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ORIGINAL ARTICLE

# Strain-independent global effect of hippocampal proteins in mice trained in the Morris water maze

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**Abstract** A series of individual proteins have been linked to performance in the Morris water maze (MWM) but no global effects have been reported. It was therefore the aim of the study to show which proteins were strain-independent, global factors for training in the MWM. Strains C57BL/6J, *apodemus sylvaticus* and PWD/PhJ were used. MWM and gels from trained animals were from a previous own study and corresponding yoked groups were generated. Hippocampal proteins were extracted and run on two-dimensional gel electrophoresis. Spots with different expressional levels between trained and yoked groups were punched and identified by mass spectrometry (nano-LC-ESI-MS/MS, ion trap). Two-way ANOVA with two factors (strain and training) was carried out and a Bonferroni test was used to compare groups. 12 proteins from several pathways and cascades showed different levels in trained mice versus corresponding yoked animals in all strains tested. Four out of these proteins were verified by immunoblotting: beta-synuclein, profilin 2, nucleoside diphosphate kinase A (NME1) and isocitrate dehydrogenase 3. Four proteins verified by immunoblotting could be shown to be involved in training in the MWM as a global effect, independent of the strain tested.

**Keywords** Morris water maze · Hippocampal proteins · Beta-synuclein · Profilin-2 · Nucleoside diphosphate dehydrogenase

## Introduction

The Morris water maze (MWM) is widely used to investigate spatial learning and memory (L&M) and a series of investigations linking individual proteins to spatial memory processes were carried out so far (Fukuda et al. 2007; Tan 2009): Synaptosomal-associated protein-25 (Li et al. 2010), diacylglycerol kinase (Shirai et al. 2010), cyclin-dependent kinase-like 5 (Cuadrado-Tejedor et al. 2011), Spred1 (Denayer et al. 2008), phosphorylated protein kinase A (Sunyer et al. 2009), dual specificity tyrosine-regulated kinase-1A (Arque et al. 2008) and ElF2alpha kinase GCN2 (Costa-Mattioli et al. 2005). Moreover, it has been demonstrated that deficiency of several proteins led to impairment of spatial memory in the MWM including NDRG4 (Yamamoto et al. 2011), ATRX gene, a chromatin-remodeling factor (Nogami et al. 2011), phosphorylated cyclic AMP response element-binding protein (Jung et al. 2010), endothelin-converting enzyme-2 (Rodriguiz et al. 2008), S6K1 and S6K2 (Antion et al. 2008), the UvCK isoform of creatine kinase (Streijger et al. 2004), apolipoprotein C–I (Abildayeva et al. 2008) and ARMS/Kidins220

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scaffold protein (Duffy et al. 2011). Overexpression of a series of individual proteins was linked to impaired spatial memory in the MWM: a selective role for over-expression of two different forms of the alpha-secretase ADAM10 in L&M in mice as evaluated by the MWM was suggested (Schmitt et al. 2006). Impairment of performance in the MWM by over-expressed myristoylated alanine-rich C kinase substrate (MARCKS) was demonstrated by McNamara et al. (2005). Ahn et al. (2006) have shown that DYRK1A BAC transgenic mice show altered synaptic plasticity with L&M defects in the MWM.

Hippocampal protein changes in spatial memory retrieval in the MWM are well documented, that generated maps or patterns of spatial memory-related proteins (Patil et al. 2010a, b, 2011). A series of signaling proteins from different pathways were linked to performance in the MWM including proteins from the Ras signaling pathway (Brambilla et al. 1997), the WNT signaling (Tabatadze et al. 2011), protein kinase C and PKA signaling (Holahan and Routtenberg 2008; Sunyer et al. 2009) and the MAP kinase signaling pathway (Selcher et al. 1999), to name a few.

Although previous studies have shown strain-dependence in L&M in the MWM (Brooks et al. 2005; Moy et al. 2007; Nguyen et al. 2000; Patil et al. 2009), there is no information and data to answer the question which hippocampal proteins are strain-independent in the MWM or other paradigms of spatial memory, i.e. which proteins are *global factors* linked to memory training in the MWM.

It was therefore decided to test three different mouse strains, trained and yoked controls, in a MWM protocol and to carry out a gel-based proteomic approach with appropriate statistical handling, in order to find hippocampal proteins that are strain-independent but depend on training in the MWM.

## Materials and methods

### Animals

Male, 12–14-week-old C57BL/6J, *apodemus sylvaticus* (AS) and PWD/PhJ mice were used and housed in their individual home cages before experiments. An autoclaved standard rodent diet (Altromin 1314ff) and water were available ad libitum. Room temperature was 22°C ± 1°C, and relative humidity was 50 ± 10%. The light–dark rhythm was 14:10. Ventilation with 100% fresh air resulted in an air change rate of 15 times per hour. The room was illuminated with artificial light at an intensity of about 200 lx in 2 m from 5 a.m. to 7 p.m. Behavioral tests were performed between 8 a.m. and 1 p.m. Experiments were done under license of the federal ministry of education, science, and culture, which includes an ethical evaluation of the project (Project: BMWF-66.009/0152-C/GT/2007). Housing and maintenance of animals were in compliance with European and national regulations (Patil et al. 2011).

### Morris water maze (MWM) studies

Spatial memory was evaluated in the MWM on the identical three mouse strains as reported earlier (Patil et al. 2010b, 2011) in order to evaluate hippocampal protein levels in three individual strains showing strain-dependent protein expression. Yoked mice from the three strains that spent the same time in the MWM, but were not trained (absence of a platform during the whole session) were herein used in the MWM in order to show global training effects of proteins (reprinted from Sudarshan S. Patil, Sanjay V. Boddul, Konstantin Schlick, Sung Ung Kang, Sonja Zehetmayer, Harald Höger, Gert Lubec. Differences in hippocampal protein levels between C57Bl/6J, PWD/PhJ, and AS are paralleled by differences in spatial memory. Hippocampus 21(7):714-723, Copyright (2011), with permission from Wiley–VCH). These yoked mice were newly introduced into MWM studies.

### Hippocampal samples

Animals trained in the MWM and yoked controls were used. For chemical analysis, animals were killed by decapitation 6 h following the probe trial on day 12. Twenty hippocampal tissue samples per group were taken from three mouse strains and immediately frozen in liquid nitrogen and stored at −80°C. The freezing chain was never interrupted. All efforts were made to minimize animals suffering.

### Sample preparation

Sample preparation was essentially conducted as described (Zheng et al. 2009). Hippocampi were homogenized and suspended in 1.2-mL sample buffer (20 mM Tris, 7 M urea, 2 M thiourea, 4% w/v CHAPS, 10 mM 1,4-dithioerythritol, 1 mM EDTA, 1 mM PMSF, 1 tablet Complete™ from Roche Diagnostics, and 0.2% v/v phosphatase inhibitor cocktail from Calbiochem). After sonication of the suspension ~30 s on ice, the suspension was left at 21°C for 1 h and centrifuged at 14,000×*g* for 60 min at 12°C. Desalting was carried out with an Ultrafree-4 centrifugal filter unit with a cutoff molecular weight of 10,000 Da (Millipore, Bedford, MA) at 3,000×*g* at 12°C until the eluted volume was about 4 ml and the remaining volume reached 100–200 µL. The protein content of the supernatant was determined by the Bradford (1976) assay.

### Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2-DE) was performed essentially as reported previously (John et al. 2009). The first dimension of gel electrophoresis was carried out using 700 µg of proteins applied to immobilized pH 3–10 nonlinear gradient strips (Immobiline™ Dry strips, GE Healthcare) with a horizontal electrophoretic apparatus (Ettan IPGphore 3, GE Healthcare). The isoelectric focusing started at 200 V, and the voltage was gradually increased to 8,000 V at 4 V/min and kept constant for a further 3 h (~150,000 Vh totally). Prior to the second dimensional run, strips were equilibrated twice for 15 min with gentle shaking in 10 ml of SDS equilibration buffer (50 mM pH 8.8 Tris–HCl, 6 M urea, 30% v/v glycerol, 2% w/v SDS, and trace of bromophenol blue). DTT (1%, w/v) was added at the first incubation for 15 min and 4% (w/v) iodoacetamide instead of DTT at the second incubation step for 15 min. The second-dimensional separation was performed on 10–16% gradient SDS-PAGE. After protein fixation for 12 h in 50% methanol and 10% acetic acid, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 8 h, and excess of dye was washed out from the gels with distilled water. Molecular masses were determined by running precision protein standard markers (Bio-Rad Laboratories, Hercules, CA, USA) covering the range of 10–250 kDa. Isoelectric point values were determined as given by the supplier of the immobilized pH-gradient strips (Chen et al. 2010).

### Quantification of protein levels

Two-dimensional gel electrophoresis gels were washed with distilled water to remove excess dye, and gels were scanned with an Image Scanner (GE Healthcare, Chalfont St Giles, Buckinghamshire, UK). Image analysis and quantification of spots were performed with Proteomweaver software (Definiens, Munich, Germany). For quantification, gel images from trained mice (C57BL/6J, $n = 15$; PWD/PhJ, $n = 16$, AS, $n = 16$) and yoked controls (C57BL/6J, $n = 13$; PWD/PhJ, $n = 16$, AS, $n = 16$) were outlined.

Behavioral data sets from the above-mentioned publication were corrected for those animals that showed gels of hippocampal proteins that could be used for the quantification because of highest quality: from originally 20 hippocampi per group, a lower number (see Results section) was used for the protein experiments, because insufficient protein separation in a few gels did not allow the use for quantification.

Quantification data from Proteomweaver were processed as described in statistical analysis. The percentage of the volume of the spots representing a certain protein was determined in comparison with the total proteins present in the 2-DE gel (Langen et al. 1999). The quantified protein spots from trained mice (C57BL/6J, PWD/PhJ, and AS) and yoked mice were excised manually based on the observed statistical difference, and analysis was carried out as described in the next steps.

### Protein identification with nano-LC-ESI-CID/ETD-MS/MS

Protein identification with nano-LC-ESI-CID/ETD-MS/MS was performed as described (Monje et al. 2011). Protein spots of interest (13 spots) were excised and put into 0.5-mL protein lobind tubes (Eppendorf, Hamburg, Germany). They were initially washed with 10 mM ammonium bicarbonate and then with 50% 20 mM ammonium bicarbonate/50% acetonitrile for 30 min with occasional vortexing. The step above was repeated until the color disappeared. Hundred microliters of acetonitrile were added to each tube to cover the gel piece completely and incubated for at least 5 min. Gel pieces were dried completely in a Speedvac Concentrator 5301 (Eppendorf, Hamburg, Germany). The dried gel pieces were re-swollen and in-gel digested with 12.5 ng/µl trypsin (Promega, Madison, WI) solution buffered in 25 mM ammonium bicarbonate. Gel pieces were incubated for 16 h (overnight) at 37°C. Supernatants were transferred to new 0.5-mL tubes, and gel pieces were extracted again subsequently with 20 µL of 1% formic acid/5 mM OGP, 20 µL of 0.1% formic acid, and 20 µL of 0.1% formic acid/20% acetonitrile each time for 15 min on a shaker. Samples in extraction buffer were pooled in a 0.5-mL tube.

Forty microliters of extracted peptides were analyzed by nano-LC-ESI-CID/ETD-MS/MS. The HPLC used was an Ultimate 3000 system (Dionex Corporation, Sunnyvale, CA) equipped with a PepMap100 C-18 trap column (300 µm × 5 mm) and PepMap100 C-18 analytic column (75 µm × 150 mm). The gradient was (A = 0.1% formic acid in water, B = 0.08% formic acid in acetonitrile) 4–30% B from 0 to 105 min, 80% B from 105 to 110 min, and 4% B from 110 to 125 min. The flow rate was 300 nL/min. A HCT ultra ETD II PTM discover system (Bruker Daltonics, Bremen, Germany) was used to record peptide spectra over the mass range of $m/z$ 350–1,500 and MS/MS spectra in information-dependent data acquisition over the mass range of $m/z$ 100–2,800. Repeatedly, MS spectra were recorded followed by four data-dependent CID-MS/MS spectra and four ETD-MS/MS spectra generated from the three highest intensity precursor ions. An active exclusion of 0.4 min after two spectra was used to detect low-abundant peptides. The voltage between ion spray tip and spray shield was set to 1,400 V. Dry nitrogen

gas was heated to 150°C, and the flow rate was 10 L/min. The collision energy was set automatically according to the mass and charge state of the peptides chosen for fragmentation. Multiple-charged peptides were chosen for MS/MS experiments due to their good fragmentation characteristics. MS/MS spectra were interpreted, and peak lists were generated by Data Analysis 4.0 (Bruker Daltonics, Bremen, Germany). All the searches were done using the software MASCOT 2.2.04 (Matrix Science, London, UK) against latest UniProtKB database for protein identification. Searching parameters were set as follows: enzyme selected as trypsin with two maximum missing cleavage sites, species limited to mouse, a mass tolerance of 0.2 Da for peptide tolerance, 0.2 Da for MS/MS tolerance, fixed modification of carbamidomethyl(C), and variable modification of methionine oxidation and phosphorylation (Tyr, Thr, and Ser). Positive protein identifications were based on a significant MOWSE score. After protein identification, an error-tolerant search was done to detect unspecific cleavage and unassigned modifications. Protein identification and modification information returned from MASCOT were manually inspected and filtered to obtain confirmed protein identification and modification lists.

### Immunoblotting

To verify main findings from the gel-based mass spectrometrical procedure, immunoblotting was carried out in trained versus yoked mice.

After the determination of protein concentration, samples containing 10 μg of protein were prepared. Samples were denatured at 95°C for 5 min and loaded onto 15% SDS gels. Electrophoresis was performed with a Bio-Rad Electrophoresis System (Bio-Rad, Criterion™ Cell, Hercules, CA) and proteins separated on the gel were transferred (Bio-Rad, Transfer Blot SD Cell, Hercules, CA) onto PVDF membranes that were probed with mouse monoclonal antibody NME1/NDKA (1:1,000; Cell Signaling Technologies Inc, Danvers, MA, USA), monoclonal antibody beta Synuclein (ab76111, 1:5,000; Abcam, Cambridge, UK), polyclonal antibody isocitrate dehydrogenase IDH3A (ab58641, 1:5,000; Abcam, Cambridge, UK),and polyclonal antibody Profilin 2 (ab96676, 1:5,000; Abcam, Cambridge, UK). Detection was carried out with horseradish peroxidase-coupled secondary antibodies: anti-rabbit IgG (Cell Signaling Technologies Inc, Danvers, MA, USA) according to the supplier's protocol. Membranes were developed with the ECL plus Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK). Densities of immunoreactive bands were measured by Image J software program (http://rsb.info.nih.gov/ij/). GAPDH immunoreactivity was used as a loading control.

### Statistical analysis

Changes in hippocampal protein levels were evaluated using two-way analysis of variance (ANOVA) with the two factors, *strain* (AS, PWD/PhJ and C57BL/6) and *training* (trained vs. yoked control) and post hoc pairwise comparisons using Tuckey-HSD (Li et al. 2009; Uddin et al. 2010). For high stringency, an alpha-level cut-off was set to 0.001. For immunoblotting, independent student *t* test were carried out to compare trained and yoked mice in three different strains. All calculations were done employing SPSS 17.0 (SPSS GmbH Software, München, Germany).

### Pathway analysis

Differentially expressed proteins were analyzed further by bioinformatic pathways analysis [Ingenuity Pathway Analysis (IPA); Ingenuity® Systems, Mountain View, CA; http://www.ingenuity.com]. IPA constructs hypothetical protein interaction clusters on the basis of a regularly updated "Ingenuity Pathways Knowledge Base." The Ingenuity Pathways Knowledge Base is a very large curated database that consists of millions of individual relationships between proteins, culled from the biologic literature. These relationships involve direct protein interactions, including physical binding interactions, enzyme substrate relationships, and cis–trans relationships in transcriptional control. The networks are displayed graphically as nodes (individual proteins) and edges (the biologic relationships between the nodes).

Therefore, data set containing the identifiers of differentially expressed protein kinases and protein phosphatases identified by protein microarrays with subsequent immunoblot verification is uploaded into IPA. IPA then builds hypothetical networks from these proteins, and other proteins from the database that are needed to fill out a protein cluster. Network generation is optimized for inclusion of many proteins from the input expression profile as possible and aims for highly connected networks (Hoorn et al. 2005; Raponi et al. 2004; Siripurapu et al. 2005).

## Results and discussion

Three mouse strains performed training in the MWM (Patil et al. 2011) and the added corresponding yoked (untrained) controls were kept for the same time in the MWM.

Global effects i.e. strain-independent expressional changes linked to memory training in the MWM, were observed for 12 proteins. These proteins were from different protein networks as protein synthesis (eukaryotic translation initiation factor 4H, ribosomal protein SA),

**Table 1** Protein identification data on global factor proteins verified by western blotting

| Accession number | Protein name | MS/MS score | Match pept. | Seq. Cov. (%) | MS/MS peptides | Theor. MW (Da) | Theor. pI |
|---|---|---|---|---|---|---|---|
| Q91ZZ3 | SYUB_MOUSE Beta-synuclein | 559 | 7 | 97.7 | -.MDVFMKGL.S<br>K.EGVVAAAEK.T<br>K.QGVTEAAEK.T K.EGVLYVGSK.T K.TSGVVQGVASVAEK.T<br>K.TKEQASHLGGAVFSGAGNI AAATGLVK.K<br>L.VKKEEFPTDLKPEEVAQEAAEE PLIEPLMEPEGESY.E<br>Y.EDSPQEEYQEYEPEA.- | 14,043 | 4.38 |
| Q9JJV2 | PROF2_MOUSE Profilin-2 | 421 | 8 | 72.1 | M.AGWQSY.V<br>Y.VDNLMCDGCCQEAAIVGY.C<br>Y.CDAKYVW.A<br>Y.VWAATAGGVF.Q<br>K.DREGFFTNGLTLGAK.K<br>K.KCSVIRDSLYVDGDCTMDIR.T<br>K.SQGGEPTYNVAVGR.A<br>R.VLVFVMGK.E | 15,364 | 6.55 |
| P15532 | NDKA_MOUSE Nucleoside diphosphate kinase A | 485 | 10 | 78.3 | R.TFIAIKPDGVQR.G<br>R.GLVGEIIK.R<br>K.FLQASEDLLK.E<br>K.DRPFFTGLVK.Y<br>K.YMHSGPVVAMVWEGLNVVK.<br>R.VMLGETNPADSKPGTIR.G<br>R.GDFCIQVGR.N<br>R.NIIHGSDSVK.S<br>K.EISLWFQPEELVEYK.S<br>K.SCAQNWIYE.- | 17,311 | 6.84 |
| Q9D6R2 | IDH3A_MOUSE Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial | 1,255 | 23 | 68 | F.AGGVQTVTL.I<br>K.APIQWEER.N<br>R.NVTAIQGPGGK.W<br>K.WMIPPEAK.E<br>W.MIPPEAKESMDKNKMGL.K<br>L.KGPLKTPIAAGHPSMNL.<br>K.TPIAAGHPSMNLLLR.K<br>Y.ANVRPCVSIEGY.K<br>K.TPYTDVNIVTIR.E<br>R.ENTEGEYSGIEHVIVDGVVQSIK.L<br>L.ITEEASKRIAEFAFEY.A<br>R.IAEFAFEYAR.N<br>R.MSDGLFLQK.C<br>F.LQKCREVAENCKDIKF.N<br>F.NEMYLDTVCL.N<br>L.NMVQDPSQF.D<br>L.IGGLGVTPSGNIGANGVAIF.E<br>F.ESVHGTAPDIAGKDMANPTAL.L<br>R.HMGLFDHAAK.I<br>F.DHAAKIEAACF.A<br>K.IEAACFATIK.D<br>K.CSDFTEEICR.R<br>F.TEEICRRVKDLD.- | 40,069 | 6.27 |

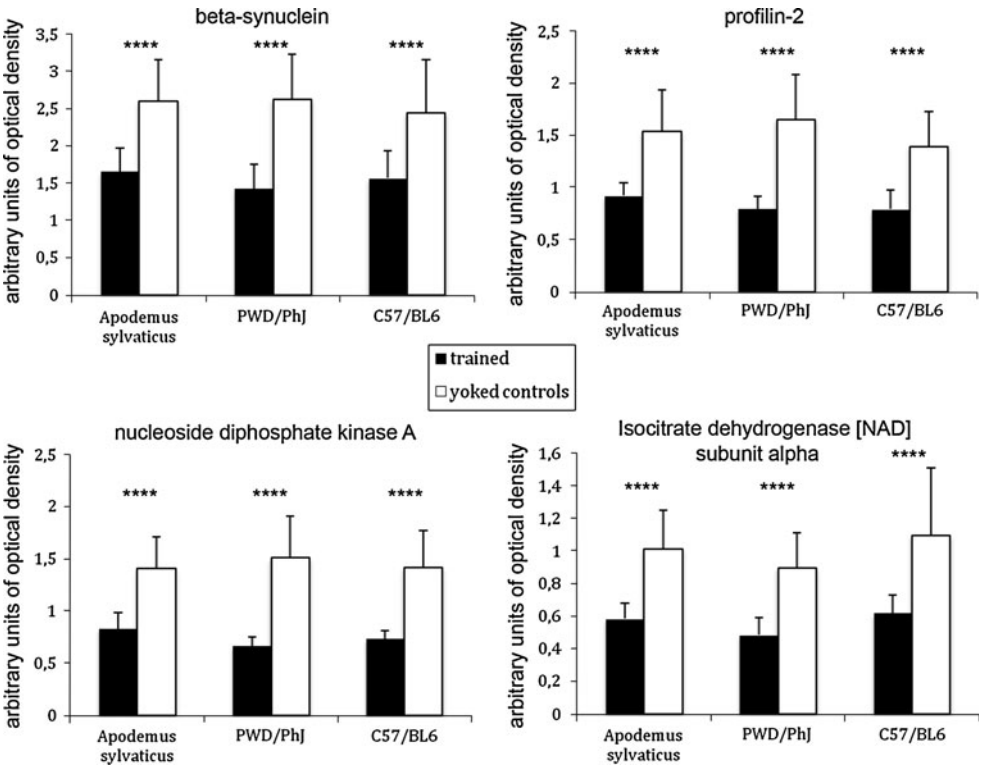


**Fig. 1** Hippocampal levels (arbitrary units of optical density) of the four proteins identified as global factors for memory training in three individual strains are shown. $****P \leq 0.0001$

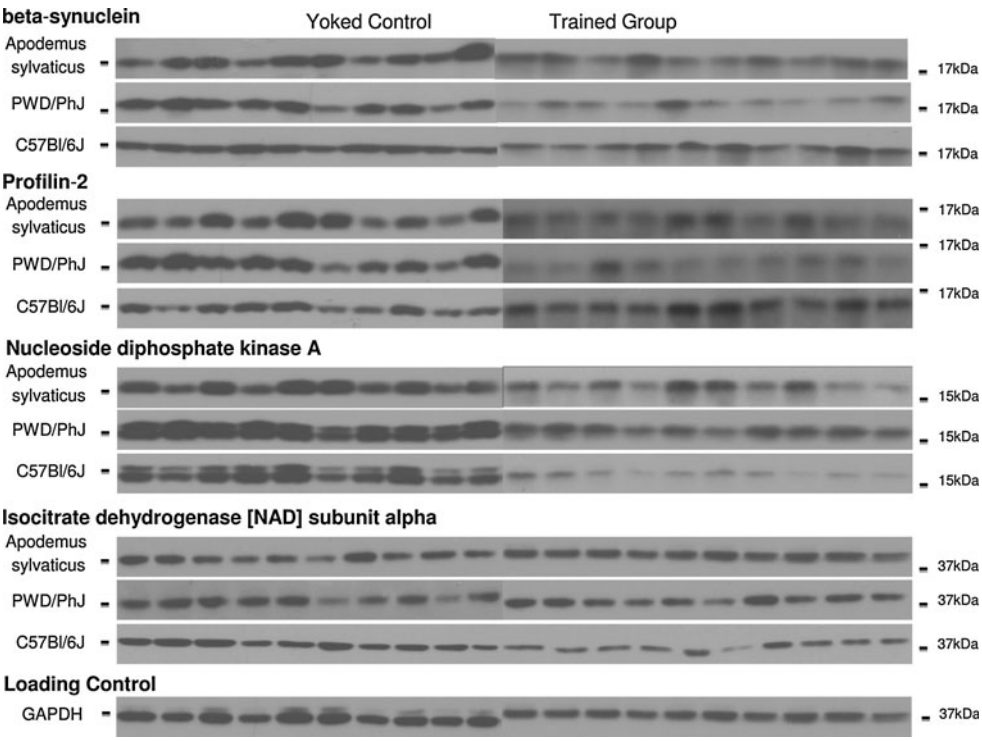


**Fig. 2** Immunochemical verification of beta-synuclein, profilin-2, nucleoside diphosphate kinase a, and isocitrate dehydrogenase subunit alpha. GAPDH was used as loading control. The findings obtained from quantifying corresponding spots from the 2DE were verified. For comparison of different blottings one sample was permanently loaded on all gels

protein degradation (ubiquitin-40S ribosomal protein S27a), protein isomerisation (protein disulfide-isomerase A3), signaling (nucleoside diphosphate kinase A, programmed cell death 6-interacting protein), intermediary metabolism (malate dehydrogenase, ATP synthase subunit d, isocitrate dehydrogenase subunit alpha), a protein involved in neurodegeneration (beta-synuclein) and cytoskeleton organisation (clathrin light chain B and profilin-2,

represented by two expression forms). The statistical results following stringent criteria of $P < 0.001$ for the individual proteins as global factors for training in the MWM are shown in supplemental Table 1.

Identification results by mass spectrometry for the four immunochemically verified proteins are shown in Table 1. As shown in supplemental Fig. 1, hippocampal proteins were well separated and proteins are given in the map as UniProtKB accession numbers. Identification results indicating protein accession numbers (UniProtKB), protein name, MS/MS scores, number of matched peptides, sequence coverages (ranging from 49.3 to 97.7%), sequence of MS/MS peptides, theoretical MW and theoretical pI are listed in supplemental Table 2; data on the four verified proteins are provided in Table 1. Only 4 out of these 12 proteins were verified by immunoblotting and are herein further discussed.

Hippocampal levels (arbitrary units of optical density) of the four proteins identified as global factors for memory training in three individual strains are shown in Fig. 1. The immunoblots showing verification of differences of the four proteins between trained and yoked controls are given in Fig. 2. In addition, statistical analysis of immunoblots with mean, standard deviation and $P$ value from independent student $T$ test between trained and yoked groups of three strains is given in Table 2.

Altered levels of beta-synuclein and NME1 have been reported to be dysregulated in neurological disorders with mental deficits; profilin-2 is an essential component of the dendritic spine formation and the isocitrate dehydrogenase 3 may be required for provision of energy for hippocampal cognitive functions. The current report reveals for the first time a global effect and this work may be important for design and interpretation of strain-independent cognitive studies.

The synuclein family of proteins consists of synucleins alpha, beta, gamma. Beta-synuclein is co-expressed with alpha-synuclein at presynaptic nerve terminals. It is subject to phosphorylation by Ca(2+) calmodulin protein kinase II (Sopher et al. 2001), Polo-like kinases (Mbefo et al. 2010),

**Table 2** Statistical analysis of immunoblots with mean, standard deviation and $P$ value from independent student $T$ test between trained and yoked groups of three strains

| Protein strain | | $N$ | Mean ± SD | $P$ value |
|---|---|---|---|---|
| Beta_synuclein | | | | |
| *Apodemus sylvaticus* | Trained mice | 20 | 1.80 ± 0.55 | 0.05 |
| | Yoked controls | 20 | 2.07 ± 0.50 | |
| PWD/PhJ | Trained mice | 20 | 1.83 ± 0.74 | 0.0001 |
| | Yoked controls | 20 | 6.33 ± 1.71 | |
| C57Bl/6J | Trained mice | 20 | 2.22 ± 0.57 | 0.0001 |
| | Yoked controls | 20 | 3.96 ± 0.36 | |
| Profilin-2 | | | | |
| *Apodemus sylvaticus* | Trained mice | 20 | 3.10 ± 1.37 | 0.1143 |
| | Yoked controls | 20 | 3.72 ± 1.78 | |
| PWD/PhJ | Trained mice | 20 | 3.43 ± 0.79 | 0.0001 |
| | Yoked controls | 20 | 7.22 ± 2.55 | |
| C57Bl/6J | Trained mice | 20 | 2.56 ± 0.61 | 0.0001 |
| | Yoked controls | 20 | 6.39 ± 1.61 | |
| Isocitrate dehydrogenase (NAD) subunit alpha | | | | |
| *Apodemus sylvaticus* | Trained mice | 20 | 0.79 ± 0.27 | 0.0001 |
| | Yoked controls | 20 | 2.82 ± 1.47 | |
| PWD/PhJ | Trained mice | 20 | 1.32 ± 0.20 | 0.0001 |
| | Yoked controls | 20 | 1.95 ± 0.50 | |
| C57Bl/6J | Trained mice | 20 | 0.50 ± 0.10 | 0.12 |
| | Yoked controls | 20 | 0.63 ± 0.22 | |
| Nucleoside diphosphate kinase A | | | | |
| *Apodemus sylvaticus* | Trained mice | 20 | 1.63 ± 0.31 | 0.0169 |
| | Yoked controls | 20 | 2.08 ± 0.84 | |
| PWD/PhJ | Trained mice | 20 | 3.71 ± 1.04 | 0.0018 |
| | Yoked controls | 20 | 4.70 ± 0.99 | |
| C57Bl/6J | Trained mice | 20 | 1.46 ± 0.32 | 0.0001 |
| | Yoked controls | 20 | 3.09 ± 0.49 | |

C-terminal Src kinase homologous kinase CHK (Ia et al. 2011), G protein-coupled receptor kinases (Pronin et al. 2000) and is important for neural plasticity.

Beta-synuclein regulates Akt (serine threonine kinase Akt) activity and can be therefore considered as a major player in memory mechanisms (Hashimoto et al. 2004a): Akt has been shown to be a key element in spatial memory processes (Chao et al. 2007; Chen et al. 2011; Horwood et al. 2006; Mizuno et al. 2003). In mice lacking all three members of the synuclein family, a series of neurochemical and behavioral changes were observed but spatial memory was not evaluated (Anwar et al. 2011).

A beta-synuclein mutation is linked to memory deficits in a transgenic mouse model (Fujita et al. 2010) and abnormal beta-synuclein brain levels are associated with Parkinson's disease (Beyer et al. 2011). Moreover, beta-synuclein may play a role for Parkinson's disease and dementia with Lewy bodies and an experimental treatment approach in transgenic mice might have potential for the development of corresponding therapies (Hashimoto et al. 2004b).

Herein, we observed that in a strain-independent way beta-synuclein levels were a global factor for memory training in the MWM.

The identification of profilin-2 as global factor for memory training is compatible with so far known functions of this molecule: it contributes to synaptic vesicle exocytosis i.e. neural transmission and is involved in cognitive function albeit not in memory processes (Pilo Boyl et al. 2007). In addition, profilin is involved in synaptic plasticity (Ackermann and Matus 2003) and in a fear memory setting, profilin is driven into amygdala dendritic spines, structures considered important for memory formation (Lamprecht et al. 2006). The findings in the current study identify profilin-2 as a global factor for spatial memory and is may be indirectly supporting evidence for abovementioned previous findings.

Nucleoside diphosphate kinases are regulating several cellular functions in mammals including signaling via G proteins (Kimura et al. 2000). The enzyme is present in the postsynaptic density fraction (Satoh et al. 2002) and a role in mouse brain development was proposed (Carotenuto et al. 2006). Nucleoside diphosphate kinase a levels were shown to be significantly decreased in brain of patients with Alzheimer's disease (Kim et al. 2002) and it may be speculated that this kinase may be associated with the memory loss in this disorder.

Herein, we show for the first time a link to spatial memory training identifying nucleoside diphosphate kinase a as a global factor.

As to isocitrate dehydrogenase as a global factor, we may propose that this key regulatory enzyme in the Krebs cycle (Weiss et al. 2000) is essential for energy provision to serve memory functions.

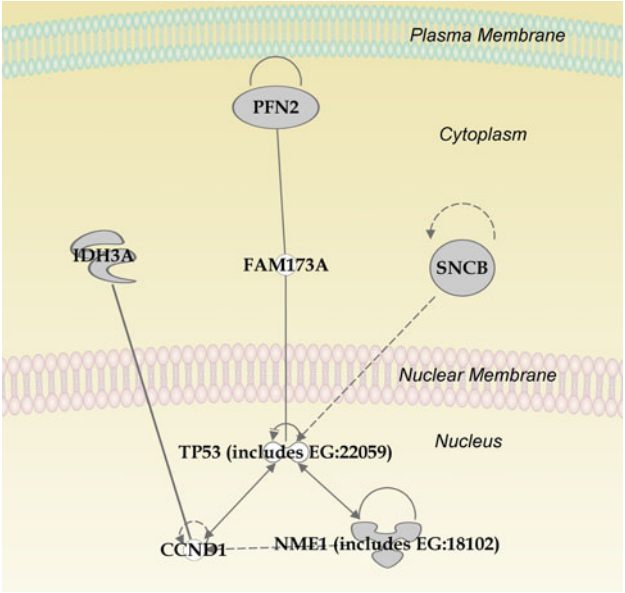


**Fig. 3** A protein network was constructed and the interactions are shown. A *solid line* indicates direct interaction, the *dashed line* is indicating indirect regulation or activation. *PFN2* profilin-2, *IDH3A* isocitrate dehydrogenase subunit alpha, *SNCB* beta-synuclein, *NME1* nucleoside diphosphate kinase a, *CCND1* cyclin D1, *FAM173A* family with sequence similarity 173, member A

A network was created from the four proteins (Fig. 3) which proposes strong links of all global factors for memory training to TP53: Profilin-2 shows protein–protein interaction with FAM173A which in turn interacts with TP53 (Stelzl et al. 2005). The nature of the link between beta-synuclein and TP53 is that beta-synuclein displays an antiapoptotic p53-dependent phenotype (da Costa et al. 2003). Cyclin D1 is a part of the interactome in human cancers and is thus linked to TP53 (Jirawatnotai et al. 2011) and by involvement in p53-mediated proliferation of primary embryonic fibroblasts (Guo and Zheng 2004). Finally, nucleoside diphosphate kinase is potentiating P53 by direct interaction (Jung et al. 2007).

Nucleoside diphosphate kinase in turn is able to induce cell cycle arrest and apoptosis in lymphocytes (Choudhuri et al. 2010). The interaction between nucleoside diphosphate kinase a and serine–threonine kinase receptor-associated protein, that negatively regulates TGF-beta signaling has been reported (Seong et al. 2007)—no functional relevance for memory training could be suggested from literature.

Taken together, four hippocampal proteins have been identified as global factors for memory training in a strain-independent manner. One would therefore expect that these spatial memory training-related proteins could be important for interpretation of previous and for the design of future studies on spatial memory. These finding are also challenging previous findings of proteins "linked to

memory" when only one mouse strain was used because aberrant levels or expressions of this protein may simply indicate a strain-effect. Further studies on global effects ruling out strain-dependence of protein expression are carried out in our laboratory to generate a network of unambiguously memory-related proteins.

**Conflict of interest** The authors declare that there are no financial/commercial conflicts of interest.

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