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The rat brain hippocampus proteome

Michael Fountoulakis [a, b, *], George T. Tsangaris [b], Antony Maris [b], Gert Lubec [c]

[a] *F. Hoffmann-La Roche Ltd., Center for Medical Genomics, Basel, Switzerland*
[b] *Foundation for Biomedical Research of the Academy of Athens, Center of Basic Research, Soranou Ephessius 4, 11527 Athens, Greece*
[c] *University of Vienna, Medical Faculty, Department of Pediatrics, Vienna, Austria*

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Abstract

The hippocampus is crucial in memory storage and retrieval and plays an important role in stress response. In humans, the CA1 area of hippocampus is one of the first brain areas to display pathology in Alzheimer's disease. A comprehensive analysis of the hippocampus proteome has not been accomplished yet. We applied proteomics technologies to construct a two-dimensional database for rat brain hippocampus proteins. Hippocampus samples from eight months old animals were analyzed by two-dimensional electrophoresis and the proteins were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The database comprises 148 different gene products, which are in the majority enzymes, structural proteins and heat shock proteins. It also includes 39 neuron specific gene products. The database may be useful in animal model studies of neurological disorders.
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1. Introduction

Proteomics finds a wide application in neuroscience nowadays [1–4]. It has mainly been used for protein screening in brain tissue in healthy and diseased states for the detection of drug targets and diagnostic markers [5–11]. Proteomics is the ideal tool for studying protein–protein interactions and post-translational modifications. Moreover, it has been applied in the generation of two-dimensional (2D) protein databases which are essential in the quantification of alterations in the protein levels resulting from the various disorders or the effect of external factors [2,12–16]. Previously, neuroproteomics has mainly been applied to the analysis of total brain tissue [17,18]. Such an analysis provides us with a general pattern of the proteins expressed in all brain regions. Detection of proteins involved in certain disorders demands the analysis of specific brain regions, the preparation of subfractions and the isolation of organelles, each containing a lesser number of total proteins [4,19].

The hippocampus is a cytoarhitecturally distinct structure folded into the cerebral cortex. It has been shown to be involved in the integration of information arriving from different sensory organs and associated areas and is essential for memory storage and retrieval, playing an important role in declarative memory. It has a high abundance of glucocorticoid receptors and through their actions it serves as an integral part of the feedback loop responsible for terminating glucocorticoid release during stress response [20]. In humans, the CA1 area of hippocampus is one of the first brain areas to display pathology in Alzheimer's disease [21]. Furthermore, magnetic resonance imaging (MRI) studies in humans have demonstrated hippocampal atrophy in certain diseases such as dementias, recurrent major depression and Cushing's disease [22]. There exist several proteomics studies for rat hippocampus [23,24]. Here we report a detailed two-dimensional database for the rat hippocampus proteome and the identification of the products of 148 different genes.

* Corresponding author. Tel.: +30 210 6597069; fax: +30 210 6597545.
E-mail address: mfountoulakis@bioacademy.gr (M. Fountoulakis).

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2. Experimental

2.1. Materials

Immobilized pH-gradient (IPG) strips and IPG buffers were purchased from Amersham Biosciences (Uppsala, Sweden). Acrylamide/piperazine-di-acrylamide (PDA) solution (37.5:1, w/v) was purchased from Biosolve Ltd. (Valkenswaard, The Netherlands) and the other reagents for the polyacrylamide gel preparation from Bio-Rad Laboratories (Hercules, CA, USA). CHAPS was obtained from Roche Diagnostics (Mannheim, Germany), urea from AppliChem (Darmstadt, Germany), thiourea from Fluka (Buchs, Switzerland), 1,4-dithioerythritol (DTE) and EDTA from Merck (Darmstadt, Germany) and tributylphosphine (TBP) from Pierce Biotechnology (Rockford, IL, USA). The reagents were kept at 4 °C. Brain samples were derived from four Sprague–Dawley eight months old rats.

The animals were killed by decapitation and the brains were rapidly dissected and the hippocampus removed. The tissue was immediately frozen in liquid nitrogen and stored at −80 °C until use. The experiments were performed in accordance with the regional legal regulations.

2.2. Two-dimensional gel electrophoresis

Hippocampus tissue (0.1 mg) was suspended in 0.5 ml of 20 mM Tris, 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM 1,4-dithioerythritol, 1 mM EDTA and a mixture of protease inhibitors (1 mM PMSF and 1 tablet Complete^{TM} (Roche Diagnostics) per 50 ml of suspension buffer) and phosphatase inhibitors (0.2 mM Na_2VO_3 and 1 mM NaF). The suspension was sonicated for approximately 30 s and centrifuged at 150 000 × g for 45 min. The protein content in the supernatant was determined using the Coomassie blue method [25]. The protein concentration was approximately 10 mg/ml.

Two-dimensional gel electrophoresis was performed as previously reported [26,27]. Briefly, samples were applied on immobilized pH 3–10 nonlinear gradient strips (18 cm). Focusing started at 200 V and the voltage was gradually increased to 5000 V at 3 V/min and kept constant for a further 6 h. The second-dimensional separation was performed in 12% SDS–polyacrylamide gels. The gels (180 mm × 200 mm × 1.5 mm) were run at 50 mA per gel, in an ETTAN apparatus (Amersham Biosciences). After protein fixation with 50% methanol, containing 5% phosphoric acid for 2 h, the gels were stained with colloidal Coomassie blue (Invitrogen, Paisley, Scotland) for 16 h. Excess of dye was washed from the gels with H_2O and the gels were scanned in an Agfa DUOSCAN densitometer (resolution, 400 dpi). The percentage of the spot(s) volume representing a certain protein was determined in comparison with the total proteins present in the 2D gel, using the ImageMaster software (Amersham Biosciences).

2.3. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS)

MALDI-TOF-MS analysis was essentially performed as described [4,28]. The spots were excised and destained with 30% acetonitrile in 50 mM ammonium bicarbonate and dried in a Speedvac evaporator. Each dried gel piece was rehydrated with 5 μl of 1 mM ammonium bicarbonate, containing 50 ng trypsin (Roche Diagnostics). After 16 h at room temperature, 20 μl of 50% acetonitrile, containing 0.3% trifluoroacetic acid were added to each gel piece and incubated for 15 min with constant shaking. The peptide mixture (1.5 μl) was simultaneously applied with the matrix solution (1 μl), consisting of 0.025% α-cyano-4-hydroxycinnamic acid (Sigma) and the standard peptides des-Arg-bradykinin (Sigma, St. Louis, MI, 904.4681 Da) and adrenocorticotropic hormone fragment 18–39 (Sigma, 2465.1989 Da) in 65% ethanol, 35% acetonitrile and 0.03% trifluoroacetic acid. The samples were analyzed in a time-of-flight mass spectrometer (Ultraflex, Bruker Daltonics, Bremen, Germany). Peptide matching and protein searches were performed automatically as described [29]. The peptide masses were compared with the theoretical peptide masses of all available proteins from all species. Monoisotopic masses were used and a mass tolerance of 0.0025% was allowed. Unmatched peptides or miscleavage sites were not considered for protein identification.

3. Results

3.1. Two-dimensional gel analysis

Hippocampus extracts from eight months old rats were separated by 2D electrophoresis and the protein spots were visualized following stain with colloidal Coomassie blue. Four samples were analyzed in duplicate. The protein profiles were similar with minor differences which were due to allelic differences and artifacts of the technology. Fig. 1 shows a representative example of the hippocampus proteins separated in a 2D gel, where 1 mg of total protein was applied. Approximately 2500 spots were counted in each 2D gel using the 2D ImageMaster software. The proteins were identified by MALDI-TOF-MS on the basis of peptide mass matching [30], following in-gel digestion with trypsin. The peptide masses were matched with the theoretical peptide masses of all proteins from all databases. Approximately 800 spots from two gels were analyzed. The analysis resulted in the identification of 148 different gene products (Table 1).

In Table 1, the SWISS-PROT accession numbers are listed as well as the abbreviated and full names of the proteins, the theoretical p*I* and MW values, as well as data from the mass spectrometry analysis, i.e. the numbers of matches and the protein amino acid sequence coverage by the matching peptides. The introduction of internal peptide standards to correct the measured masses allowed the use of very narrow windows of mass tolerance (0.0025%), increasing thus the confidence

Table 1
Rat brain hippocampus proteins

Number	Name	Full name	p*I*	MW	Matches	Sequence coverage (%)	Function	Subcellular location
O08709	AOP2_MOUSE	Antioxidant protein 2 (EC 1.11.1.7) (nonselenium glutathione peroxidase)	5.8	24837	5	25	Enzyme (redox regulation)	Cytoplasmic, lysosomal, lung secretory organelles
O15144	AR34_HUMAN	ARP2/3 complex 34 kDa subunit (p34-arc)	7.4	34425	5	20	Structural protein	Cytoskeleton
P01946	HBA_RAT	Hemoglobin alpha-1 and alpha-2 chains	8.0	15358	5	52	Oxygen transport	
P02091	HBB1_RAT	Hemoglobin b chain, major form	8.2	15952	7	53	Oxygen transport	
P02551	TBA1_RAT	Tubulin alpha-1 chain	4.8	50787	10	29	Structural protein	Microtubule
P02570	ACTB_RAT	Actin, cytoplasmic 1 (beta-actin)	5.2	42051	7	25	Cell motility	Cytoplasmic
P02571	ACTG_RAT	Actin, cytoplasmic 2 (gamma-actin)	5.3	42107	8	25	Cell motility	Cytoplasmic
P02650	APE_RAT	Apolipoprotein E precursor (APO-E)	5.1	35788	8	25	Mediates the binding, internalization, and catabolism of lipoprotein particles	Secreted
P02770	ALBU_RAT	Serum albumin precursor	6.4	70669	11	23	Regulation of the osmotic pressure of blood	Secreted
P04182	OAT_RAT	Ornithine aminotransferase (EC 2.6.1.13) (ornithine-oxo-acid aminotransferase)	7.0	48701	6	18	Enzyme	Mitochondrial matrix
P04642	LDHM_RAT	L-Lactate dehydrogenase m chain (EC 1.1.1.27)	8.3	36712	6	17	Enzyme	Cytoplasmic
P04691	TBB1_RAT	Tubulin beta chain (t beta-15)	4.6	50387	10	19	Structural protein	Microtubule
P04764	ENOA_RAT	Alpha enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase)	6.5	47297	9	25	Enzyme	Cytoplasmic
P04797	G3P_RAT	Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) (gapdh)	8.3	35967	5	19	Enzyme	Cytoplasmic
P04901	GBB1_MOUSE	Guanine nucleotide-binding protein g(i)/g(s)/g(t) beta subunit 1	5.9	38151	5	15	Signaling systems	
P04906	GTP_RAT	Glutathione *S*-transferase P (chain 7) (EC 2.5.1.18)	7.4	23521	5	35	Enzyme	Cytoplasmic
P05065	ALFA_RAT	Fructose-bisphosphate aldolase (EC 4.1.2.13) a (muscle)	8.1	39652	9	34	Enzyme	Cytoplasmic
P05197	EF2_RAT	Elongation factor 2 (ef-2)	6.8	96192	7	9	Promotes the GTP-dependent translocation	Cytoplasmic
P05218	TBB5_MOUSE	Tubulin b-5 chain	4.6	50095	11	29	Structural protein	Microtubule
P05370	G6PD_RAT	Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49) (g6pd)	6.3	59662	8	17	Enzyme	Cytoplasmic
P05708	HXK1_RAT	Hexokinase, type i (EC 2.7.1.1) (hk i) (brain form hexokinase)	6.6	103563	10	11	Enzyme	Outer mitochondrial membrane
P06761	GR78_RAT	78 kDa glucose regulated protein (immunoglobulin heavy chain binding protein)	4.9	72473	5	12	Facilitating assembly of protein complexes in ER	Endoplasmic reticulum lumen
P07323	ENOG_RAT	Gamma enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (2-phospho-D-glycerate hydro-lyase, neural enolase)	4.9	47379	10	27	Enzyme	Cytoplasmic

Table 1 (*Continued*)

Number	Name	Full name	p*I*	MW	Matches	Sequence coverage (%)	Function	Subcellular location
P07335	KCRB_RAT	Creatine kinase, b chain (EC 2.7.3.2) (b-ck)	5.4	42970	9	25	Enzyme	Cytoplasmic
P07632	SODC_RAT	Superoxide dismutase (Cu–Zn) (EC 1.15.1.1)	6.3	15941	5	38	Enzyme	Cytoplasmic
P08009	GTM3_RAT	Glutathione *S*-transferase yb3 (EC 2.5.1.18) (chain 4)	7.4	25704	5	26	Enzyme	Cytoplasmic
P08081	CLCA_RAT	Clathrin light chain a (brain and lymphocyte lca)	4.2	27078	4	13	Structural, membrane traffic	Cytoplasmic face of coated pits and vesicles
P08109	HS7C_RAT	Heat shock cognate 71 kDa protein	5.3	71055	8	17	Molecular chaperone	Cytoplasmic
P08113	ENPL_MOUSE	Endoplasmin (94 kDa glucose-regulated protein)	4.6	92703	10	11	Molecular chaperone	Endoplasmic reticulum lumen
P08129	PP1A_RAT	Serine/threonine protein phosphatase pp1-alpha 1 catalytic subunit	6.3	38229	9	32	Enzyme	Cytoplasmic
P08461	ODP2_RAT	Dihydrolipoamide acetyltransferase (e2) of pyruvate dehydrogenase (EC 2.3.1.12)	5.8	59126	9	19	Enzyme	Mitochondrial matrix
P09117	ALFC_RAT	Fructose-bisphosphate aldolase c (EC 4.1.2.13) (brain) (brain-type aldolase)	7.1	39527	8	30	Enzyme	Cytoplasmic
P09329	KPR1_RAT	Ribose-phosphate pyrophosphokinase i (EC 2.7.6.1)	7.0	35194	6	26	Enzyme	Cytoplasmic
P09606	GLNA_RAT	Glutamine synthetase (EC 6.3.1.2) (glutamate–ammonia ligase)	7.0	42981	6	20	Enzyme	Cytoplasmic
P10111	CYPH_RAT	Peptidyl-prolyl *cis–trans* isomerase A (EC 5.2.1.8)	8.2	17959	7	37	Enzyme	Cytoplasmic
P10719	ATPB_RAT	ATP synthase beta chain, mitochondrial precursor (EC 3.6.1.34)	5.1	56318	8	19	Enzyme	Mitochondrial
P10860	DHE3_RAT	Glutamate dehydrogenase precursor (EC 1.4.1.3)	8	61731	12	22	Enzyme	Mitochondrial matrix
P11598	ER60_RAT	Probable protein disulfide isomerase er-60 precursor (EC 5.3.4.1) (erp60)	6.1	57043	10	27	Enzyme	Endoplasmic reticulum lumen
P11980	KPY1_RAT	Pyruvate kinase, m1 (muscle) isozyme (EC 2.7.1.40)	7.0	58163	15	33	Enzyme	Cytoplasmic
P11981	KPY2_RAT	Pyruvate kinase, m2 isozyme (EC 2.7.1.40)	7.4	58183	11	27	Enzyme	Cytoplasmic
P12839	NFM_RAT	Neurofilament triplet M protein (160 kDa neurofilament protein) (NF-M)	4.6	95716	10	13	Maintenance of neuronal caliber	Cytoskeleton, nuclear envelope
P13221	AATC_RAT	Aspartate aminotransferase, cyt (EC 2.6.1.1) (glutamate oxaloacetate transaminase-1)	6.7	46396	7	19	Enzyme	Cytoplasmic
P13353	P2AA_RAT	Serine/threonine protein phosphatase pp2a-alpha, catalytic subunit (EC 3.1.3.16)	5.3	36155	6	28	Enzyme	Cytoplasmic
P13668	STHM_RAT	Stathmin (phosphoprotein p19)	5.9	17146	4	27	Pathways regulation	Cytoplasmic
P13795	SN25_RAT	Synaptosomal-associated protein 25 (snap-25) (super protein) (sup)	4.5	23528	4	26	Regulation of neurotransmitter release	Membrane
P14152	MDHC_MOUSE	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37)	6.5	36494	5	18	Enzyme	Cytoplasmic

Table 1 (*Continued*)

Number	Name	Full name	p*I*	MW	Matches	Sequence coverage (%)	Function	Subcellular location
P14668	ANX5_RAT	Annexin v (lipocortin v) (endonexin ii) (placental anticoagulant protein i)	4.8	35648	5	13	Anticoagulant protein	Placenta, membrane
P14669	ANX3_RAT	Annexin iii (lipocortin iii) (placental anticoagulant protein iii) (pap-iii)	6.4	36527	6	22	Phospholipase A2 inhibitor and anticoagulant protein	Placenta, membrane
P14701	TCTP_MOUSE	Translationally controllled tumor protein (TCTP) (p23) (p21)	4.6	19563	4	27		Cytoplasmic
P15999	ATPA_RAT	ATP synthase alpha chain, mitochondrial precursor (EC 3.6.1.34) (fragment)	10.0	58904	12	24	Enzyme	Mitochondrial inner membrane
P16086	SPCN_RAT	Spectrin alpha chain, brain (spectrin, non-erythroid alpha chain) (fragment)	5.7	118671	5	5		Cytoskeleton
P16446	PPI1-RAT	Phosphatidylinositol transfer protein a isoform (PI-TP-a)	6.3	31984	9	30	Enzyme	Cytoplasmic
P17694	NUCM_BOVIN	NADH-ubiquinone oxidoreductase 49 kDa subunit (EC 1.6.5.3) (EC 1.6.99.3)	6.3	49484	6	12	Enzyme	Mitochondrial inner membrane
P18344	TPMZ_RAT	Tropomyosin alpha chain, brain-3 (tmbr-3)	4.5	28383	6	28	Structural, regulating protein	Cytoskeleton
P18418	CRTC_RAT	Calreticulin precursor (crp55) (calregulin) (erp60) (calcium-binding protein)	4.2	48136	5	12	Chaperone, calcium ion binding	Endoplasmic reticulum lumen
P18669	PMGB_HUMAN	Phosphoglycerate mutase, brain form (EC 5.4.2.1) (pgam-b) (EC 5.4.2.4) (EC 3.1.3.13)	7.2	28768	6	32	Enzyme	Cytosol
P19227	P60_RAT	Mitochondrial matrix protein p1 (heat shock protein 60) (hsp-60) (groel protein)	6.0	61088	6	15	Chaperone, mitochondrial protein import, macromolecular assembly	Mitochondrial matrix
P19234	NUHM_RAT	NADH-ubiquinone dehydrogenase 24 kDa subunit (EC 1.6.5.3) (EC 1.6.99.3) (fragment)	6.4	26853	6	32	Enzyme	Mitochondrial inner membrane
P19527	NFL_RAT	Neurofilament triplet L protein (68 kDa neurofilament protein) (NF-L)	4.4	61224	10	21	Maintenance of neuronal caliber	Cytoskeleton, nuclear envelope
P19804	NDKB_RAT	Nucleoside diphosphate kinase b (EC 2.7.4.6) (ndk b) (ndp kinase b)	7.5	17385	5	36	Enzyme	Cytoplasmic and plasma membrane
P21575	DYN1_RAT	Dynamin-1 (d100) (dynamin, brain) (b-dynamin)	6.7	96209	12	15	Enzyme	Microtubule-associated
P21670	PRC9_RAT	Proteasome component C9 (EC 3.4.99.46)	7.7	29764	6	26	Enzyme (Protease)	Cytoplasmic and nuclear
P21707	SYT1_RAT	Synaptotagmin I (p65)	8.4	47764	5	13	Regulatory protein	Synaptic vesicles and chromaffin granules
P21796	POR1_HUMAN	Voltage-dependent anion-selective channel protein 1	9.0	30736	6	38	Channel activity	Outer membrane of mitochondria and plasma membrane
P22626	ROA2_HUMAN	Heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNP A2, B1)	9.8	37463	14	47	RNA binding	Nuclear; component of ribonucleosomes
P23506	PIMT_MOUSE	Protein-L-isoaspartate (D-aspartate) *o*-methyltransferase (EC 2.1.1.77)	7.8	24544	4	21	Enzyme	Cytoplasmic

Table 1 (*Continued*)

Number	Name	Full name	p*I*	MW	Matches	Sequence coverage (%)	Function	Subcellular location
P23565	AINX_RAT	Alpha-internexin (alpha-inx)	5.0	56252	12	28	Maintenance of neuronal caliber	Cytoskeleton, nuclear envelope
P24142	PHB_RAT	Prohibitin (b-cell receptor associated protein 32) (bap 32)	5.5	29858	9	46	Cell proliferation	Cytoplasmic
P24155	MEPD_RAT	Thimet oligopeptidase (EC 3.4.24.15) (endo-oligopeptidase a) (endopeptidase 24.15)	5.6	78931	8	14	Enzyme	Cytoplasmic
P25113	PMGB_RAT	Phosphoglycerate mutase, brain form (EC 5.4.2.1) (EC 5.4.2.4) (EC 3.1.3.13)	6.7	28553	5	29	Enzyme	
P25388	GBLP_RAT	Guanine nucleotide-binding protein beta subunit-like protein 12.3	7.6	35510	6	24	Intracellular receptor, signal transduction	Cytoplasmic
P25809	KCRU_RAT	Creatine kinase, ubiquitous mitochondrial (EC 2.7.3.2)	8.6	47398	5	11	Enzyme	Mitochondrial inner membrane; outer side
P26040	EZRI_MOUSE	Ezrin (p81) (cytovillin) (villin-2)	6.0	69285	8	12	Structural	Cytoplasmic
P26516	PRSC_MOUSE	26S proteasome regulatory subunit s12 (proteasome subunit p40) (mov34 protein)	6.8	36574	5	22	Regulatory protein	
P27139	CAH2_RAT	Carbonic anhydrase ii (EC 4.2.1.1) (carbonate dehydratase ii)	7.4	29135	8	35	Enzyme	Cytoplasmic
P27605	HPRT_RAT	Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) (hgprt) (hgprtase)	6.5	24689	4	23	Enzyme	Cytoplasmic
P28480	TCPA_RAT	T-complex protein 1, alpha subunit (tcp-1-alpha) (cct-alpha)	6.1	60834	11	22	Molecular chaperone	Cytoplasmic
P28663	SNAB_MOUSE	Beta-soluble NSF attachment protein (snap-beta) (snap-alpha homolog) (fragment)	5.1	28122	8	40	Transport	Cytoplasmic peripheral membrane
P28746	RAN_MOUSE	GTP-binding protein RAN (TC4)	7.5	24578	6	31	Nucleocytoplasmic transport, signal transduction	Nuclear, cytoplasmic during mitosis
P29354	GRB2_RAT	Growth factor receptor-bound protein 2 (grb2 adaptor protein) (ash protein)	6.3	25304	6	26	Signal transduction, cell-cell signaling, adaptor protein	Plasma membrane
P31016	SP90_RAT	Presynaptic density protein 95 (synapse-associated protein 90)	5.8	80757	5	9	Neurogenesis	Cytoplasmic, synaptic junctions
P31044	PBP_RAT	Phosphatidylethanolamine-binding protein (23 kDa morphine-binding protein) (p23k)	5.6	20902	6	47	Binding protein, membrane remodeling	Cytoplasmic and membrane-bound
P31146	CORO_HUMAN	Coronin-like protein p57	6.7	51678	5	10	Signal transduction	Cytoskeleton
P34022	RANG_MOUSE	Ran-specific GTPase-activating protein (Ran binding protein)	5.0	23738	5	36	Signal transduction, nuclear transport	
P34058	HS9B_RAT	Heat shock protein hsp 90-beta (hsp 84)	4.9	83475	8	14	Chaperone	Cytoplasmic

Table 1 (*Continued*)

Number	Name	Full name	p*I*	MW	Matches	Sequence coverage (%)	Function	Subcellular location
P34067	PRCH_RAT	Proteasome component M3 (macropain subunit M3) (EC 3.4.99.46)	6.8	25891	5	28	Enzyme	Cytoplasmic and nuclear
P35214	143G_RAT	14-3-3 protein gamma (protein kinase c inhibitor protein-1) (kcip-1)	4.6	28324	9	32	Intracellular signaling, signal transduction, cell cycle	Cytoplasmic
P35704	TDX1_RAT	Thioredoxin peroxidase 1 (thiol-specific antioxidant protein)	5.3	21941	6	39	Redox regulation	Cytoplasmic
P37140	PP1B_RAT	Serine/threonine protein phosphatase pp1-beta	6.1	37960	5	19	Enzyme, cell cycle	
P37377	SYU1_RAT	Alpha-synuclein, forms 1 and 3	4.6	14506	4	41	Regulatory protein, membrane stability	Cytoplasmic
P37996	ARL3_RAT	ADP-ribosylation factor-like 3 (ARD3)	7.3	20614	6	45	Intracellular traffic	Cytoplasmic, Golgi apparatus
P38983	RSP4_RAT	40S ribosomal protein sa (p40) (34/67 kDa laminin receptor)	4.6	32917	7	30	RNA binding	Cytoplasmic
P42024	ACTZ_HUMAN	Alpha-centractin (centractin) (centrosome-associated actin homolog) (actin-rpv)	6.6	42700	7	25	Structural	Cytoskeleton, microtubule complex
P42123	LDHH_RAT	L-Lactate dehydrogenase h chain (EC 1.1.1.27) (ldh-b)	6.0	36743	9	29	Enzyme	Cytoplasmic
P42655	143E_RAT	14-3-3 protein epsilon (mitochondrial import stimulation factor l subunit	4.5	29326	10	38	Intracellular signaling, signal transduction, cell cycle	Cytoplasmic
P42669	PUR_MOUSE	Transcriptional activator protein pur-alpha (single-stranded dna-binding protein alpha)	6.4	34976	5	14	Transcription activator	Nuclear
P43035	LIS1_RAT	Platelet-activating factor acetylhydrolase ib alpha subunit (EC 3.1.1.47)	7.3	47079	7	24	Enzyme	Cytoplasmic
P46096	SYT1_MOUSE	Synaptotagmin i (p65)	8.8	47729	7	19	Regulatory protein, membrane interaction	Synaptic vesicles and chromaffin granules
P46460	NSF_MOUSE	Vesicular-fusion protein nsf (*n*-ethylmaleimide-sensitive fusion protein)	6.9	83083	7	9	Transport	Cytoplasmic
P46462	TERA_RAT	Transitional endoplasmic reticulum atpase	5.0	89976	7	10	Enzyme	Cytoplasmic
P47728	CART_RAT	Calretinin	4.8	31498	7	21	Calcium-binding protein	
P47754	CAZ2_MOUSE	F-actin capping protein alpha-2 subunit (capz)	5.7	33117	6	30	Structural protein	Cytoskeleton
P47757	CAPB_MOUSE	F-actin capping protein beta subunit isoforms 1 and 2 (capz)	5.5	31610	6	25		
P47819	GFAP_RAT	Glial fibrillary acidic protein, astrocyte (gfap)	5.2	49969	9	22		
P47860	K6PP_RAT	6-Phosphofructokinase, type C (EC 2.7.1.11) (phosphofructokinase 1) (fragment)	7.1	86515	6	7	Enzyme	
P47942	DPY2_RAT	Dihydropyrimidinase related protein-2 (drp-2) (turned on after division, 64 kDa protein)	6.3	62637	8	20	Axon elaboration	Membrane associated
P48500	TPIS_RAT	Triosephosphate isomerase (EC 5.3.1.1) (tim)	6.8	27285	7	37	Enzyme	

Table 1 (*Continued*)

Number	Name	Full name	p*I*	MW	Matches	Sequence coverage (%)	Function	Subcellular location
P49410	EFTU_BOVIN	Elongation factor Tu, mitochondrial precursor	7.2	49709	10	32	Translation	Mitochondrial
P49819	DLDH_CANFA	Dihydrolipoamide dehydrogenase precursor (EC 1.8.1.4)	7.8	54689	5	11	Enzyme	Mitochondrial matrix
P50137	TKT_RAT	Transketolase (EC 2.2.1.1) (tk)	7.5	68341	6	17	Enzyme	
P50399	GDIB_RAT	Rab gdp dissociation inhibitor beta (rab gdi beta) (gdi-2)	5.7	51165	7	17	Regulating protein	Cytoplasmic and membrane-associated
P50408	VATF_RAT	Vacuolar ATP synthase subunit f (EC 3.6.1.14) (v-atpase f subunit)	5.7	13361	5	46	Enzyme	
P50516	VATA_MOUSE	Vacuolar ATP synthase catalytic subunit A (EC 3.6.1.34) (V-ATPase)	5.7	68566	10	19	Enzyme	
P50554	GABT_RAT	4-Aminobutyrate aminotransferase, mitochondrial (EC 2.6.1.19) (gaba transaminase)	8.9	57177	7	17	Enzyme	Mitochondrial matrix
P51635	ALDX_RAT	Alcohol dehydrogenase (NADP(+)) (EC 1.1.1.2) (aldehyde reductase)	7.3	36579	8	28	Enzyme	
P52873	PYC_RAT	Pyruvate carboxylase precursor (EC 6.4.1.1) (pyruvic carboxylase) (pcb)	6.7	130348	12	12	Enzyme	Mitochondrial matrix
P53534	PHS3_RAT	Glycogen phosphorylase, brain form (EC 2.4.1.1) (fragment)	6.7	96854	7	10	Enzyme	
P54311	GBB1_RAT	Guanine nucleotide-binding protein b subunit	5.6	38167	7	28	Signal transduction	
P54314	GBB5_RAT	Guanine nucleotide-binding protein beta subunit 5 (transducin beta chain 5)	6.0	39504	6	23	Signal transduction	
P54921	SNAA_RAT	Alpha-soluble NSF attachment protein (snap-alpha)	5.0	33546	6	28	Transport between the endoplasmic reticulum and the Golgi apparatus	Cytoplasmic peripheral membrane protein
P55051	FABP_RAT	Fatty acid-binding protein, brain (b-fabp) (brain lipid-binding protein) (blbp)	5.4	15008	4	41	Transport, carring protein	Cytoplasmic
P70349	IPK1_MOUSE	Hint protein (protein kinase c inhibitor 1) (pkci-1)	6.9	13751	5	52	Enzyme	Cytoplasmic and nuclear.
P80254	DOPD_RAT	D-Dopachrome tautomerase	6.5	13107	7	82	Enzyme	Cytoplasmic
P80314	TCPB_MOUSE	T-complex protein 1, beta subunit (tcp-1-beta) (cct-beta)	6.4	57753	12	38	Molecular chaperone	Cytoplasmic
P97532	THTM_RAT	3-Mercaptopyruvate sulfurtransferase (EC 2.8.1.2)	6.3	33073	6	21	Enzyme	Cytoplasmic, mitiochondrial
Q00981	UBL1_RAT	Ubiquitin carboxyl-terminal hydrolase isozyme l1 (EC 3.1.2.15) (uch-l1)	5.0	25108	5	26	Enzyme	Cytoplasmic
Q01986	MPK1_RAT	Dual specificity mitogen-activated protein kinase 1 (EC 2.7.1.)	6.6	43648	6	16	Enzyme	
Q02218	ODO1_HUMAN	2-Oxoglutarate dehydrogenase E1 component (EC 1.2.4.2)	7.1	114600	6	7	Enzyme	Mitochondrial matrix
Q04760	LGUL_HUMAN	Lactoylglutathione lyase (EC 4.4.1.5) (methylglyoxalase) (ketone–aldehyde mutase)	5.2	20803	4	15	Enzyme	

Table 1 (*Continued*)

Number	Name	Full name	p*I*	MW	Matches	Sequence coverage (%)	Function	Subcellular location
Q05982	NDKA_RAT	Nucleoside diphosphate kinase a (EC 2.7.4.6) (metastasis inhibition factor nm23).	6.3	17295	7	55	Enzyme	Cytoplasmic and nuclear
Q07244	ROK_RAT	Heterogeneous nuclear ribonucleoprotein k (hnrnp k)	5.3	51229	6	15	RNA-binding protein	Cytoplasmic and nuclear; nucleoplasm
Q13347	IF34_HUMAN	Eukaryotic translation initiation factor 3 delta subunit (eif3 p36)	5.5	36877	5	18	Translation	Cytoplasm, cytosol, ribosome
Q16781	UBCC_HUMAN	Ubiquitin-conjugating enzyme e2−17 kDa (EC 6.3.2.19) (ubiquitin-protein ligase)	6.5	17183	4	29	Enzyme	
Q16781	UBCC_HUMAN	Ubiquitin-conjugating enzyme e2−17 kDa (EC 6.3.2.19)	6.5	17183	6	43		
Q28480	IDHA_MACFA	Isocitrate dehydrogenase (nad), mitochondrial subunit alpha (EC 1.1.1.41)	6.4	38030	8	27	Enzyme	Mitochondrial
Q61316	HS74_MOUSE	Heat shock 70 kDa related protein AGP-2	5.0	94871	12	16		Cytoplasmic
Q61553	FASC_MOUSE	Fascin	6.6	55112	9	24	Structural	Cytoplasmic
Q62048	PE15_MOUSE	Astrocytic phosphoprotein pea-15	4.8	15101	4	34	Apoptosis regulator (inhibitor)	Associated with microtubules
Q62689	JAK2_RAT	Tyrosine-protein kinase jak2 (EC 2.7.1.112) (janus kinase 2) (jak-2)	7.3	132041	5	4	Enzyme	Intracellular, possibly membrane associated
Q62950	DPY1_RAT	Dihydropyrimidinase related protein-1 (drp-1) (collapsin response mediator protein 1)	7.1	62498	8	15		Cytoplasmic
Q62951	DPY4_RAT	Dihydropyrimidinase related protein-4 (drp-4) (collapsin response mediator 3) (fragment)	6.8	61617	7	16		Cytoplasmic
Q63228	GLMB_RAT	Glia maturation factor b (GMF-b)	5.2	16765	6	37		
Q63270	IRE1_RAT	Iron-responsive element binding protein 1 (irp1) (aconitate hydratase) (EC 4.2.1.3)	7.1	98749	8	10	Enzyme	Cytoplasmic
Q63754	SYUB_RAT	Beta-synuclein (phosphoneuroprotein 14)	4.3	14495	4	40	Regulatory protein, membrane stability	Cytoplasmic
Q64320	STXBP1_RAT	Syntaxin-binding protein 1 (unc-18 homolog) (unc-18a) (unc-18-1)	7.0	67925	5	7	Regulating protein	Cytoplasmic and membrane-associated
Q64559	THCC_RAT	Cytosolic acyl coenzyme A thioester hydrolase (EC 3.1.2.2) (Brain acyl-CoA hydrolase)	7.5	37936	6	15	Enzyme	Cytoplasmic
Q64640	ADK_RAT	Adenosine kinase (EC 2.7.1.20) (ak) (adenosine 5′-phosphotransferase)	6.7	37630	6	14	Enzyme	
Q99798	ACON_HUMAN	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (citrate hydro-lyase)	7.5	86346	8	11	Enzyme	Mitochondrial

Proteins from the hippocampus of eight months old rats were extracted and separated by 2D electrophoresis as described in Section 2. The proteins were identified by MALDI-TOF-MS, following in-gel digestion with trypsin. The spots representing the identified proteins are indicated in Fig. 1 and are designated with their SWISS-PROT accession numbers. The accession number (column number), the abbreviated name (name), the full name (full name), the theoretical p*I* (p*I*) and MW (MW) values, as well as the matches (matches) and the protein amino acid sequence coverage by the matching peptides (sequence coverage) are given. The amino acid sequence coverage provides an indication of confidence of identification. The protein with the highest sequence coverage was selected.

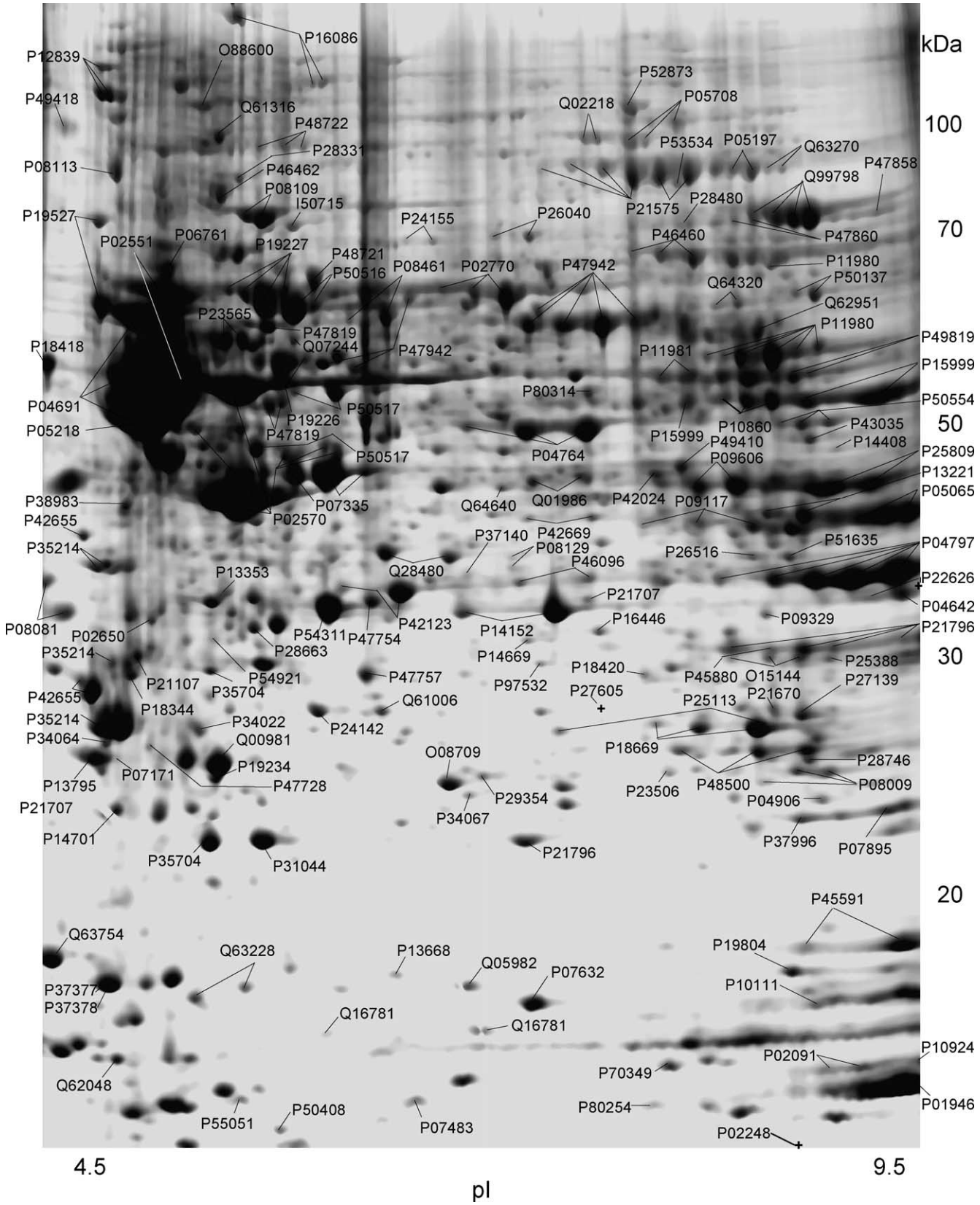

Fig. 1. Two-dimensional gel analysis of the rat hippocampus proteins. The proteins from the whole hippocampus of an eight months old rat were extracted and separated on a pH 3–10 nonlinear IPG strip, followed by a 12% SDS–polyacrylamide gel, as stated under Section 2. The gel was stained with Coomassie blue. The spots were analyzed by MALDI-TOF-MS. The proteins identified are designated with their SWISS-PROT accession numbers. The names of the proteins are listed in Table 1 (not all listed proteins are shown in Fig. 1).

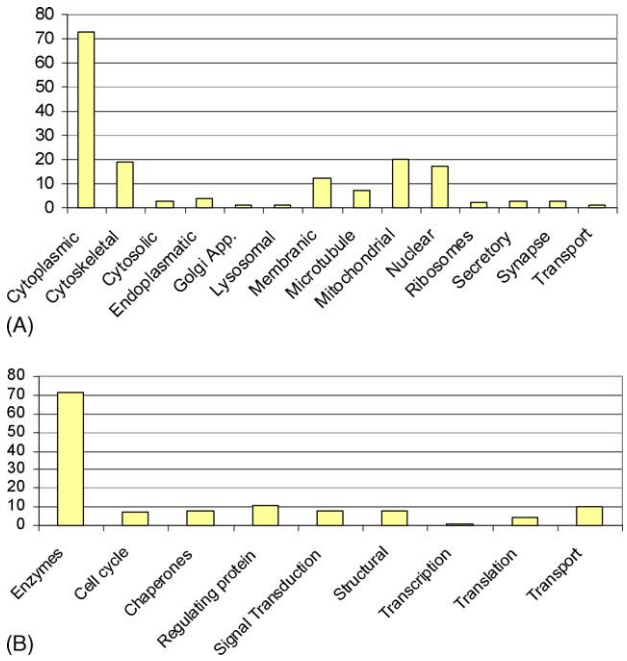

Fig. 2. Subcellular location (A) and function (B) of the hippocampus proteins identified in the present study.

of identification. A minimal number of five matches were used for protein search. In most cases, a larger number of matching peptides were found. In some cases, mainly for low molecular mass proteins, that usually deliver few peptides [31], the identification was based on four matches. However, these were the major peptides and the theoretical and observed p*I* and MW values were in good agreement. In many cases, no identification could be assigned, most likely because of technical limitations, i.e. low signals, loss of spots or no signal acquisition for one of the standard peptides.

3.2. Protein function and subcellular location

The proteins of Table 1, which are mainly the major visible components of Fig. 1, can be classified into several functional groups. Nine of them are structural proteins, like actin, tubulin chains and neurofilaments. About 71 are enzymes or enzyme chains with various catalytic activities, 11 are regulating proteins and 10 have transport functions (Fig. 2A). One hundred and eight proteins of Table 1 with various cellular functions, including enzymes, transcription factors, translation factors, etc., are ubiquitously expressed, 15 proteins (10%) have been detected in a variety of organs, including the nervous system, whereas 39 proteins (23%) are specifically expressed in the central nervous system (CNS). A high percentage of the identified proteins are localized in the cytoplasm (44%), about 11% are localized in the nucleus, 12% in mitochondria or mitochondrial membranes, 7% are membrane proteins and the remaining are localized in various organelles (Fig. 2B). In Table 1, the annotated function and subcellular location of the hippocampus proteins are given.

4. Discussion

Targeted proteome analysis refers to the construction of protein databases for specific organs, in healthy and pathological situations. Its goal is the elucidation of functional and structural specificities and could be useful in the detection of novel drug targets and early disease markers. In the present study, we applied proteomics tools, 2D electrophoresis followed by mass spectrometry, to construct a two-dimensional database for rat hippocampus proteins. Two-dimensional databases are useful tools to compare data between control and disease and between different laboratories [4]. The analysis of hippocampal proteins represents a region-specific proteomic study. The present database comprises 148 hippocampus proteins, of which about one-fourth are neuron specific. Comparison with published data showed that only 47 of the proteins of Table 1 had previously been reported to belong to the whole brain proteome, whereas 103 proteins seem to belong in the hippocampus proteome [32]. Furthermore, 58 out of 148 proteins of this study had previously been identified in the hippocampal regions CA1 and CA3 [23,24]. Ninety-two proteins were identified for the first time in adult rat hippocampus.

The hippocampus plays an important role in memory storage and retrieval and has an extensive ability in exchanging information with other parts of the brain and sensory organs. Of the proteins identified in hippocampus (Table 1), six have been linked to signal regulation and neural transmittance, synaptosomal-associated protein 25 kDa (SNAP-25), syntaxin-binding protein 1 (STXBP1), synaptotagmin 1 (SYT1), clathrin light chain A (CLCA), dynamin 1 (DYN1), and beta-soluble NSF attachment protein (SNAB). SNAP-25 is highly expressed in neurons of the neocortex, hippocampus, piriform cortex, anterior thalamic nuclei, pontine nuclei, and granule cells of the cerebellum [33]. The role of SNAP-25 in diseases has not yet been clarified; accumulated data indicate that this protein plays a key role in hippocampus function. Specifically, it has been suggested that in schizophrenia, the molecular pathology of the hippocampus involves presynaptic components associated with pathological synapses and abnormal exocytosis indicated by reduced amounts of SNAP-25 in hippocampus and elevated amounts in cerebrospinal fluid [34,35]. Changes in SNAP-25 expression levels represent early markers of synaptic loss [11,36]. The levels of that protein were found to decrease in the Alzheimer's disease and Down syndrome brain [37].

Neural transmission requires a balance between synaptic vesicle exocytosis and endocytosis, while membrane fusion begins with the binding of the vesicles to the target membrane via receptors. The process of exocytosis relies on three SNARE proteins, syntaxin (STX) and SNAP-25 on the target plasma membrane and synaptobrevin on the vesicular membrane. The plasma membrane proteins syntaxin and SNAP-25 interact with high affinity and in an equimolar stoichiometry to form a stable dimer in the pathway to the ternary SNARE

complex [38]. STXBP1 participates in the determination of the specificity of the intracellular fusion reactions in a 1:1 ratio with the syntaxins 1, 2 or 3 but not 4 [39]. STXBP1 is highly expressed in the brain and the spinal cord. Faint levels are detectable on embryonic day 14, rising in later embryonic ages and peaking on postnatal day 7 [40,41].

The process of endocytosis is complex and involves the interaction of several molecules like phosphoinositides (PIP_2), clathrin and clathrin adaptors, dynamin 1, synaptotagmin 1 and amphiphysin. Plasma membrane proteins can be removed selectively and efficiently from the cell surface through endocytic uptake by clathrin-coated vesicles. Dynamin 1 and CLCA are included in our 2D hippocampus database. Dynamin 1 is a 100-kDa GTPase that controls a variety of vesicular budding events including the detaching of clathrin-coated vesicles from the plasma membrane, synaptic clathrin-driven recycling pathway, receptor-mediated endocytosis, caveolae internalization, phagocytosis, and secretory vesicle budding from the trans-Golgi network [42]. In mammals, three closely related dynamin genes are expressed in a tissue-specific manner. Dynamin 1 is almost exclusively expressed in neurons, dynamin 2 is found in the brain but is also widely expressed among other tissues and dynamin 3 was initially identified in testis but is also found in brain, lung, and heart. Because of its restriction to neurons, dynamin 1 has been assumed to be the synaptic isoform of dynamin expressed in neurons after maturation and in the adult brain [43–45]. Although the majority of studies implicate dynamin in endocytosis, there is evidence that dynamin may exert additional functions in cell physiology. Thus, recent data indicate that dynamin is a high-affinity substrate for calcineurin, and its GTPase activity is determined by the balance between PKC-mediated phosphorylation and calcineurin-dependent dephosphorylation. Further studies suggest that there is a direct functional link of dynamin to the actin cytoskeleton, reporting that dynamin is co-localized with actin filaments and directly binds regulatory components of the actin cytoskeleton [46,47]. Sugita et al. [48] identified in the rat brain a protein complex associated with the cytoplasmic domain of the chloride intracellular channel CLIC4 (p64H1), which includes dynamin 1, alpha-tubulin, beta-actin, creatine kinase (KCRB) and two 14-3-3 isoforms. It is interesting that all these proteins, except of CLIC4, are included in Table 1, suggesting that interaction of dynamin, cytoskeletal proteins and 14-3-3 proteins might be relevant to hippocampus functions.

Synaptotagmin 1 belongs to the synaptotagmin family, which are synaptic vesicle membrane proteins found in abundance in nerve cells and some endocrine cells. They have a regulatory role in membrane interactions during trafficking of synaptic vesicles at the active zone of the synapse. That vesicle-associated calcium-binding protein predominantly expressed in rostral, phylogenetically younger brain regions, and in some endocrine tissues, seems to be important in the docking and fusion of synaptic vesicles with the plasma membrane, required for the tight temporal coupling between calcium ion influx and synaptic vesicle fusion [49,50]. Finally, SNAB is expressed in cerebellar cortex, hippocampus, dentate gyrus, and weakly expressed in the putamen, the thalamus and the brain stem.

Neurotransmitter release and some other brain functions, like short- and long-term modulation of synaptic efficacy, are regulated by calcium ions. Calcium functions are mediated by calcium-binding proteins, including a group of proteins known as neuronal calcium sensor (NCS) proteins [51]. The calcium-dependent membrane association reversibly localizes NCS proteins to distinct cellular signalling compartments and may exist a critical mechanism for the coordinated regulation of signalling cascades [52]. In normal human hippocampus, visilin-like protein 1 (VIS1, VILIP-1) [53,54] has been detected in multiple pyramidal cells (subfields CA1 and CA4 pyramidal cells) and interneurons (especially hilar interneurons), while a portion (60%) of VILIP-1-expressing interneurons co-express calretinin (CART) which is included in our database [55,56]. Reduction in VILIP-1-expressing cells has been linked to the pathophysiology of Alzheimer's disease [57]. A fast and reversible translocation of VILIP-1 to specialized membrane structures has been observed after a depolarizing stimulus or activation of glutamate receptors in hippocampal neurons [52]. Although the functions of VILIP-1 are not known, recent data have shown that, apart of calcium regulation, it plays a crucial role in regulating cAMP levels, cell signalling, and differentiation [58].

The proteins alpha-synuclein 1, and 3 and beta-synuclein are also included in our list (Table 1). Synucleins (SYU) are specifically expressed in the brain (hippocampus, brainstem and cortex) and are possibly involved in neuronal plasticity. They are mainly located in presynaptic terminals, whereas alpha-synuclein is specifically expressed in neuronal cell bodies and synapses. Normal cellular functions have not been found for any of the synucleins, although some data suggest a role in the regulation of membrane stability and/or turnover. Alpha-synuclein was previously found to be accumulated abnormally in Parkinson's disease, Alzheimer's disease, and several other neurodegenerative disorders. Additionally, mutations in alpha-synuclein were found to be associated with rare familial cases of early-onset of Parkinson's disease [59,60].

Furthermore, our list includes three proteins closely related to memory and learning, phosphatidylethanolamine-binding protein (PBP) and serine/threonine protein phosphatase PP1 alpha and beta isoforms (PP1A and PP1B). PBP is highly expressed in the brain and is the major component of epididymal secretions. It belongs to the hippocampal cholinergic neurostimulating peptides and is involved in the function of the presynaptic cholinergic neurons of CNS [61,62]. Recently, PP1 received a great attention as a regulator of learning and memory and as a potential mediator of cognitive decline during aging. Genoux et al. [63] demonstrated that PP1 determines the efficacy of learning and memory by limiting acquisition and favouring memory decline. They found that inhibition of PP1 prolongs memory when induced after learning, while enhanced learning correlates with

increased phosphorylation of the transcription factor cyclic AMP-dependent response element binding protein (CREB).

Two additional proteins of Table 1 show phosphatase activity, the vacuolar ATP synthase subunit F (VATF, F1-ATPase) and the vacuolar ATP synthase catalytic subunit A (VATA, V-ATPase). They are important in the regulation of the intracellular concentrations of phosphate, ATP and ADP. In the neuronal system, ATP and adenosine have been linked to pain and the control of pain. Adenosine kinase (ADK) seems to be important for the regulation of the intracellular ATP concentration. ADK phoshorylates adenosine and other intracellular adenine nucleotides, serving as a potential regulator of the concentration of extracellular adenosine and intracellular adenine nucleotides, thus preventing the formation of toxic levels of adenosine within the cell [64–66].

A number of structural proteins were also identified, including members of the intermediate filament (IF) and of actin filament. IFs are primordial components of the cytoskeleton and the nuclear envelope, subdivided into five major subgroups. Four IF proteins are included in Table 1, glial fibrillary acidic protein (GFAP), member of the IF type III subgroup, and three members of the IF type IV subgroup, neurofilament NF-L, neurofilament NF-M, and α-internexin (AINX). GFAP is a cell-specific marker that during the development of the central nervous system, distinguishes astrocytes from other glial cells. It has been suggested that expression of GFAP as well as GFAP isotypes may be related to memory retention in rats [67,68]. Higher levels of GFAP were observed in the AD and DS brain [34]. The neurofilaments NF-L and NF-M are involved in the maintenance of neuronal caliber. Phosphorylation seems to play a major role in the function of the larger neurofilament polypeptides such as NF-M, the levels of phosphorylation being altered developmentally and coincident with a change in the neurofilament function. Further it is thought that phosphorylation of NF-M results in the formation of interfilament cross bridges that are important in the maintenance of axonal caliber. During development of the mammalian nervous system, α-internexin mRNA and protein are expressed earlier and more abundantly than the neurofilament NF-L, M and H proteins. Because of its early expression, it has been suggested that α-internexin may stabilize neurons and their processes and provide a scaffold for the coassembly of other IF proteins during development. In the mature nervous system, α-internexin shows a distribution pattern restricted to neurons, partially overlapping but distinct from that of the NF-L, M and H proteins. Although many larger neurons express α-internexin along with the three NF-L, M and H proteins, in some mature neurons, α-internexin is the only intermediate filament protein expressed, suggesting a role for this protein in neuron maturation and neuronal regeneration after injury [69].

Several structural proteins, related to the actin filament and to the cytoskeleton, are included in our list, beta-actin (ACTB), gamma-actin (ACTG), centractin (ACTZ), F-actin capping protein alpha-2 subunit (CAPZ), F-actin capping protein beta subunit (CAPB), fascin (FASC), ARP2/3 complex 34 kDa (AR34), ezrin (EZRI), tropomyosin (TPMZ) and tubulin b-5 chain (TBB5). Fascin belongs to a structurally unique and evolutionarily conserved group of actin cross-linking proteins participating in cell architecture and cell-matrix adhesion, cell interaction and cell migration [70]. Tropomyosin is implicated in stabilizing cytoskeleton actin filaments, while ezrin crosslinks actin filaments with plasma membranes [71]. The complexity of the function of these proteins and the possible crosslink between them and other identified proteins (such as phosphatases and kinases) suggest that these proteins may have a more extensive and important role in the hippocampus function, possibly participating in information storage and memory.

Lipids as well as their metabolism seem also to be important for the brain and hippocampus development and function, as indicated by the number of identified lipid-related proteins. To the lipid-related proteins, belongs acyl-CoA hydrolase (THCC), which has an important physiological function in brain by modulating the cellular levels of fatty acyl-CoA ligands for certain transcription factors as well as for the substrates for fatty acid metabolizing enzymes, contributing to lipid homeostasis. Platelet-activating factor (PAF) is a biologically active lipid mediator acting through a specific receptor (PAF receptor). It has been shown that PAF is produced in the brain from its precursor, while PAF receptor is expressed in neurons and microglia [72]. PAF is degraded and inactivated by a specific hydrolase, PAF acetylhydrolase (PA). The alpha PAF acetylhydrolase subunit (LIS1) was identified. LIS1 has been detected in most tissues, with highest expression in the brain. It seems to be important for neural cell differentiation, as it is already expressed by the time of neurulation and is abundant in the developing central and peripheral nervous system, with major sites including the neuroepithelium of the fore-, mid-, and hindbrain, the spinal cord, the dorsal root, and the cranial ganglia [73]. LIS1 is the regulatory subunit of PA, a pivotal molecule linking the PAF action to the neuronal cell migration, as it has been previously shown that the migration of the cerebellar granule cells and the differentiating neurons are regulated by PAF [72,74]. Furthermore, LIS1 deletion causes the human neuronal migration disorder type I lissencephaly, a cortical malformation disorder characterized by disorganized cortical layers and gyral abnormalities and associated with severe cognitive impairment and epilepsy [75]. Additionally, recent studies indicated that PA is related to Parkinson's disease. PA and fatty acid-binding protein (FABP), also included in our map, were found to interact with *N*-methylated beta-carbolines which are the structural and functional analogs of the parkinsonian-inducing toxin [76]. FABP is a cytosolic protein involved in fatty acid uptake, transport, and targeting, modulating fatty acid concentration. In this way, it influences the function of enzymes, membranes, ion channels, receptors, gene expression and cellular growth and differentiation [77]. Recent data indicate that FABP is required for the establishment of the radial glial fiber system in developing brain, a system that is necessary for the migration of immature neurons to establish

cortical layers, possibly through the transport of hydrophobic ligands with potential morphogenic activity during CNS development. Pyruvate carboxylase (PYC) was also detected, which catalyzes in a tissue-specific manner the initial reactions of glucose (liver, kidney) and lipid biosynthesis from pyruvate (adipose tissue, liver and brain).

Adult neural stem cells can be detected in various regions of the rat brain and it is well established that the structural basis of hippocampal plasticity involves neural stem cells differentiation [78]. Several proteins previously identified in neural stem cells were identified, like SYT1, LYS1, FABB, GLMB, STHM, SN25, GFAP, DPY1, DPY2, TYO3, PA1B and SYUB. The results suggest the existence of a neural stem cell population within hippocampus which seems to be responsible for the neurogenesis and differentiation related to hippocampus plasticity.

Finally three proteins related to hippocampus protection, thioredoxin peroxidase 1 (TDX1), astrocytic phosphoprotein (PE15) and antioxidant protein 2 (AOP2), we identified, as well as two enolase isoenzymes (ENOA and ENOG), a protein that plays a role in the repair and/or degradation of damaged proteins, protein-L-isoaspartate (PIMT), and a neuron specific ubiquitin recycling enzyme (UBL1).

In summary, we constructed a 2D reference protein map for rat hippocampus, including 148 different gene products, of which about 70% appear to belong to the hippocampus proteome and one-fourth are neuron specific. The hippocampus is essential for memory storage and retrieval and its physiological function includes information transmission as signal from other parts of the brain. The results suggest the existence of a neural stem cell population in hippocampus possibly involved in neurogenesis and differentiation related to hippocampus plasticity. Further studies on hippocampus, involving enrichment of low-abundance gene products, will strengthen our knowledge, concerning the causes of pathological situations such as Parkinson's disease, Alzheimer's disease and schizophrenia, related to hippocampus dysfunction.

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