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Increased Hippocampal Expression of the Divalent Metal Transporter 1 (DMT1) mRNA Variants 1B and +IRE and DMT1 Protein After NMDA-Receptor Stimulation or Spatial Memory Training

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Abstract Iron is essential for crucial neuronal functions but is also highly toxic in excess. Neurons acquire iron through transferrin receptor-mediated endocytosis and via the divalent metal transporter 1 (DMT1). The N-terminus (1A, 1B) and C-terminus (+IRE, −IRE) splice variants of DMT1 originate four protein isoforms, all of which supply iron to cells. Diverse physiological or pathological conditions induce differential DMT1 variant expression, which are cell-type dependent. Hence, it becomes relevant to ascertain if activation of neuronal plasticity processes that require functional *N*-methyl D-aspartate (NMDA) receptors, including in vitro stimulation of NMDA receptor-mediated signaling and spatial memory training, selectively modify DMT1 variant expression. Here, we report for the first time that brief (5 min) exposure of primary hippocampal cultures to NMDA (50 μM) increased 24 h later the expression of DMT1-1B and DMT1+IRE, but not of DMT1−IRE mRNA. In contrast, endogenous DMT1 mRNA levels remained unaffected following 6 h incubation with brain-derived nerve factor. NMDA (25–50 μM) also enhanced DMT1 protein expression 24–48 h later; this enhancement was abolished by the transcription inhibitor actinomycin D and by the NMDA receptor antagonist MK-801, implicating NMDA receptors in de novo DMT1 expression. Additionally, spatial memory training enhanced DMT1-1B and DMT1+IRE expression and increased DMT1 protein content in rat hippocampus, where the exon1A variant was not found. These results suggest that NMDA receptor-dependent plasticity processes stimulate expression of the iron transporter DMT1-1B+IRE isoform, which presumably plays a significant role in hippocampal spatial memory formation.

Keywords Iron transport · Synaptic plasticity · mRNA splicing variants · DMT1 protein · Morris water maze · Spatial learning · BDNF · Hippocampal pyramidal neurons

Abbreviations

ACSF	Artificial cerebrospinal fluid
Act D	Actinomycin D
BDNF	Brain-derived neurotrophic factor
DMT1	Divalent metal transporter 1
GFAP	Glial fibrillary acidic protein
IRE	Iron response element
IRP	Iron regulatory protein
MAP2	Microtubule-associated protein 2
MWM	Morris Water Maze
NF-Y	Nuclear factor Y
NFκB	Nuclear factor kappa B
NMDA	*N*-Methyl D-aspartate

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PBS Phosphate buffered saline
ROS Reactive oxygen species

Introduction

Iron deficiency during infancy leads to lower cognitive performance in adulthood (Lozoff 2000). In animal models, nutritional iron deficiency interferes with hippocampus-depending learning (McEchron and Paronish 2005; Ranade et al. 2008) and synaptic plasticity (Jorgenson et al. 2005). These functional failings have been ascribed to the iron requirements of metabolic pathways involved in neurotransmitter synthesis and myelin formation (Youdim et al. 1980; Taneja et al. 1986; Kwik-Uribe et al. 2000). Conversely, excessive iron accumulation impairs normal neuronal functions and induces neuronal death (Cheah et al. 2006; Salazar et al. 2006, 2008; Du et al. 2009).

The main players of cellular iron homeostasis comprise: the transferrin receptor (Griffiths and Crossman 1996; Moos et al. 1998), the divalent metal transporter DMT1 (Burdo et al. 1999, 2001; Williams et al. 2000), the iron storage protein ferritin (Benkovic and Connor 1993), and the iron export transporter ferroportin (Wu et al. 2004), all of which are well expressed in different areas of the brain. In the particular case of DMT1, which mediates ferrous iron uptake into cells (Gunshin et al. 1997), in situ hybridization studies in developing rat brain showed DMT1 mRNA localization in striatum, cortex, hippocampus, and cerebellum (Williams et al. 2000). Immunohistochemistry analysis of adult rat brain showed DMT1 protein expression in striatum, cerebellum, and thalamus, as well as in vascular cells throughout the brain and ependymal cells in the third ventricle (Burdo et al. 2001).

The mammalian DMT1 gene (SLC11A2; Nramp2) undergoes alternative splicing. The 1A and 1B mRNA DMT1 variants originate from alternative splicing at the 5′ end (exons 1A and 1B), while the +IRE or −IRE variants originate from the 3′ end (exons 16/16A and 17) (Hubert and Hentze 2002). These variants give rise to four DMT1 protein isoforms, all of them active in Fe^{2+} transport (Ludwiczek et al. 2007). It is generally accepted that the two +IRE isoforms are post-transcriptionally regulated by the IRE/IRP system (Pantopoulos 2004). Knowledge of differential transcriptional regulation of DMT1 expression is emerging. Both the inflammatory cytokine nuclear factor kappa B (NFκB) and the nuclear factor Y (NF-Y) regulate DMT1-1B expression in embryonic carcinoma cells (Paradkar and Roth 2007). In contrast, hypoxia upregulates expression of the 1A mRNA variant in PC12 cells, presumably through activation of hypoxic response elements in its promoter region (Lis et al. 2005).

DMT1 mediates iron uptake into neurons, where cellular iron levels are essential for crucial neuronal functions but highly toxic in excess (Hidalgo et al. 2007; Hidalgo and Nuñez 2007; Pelizzoni et al. 2008). Interestingly, a drug that causes experimental Parkinson's disease (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) upregulates DMT1+IRE protein expression in mice ventral mesencephalon, where it increases neuronal death presumably through abnormal increases in cellular iron content (Salazar et al. 2008). Additionally, DMT1−IRE mediates L-DOPA neurotoxicity in primary cortical neurons (Du et al. 2009). Consequently, to understand the role of neuronal iron in health and disease, it becomes important to determine which DMT1 isoforms are expressed in neurons, and how neuronal activity regulates their expression.

In this work, we report for the first time that *N*-methyl D-aspartate (NMDA) addition to primary hippocampal cultures, or spatial memory training, selectively stimulate hippocampal expression of the DMT1 splicing variants 1B and +IRE, and increased DMT1 protein content. These results suggest that enhanced expression of the iron transporter 1B+IRE DMT1 isoform has a role in hippocampal-dependent long-lasting plasticity changes.

Methods

Hippocampal Cultures

Primary cultures were prepared from hippocampus dissected from Sprague Dawley rats at embryonic day 18 (Kahlert et al. 2005). Cells, plated in minimum essential medium plus 10% horse serum, were grown subsequently at 37°C under 5% CO_2 in serum-free Neurobasal medium supplemented with GIBCO^{TM} B27 serum-free and 2 mM GlutamaxTM (Invitrogen). To inhibit glial cell proliferation, 5-fluoro-2′-deoxy-uridine (1.4 mg/l) plus uridine (3.5 mg/l) were added for 24 h at the second day of culture.

Immunocytochemistry and Immunohistochemistry

Hippocampal cells were fixed for 20 min in PBS containing 4% paraformaldehyde, 4% sucrose and permeabilized for 10 min with 0.1% Triton X-100 in phosphate buffered saline (PBS). For immunodetection, cells were incubated overnight at 4°C with polyclonal rabbit anti-PanDMT1 antibody prepared against a peptide common to all isoforms (Salazar et al. 2008), with anti-β-Tubulin III (Sigma) or with MAP2 antibody (Chemicon). Glial cells were stained with an antibody against glial fibrillary acidic

protein (GFAP) (DAKO). Cells were subsequently incubated with Alexa Fluor® 488 anti-rabbit or Alexa Fluor® 635 anti-mouse as secondary antibody. Brain tissue fixation and immunohistochemistry were performed as described (Haeger et al. 2006), except that coronal slices were incubated at 4°C overnight with PanDMT1 and MAP2 antibodies. Sections were incubated at room temperature for 2 h in PBS buffer containing 0.2% gelatin and 0.1% Triton X-100 plus the Alexa antibodies defined above, rinsed in PBS, and mounted with glycerol. All fluorescence images were obtained in a confocal microscope (Carl Zeiss LSM Pascal 5, Zeiss, Oberkochen, Germany), and digitally acquired using LSM software (Zeiss). Controls performed without primary or secondary antibody yielded only background staining.

Western Blot Analysis

Cells extracts prepared as described (Aguirre et al. 2005) were resolved in 10% Laemmli SDS-polyacrylamide gels, transferred to PDVF membranes (Millipore) and incubated overnight with PanDMT1 primary antibody. To correct for loading, membranes were stripped and re-probed for β-actin. The IMAGE J image program (National Institutes of Health, USA) was used to quantify optical band density.

Incubation with NMDA or Brain-Derived Neurotrophic Factor (BDNF)

One hour before NMDA addition, the culture medium was replaced by Neurobasal-B27 without antioxidants. Cells were then incubated for 5 min with NMDA (25 or 50 μM), 10 μM D-Serine in ACSF plus 0.62 mM $MgCl_2$. Control cells were incubated similarly without NMDA. Cells were subsequently incubated 1 h in Neurobasal-B27 medium without antioxidants plus 23 h in the original culture medium prior to RNA isolation or western blot analysis. Alternatively, cultures were incubated in supplemented Neurobasal-B27 medium for 6 h with BDNF (50 ng/ml) and DMT1 mRNA expression was determined right after incubation.

RNA Isolation and RT-PCR

Total RNA was isolated using Trizol reagent (Invitrogen). DNAase digestion (DNA-free™ Kit, Ambion) was included to remove any contaminating genomic DNA. RNA purity was assessed by the 260/280 absorbance ratio and RNA integrity by gel electrophoresis. cDNA was synthesized from 0.5 μg total RNA using ImProm-II™ Reverse Transcriptase (Promega). Twenty-five nanograms of cDNA was used in 20 μl final volume for PCR amplification using an Applied Biosystem Thermal cycler. Amplification of the mRNA for all four DMT1 isoforms was done for 35 cycles; each cycle included 15 s at 94°C, 15 s at 60°C (or 58°C for DMT1 1A and 1B isoforms), and 15 s at 72°C. After these cycles, a final 10 min incubation step at 72°C was added. Primers for DMT1+IRE and DMT1−IRE cDNAs, constructed based on the rat DMT1 sequence (GenBank #AF008439 and #AF029757), were: Forward, 5′-CTGAG CGAAGATACCAGCG-3′ and 5′-GTCTTCCAAGATGT GGAGCA-3′; Reverse, 5′-GGAGCCATCACTTGACCAC AC-3′ and 5′-ATCTTACCCAAACTGGCACG-3′, respectively. The selected forward primers for the DMT1 1A and 1B variants were as previously described (Hubert and Hentze 2002; Lis et al. 2005): 5′-TCCGATGGGGAAGAA GCAGCC-3′ for DMT1 1A and 5′-CAATCACGGGA GGGCAGGAG-3′ for DMT1-1B. The same reverse primer 5′-CTAGGTAGGCAATGCTCATAAGAAAGCCAGG-3′ was used for both variants. The β-actin primers used were: forward 5′-TCTACAATGAGCTGCGTGTG-3′ and reverse 5′-TACATGGCTGGGGTGTTGAA-3′. The amount of cDNA was determined by β-actin amplification using real time PCR, performed in a thermocycler (Stratagene MX3000P) using the DNA binding dye SYBR green (Invitrogen). A graph digitizing software was used for densitometry analysis of RT-PCR products. The 380-bp DMT1-1B PCR fragment obtained after RT-PCR amplification of the total RNA obtained from cultured hippocampal rat cells (Fig. 1, lane 1), was extracted from the agarose gel by means of a gel extraction kit (Qiagen). This product was directly sequenced (ABI PRISM 310; ABI, Foster City, CA, USA) using the forward and reverse primers for DMT1-1B described above, and was aligned with the 5′ end of mouse DMT1-1B (MM001146161) using the Cluscalw2 software (EMBL-EBI).

Morris Water Maze Training

Male Sprague Dawley rats were trained once daily using a circular water maze (1.6 m diameter, 75 cm deep) endowed with spatial cues and a hidden platform placed at a fixed location; each trial involved three 1 min sessions, separated by 20 min intervals. Training was continued for six consecutive days, followed by 2 days off; to evaluate memory retention, one additional day without platform was added. The whole hippocampus was removed 6 h after the ninth day trail. Escape latency was measured as described (De Ferrari et al. 2003). Control animals were subjected for four consecutive days to three swimming trials daily with the cued platform; the platform location varied daily. At day four, the whole hippocampus was removed 6 h after the last trial.

All experiments described here were carried out under the guidelines of National Institutes of Health, USA, regulations for the Care and Use of Animals for Scientific

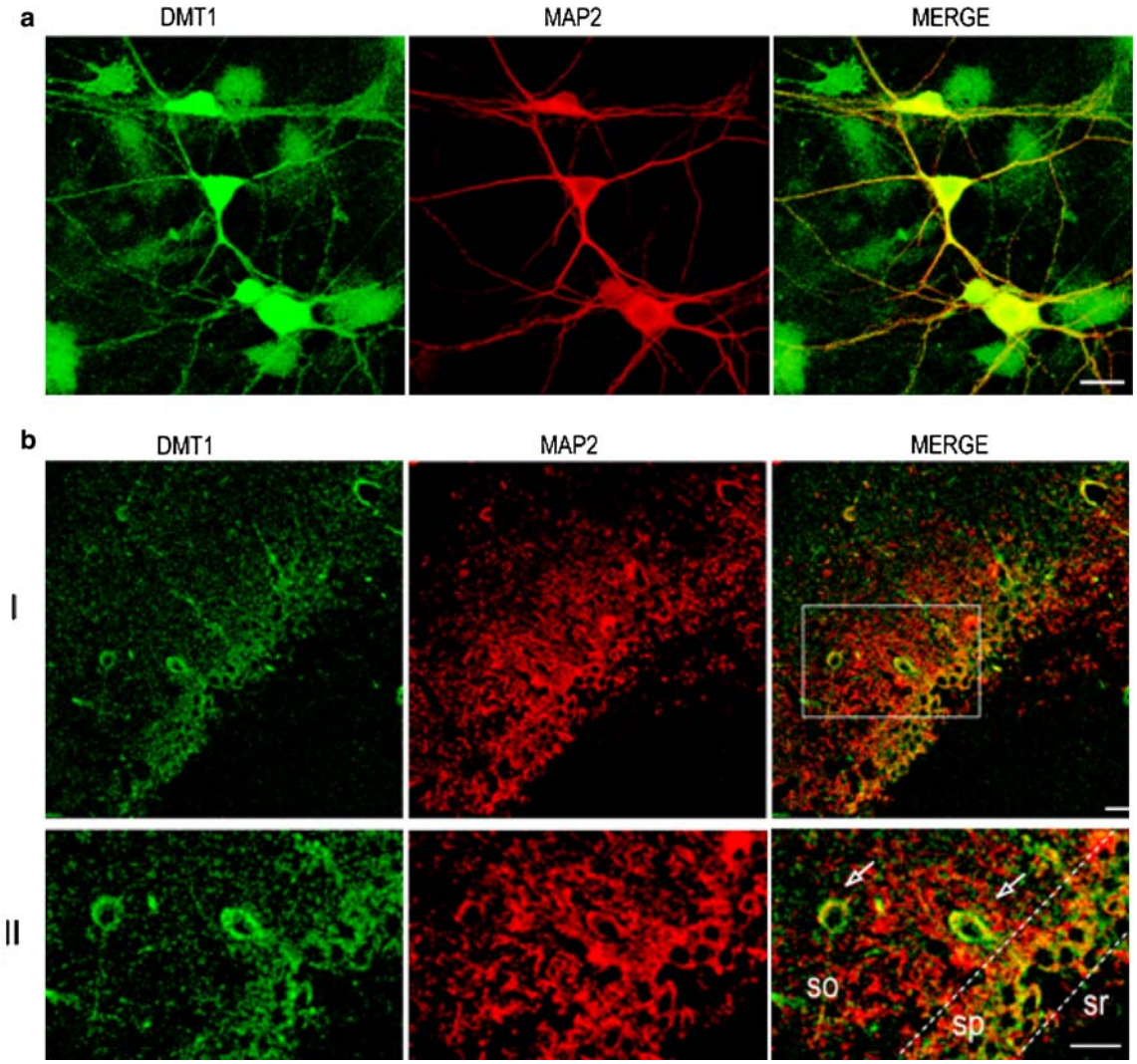

Fig. 1 Confocal analysis of hippocampal DMT1 protein expression: **a** Co-immunofluorescence of primary hippocampal cells cultured for 14 days, fixed and reacted with MAP2 antibody (*red*) and rabbit PanDMT1 antibody (*green*). Merge: The image shows DMT1 expression in pyramidal and granular neurons. **b** Co-immunofluorescence of DMT1 and the neuronal marker MAP2 in the CA1 region of rat hippocampus. Coronal brain slices were incubated with specific PanDMT1 or MAP2 antibody. *I* show the CA1 region of the hippocampus, *II* shows the magnification of the inset defined in *I*. The immunoreactions show DMT1 expression (*green*) in CA1 pyramidal neurons (*red*) of the stratum oriens (so; see *arrows*) and stratum pyramidalis (sp). *Sr* stratum radiatum. Calibration bar, 20 μm

Purposes; all protocols were approved by the Bioethics Committee, F. Medicine, Universidad de Chile.

Results

Hippocampal DMT1 Expression

Previous reports have described DMT1 mRNA expression in the hippocampus (Williams et al. 2000), and DMT1 protein expression in glial cells (Huang et al. 2006), but to our knowledge no published data are available showing expression of DMT1 protein or mRNA variants in hippocampal neurons. To address this issue, we analyzed DMT1 expression in primary hippocampal cultures highly enriched in neurons (Supplementary Fig. 1a) and hippocampal tissue. We report for the first time that primary hippocampal neurons and hippocampal tissue contain significant DMT1 protein levels (Fig. 1) and express the 1B, +IRE, and −IRE DMT1 splicing variants (Fig. 2). Thus, immunofluorescence analysis indicated that the primary hippocampal cultures used here possess appreciable DMT1 protein levels (Fig. 1a). Likewise, analysis of adult rat hippocampus sections by immunofluorescence revealed strong DMT1 protein expression in CA1 pyramidal neurons of the pyramidalis and oriens stratum (Fig. 1b).

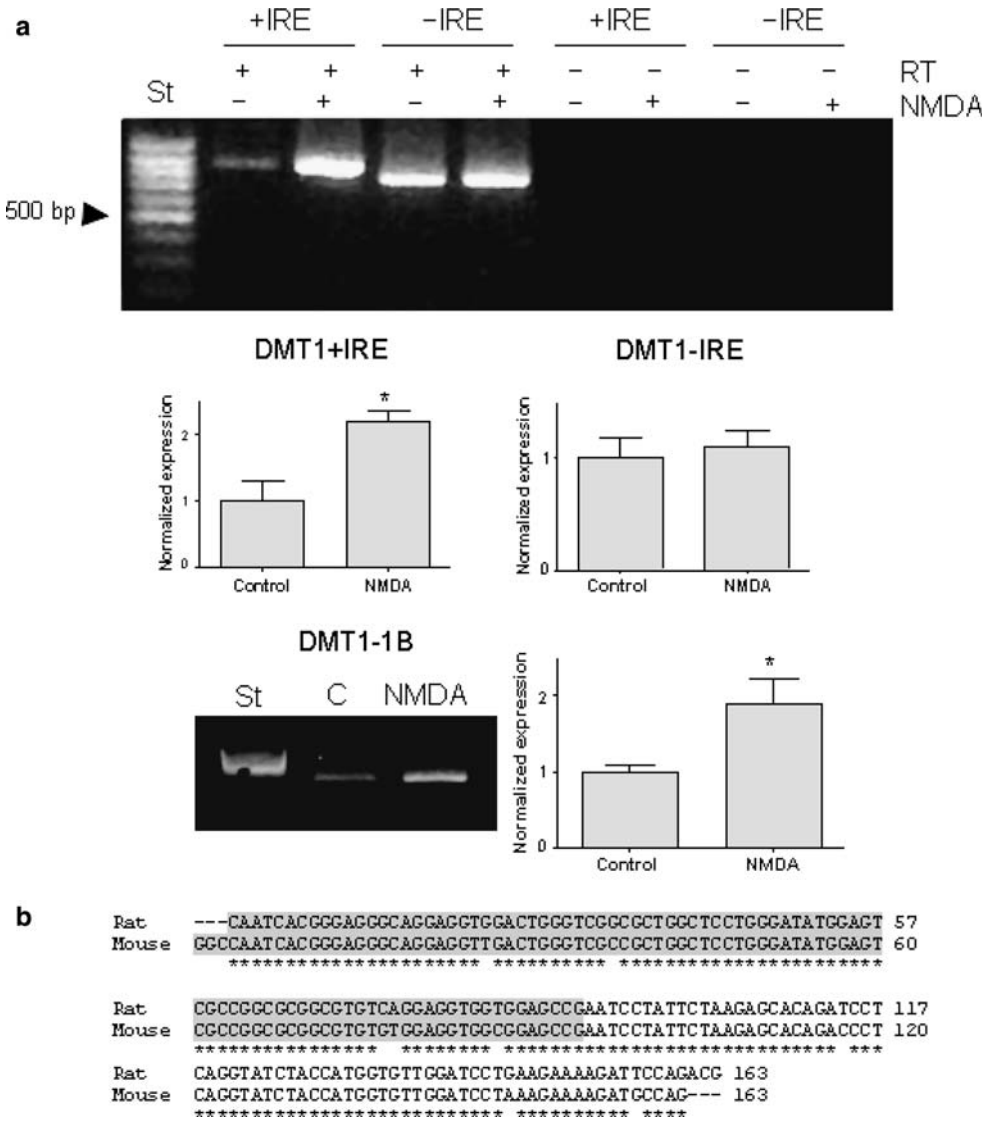

Fig. 2 NMDA increases 1B and +IRE DMT1 mRNA expression in primary hippocampal cultures. **a** RT-PCR analysis of RNA extracted from cultured hippocampal cells. Gels and quantitative densitometry analyses, normalized with β-actin, show increased +IRE and 1B DMT1 mRNA variants in cultures incubated with NMDA (5 min, 50 μM), whereas endogenous −IRE expression was not affected. St: 100 bp DNA ladder. −RT: mock synthesis of cDNA in the absence of reverse transcriptase (RT). Data, expressed as fold of control, represent mean ± SEM. * $P < 0.05$ ($n = 3$), evaluated with one-tailed paired Student's t test. **b** Alignment of the partial sequences for exon 1B and exon 2 of rat and mouse DMT1-1B mRNA. The mouse sequence was previously described (NM001146161). We reported the rat sequence from the present work to NIH Genebank (GQ16122).The sequence highlighted in gray shows the partial sequence of exon 1B of DMT1-1B mRNA

NMDA Increases the Expression of the Iron Transporter DMT1

Activation of hippocampal NMDA receptors promotes the expression of genes required to maintain synaptic plasticity for prolonged times (Platenik et al. 2000; Deisseroth et al. 2003; Tabuchi 2008). To our knowledge, no data are available in the literature describing the effects of NMDA on DMT1 expression. Here, we report that NMDA receptor activation, induced by incubation with 50 μM NMDA for 5 min, produced a significant increase in 1B and +IRE DMT1 mRNA expression when measured 24 h later, whereas the endogenous −IRE mRNA levels remained unchanged (Fig. 2a). The primary cultures used here did not possess measurable levels of the DMT1 1A splicing variant (data not shown). In addition, we report here (Fig. 2b) a partial sequence of the rat 1B-DMT1 exon, which after sequence alignment revealed 94% identity with the mouse exon 1B sequence.

Incubation for 5 min with 50 μM NMDA produced 24 h later a moderate (1.2-fold) but significant increase in DMT1 protein content (Fig. 3a, left). DMT1 protein expression, measured 48 h after incubation with 25 μM NMDA for 5 min, increased 2.4-fold (Fig. 3a, right). Preincubation of cells for 1 h with the NMDA receptor inhibitor MK-801 (10 μM) before NMDA addition abolished the NMDA-induced DMT1 protein increase (Fig. 3b), indicating that NMDA receptors mediate this response. Furthermore, preincubation with 1.5 μM actinomycin D also abolished NMDA-induced DMT1 protein expression (Fig. 3b). These results strongly suggest that NMDA receptor stimulation promotes de novo DMT1 gene expression in primary hippocampal cultures.

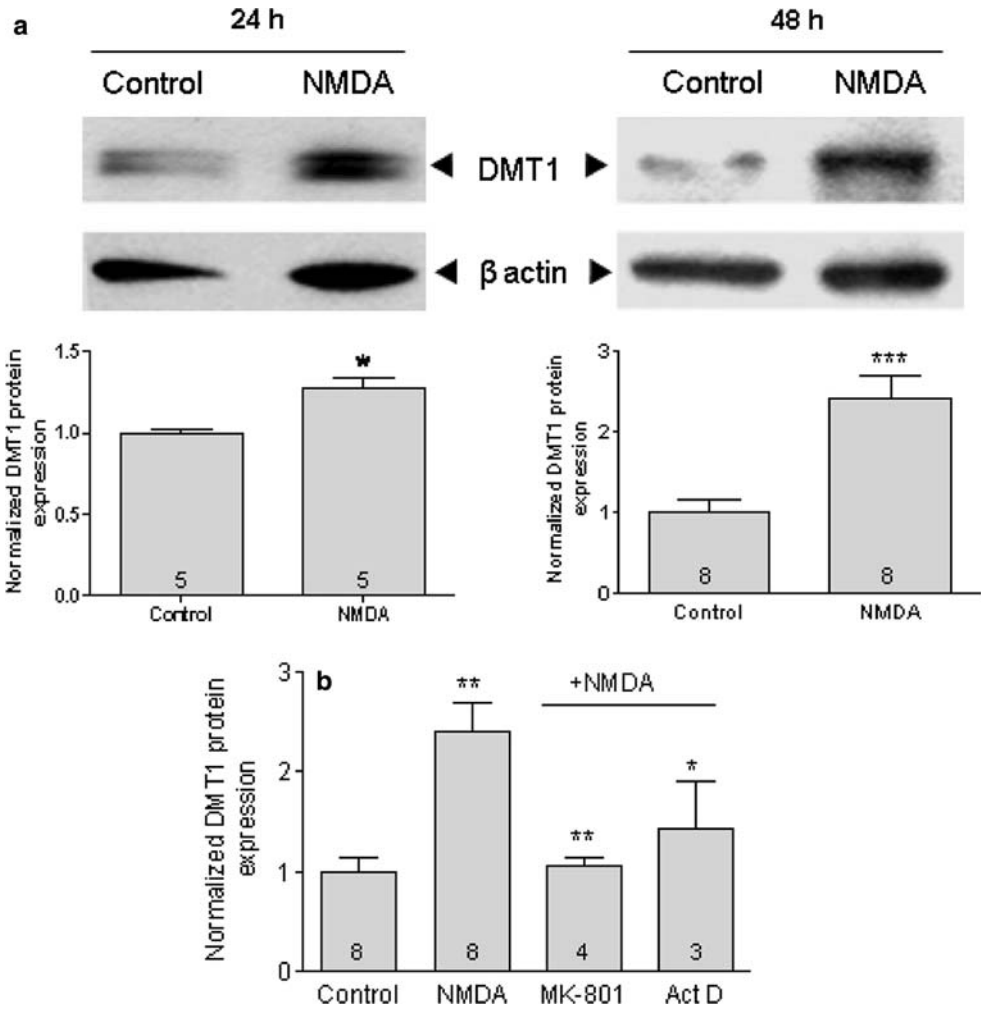

Fig. 3 NMDA increases DMT1 protein levels in primary hippocampal cultures. **a** Hippocampal cells were incubated for 5 min with 50 or 25 μM NMDA; cell extracts were collected after 24 or 48 h of culture, respectively. NMDA stimulation increased total DMT1 protein content, as shown in western blots of cell extracts obtained 24 and 48 h after incubation with NMDA. *β*-actin was used as loading control. The number of experiments is indicated in each bar. Data are given as mean ± SEM. Statistical differences were analyzed by one-tailed paired Student's *t* test. * $P < 0.05$, *** $P < 0.001$. **b** Previous to 25 μM NMDA stimulation, hippocampal cells were incubated for 1 h with the NMDA receptor antagonist MK-801 (10 μM) or the transcription inhibitor Actinomycin D (Act D, 1.5 μM) and the extract was obtained 48 h post-NMDA stimulus. The number of experiments is indicated in each bar. Statistical analysis: One-way Anova, followed by Newman Keuls post-test

The enhanced expression of DMT1 protein probably reflects increased expression of the DMT1-1B+IRE isoform since the 1A mRNA variant was not detected in the cultures. The PanDMT1 antibody used does not distinguish between the different DMT1 protein isoforms, so the increase in DMT1-1B+IRE content may be higher than estimated here.

In contrast to the stimulatory effects of NMDA observed at 24 and 48 h, incubation of primary cultures for 6 h with BDNF, a neurotrophin functionally stimulated by NMDA receptor activation (Caldeira et al. 2007), did not increase expression of the 1B, +IRE, and −IRE DMT1 variants when measured right after the incubation period (Fig. 4). Yet, incubation with BDNF for 6 h significantly stimulates transcription of several synaptic protein genes (Ring et al. 2006). Accordingly, our results suggest that BDNF does not stimulate DMT1 expression, or that if it does, this stimulation occurs at times longer than 6 h.

In agreement with the above findings in cultured cells, the hippocampus of adult rats displayed significant endogenous levels of DMT1+IRE, DMT1−IRE, and DMT1-1B mRNA variants (Fig. 5a), whereas DMT1-1A mRNA was not detected (Fig. 5b). NMDA receptor activation is a key step of spatial memory formation (Robbins and Murphy 2006). Accordingly, we investigated if training in a Morris water maze modified hippocampal DMT1 expression. As observed in NMDA-stimulated primary cultures, a significant increase in the +IRE and 1B mRNA variants occurred following spatial training of rats while DMT1−IRE mRNA remained unaffected (Fig. 5a). Immunohistochemistry analysis showed significant DMT1 protein expression in CA1 pyramidal neurons of adult rat hippocampus (Fig. 1b). Interestingly, after spatial memory training DMT1 protein content in hippocampal extracts augmented >2.5-fold, as revealed by western blot analysis (Fig. 5c).

Discussion

Scant information is available regarding the role of DMT1 in normal or pathological neuronal function. Preliminary

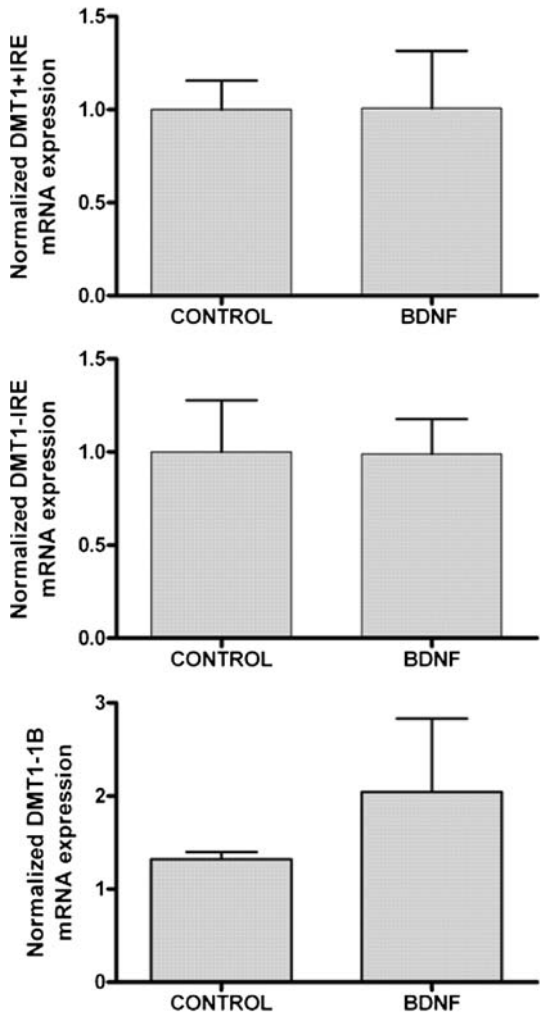

Fig. 4 Lack of effect of BDNF on DMT1 mRNA variant expression in hippocampal cultures. RT-PCR analysis of RNA extracted from cultured hippocampal cells incubated for 6 h with BDNF (50 ng/ml). Quantitative densitometry analysis, normalized with β-actin, revealed that expression of 1B, +IRE, and −IRE DMT1 mRNA variants did not undergo significant changes after BDNF incubation. Data, expressed as fold of control, represent mean ± SEM ($n \geq 3$). Significance was evaluated with one-tailed paired Student's t test

results from our group[1] showed that incubation of primary cultures with NMDA or spatial memory training of rats in the MWM increased hippocampal DMT1+IRE mRNA and protein expression. While this work was in progress, deficient spatial learning in a conditionally targeted DMT1 knockout mouse and increased DMT1 mRNA expression after spatial memory training was reported (Carlson et al. 2009); however, the DMT1 variants induced by training were not identified. Expanding on the above results, we report here that hippocampal cultures briefly exposed for 5 min to the glutamate receptor agonist NMDA displayed 24 h later significantly enhanced expression of the +IRE and 1B mRNA variants, coupled with increased DMT1 protein content, which increased even further at 48 h.

Previous reports indicate that NMDA stimulates signaling cascades that enhance iron uptake in cortical neurons (Cheah et al. 2006), and that iron chelation with desferrioxamine impairs tetanically induced long-term potentiation at hippocampal CA1 neurons (Hidalgo et al. 2007), a process known to involve NMDA receptors (Platenik et al. 2000). Hence, the significant up-regulation of DMT1 expression following NMDA receptor stimulation reported here, suggests that hippocampal neurons increase DMT1 protein expression after synaptic stimulation to improve their iron uptake capacity, and thus ensure an adequate iron supply for plasticity processes. Our results also suggest that enhanced transcription of the 1B variant, plus selective expression of the +IRE protein isoform underlie this increase.

In agreement with the above results in primary hippocampal cultures, we show here for the first time that training rats in a spatial memory task, which entails NMDA receptor activation (Robbins and Murphy 2006), also increased DMT1+IRE and DMT1-1B mRNA expression and increased more than 2.5-fold total DMT1 protein content in the CA1 hippocampal region. Furthermore, the increased expression of the DMT1 variants 1B and +IRE after spatial memory training strongly suggests that hippocampal neurons up-regulate the expression of the DMT1-1B+IRE protein isoform during training.

The induction of DMT1 1B and +IRE mRNA expression reported here may arise from transcriptional upregulation initiated by NMDA binding to its receptor. Additionally, NMDA receptor stimulation may activate signaling pathways that selectively stabilize these particular mRNA splicing variants. Both pathways would increase DMT1 protein expression. The DMT1-1B promoter contains an NFκB response element (Paradkar and Roth 2006). Interestingly, in cerebellar neurons NFκB-dependent transcription is activated by calcium (Lilienbaum and Israel 2003) and by NMDA itself (Lipsky et al. 2001), whereas many studies have reported activation of NFκB by cellular ROS (Gloire et al. 2006). Accordingly, the intracellular increases in calcium (Lerea et al. 1992) and ROS (Kahlert et al. 2005) produced by NMDA receptor stimulation may jointly stimulate NFκB-dependent transcription of the DMT1 gene in hippocampal neurons. Yet, a direct demonstration of NMDA receptor involvement in learning-induced DMT1 expression is not a trivial matter. NMDA receptor inhibition induces alterations in spatial memory formation (Robbins and Murphy 2006; Enomoto et al. 2008) and provokes schizophrenia-like symptoms (Tamminga et al. 2003). Thus, if NMDA receptor

[1] Haeger P., Muñoz P., Carrasco M. A., Núñez, M. T. & Hidalgo C. Increased RyR and DMT1 expression in rat hippocampus after spatial memory training, 38th annual meeting of the Society for Neuroscience, Washington, USA, November 15–19, 2008.

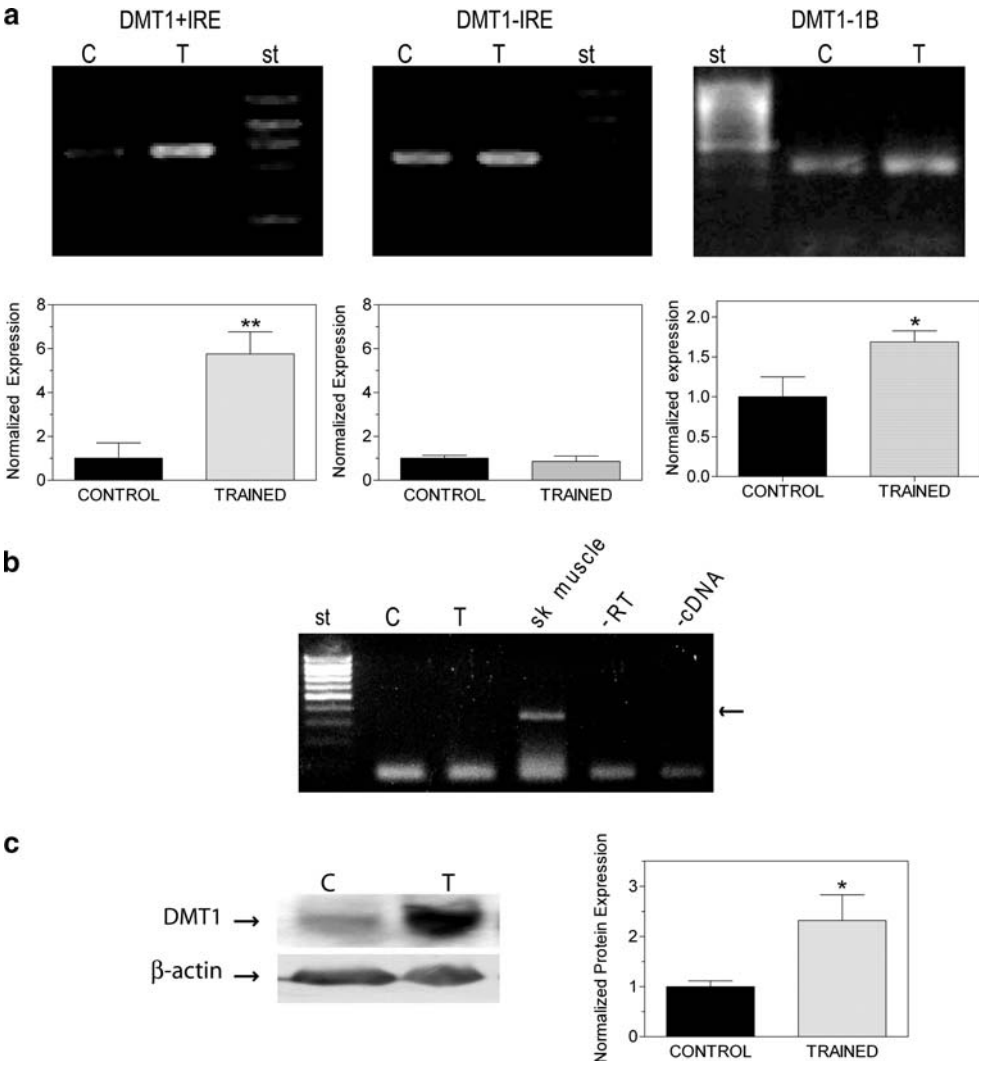

Fig. 5 Spatial training increases 1B and +IRE DMT1 mRNA and protein expression in rat hippocampus. **a** RT-PCR of RNA extracted from hippocampus of control (C) or trained (T) rats. Quantitative densitometry analysis, normalized with β-actin, shows an increase over the endogenous levels of the +IRE and 1B DMT1 mRNA variants after spatial memory training, whereas −IRE expression was not affected. **b** In contrast to skeletal (sk) muscle, hippocampal tissue did not express the DMT1-1A variant (*arrow*). **c** Western blot quantification by densitometry shows a significant increase of DMT1 protein content after spatial training. St: 100 bp DNA ladder. −RT: as a control, cDNA was synthesized in the absence of RT. −cDNA: PCR without cDNA. Data, expressed as fold of control, represent mean ± SEM. ** $P < 0.01$ ($n = 4$), * $P < 0.05$ ($n = 5$), evaluated with unpaired Student's t test

antagonists precluded stimulation of DMT1 expression after training, it would not be possible to distinguish if this effect was caused by NMDA receptor inhibition or by a deficit in memory formation.

The current findings open interesting new avenues of research. Increased DMT1 expression leading to increased iron uptake (Wardrop and Richardson 2000), as well as differential expression of DMT1 variants by inflammatory stimuli, hypoxia, and neurotoxic agents, has been reported in diverse cell types (Lis et al. 2005; Paradkar and Roth 2006, 2007). This differential expression suggests that cell-specific transcriptional control mechanisms regulate the expression of the different DMT1 variants, which ultimately contribute to determine cellular iron content in physiological and pathological conditions, including some neurodegenerative diseases (Jellinger et al. 1990; Salazar et al. 2008; Youdim 2008; Brar et al. 2009). The particular mechanisms, however, whereby DMT1-mediated iron uptake participates in normal plasticity processes or neurodegeneration remain unknown. Hence, future studies should address the particular regulatory mechanisms, including calcium-mediated signal integration systems, through which NMDA receptor stimulation and spatial memory training induce the selective expression of the DMT1-1B+IRE isoform.

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