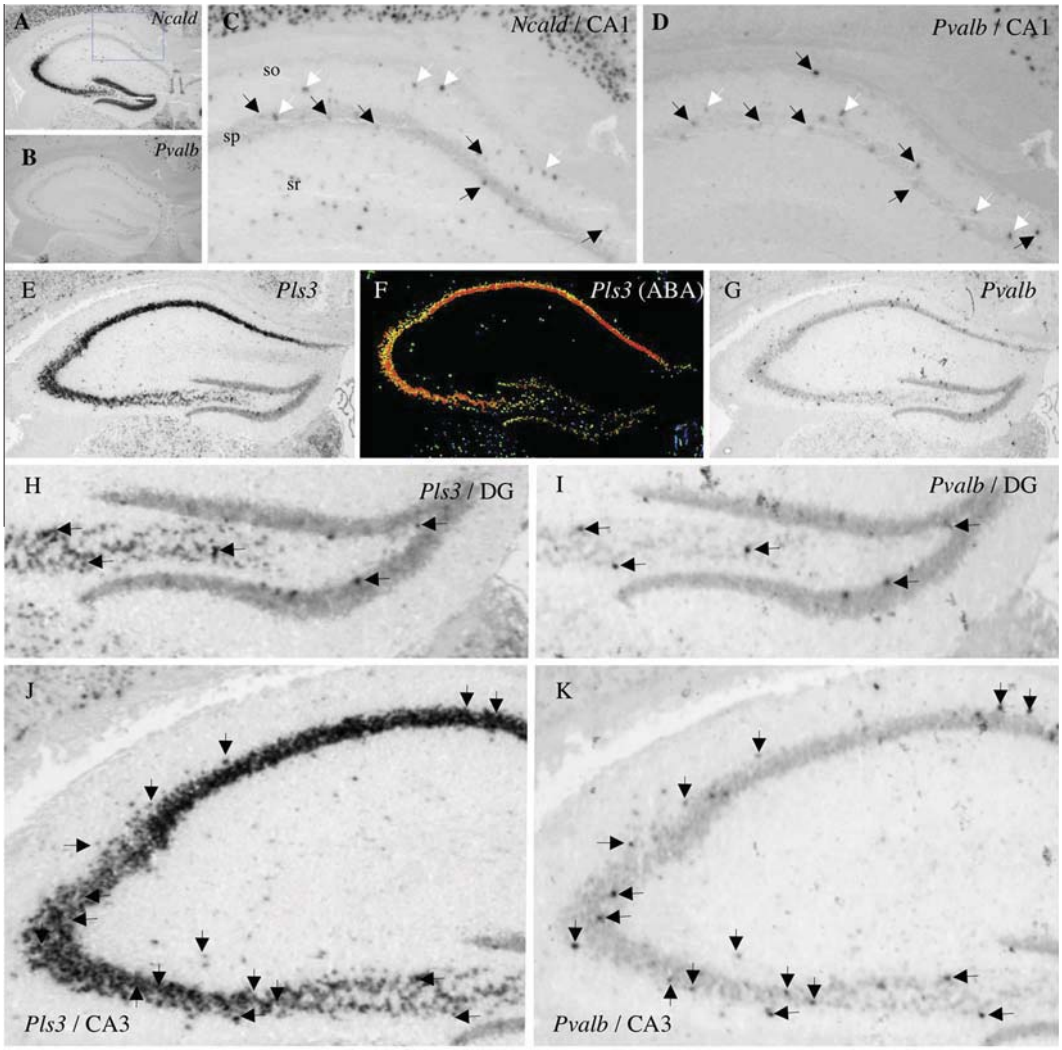

Fig. 6. Co-expression of *Ncald* and *Pls3* with *Pvalb* in the hippocampus. (A–D) *In-situ* hybridization to *Ncald* (A, C) and *Pvalb* (B, D) on adjacent coronal mouse brain sections. Panels C to D are higher magnifications of panels A and B, respectively. Black arrows point to neurons co-expressing both genes in CA1sp and so. Some cells (white arrows) are only *Pvalb*, respectively *Ncald* positive. (E–K) *In situ* hybridization results for *Pls3* (E, H, J) and *Pvalb* (G, I, K) on adjacent coronal mouse brain sections. Panel F shows *Pls3* ISH image taken from the ABA. Panels H–K are higher magnifications of panels E and G. Arrows point to neurons co-expressing both genes, in dentate gyrus sg (granular cell layer) and po (polymorph cell layer), and in the pyramidal layer of regions CA1/2/3.

Ncald also co-exists with CalR in the opl. (2) In the glomerular layer: Ncald-immunoreactive neurons are mostly CalR-positive and Calb-D28K-negative. (3) In the mitral and granular layers: little or no coexistence with CalR and Ncald can be observed. Analyzing co-expression with these three EF-hand proteins in the cerebral cortex, we found no co-expression of Hpcal4 with Parv and CalR, whereas Hpcal4 is expressed in Calb-D28K immunoreactive neurons in the upper cortical layers (2/3), but not in scattered neurons in the lower layers (5/6). For Ncald, we did not observe co-expression with any of the three markers in the cerebral cortex (data not shown).

Expression pattern of Tescalcin

Tescalcin, a member of the calcineurin homologous protein family, is expressed both in embryonic and adult mouse (Guttierez-Ford et al., 2003; Bao et al., 2009).

Based on ABA ISH images, the *Tesc* mRNA is expressed in the following brain areas (see also the different Tables): olfactory bulb (weak expression in gr layer); cerebral cortex (layers 2/5/6); striatum; strong expression in the hippocampus; little or no expression in the HY, excepting the suprachiasmatic nucleus (SCH) and sparse cells in the zona incerta (ZI); thalamus (strong in medial habenula (MH), weaker in anterodorsal thalamic nucleus (AD)); midbrain (moderate to strong expression in red nucleus (RN), oculomotor nucleus (III), midbrain trigeminal nucleus (MEV), weaker in inferior colliculus (IC) and dorsal raphé nucleus (DR)); pons (strong expression in motor nucleus of trigeminal nerve (V), PRN, TRN, weaker in pontine gray (PG), nucleus of the lateral lemniscus (NLL), nucleus of the trapezoid body (NTB) and principal sensory nucleus of the trigeminal (PSV)); medulla (expression scattered throughout the medulla, stronger in several nuclei including abducens nucleus (VI), facial motor nucleus (VII), hypoglossal nucleus (XII), nucleus

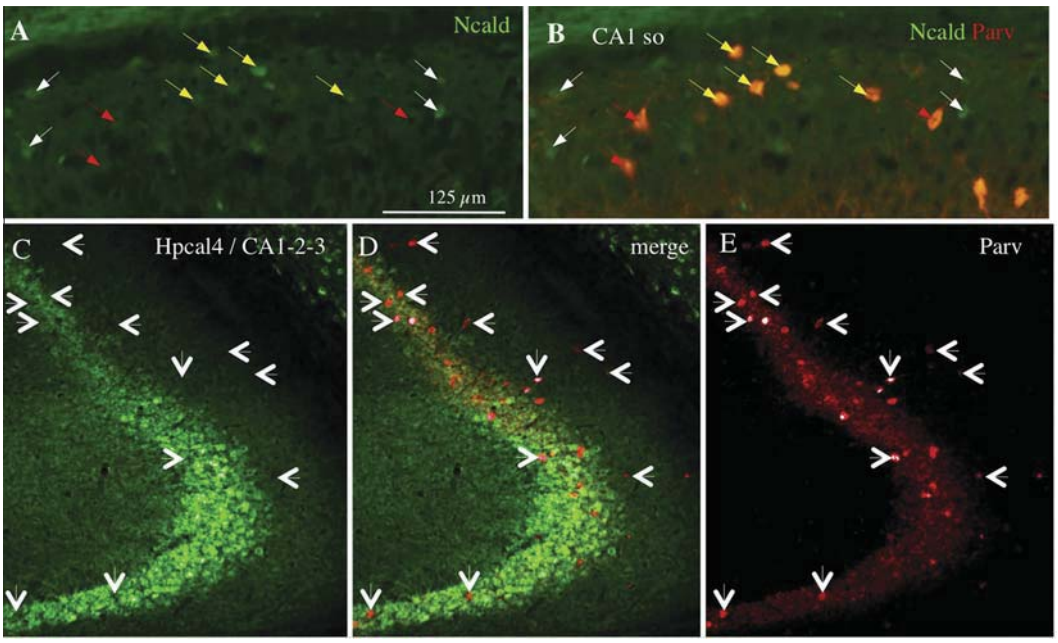

Fig. 7. Ncald protein, but not Hpcal4, is co-expressed in Parv-immunoreactive neurons in the hippocampus. (A–B) Ncald immunoreactivity in the hippocampus. Co-expression with Parv is visible in some neurons in the pyramidal and oriens layers (so) of the CA1 (yellow arrows), while some other cells are only Parv (red arrows), respectively Ncald positive (white arrows). Panel (B) is a merged image with both Ncald (green) and Parv (red) immunostainings. (C–E) Hpcal4 (C) and Parv (E) immunostaining in the CA1/2/3 regions. Hpcal4 is not detected in Parv immunoreactive neurons (marked with arrows), both in the pyramidal layer and stratum oriens. Panel E is the merge image. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ambiguus (AMB), lateral reticular nucleus (LRN)); cerebellum (gr and Pu layers). At the protein level, similar expression patterns were observed, in particular the areas of stronger expression sites, including the striatum, hippocampus, medial habenula and several well-defined nuclei in the midbrain, pons and medulla (MEV, NTB, V, VII) (Fig. 11).

DISCUSSION

Attempts to understand the structural complexity of the brain by a systematic ordering of its manifold neuronal populations have involved the instrumentation of several, mostly independent, criteria, such as cell morphology, cellular connectivity, electrophysiological properties and the expression of specific markers. Implementation of the latter system of categorization led, for example, to the identification of glutamatergic and GABAergic interneurons on the basis of their expression of the eponymous neurotransmitters (Seguela et al., 1984; Storm-Mathisen and Ottersen, 1987; Somogyi and Klausberger, 2005; DeFelipe et al., 2013). The neuropeptides (e.g., cholecystokinin, VIP and somatostatin) represent another group of successful markers. However, in some instances, detectable levels of the peptide in the cell body cannot be achieved other than by a colchicine-induced inhibition of its cytoplasmic transport. An exception worthy of note is the peptide hypocretin/orexin (Peyron et al., 1998), which is exclusively expressed in a circumscribed hypothalamic neuronal population. Antibodies against hypocretin/orexin have been employed not only to elucidate the morphological features of this sub-population of neurons but also to study their connectivity and hence putative function within the neuronal ensemble.

In the Introduction section, four members of the superfamily of EF-CaBPs were named as being useful neuronal markers [Calb-D28K, CalR, Parv (Baimbridge et al., 1992; Schwaller, 2009, 2010) and Scgn (Alpar et al., 2012)]. This attribute partially reflects their high cellular concentration and preferentially cytosolic distribution. Immunohistochemical staining after treatment with antibodies against these CaBPs has permitted a delineation of the soma, the dendrites, the axon and the endings of the targeted cells. The use of antibodies against these four EF-CaBPs has been helpful in revealing (1) specific subdivisions of the brain [e.g., the patch-matrix mosaic in the basal ganglia using antibodies against Calb-D-28k (Gerfen et al., 1987)] and the extended amygdala using anti-Scgn antibodies (Mulder et al., 2010)]; (2) new'' brain nuclei, such as the hypothalamic Parv-immunoreactive PV1/Foxb1-nucleus (Celio, 1990; Girard et al., 2011; Meszar et al., 2012; Bilella et al., 2014)]; and (3) a rare and ''new'' type of disinhibitory interneurons using CalR antibodies (Gulyas et al., 1992)]. These four EF-CaBPs alone have had an important impact on neuroscience, and we suspected that the EF-hand family contains other ''rare pearls'' and that it is under-represented among the accepted neuronal markers. For example, the S100A6 protein (also called calcyclin) was identified very recently as a specific marker for neural stem cells and astrocyte precursors in the hippocampus (Yamada and Jinno, 2014). It was for this reason that the meticulous, data-mining analysis of the *in-situ*-hybridization images in the ABA was undertaken. Our primary goal was to establish a repertoire of

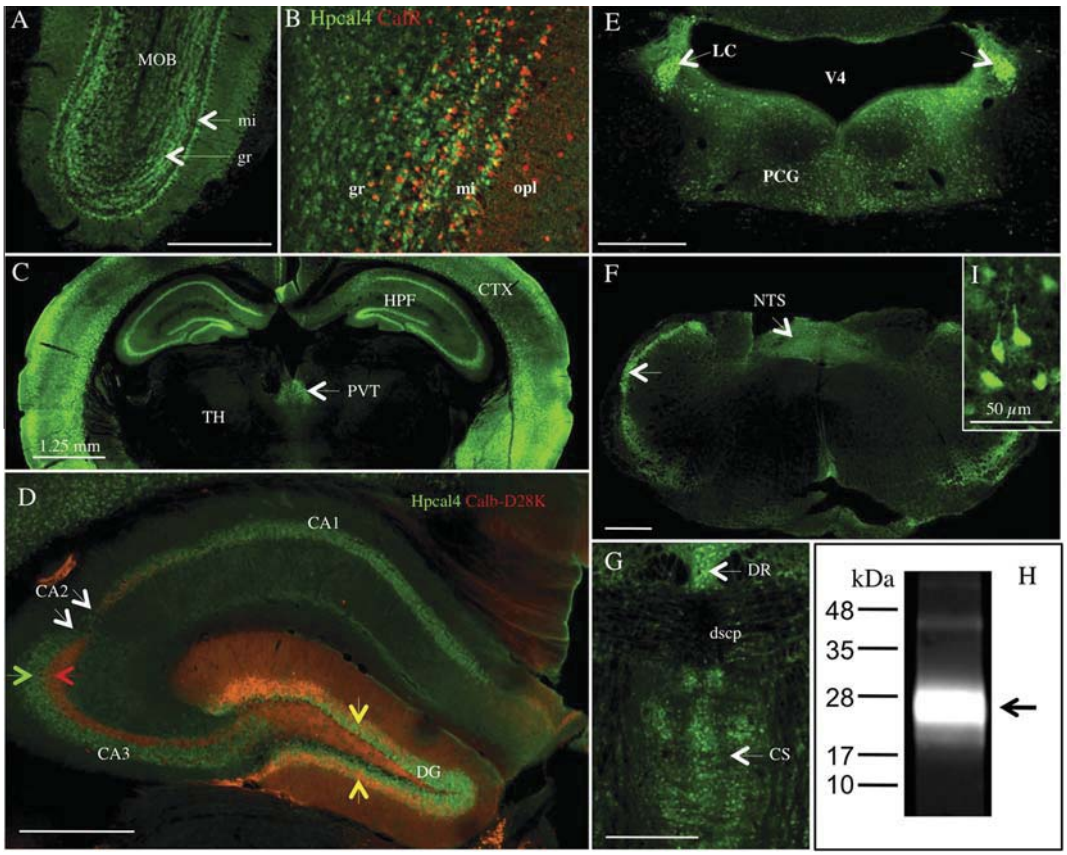

Fig. 8. Hpcal4 protein expression in the mouse brain. (A) Hpcal4 staining is detected in the main olfactory bulb (MOB), both in mitral (mi) and granular (gr) layers. (B) Note that Hpcal4 immunoreactivity is detected mostly in CalR-negative neuronal populations, but also exists in some CalR-positive ones. (C) Staining is also detected in the cerebral cortex (CTX) (stronger in layers 2/3 and 5/6), in the paraventricular nucleus of the thalamus (PVT) and the hippocampus (HPF). (D) Double immunostaining for Calb-D28K (red) and Hpcal4 (green). A large contingent of granule cells of the DG expresses both proteins (yellow arrows). Notice that Calb-D28K immunoreactivity is of much lower intensity in CA1 compared to DG. Red arrow points to Calb-D28K immunoreactivity in axonal projections from cell bodies localized in DG, while Hpcal4 antibody stains CA3 pyramidal cell bodies (green arrow). White arrows delimitate the CA2 region, highlighting a fainter Hpcal4 staining. (E) The locus coeruleus (LC) expresses a strong Hpcal4 signal. Lower staining intensity can be detected in the pontine central gray (PCG; E), the nucleus of the solitary tract (NTS; F), the dorsal raphé nucleus (DR; G), the superior central raphé nucleus (CS; G). In panel F, arrow points to staining in the lateral-most region of the spinal nucleus of the trigeminus, interpolar part (SPVII). In panel I, higher magnification of PAG neurons shows that Hpcal4 immunoreactivity can be detected both in the soma and dendrites. (H) Anti-Hpcal4 antiserum detects a band at 25 kDa in mouse brain extract. Scale bar = 500 μm, unless differently specified. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

expression profiles in the murine brain for the genes encoding the EF-CaBPs, with a view to affording a basis for more specialized experimental investigations. Our second goal was to ascertain which of the 249 EF-CaBPs might serve as putative neuronal (or glial) markers. Actually, notwithstanding the suggestivity of the data, the mRNA-expression profile of a particular gene as reported in the ABA is no guarantee that an antibody against the corresponding encoded protein will be a useful neuro-anatomical tool. *In-situ*-hybridization yields a signal that is confined to the cell bodies; it elicits no information appertaining to the precise expression profile of the protein within a neuron. Moreover, one cannot discount regulation at the translational and/or post-translational levels. Consequently, *in-situ*-hybridization signals do not necessarily correspond to the protein-expression levels. This is the case with S100A6; although the protein is detected by immunostaining in the neuronal precursors of the subgranular zone of the DG (Yamada and Jinno, 2014), no ISH signal is documented in the ABA. Discrepancies might also exist for genes that encode various extracellular-matrix proteins, for example *Smoc1 and 2, Sparc, Sparcl*, *Spock1, 2 and 3*. Although the cell bodies are revealed by ISH, the proteins themselves infiltrate the extracellular space, partially accounting for the perineuronal net around distant neurons, such as was shown for tenascin-R (Wintergerst et al., 2001). As is revealed in Table 2, most of the EF-CaBP-encoding genes are expressed in numerous areas, mainly in neurons but sometimes also in glial cells (*S100b, S100A16, 4732418C07Rik*), choroid plexus (*Actn1*) or meninges (*Sparc*). Those that are ubiquitously expressed can be distinguished from those that manifest a more restricted and complex pattern of expression, with elevated levels in only a few, well-defined areas. The latter members are considered to be the most promising marker candidates (Table 2, 3d column). Notable among these, are neurocalcin-δ, hippocalcin-like 4 or tescalcin. For these members, the protein-expression patterns were analyzed immunohistochemically. On the basis of the cytoplasmic staining patterns that were thereby revealed, antisera against these three EF-CaBPs might indeed prove to be

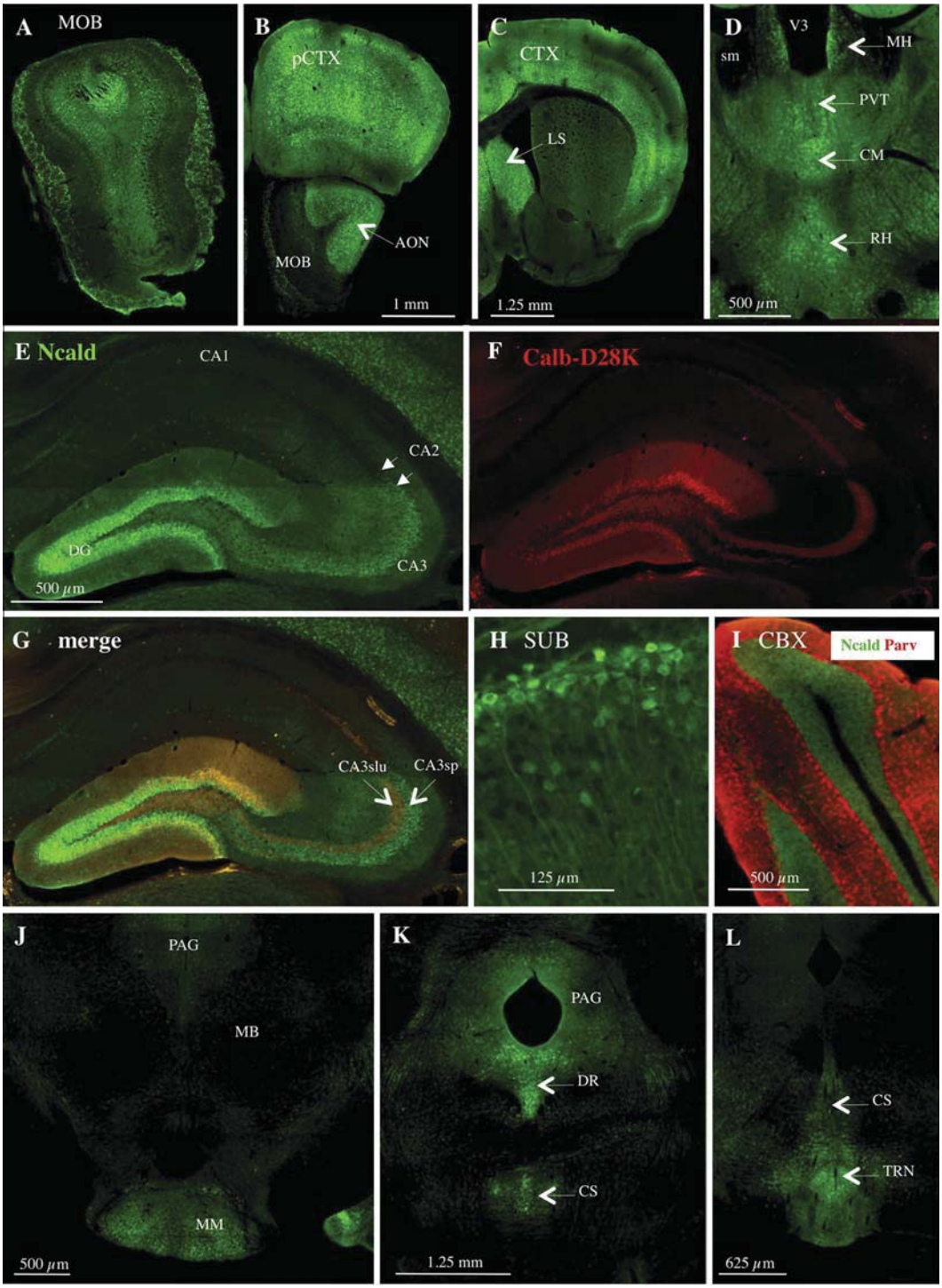

Fig. 9. Ncald protein expression in the mouse brain. (A) Ncald staining of moderate intensity is detected in the main olfactory bulb (MOB). (B) Staining in CTX layers 2/3 (pCTX: prefrontal cortex) and anterior olfactory nucleus (AON). (C) Labeling of the lateral septal nucleus (LS) and absence of labeling in the upper layers of the CTX. (D) Thalamus with labeling in the medial habenula (MH), paraventricular nucleus (PVT), central medial nucleus of the thalamus (CM) and rhomboid nucleus (RH). (E) Hippocampus with strong labeling in DG granular layer and CA3 pyramidal layer, low intensity in CA2 (delimitated by white arrows), and present only in very few cells in CA1 (stratum oriens layer). (F) Same section as in (E), but incubated with an antibody to Calb-D28K (red). Panel G is the merged image. Ncald-immunoreactivity is detected in cell bodies in the pyramidal cell layer of CA3, but also in neuronal projections co-stained with Calb-D28K in the stratum lucidum (slu) (arrows in panel G). Note that a large number of granular cells in the dentate gyrus express both proteins. (H) Staining in the subiculun (SUB), highlighting Ncald immunoreactivity both in cell bodies and neuronal processes. (I) double immunostaining for Parv (red) and Ncald (green) in the cerebellum, expressed respectively in the molecular and granular cells layer. (J) staining in the hypothalamus with mammillary nucleus (MM) highlighted. (K) staining in the midbrain (MB) highlighting the periaqueductal gray (PAG), the dorsal raphé nucleus (DR) and the superior central raphé-nucleus (CS). (L) Weak-to-moderate labeling of the tegmental reticular nucleus (TRN). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

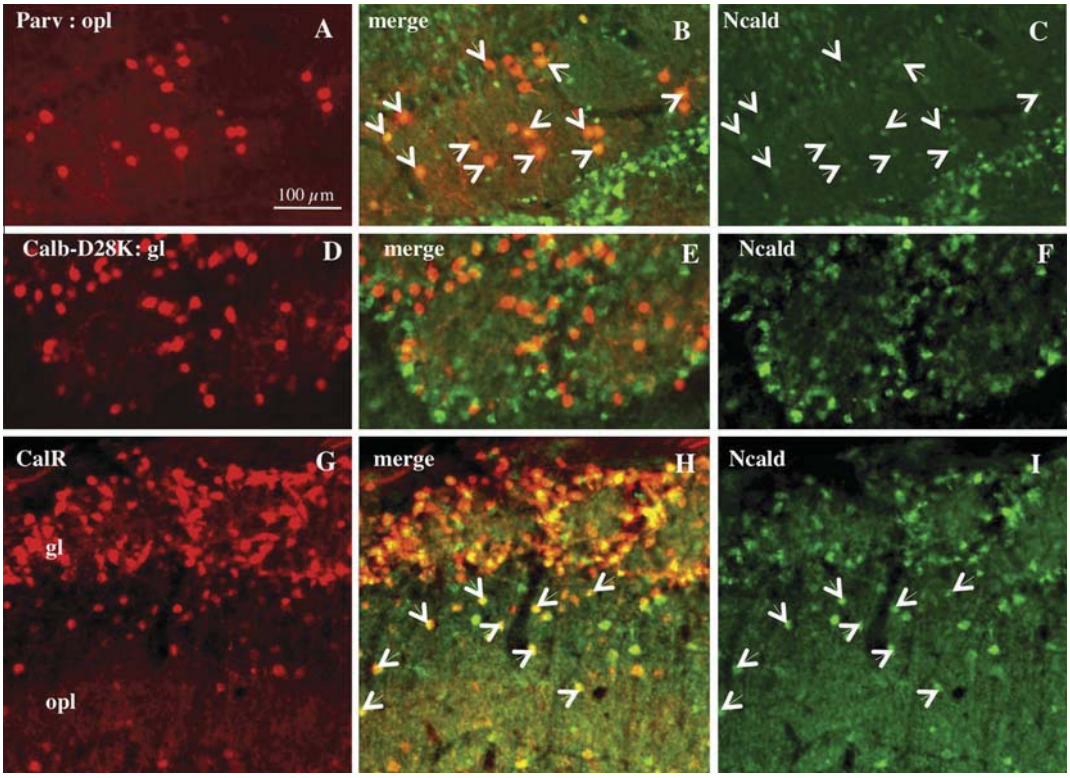

Fig. 10. Ncald immunoreactivity in the main olfactory bulb. Co-immunostaining experiments in the main olfactory bulb for Ncald (green) (C, F, I) and each of the three EF-CaBPs (red): Parv (A), Calb-D28K (D) and CalR (G). Central panels (B, E, H) are merged images. Arrows in panels (B) and (C) highlight example neurons immunoreactive for both Ncald and Parv in the outer plexiform layer (opl). Arrows in panels (H) and (I) highlight example neurons immunoreactive for both Ncald and CalR in the opl. Notice that in both cases, a significant proportion of Ncald-positive neurons are negative for Parv or CalR. In the glomerular layer (gl), Ncald immunoreactivity pattern mostly overlaps with CalR (H), but not Calb-D28K (E). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

useful for the tagging of specific neuronal populations. Hpcal4, for example, is expressed in the cells of several brain nuclei that are involved in the control of sleep–wake cycles, including DR, LC, VTA, pontine central gray (PCG) and hypothalamus (HY) (Brown et al., 2012). Tesc shows strong expression in several brainstem nuclei known to be involved in whisker circuits in rodents (trigeminal nuclei MEV, V; facial motor nucleus) (Sehara and Kawasaki, 2011; Petersen, 2014).

The mitral cells in the OB, the CA1-pyramidal cells of the hippocampus and the cerebellar Purkinje cells, were revealed to express between 80 (mitral- and Purkinje-cells) and 100 (pyramidal cells) different EF-hand genes thereby indicating that their functional competence greatly depends upon Ca^{2+}. The same may hold true also for interneurons. However, owing to their heterogeneity and scattered distribution, the study of these cells is more complex; for example in the Stratum oriens of the hippocampus - a region that is known to be populated exclusively by interneurons -, scattered cells express 51 different EF-CaBP-encoding genes, which are probably localized in different subpopulations (Table 6). The same genes are often expressed in interneurons that are lodged in the periglomerular layers of the OB (16 genes), in the external plexiform layer of the OB (five genes) and, in the molecular layer of the CB (six genes). Particularly promising will be the comparison with the four classical markers Calb-D28K, CalR, Parv, Scgn with the expression of Capns1, Eps15, Ncs1, Necab2, Sparc, Spock1/2/3, Vsnl1 and Zzef1 proteins, which are found in various combinations in the aforementioned interneuron-rich regions. In the cerebral cortex, all genes that are expressed in sparse/scattered cells in several or all of the layers may be markers for interneurons (48 different genes). *Sparcl1* may be a new marker of Cajal-Retzius cells in layer 1 and *Tnnc1* is one of the only neuronal EF-CaBP-encoding gene that is enriched in layer 4 neurons of the somatosensory barrel cortex. Single layer markers include *Cabp1* for layer 2 and 5a whereas *Sparcl1* is expressed in all layers except number 4.

Some genes selectively reveal specific cells, nuclei or layers in the brain, which affords the unique opportunity of investigating their development and functions by genetic targeting and manipulation. For example, *Nkd2* occurs only in CA3-pyramidal cells and in layer-2 cells of the piriform cortex. *Myl* 4 is expressed in layers 5 and 6 of the isocortex, in the amygdala and in the ventral pallidum. *Necab3* is strongly expressed in the lateral habenula (LH) and in the upper and the lower isocortical layers. *Pls3* occurs specifically in the endopiriform nucleus (END) and in the claustrum. *Rcn1* is expressed specifically in the medial-posterodorsal and in layer 2 of the cortical posterior amygdalar nucleus; it also occurs in the Edinger–Westphal nucleus as already shown by

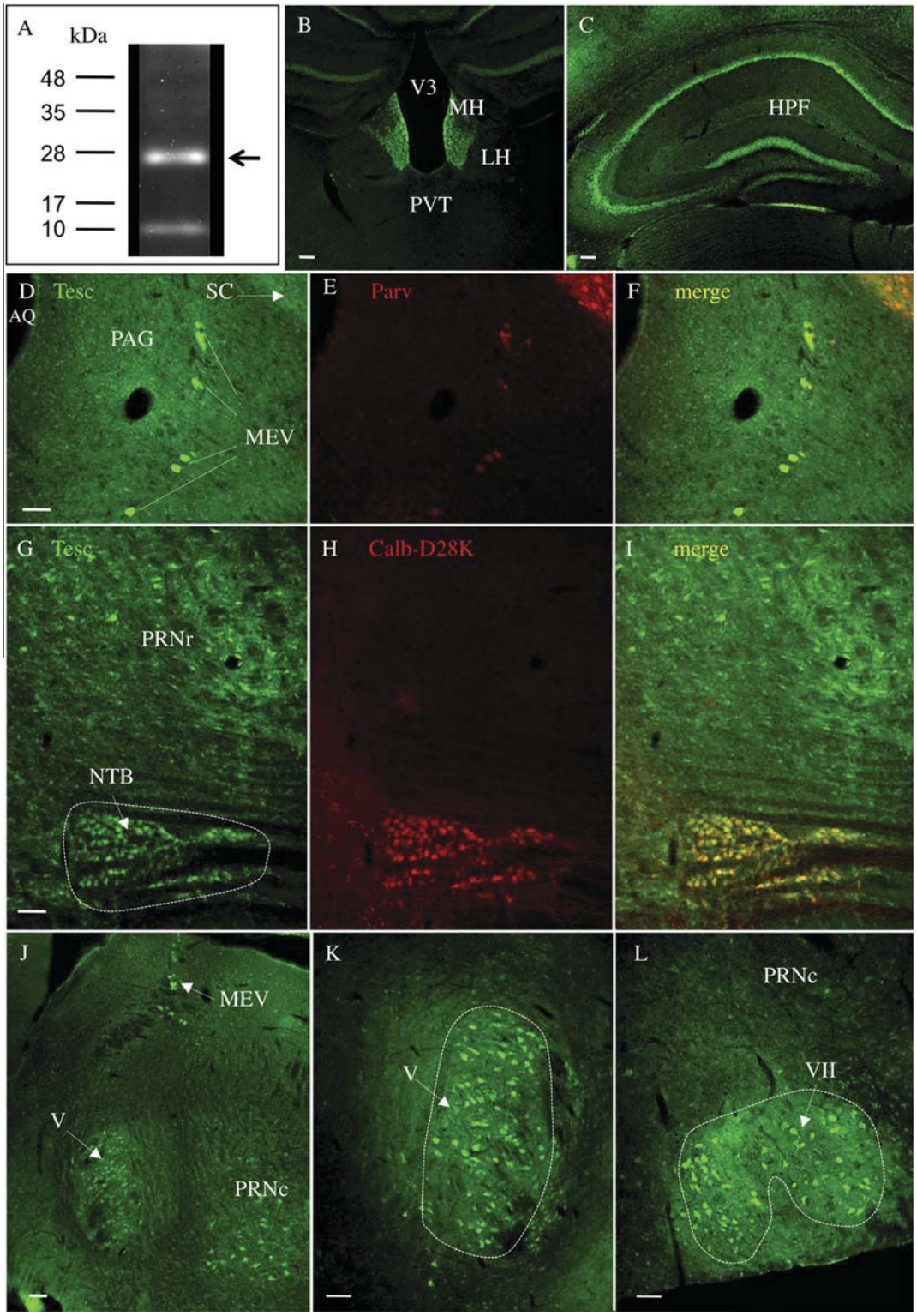

Fig. 11. Tesc protein expression in the mouse brain. (A) Immunoblotting, showing the specificity of the anti-Tesc immunoserum for 25-kDa tescalcin protein (arrow) in mouse brain extracts. (B–L) Immunostaining experiments highlighting some preferential sites of Tesc immunoreactivity. (B) Medial habenula (MH); (C) hippocampus (HPF); (D, J) trigeminal nucleus (MEV) (notice the co-expression with Parv, Panels (D–(E)); (G) nucleus of the trapezoid body (NTB) (notice the co-expression with Calb-D28K, Panels (G)–(I)); (J, K) motor nucleus of the trigeminal (V); (L) facial motor nucleus (VII). *Other abbreviations:* V3: third ventricle; SC: superior colliculus; PAG: periaqueductal gray; PRNr/c (pontine reticular nucleus rostral/caudal part); PVT: paraventricular nucleus of the thalamus. Scale bar = 100 μm.

others (Giardino et al., 2012). *Fstl5* is expressed only in the sparse population of neurons in the CA3 region [but see also (Masuda et al., 2014)], and the SCH expresses two genes at high levels (*Nkd1* and *Plch2*).

A remarkable continuous, single layer of cells expressing the six EF-hand genes *Drp2, Rhbdl3, Rcvrn, Ryr2, Sdf4* and *Sri*, is aligned in parallel to the Purkinje cell layer at the edge of the granular layer, close to the white matter. To our knowledge such cells have not been reported in the recent literature [but see (Golgi, 1903; Cajal, 1955)] and a clarification of their identity must await the generation of specific antibodies and the performance of detailed immunohistochemical studies.

In conclusion, this study reports on a meticulous analysis of the mRNA-expression profiles of the EF-CaBP-encoding genes in the murine brain, which was based on an evaluation of the *in-situ*-hybridization images in the ABA. The purpose of the study was to identify potentially useful markers for specific neurons, as well as for specific brain nuclei, areas and layers, and possibly also for specific functional systems. Our findings afford an insight into the organizational complexity of the EF-CaBPs, which act as intracellular Ca^{2+}-sensors or buffers and translate short-lived changes in the intracellular Ca^{2+}-concentration into meaningful cellular messages. Several investigations have addressed the potential roles of EF-CaBPs other than Calb-D28K, CalR and Parv in neuropathologies like Alzheimer's disease [Vsnl1 and Hpcal1 (Braunewell, 2012; Plc: Popovics and Stewart, 2012); Ryanodine receptors (Del Prete et al., 2014); S100a9 (Chang et al., 2012)], schizophrenia [Cabp1 (Bernstein et al., 2007)] or traumatic brain injury [neuronal calpains (Saatman et al., 2010; Liu et al., 2014)]. Thus, collecting data on this family of Ca^{2+}-binding proteins might also be medically relevant, because of their crucial roles in regulating Ca^{2+} homeostasis, a process perturbed in several neurological and psychiatric diseases.

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