

Nurr1 Protein Is Required for *N*-Methyl-D-aspartic Acid (NMDA) Receptor-mediated Neuronal Survival*[§]

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Background: The mechanism involved in activity-dependent survival of neurons in the central nervous system is not fully understood.

Results: Nurr1 is involved in excitatory transmission-dependent survival of glutamatergic neurons by acting downstream CREB and upstream of BDNF.

Conclusion: Nurr1 activation mediates activity-dependent survival of glutamatergic neurons.

Significance: A novel function of Nurr1 in activity-dependent survival of glutamatergic neurons is reported.

NMDA receptor (NMDAR) stimulation promotes neuronal survival during brain development. Cerebellar granule cells (CGCs) need NMDAR stimulation to survive and develop. These neurons differentiate and mature during its migration from the external granular layer to the internal granular layer, and lack of excitatory inputs triggers their apoptotic death. It is possible to mimic this process *in vitro* by culturing CGCs in low KCl concentrations (5 mM) in the presence or absence of NMDA. Using this experimental approach, we have obtained whole genome expression profiles after 3 and 8 h of NMDA addition to identify genes involved in NMDA-mediated survival of CGCs. One of the identified genes was *Nurr1*, a member of the orphan nuclear receptor subfamily Nr4a. Our results report a direct regulation of Nurr1 by CREB after NMDAR stimulation. ChIP assay confirmed CREB binding to *Nurr1* promoter, whereas CREB shRNA blocked NMDA-mediated increase in *Nurr1* expression. Moreover, we show that Nurr1 is important for NMDAR survival effect. We show that Nurr1 binds to *Bdnf* promoter IV and that silencing *Nurr1* by shRNA leads to a decrease in brain-derived neurotrophic factor (BDNF) protein levels and a reduction of NMDA neuroprotective effect. Also, we report that Nurr1 and BDNF show a similar expression pattern during postnatal cerebellar development. Thus, we conclude that Nurr1 is a downstream target of CREB and that it is responsible for the NMDA-mediated increase in BDNF, which is necessary for the NMDA-mediated prosurvival effect on neurons.

During brain development, neuronal activity suppresses apoptosis and promotes survival of neurons, adjusting neuronal population to its connection patterns. The role of the NMDA subtype of glutamate receptors (NMDAR)⁵ supporting neuronal survival during CNS development is well documented, and it has been reported that basal NMDAR activity suppresses neuronal apoptosis in the somatosensory thalamus, the hypothalamus, and the dentate gyrus and in CA1 in the hippocampus, among other brain areas (1–3). The trophic effect of NMDAR stimulation is especially necessary for the survival and differentiation of cerebellar granule cells (CGCs) (4).

CGCs are generated during the postnatal development of the cerebellum in the external granule layer and migrate to the internal granule layer to become mature CGCs (5, 6). During its migration, they need glutamatergic stimulation from the mossy fibers. The stimulation of NMDAR is necessary for the maintenance of these neurons during cerebellum development (7–9). Failure to receive mossy fiber inputs will drive CGCs to apoptotic death (10, 11). This scenario can be mimicked *in vitro* in primary cultures of CGCs. When these neurons are grown in low potassium concentration (5 mM KCl; K5), they undergo spontaneous apoptosis. Nevertheless, they will develop and survive in the presence of high potassium concentrations (25 mM KCl; K25) or NMDA (4, 12, 13).

It has been widely reported that the activation of survival signaling kinases, including Akt and ERK (14, 15), and the suppression of proapoptotic kinases, such as glycogen synthase kinase-3 β (GSK-3 β) or JNK (16, 17), are involved in neuronal survival. The regulation of these kinases modulates the activity of some transcription factors that control the expression of pro- and antiapoptotic genes. In CGCs, NMDA-mediated prosur-

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[§] This article contains supplemental Tables I–III and Figs. 1 and 2.

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⁵ The abbreviations used are: NMDAR, *N*-methyl-D-aspartate receptor; BDNF, brain-derived neurotrophic factor; CGC, cerebellar granule cells; CREB, cAMP-response element-binding protein; VIP, vasoactive intestinal peptide; K5, 5 mM KCl; K25, 25 mM KCl; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; DIV, days *in vitro*; Q-RT-PCR, quantitative real-time PCR; P, postnatal day.

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vival effect seems to be dependent on the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (13, 18) and the activation of CREB (19). Other transcription factors, for example MEF2, FKHL1, DeltaNp73, or Sp1/3, have also been reported to be involved in neuronal survival (20–23). However, whether these or other factors are involved in the signaling related to the NMDAR-mediated prosurvival effect is still poorly understood.

In the present study, we have analyzed the expression changes in response to NMDA treatment in cultured CGCs. By comparative analysis, we have identified Nurr1 as a candidate to mediate the NMDAR prosurvival response. In fact, we report that NMDA induction of CREB activation promotes *Nurr1* expression and consequent up-regulation of its protein levels. In addition, silencing of *Nurr1* results in a reduction in brain-derived neurotrophic factor (BDNF) levels that correlates with the decrease in the NMDA-mediated neuroprotection in these cells.

EXPERIMENTAL PROCEDURES

Cell Culture—Granule cell cultures were prepared from dissociated cerebella of 8-day-old Wistar rats as described previously (4). Cells were plated (3×10^3 cells/mm²) in Eagle's basal medium (Sigma-Aldrich) and 5 or 25 mM KCl (K5, K25) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich), 25,000 units of penicillin, and 25 mg of streptomycin (PAN Biotech Inc.). 10 μ M cytosine- β -D-arabino-furanoside was added to the cultures 24 h after plating to prevent proliferation of non-neuronal cells. Neurons were plated onto poly-L-lysine coated 48-well plates for measurement of cell viability and immunocytochemistry, on 35-mm culture dishes for RNA extraction and Western blotting, on 24-well plates for luciferase assays, and on 100-mm plates for ChIP assays. At 2 days *in vitro* (DIV), K5 neurons were treated or not with NMDA (100 μ M, Sigma-Aldrich) or K25. The procedures followed were in accordance with guidelines of the Comissió d'Ètica en l'Experimentació Animal i Humana of the Universitat Autònoma de Barcelona.

Cell Viability—Neuronal viability was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. Cells were incubated with MTT (0.2 mg/ml) for 45 min at 37 °C. The blue formazan derivative was solubilized in 100 μ l of dimethyl sulfoxide, and the dual wavelength was measured at 560 and 620 nm in a Labsystems Multiskan plate reader. Data are presented as mean \pm S.E. of values obtained from three or four independent experiments performed in triplicates.

Fluorescence Analysis of Apoptotic Nuclei—Apoptosis was assessed by nuclear DNA staining with Hoechst 33258. Cells were washed twice in Tris-buffered saline (TBS) 24 h after the treatment, fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 45 min at 4 °C, and stained with 1 μ g/ml Hoechst 33258 (Molecular Probes) for 5 min after being washed again twice in TBS. Nuclear DNA staining was observed with an inverted microscope (Nikon eclipse TE-2000-E). In every experiment, more than 1000 cells were individually examined for each experimental condition. Condensed and/or fragmented nuclei were considered as apoptotic nuclei. Data are

given as mean \pm S.E. of values obtained in three or four independent experiments performed in triplicate. Results are expressed as the percentage of apoptotic nuclei *versus* total nuclei.

Immunoblotting—CGC cultures were washed with PBS, and total protein was extracted by incubating neurons in lysis buffer containing 20 mM Tris, pH 7.5, 1% Nonidet P-40, 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, 10 μ g/ml aprotinin, 20 μ g/ml leupeptin, 1 mM activated orthovanadate. Cell lysates were sonicated for 1 min. Proteins (25 μ g) were resolved in 10% SDS-PAGE gel and transferred to nitrocellulose membranes (Amersham Biosciences). Membranes were washed with PBS/Tween (PBS with 0.05% Tween 20) and incubated for 1 h in blocking buffer (5% of nonfat dry milk in PBS/Tween except 10% of nonfat dry milk for Nurr1 and supplemented with 5% FBS for BDNF) at room temperature to block nonspecific binding. Blots were washed and incubated overnight at 4 °C with primary antibodies against CREB, phospho-Ser¹³³-CREB, and Nur77 from Cell Signaling; Nurr1 and NOR-1 from Abcam; BDNF from Santa Cruz Biotechnology; GAPDH from Ambion Inc.; and actin from Sigma-Aldrich, diluted (1:1000 for all, except GAPDH and actin 1:40,000) in blocking buffer or PBS/Tween with 0.1% BSA and 2% nonfat dry milk for BDNF. Blots were then washed and incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibodies (BD Biosciences) diluted 1:3000 in blocking buffer. Immunoreactive bands were visualized using the ECLTM Western blotting detection reagent (Amersham Biosciences, Uppsala, Sweden) and quantified by a computer-assisted densitometer. Actin and GAPDH were used as loading controls.

Immunohistochemistry—Cerebella of P9, P14, P26, and adult rats were fixed for 24 h with 4% paraformaldehyde in PBS at 4 °C and embedded in paraffin. 5- μ m sections were processed for Nurr1 labeling. Briefly, sections were rehydrated and washed twice in TBS. Endogenous peroxidase activity was inactivated with 3% H₂O₂ in TBS. After extensive washes with TBS/Tween (TBS with 0.5% Tween), the sections were incubated in blocking solution (TBS/Tween with 10% FBS). Subsequently, the sections were exposed to Nurr1 antibody (1:200 in blocking solution; Abcam) for at least 12 h at 4 °C. The sections were again rinsed extensively in TBS/Tween and incubated with anti-mouse antibody coupled to biotin (1:200 in blocking solution; Vector Laboratories) for 1 h at room temperature. After rinsing, the sections were incubated with streptavidin-peroxidase in TBS (1:300; Vector Laboratories). Peroxidase was developed with 0.05% diaminobenzidine in TBS and 0.035% H₂O₂ solution. Nuclei were stained by incubation with hematoxylin for 1 min. Sections were dehydrated and covered with DPX medium. Pictures were obtained in a NIKON Eclipse 901 microscope/Nikon digital sight camera, using 2 \times , 10 \times , 20 \times , and 40 \times objective lens.

Gene Expression Analysis—CGCs were plated in K5 and treated or not at 2 DIV with NMDA (100 μ M). After 3 and 8 h, RNA extraction was done with the RNeasy[®] Protect kit (Qiagen), and samples were sent to the genomic service of the Vall d'Hebron Hospital Research Institute to perform the Affymetrix Rat Genome 430 2.0 array. Expression data were obtained using the GCOS software and normalized with robust

multichip average. The LIMMA package was used for statistical analysis to identify up- and down-regulated genes using a multiple test-adjusted p value <0.2 . For genes with multiple probes, we selected the most informative probe showing the lowest p value. The up-regulated genes at 8 h with an increase over 2-fold and a p value <0.05 were submitted to functional annotation analysis with the DAVID program (david.abcc.ncifcrf.gov). Filters used in the functional annotation cluster were: Similarity Term overlap 6; Similarity threshold 0.70, and Classification initial group membership 5. Hierarchical clustering of the Affymetrix data was performed with the Cluster software (version 3.0) and represented with TreeView software. The clustering algorithm (unweighted pair group method with arithmetic mean) was used in a centroid linkage fashion on the probes showing an at least 1-fold change, after being centered to the median.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)—Total RNA was extracted from CGCs at the indicated times after treatment with TRIzol (Invitrogen) according to the manufacturer's instructions. 1 μ g of total RNA was converted to first strand cDNA using the SuperScriptTM II reverse transcriptase protocol (Invitrogen) following the manufacturer's instructions. The resulting cDNA was subjected to PCR analysis. PCR cycling parameters were as follows: 94 °C for 2 min for one cycle followed by 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for 35 cycles and 72 °C for 2 min. The PCR products were stained with SYBR SafeTM (Invitrogen). A sample without RNA was always negative; neonatal rat brain RNA was used as a positive control for the products (results not shown). The actual sequences of specific primers are as follows: *Nr4a1/Nur77* (sense) 5'-CAC CTT CCT ATA CCA GCT GC-3'; (antisense) 5'-GAA GGA GAA GAA GGT TGG AG-3'; *Nr4a2/Nurr1* (sense) 5'-CCT TCA CAA CTT CCA CCA GA-3'; (antisense) 5'-AAC ACC GTA ATG CTG ACA GG-3'; *Nr4a3/Nor1* (sense) 5'-CCT GAT TCT GGA GAG CAG TG-3'; (antisense) 5'-GGC GGA GAC TGC TTG AAG TA-3'; *18s* (sense) 5'-TCA AGA ACG AAA GTC GGA GG-3' (antisense) 5'-GGA CAT CTA AGG GCA TCA CA-3'. They were used at a concentration of 0.5 μ M.

RNA Interference—Constructs for RNA interference experiments were obtained into the pSUPER.retro.puro plasmid (OligoEngine) using specific oligonucleotides of the *Nurr1* and *Creb* sequence and the scrambled sequence of the oligonucleotide of *Nurr1_1*, which was used as a control and does not recognize any rat coding sequence, indicated by capital letter, as follows: shRNA *Nurr1_1* (forward) gatccccCAT-TAAGGTAGAAGACATttcaagagaATGTCTTCTACCTT-AATGGttttt, (reverse) agctaaaaCCATTAAGGTAGAAG-ACATtctctttaaATGTCTTCTACCTTAAATGGggg; shRNA *Creb_1* (forward) gatccccCTGAAGAAGCAGCACGAAAttcaagagaTTTCGTGCTTCTTCAAGttttt, (reverse) agctaaaaCTGAAGAAGCAGCACGAAAtctctttaaTTTCGTGCTGCTTCTTCAAGggg; shRNA *Creb_2* (forward) gatccccGC-AAGAGAATGTCGTAGAAttcaagagaTTCTACGACATTCT-CTTGctttt, (reverse) agctaaaaGCAAGAGAATGTCGTAGA-AtctctttaaTTCTACGACATTCTTTCGggg; shRNA *scNurr1* (forward), gatccccAGCGAAAACTTTATCGGAttaagagaTC-CGATAAAGTTTTTCGCTttttt, (reverse) agctaaaaAGCGAA-

AAACTTTATCGGAtctctttaaTCCGATAAAGTTTTTCGCT-ggg. Oligonucleotides were obtained from Invitrogen and were cloned between BglII/HindIII sites of pSUPER.retro.puro plasmid. Lentiviral constructs were achieved by digesting EcoRI-Clal sites from pSUPER-sh to replace H1 promoter with H1-shRNA cassette in pLVTHM.

Lentiviruses were propagated using methods described previously (24) (25). Briefly, human embryonic kidney 293T (HEK293T) cells were seeded at a density of 2.5×10^6 cells in 100-mm dishes. The following day, cells were transfected with 20 μ g of pLVTHM-derived constructs, 15 μ g of pSPAX2, and 8 μ g of pMD2G. The transfection was routinely performed by the calcium phosphate transfection method (26). Cells were allowed to produce lentiviruses for 48 h. After 48 h, the medium was centrifuged at $1200 \times g$ for 5 min, and the supernatant was concentrated at $141,000 \times g$ for 120 min and then resuspended in 50 μ l of PBS containing 1% BSA and stored at -80 °C. Biological titers of the viral preparations expressed as a number of transducing units per milliliter were determined by transducing HEK293T cells in limiting dilutions. After 72 h of incubation, the percentage of green fluorescent protein (GFP)-positive cells was determined by cytometer, and viruses were used in the experiments at 1×10^6 transducing units/ml.

Lentiviral infection was performed at the day of plating. Cells remained in contact with lentivirus for 5 h, and then medium was replaced with Eagle's basal medium and 5 or 25 mM KCl supplemented with 10% FBS, 25,000 units of penicillin, and 25 mg of streptomycin. More than 75% of neurons were infected. At 2 DIV, treatments were performed as described before, and chromatin condensation was assessed at 7 DIV, respectively. Cell lysates were obtained 30 h after treatment.

Plasmid Constructs—Rat genomic DNA was used as the template for PCR to amplify the *Nurr1* promoter fragment, containing the CREB-response element reported in Ref. 27, with the forward primer A (5'-AAGAGCTCGAGAGTTACAGTCAACGGGA-3') and the reverse primer B (5'-AACTCGAGGGCTTCAGCCGAGTGATTGG-3'). The fragment was cloned into the SacI and XhoI sites of pGL3-Basic luciferase reporter vector (pGL3-Basic) to generate plasmid pGL3-Nurr1 promoter. The plasmids construct was confirmed by sequencing.

Transfection and Luciferase Assay—CGCs were plated and infected with the indicated lentivirus, and at 1 DIV, they were transfected using Lipofectamine 2000 (Invitrogen) with pGL3-Basic or pGL3-Nurr1 promoter. For luciferase assays, 0.75 μ g of each pGL3 vector and 0.25 μ g of *Renilla* plasmid were transfected in each 24-well plate well.

At 2 DIV, cells were treated with NMDA (100 μ M). 6 h after treatment, cells were washed twice with cold PBS and were lysed with 100 μ l of lysis buffer (Promega). 30 μ l of cell extract were used for a luciferase reporter assay (Dual-Luciferase[®] reporter assay system, Promega). Data are given as mean \pm S.E. of values obtained in three independent experiments performed in duplicate.

Chromatin Immunoprecipitation—CGCs were plated in K5 and treated with NMDA (100 μ M) or K25 at 2 DIV. After 90 min or 3 h, cells were washed with PBS and fixed with formaldehyde. Cells were then lysed and sonicated. 2.5 μ g of soluble chromatin were co-immunoprecipitated with 0.5 μ l of phospho-

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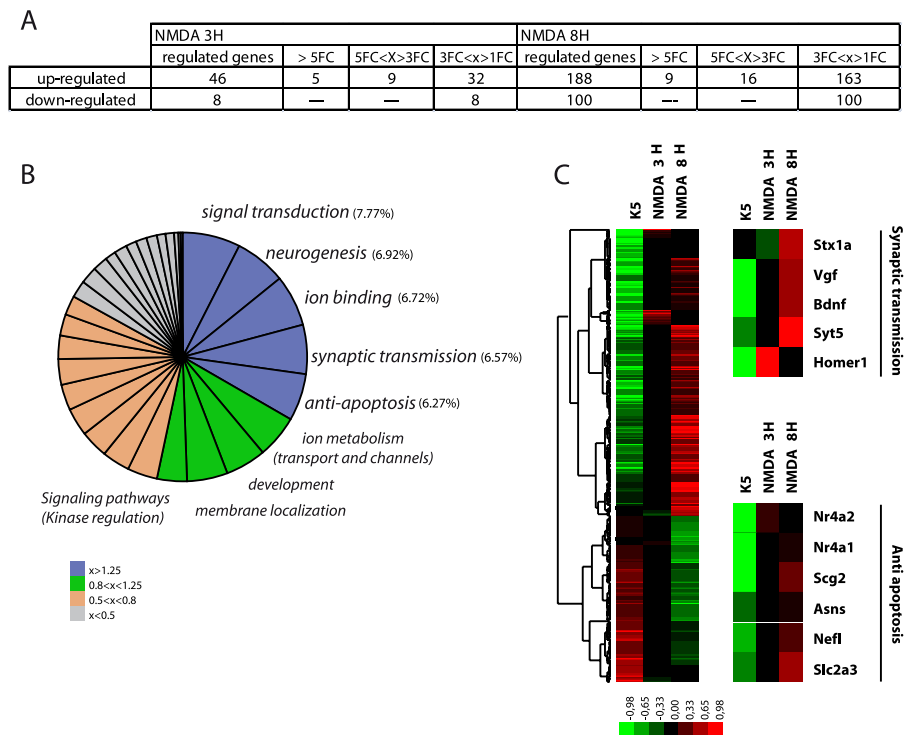


FIGURE 1. Gene expression profiling of NMDA treatment in CGCs. CGCs were plated in KCl 5 mM (K5) and treated or not at 2 DIV with NMDA (100 μ M). After 3 and 8 h, RNA extraction was done, and samples were subjected to the Affymetrix Rat Genome 430 2.0 array. The LIMMA package was used for statistical analysis to identify up- and down-regulated genes using a multiple test (adjusted p value < 0.2). **A**, table of the up- and down-regulated genes at 3 and 8 h classified by -fold change increase or decrease ($FC = -$ fold change). **B**, pie charts representing the ontological analysis of the up-regulated genes at 8 h. The most enriched programs are detailed. **C**, heat map of the up- and down-regulated genes at 8 h. Representative genes of the synaptic transmission and antiapoptotic programs are shown.

Ser¹³³-CREB antibody (Cell Signaling), Nurr1 antibody (Abcam), or rabbit IgG (Santa Cruz Biotechnology). After de-cross-linking of the DNA, samples were subjected to quantitative real-time PCR (Q-RT-PCR). Q-RT-PCR was performed on a LightCycler 480 II System using LightCycler 480 SYBR Green mix (Roche Applied Science). Reactions were carried out in triplicate, and Q-RT-PCR data were analyzed using the standard curve method. The primers used were: *Nurr1* forward, 5'-AAGTGGGCTACCAAGGTGAA-3', reverse, 5'-CTGC-CAACATGCACCTAAAG-3'; and *Bdnf* promoter IVa forward, 5'-CAAAGCATGCAATGCCCTGGAA-3', and reverse, 5'-CGGCAGCGCAGCAGTCCTCT-3', which amplify regions containing CREB binding sites, and *Bdnf* promoter III forward, 5'-GCCTGCCCTAGCCTTTACTT-3', reverse, 5'-GCCAAGCTGTCTACCTGGAT-3'; and *Bdnf* promoter IVb forward, 5'-CTCCACAGAACTTGGGTGT-3', reverse, 5'-AGGAACCCTACTCCCCTCAA-3', which amplify regions containing putative Nurr1 binding sites. Results are shown as the mean \pm S.E. of three independent experiments.

Statistical Analysis—Statistical significance was determined by one-way analysis of variance followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

NMDA-mediated Changes in Gene Expression—NMDA receptor stimulation rescues neurons from apoptosis through activation of several pathways that would finally modulate the activity of transcription factors (13, 28, 29). To analyze

changes in gene expression induced by NMDA in CGC cultures, we extracted RNA from K5 cultures treated or not with the glutamatergic agonist for 3 or 8 h at 2 DIV, and a gene expression analysis was performed with the Affymetrix platform rat genome 430 2.0 array. Expression data were obtained using GCOS software and normalized with robust multichip average. Statistical analysis was done with the LIMMA package to identify up- and down-regulated genes *versus* control conditions (K5). We considered that a gene was up-regulated or down-regulated if its expression was 1.5 \times larger or smaller than in control condition (false discovery rate < 0.2).

After exposure to NMDA, we detected 44 up-regulated genes and 7 down-regulated genes at 3 h (Fig. 1A and supplemental Table I). 188 up-regulated genes and 112 down-regulated were detected at 8 h (Fig. 1A and supplemental Table II). Moreover, we observed that almost 50% of the genes up-regulated at 3 h were still up-regulated at 8 h and that six of the seven down-regulated genes at 3 h had also a reduced expression at 8 h (supplemental Tables I and II).

To determine the meaning of the expression changes, we performed a functional annotation analysis of the data obtained at 3 and 8 h using the DAVID program (data not shown, Fig. 1B and supplemental Table III). At 3 h, the most enriched functions and categories were related to transcriptional activity and nuclear localization. By contrast, at 8 h, the expression changes reflected a more complex situation, having an enrichment of functions such as neurogenesis,

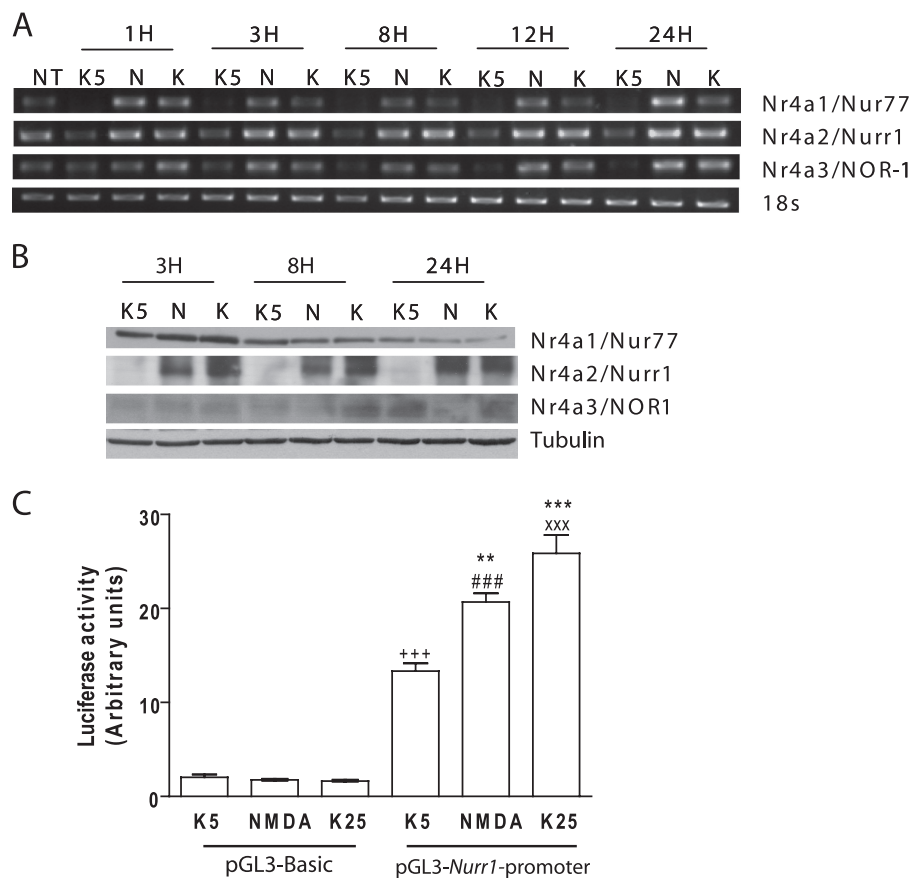


FIGURE 2. Nr4a Family members are up-regulated in response to NMDA, but only Nurr1 protein levels are increased. CGCs were plated in 25 mM KCl (NT) or K5. At 2 DIV, K5 cells were treated or not with NMDA (100 μ M) (N) or K25 (K). A and B, at the indicated times, RNA and cell lysates were obtained. RT-PCR (A) and Western blot (B) analyses were performed. Representative experiments are shown. Similar results were obtained in three independent experiments. C, CGCs were plated in K5. At 1 DIV, CGCs were transfected with pGL3-Basic or pGL3-Nurr1-promoter vectors, and at 2 DIV, K5 neurons were treated or not with NMDA (100 μ M) or K25. Luciferase assay was performed 6 h after the treatment. Results are the mean \pm S.E. of values from three independent experiments performed in duplicate. + + +, $p < 0.001$ versus K5 pGL3-Basic; ###, $p < 0.001$ versus NMDA pGL3-Basic; xxx, versus K25 pGL3-Basic; ***, $p < 0.001$ versus K5 pGL3-Nurr1-promoter; **, $p < 0.01$ versus K5 pGL3-Nurr1-promoter.

synaptic transmission, or development as well as ionic metabolism and antiapoptotic program (Fig. 1B and supplemental Table III). All of them were in agreement with the category of signal transduction and cell communication that got the higher enrichment scores (Fig. 1B and supplemental Table III).

A deeper analysis of the changes in gene expression indicated that 4.8% of the up-regulated genes demonstrated at least an increase of 5-fold in their expression, 8.5% had an increase between 3- and 5-fold, and 86.7% were increased over 1-fold but under 3-fold. In the case of down-regulated genes, all of them were between 1- and 3-fold change (Fig. 1A and supplemental Table II). In the group of genes that changed their expression over 5-fold, we found protein convertase subtilisin/kexin type 1 (*Pcsk1*), *Vgf*, FBJ murine osteosarcoma viral oncogene homolog (*Fos*), *Bdnf*, protein kinase AMP-activated γ 2 noncatalytic subunit, phosphodiesterase 10A, and the components of the Nr4a family: *Nr4a1/Nur77*, *Nr4a2/Nurr1*, and *Nr4a3/NOR-1* (supplemental Table II). In the group of genes up-regulated over 3-fold but under 5-fold, we found, for example, synaptotagmin V, stanniocalcin 1, cAMP-response element modulator (*Crem*), myocyte enhancer factor 2, polypeptide C (*Mef2C*), γ -aminobutyric acid (*Gaba-A*) receptor, subunit α 5 (*Gabra5*), potassium

voltage-gated channel, shaker-related subfamily member 4 (*Kcna4*), and metabotropic glutamate receptor 4 (see supplemental Table II for further details).

Zhang *et al.* (30) performed an exhaustive study of the gene expression changes in response to NMDAR stimulation comparing the profile between the protective and the excitotoxic stimulation in hippocampal neurons. We compared their results with our list of genes up-regulated at 3 h, and we obtained a list of candidates to participate in the NMDA pro-survival effect in our experimental model (Table I). From this list, we chose the Nr4a subfamily members because of their higher increase in expression, their potential role in apoptosis, and their unknown function in NMDA-mediated neuronal survival during cerebellum development.

Nurr1 Is Involved in NMDA-mediated Neuronal Survival—The observed increase in gene expression of the Nr4a subfamily members was further confirmed by RT-PCR. An increase in *Nr4a1*, *Nr4a2*, and *Nr4a3* was detected from 1 to 24 h after treatment with NMDA or K25 (Fig. 2A). However, when the protein levels of the different members of Nr4a family were determined, only Nr4a2/Nurr1 protein levels showed a clear and consistent increase in response to NMDA or K25. Nr4a1/Nur77 and Nr4a3/NOR-1 protein levels were not increased. In fact, a clear decrease in Nr4a1 and Nr4a3

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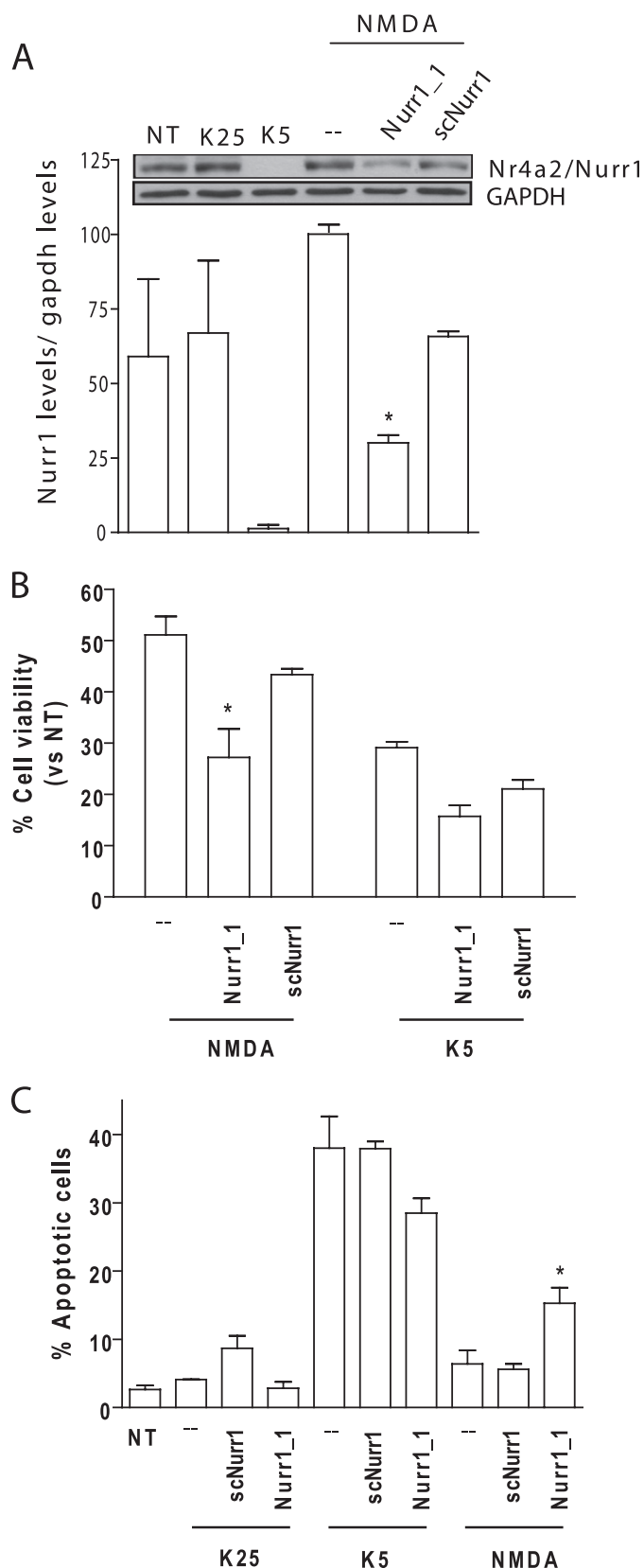


FIGURE 3. Nurr1 participates in NMDA neuroprotective effect. CGCs were plated in the presence of shRNA against Nurr1 (Nurr1_1) or a scrambled shRNA (scNurr1) as described under "Experimental Procedures." At 2 DIV, cells were treated (or non-treated; NT) with NMDA (100 μ M) or K25. *A*, upper panel, 30 h after treatment, cell lysates were obtained and subjected to Western blot with Nurr1 antibody. Lower panel, quantification of Nurr1 levels of three independent experiments are shown in the graph; *, $p < 0.05$ versus

was observed between 8 and 24 h after NMDA or K25 treatment (Fig. 2B).

To further confirm the observed induction of Nurr1 after NMDA treatment, we decided to analyze the activation of its promoter in response to NMDA or K25 treatment. After 6 h of being treated, CGCs were subjected to a luciferase assay with a plasmid containing the *Nurr1* promoter (pGL3-Nurr1promoter). Granule neurons presented an increase of *Nurr1* promoter activity in the presence of NMDA and K25 versus K5 (Fig. 2C).

Next, we analyzed the participation of Nurr1 in the effect of NMDA on neuronal survival by silencing its expression by shRNA. CGCs were plated in the presence of lentivirus vectors containing the shRNA against *Nurr1* (Nurr1_1) sequence or shRNA containing a scrambled Nurr1 sequence (scNurr1) as a control. As shown in Fig. 3A, the presence of Nurr1_1 shRNA reduces NMDA-mediated increase in Nurr1 protein levels. Moreover, the prosurvival effect of NMDA was reduced in the presence of Nurr1_1 shRNA when either cell viability or the number of apoptotic nuclei was determined (Fig. 3, B and C). By contrast, the presence of the Nurr1_1 shRNA did not block the prosurvival effect of K25 (Fig. 3C).

CREB Regulates NMDA-mediated Induction of Nurr1—It is widely supported that CREB is an important transcription factor in neuronal survival signaling. Moreover, it is also known that NMDA activates CREB and that its activation is necessary for NMDA-mediated neuronal survival (28, 29). Our results confirmed that in our experimental system, NMDA activates CREB. As shown in Fig. 4A, treatment of CGC cultures with NMDA produced a time-dependent increase in the phosphorylation levels of phospho-Ser¹³³-CREB. The increase was evident 1 h after NMDA treatment and reached its higher significance at 8 h (Fig. 4A). We analyzed whether this activation was accompanied with a higher activity of CREB on *Nurr1* promoter by performing a chromatin immunoprecipitation assay. K5 cultures were treated at 2 DIV with NMDA or K25, and 90 min after, cells were fixed to promote the cross-linking of DNA and proteins. A co-immunoprecipitation of the soluble chromatin was performed with phospho-Ser¹³³-CREB antibody. The products of de-cross-linking were subjected to Q-RT-PCR with the primers flanking the *Nurr1* promoter that contains the *Cre* binding site. We observed a clear increase in CREB binding to the *Nurr1* promoter in the presence of NMDA. An increase in the binding was observed neither in K5 nor in K25 cultures (Fig. 4B). Moreover, we assessed the promoter activity in the presence of two CREB shRNA (creb_1 and creb_2). As shown in Fig. 4C, both shRNA were able to reduce CREB levels, with the creb_1 shRNA having a greater effect. Accordingly, both shRNA inhibited NMDA-mediated activation of *Nurr1* promoter, with the creb_1 shRNA having a greater effect reducing the NMDA-mediated increase in Nurr1 protein levels than the

scNurr1. *B*, cell viability was assessed 7 DIV after treatment by MTT assay. Results are shown as mean \pm S.E. of values from three independent experiments performed in triplicate; *, $p < 0.05$ versus NMDA scNurr1. *C*, at 7 DIV, chromatin condensation was assayed by staining with Hoechst 33258. Condensed nuclei were counted and represented as percentage versus total nuclei. Results are the mean \pm S.E. from three independent experiments performed in triplicate; *, $p < 0.05$ versus NMDA scNurr1.

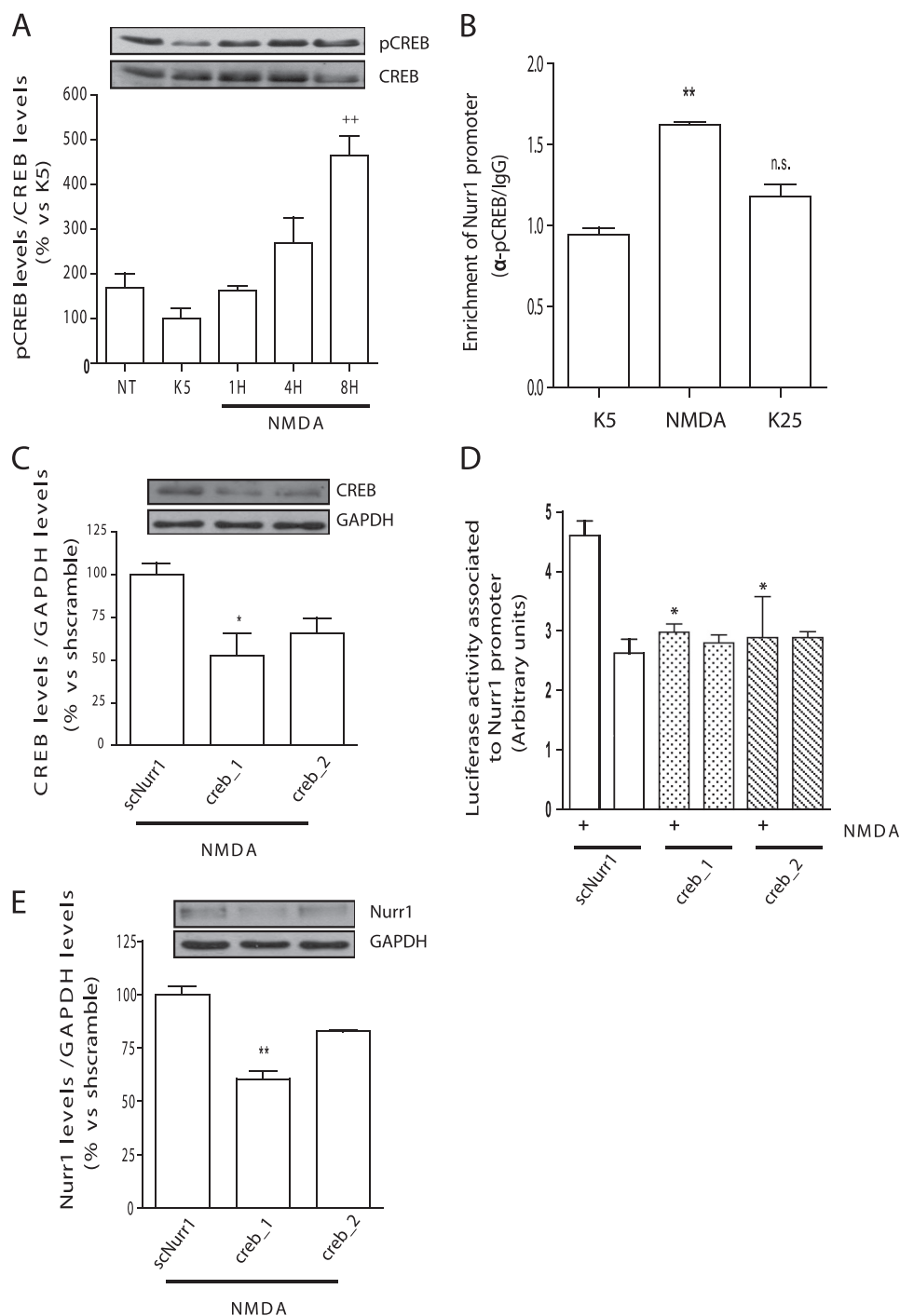


FIGURE 4. CREB regulates *Nurr1* induction by NMDA. Neurons cultured in K5 were treated (or non-treated; *NT*) with NMDA (100 μ M) or K25 at 2 DIV. **A**, phosphorylation of CREB (*pCREB*) and total CREB were determined by Western blot at the indicated times. Data are presented as mean \pm S.E. of three independent experiments; ++, $p < 0.01$ versus K5. **B**, recruitment of phospho-CREB to *nurr1*-promoter was determined 90 min after treatment by ChIP assay (see "Experimental Procedures"). Data represent -fold enrichment (mean \pm S.E.) versus K5 condition performed in three independent experiments; **, $p < 0.01$ versus K5. **C**, **D**, and **E**, cells were plated in the presence of lentiviruses containing the indicated shRNAs and treated with NMDA at 2 DIV. Cell lysates were obtained 30 h after treatment to determine CREB (**C**) or Nurr1 (**E**) levels. A luciferase assay of *Nurr1*-promoter was performed as described under "Experimental Procedures." **D**, 6 h after treatment. Data are shown as the mean \pm S.E. of three or four independent experiments. **, $p < 0.01$, and *, $p < 0.05$ versus scNurr1.

creb_2 shRNA (Fig. 4, *D* and *E*). Blockade of *Creb* expression by CREB shRNA did not modify the activity associated with *Nurr1* promoter and Nurr1 protein levels in K5 cultures (data not shown).

***Bdnf* Is Regulated by Nurr1 in Response to NMDA**—Several genes have been reported to be regulated by the transcription factor Nurr1. Most of them are related to the maturation and

maintenance of dopaminergic neurons. However, a few, such as BDNF, vasoactive intestinal peptide (VIP), or α -synuclein, have been described to be involved in CGC survival (31–33). Thus, we decided to explore whether one of these Nurr1 targeted genes was related to the prosurvival effect of NMDA on CGCs. First, we analyzed their protein levels in CGCs treated with NMDA or K25 in comparison with K5 conditions. We did not

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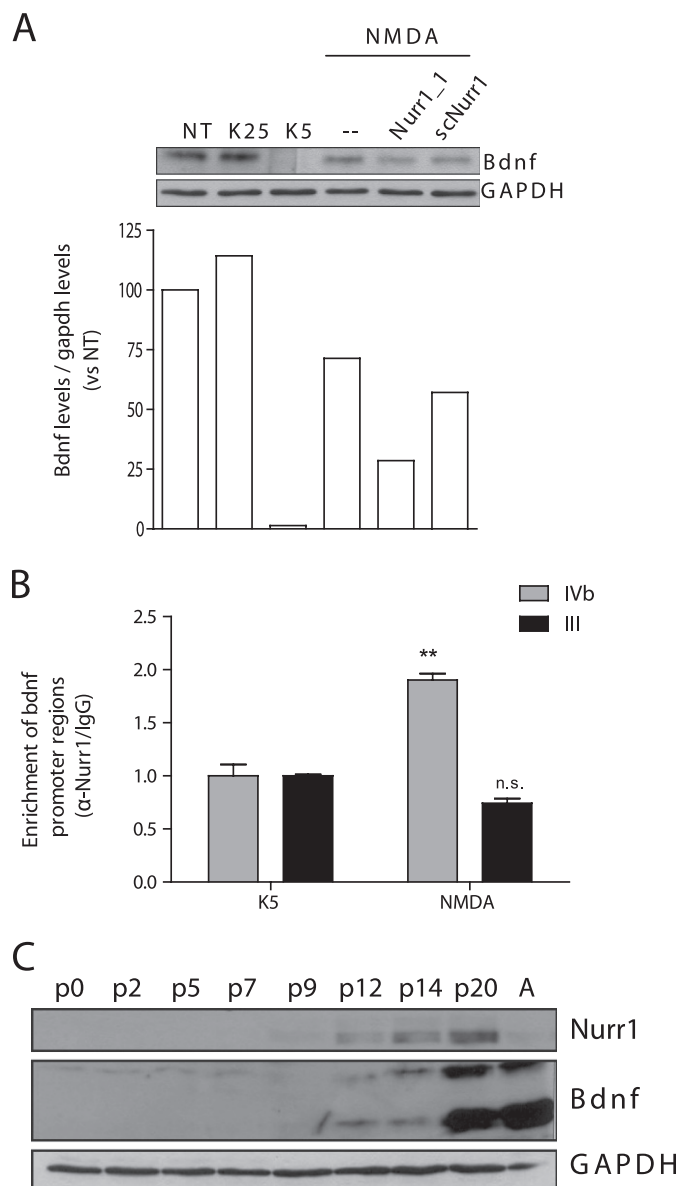


FIGURE 5. Nurr1 regulates NMDA-mediated increase in BDNF levels and has similar expression pattern to neurotrophin during postnatal cerebellum development. *A*, CGCs were plated in the presence or absence of the indicated shRNA and treated (or non-treated; NT) at 2 DIV with NMDA (100 μ M) or K25. BDNF protein levels were assessed by Western blot 30 h after treatment. Data are from a representative experiment. Three additional independent experiments showed similar results. *B*, recruitment of Nurr1 to *Bdnf* promoters III and IV was determined 90 min after treatment by ChIP assay (see "Experimental Procedures"). Data represent -fold enrichment (mean \pm S.E.) versus K5 condition performed in three independent experiments; **, $p < 0.01$ versus K5. *C*, protein extracts from different postnatal ages of rat cerebellum were subjected to Western blot analysis to determine Nurr1 and BDNF levels. A representative Western blot is shown. Three or four animals of each age were analyzed and gave similar results.

find any differences in VIP and α -synuclein when their protein levels were compared in the presence or absence of NMDA (data not shown). However, we observed that although BDNF protein levels were barely detectable in K5, NMDA dramatically increased BDNF levels (Fig. 5A). This fact was in accordance with the NMDA-mediated increase in *Bdnf* expression observed at 3 and 8 h with the Affymetrix array platform (supplemental Tables I and II). Activity-dependent *Bdnf* up-regula-

tion has been attributed to CREB activity (34). To check whether CREB was involved in NMDA- and Nurr1-mediated increase in *Bdnf* expression, we decided to perform a ChIP assay with the *Bdnf* promoter IV (35), 90 min and 3 h after NMDA or K25 treatment. We were unable to detect any significant differences at 90 min in K25-treated cells. However, a robust increase in CREB binding to *Bdnf* promoter IV was observed in K25-treated cells after 3 h. However, we did not find differences between NMDA and K5 either at 90 min or at 3 h (supplemental Fig. 1), suggesting that CREB is not the transcription factor directly involved in NMDA-mediated activation of *Bdnf* expression. Next, we assessed the levels of BDNF in the presence of Nurr1_1 shRNA to know whether NMDA-mediated BDNF induction could be due to Nurr1. As shown in Fig. 5A, reducing Nurr1 levels produced a reduction in BDNF protein levels, suggesting that Nurr1 is directly involved in NMDA-mediated induction of *Bdnf* expression in CGC cultures. This suggestion was confirmed when we assessed Nurr1 binding to *Bdnf* promoter IV by ChIP assay. Our results show that Nurr1 binding to *Bdnf* promoter IV is clearly increased in NMDA-treated cells. No changes in Nurr1 binding were observed in K25-treated cells (Fig. 5B). Moreover, NMDA-mediated Nurr1 binding to *Bdnf* promoter seems to be selective because no binding was observed to other *Bdnf* promoters such as promoter III (Fig. 5B).

Next, we performed a first approximation of the *in vivo* situation by determining the protein levels of Nurr1 and BDNF by Western blot in cerebellum lysates at different days after birth. We observed that both mature BDNF and Nurr1 protein levels increased in the cerebellum during the second postnatal week in a similar way (Fig. 5C). We went deeper in the expression analysis of Nurr1 by performing an immunohistochemistry analysis to know which cells were expressing Nurr1 and whether its expression was homogeneous in all lobes of the cerebellum. We detected Nurr1 mainly in the internal granule layer. Moreover, the expression of Nurr1 was more evident in the nodulus (lobe X) than in the other lobes of the cerebellum (Fig. 6 and supplemental Fig. 2).

DISCUSSION

NMDAR stimulation is necessary for neuronal survival and development in different cerebral areas. One example of such NMDAR dependence is CGCs. Survival of these neurons during their postnatal migration from the external granule layer toward the internal granule layer (5, 6) requires excitatory inputs from the mossy fibers acting through the NMDARs of CGCs. Failure to receive mossy fibers inputs triggers apoptotic CGC death (10, 11, 36). In culture, CGCs are also sensitive to NMDAR stimulation for their survival. When CGCs are cultured in nonpolarized culture medium containing K5, the CGCs die by apoptosis (12). However, if NMDA is added to the medium, the CGCs will survive for several days (13).

Some studies have shown that the prosurvival effect of NMDA is mediated by synaptic NMDAR (30, 37). It is believed that calcium entry through synaptic NMDAR triggers CREB activation (38) likely by stimulation of calmodulin kinases and/or MEK/ERK kinase pathways (14, 39). In CGCs,

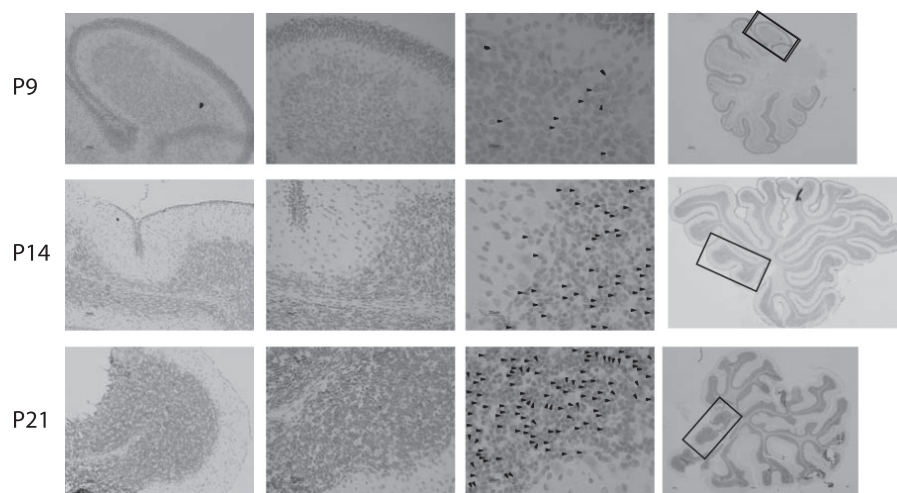


FIGURE 6. **Nurr1 expression is mainly restricted to nodulus lobe.** Sagittal sections of cerebellum from P9, P14, and P21 rats were obtained and subjected to Nurr1 immunohistochemistry. Nurr1-increased expression during the different postnatal days was clearly evident in the nodulus lobe. Representative pictures of each age are shown (10 \times , 20 \times , 40 \times , and a reconstruction of whole cerebellum of each age performed with 2 \times photographs). The squares indicate the magnified area (nodulus), and the arrows indicate cells positive to Nurr1 staining.

TABLE 1

Up-regulated genes in action potential bursting and at 3 h after NMDA treatment

A comparative analysis between the up-regulated genes at 3 h after NMDA treatment in CGC culture and action potential-bursting stimulation for 2 h in hippocampal cultures (30) shows a list of CREB-dependent genes. The symbols indicate: \uparrow >1-fold change and <3-fold change; $\uparrow\uparrow$, >3-fold change and <5-fold change; $\uparrow\uparrow\uparrow$, >5-fold change.

Gene symbol	Reference sequence	Gene title	FC at 3h
<i>Atf3</i>	NM_012912	Activating transcription factor 3	$\uparrow\uparrow$
<i>Bdnf</i>	NM_012513	Brain derived neurotrophic factor	$\uparrow\uparrow$
<i>Cpd</i>	NM_012836	Carboxypeptidase D	$\uparrow\uparrow$
<i>Creml</i>	NM_013086	cAMP responsive element modulator	$\uparrow\uparrow$
<i>Fos</i>	NM_022197	FBJ murine osteosarcoma viral oncogene homolog	$\uparrow\uparrow$
<i>Homer1</i>	NM_031707	Homer homolog 1 (Drosophila)	$\uparrow\uparrow$
<i>Nfil3</i>	NM_053727	Nuclear factor, interleukin 3 regulated	$\uparrow\uparrow$
<i>Nr4a1</i>	NM_024388	Nuclear receptor subfamily 4, group A, member 1	$\uparrow\uparrow$
<i>Nr4a2</i>	NM_019328	Nuclear receptor subfamily 4, group A, member 2	$\uparrow\uparrow$
<i>Nr4a3</i>	NM_017352	Nuclear receptor subfamily 4, group A, member 3	$\uparrow\uparrow$
<i>Pcsk1</i>	NM_017091	Proprotein convertase subtilisin/kexin type 1	$\uparrow\uparrow$
<i>Plagl1</i>	NM_012760	Pleomorphic adenoma gene-like 1	$\uparrow\uparrow$
<i>Rem2</i>	NM_022685	Rad- and gem-related GTP-binding protein 2	$\uparrow\uparrow$
<i>Snf1lk</i>	NM_021693	SNF1-like kinase	$\uparrow\uparrow$
<i>Vgf</i>	NM_030997	VGF nerve growth factor-inducible	$\uparrow\uparrow$

it has been suggested that the prosurvival effect of NMDAR stimulation also needs CREB activation (19), the release of the neurotrophin BDNF (13, 40, 41), the activation of the PI3K/Akt/PKB pathway (18, 42), and the inactivation of FOXO transcriptional activity (43). By contrast, the involvement of the MEK/ERK pathway is highly controversial (13, 28, 40).

Because the intracellular signaling triggered by synaptic NMDAR to promote neuronal survival is still only partially known, we have used DNA microarrays to have a complete picture of the NMDA-mediated differences in the transcriptome. This methodology has allowed us not only to identify single genes that could mediate the prosurvival effect of NMDAR stimulation, but also to identify which functional genetic modules are activated. Our ontological analysis revealed that functional clusters related to neuronal development as well as neurogenesis and antiapoptotic programs were enriched in NMDA-treated cells. Accordingly, we detected up-regulation of the $\alpha 1$, $\gamma 2$, and δ subunits of the GABA_A receptor and a reduction of the catalytic subunit of calcineurin, postu-

lated to be responsible for the maturation blockade of CGCs (44).⁶ Several genes, related to the development and/or survival of CGCs, were also up-regulated in NMDA-treated cells, such as *Bdnf*, neurocalcin, *Vgf*, and *Pcsk1/3* (31, 45–49). We decided to cross-check the list of genes that were up-regulated in NMDA-treated CGCs at 3 h with the list of up-regulated genes obtained by Zhang *et al.* (30) after an action potential-bursting condition. From all the genes up-regulated in both conditions (listed in Table 1), we focused our interest on the members of the Nr4a subfamily because their role in cerebellum development and NMDA-mediated neuroprotection was barely unknown. Only a previous study had suggested that Nur77 protects hippocampal neurons in culture from staurosporine and growth factor removal-induced toxicity *in vitro* and protects hippocampal neurons from kainate-induced toxicity *in vivo* (30). RT-PCR analysis confirmed the increase in the expression of all the Nr4a subfamily members. However, only Nurr1 pro-

⁶ B. Barneda-Zahonero and J. Rodríguez-Alvarez, unpublished data.

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tein levels were consistently increased after NMDA treatment. By contrast, Nur77 was down-regulated by NMDA in CGCs, precluding its effect as a prosurvival factor in these cells.

Although Nurr1 is involved in the development and differentiation of the midbrain dopaminergic neurons (50) and regulates genes such as tyrosine hydroxylase and dopamine transporter (51), the fact is that some studies have also shown that it controls the transcription of proteins such as the VIP (52); α -synuclein, (53)); or BDNF (54), which have been related to CGC survival (31–33, 45). These evidences suggested to us that Nurr1 could be a putative candidate to mediate NMDA prosurvival action. Accordingly, Nurr1 shRNA reduced NMDA protective effect, showing that endogenous Nurr1 is involved in the prosurvival signaling triggered by NMDAR. By contrast, no reduction was observed in the prosurvival effect of K25. As mentioned above, *Nurr1* expression is regulated by CREB in non-neuronal and in neuronal cells (27, 55, 56). Our data show for the first time that CREB binds to *Nurr1* promoter in response to NMDA treatment. In addition, reducing CREB levels by shRNA leads to a reduction in the *Nurr1* promoter activity as well as a decrease of Nurr1 protein levels in NMDA-treated neurons.

Next, we became interested in identifying the substrate/substrates of Nurr1 that could be responsible for the prosurvival effect of NMDA. As mentioned above, Nurr1 has been described to regulate the expression of *Bdnf* in midbrain neurons (54), and it is known that BDNF is important in migration and maintenance of CGCs during cerebellum development (45, 57, 58). Moreover, BDNF has been suggested to mediate NMDA prosurvival effect on CGCs (13, 40, 41). All these studies allowed us to hypothesize that Nurr1 could be responsible for NMDA-mediated regulation of *Bdnf*. Our data support that possibility because: (a) silencing Nurr1 expression by shRNA produced a decrease in BDNF levels in NMDA-treated neurons and (b) NMDA increases the binding of Nurr1 to *Bdnf* promoter IV. This binding seems to be specific to certain *Bdnf* promoters because no binding of Nurr1 to *Bdnf* promoter III was observed. It has been also reported that CREB could interact with the *Bdnf* promoter in cortical neurons after KCl stimulation (34). Our results indicate that in CGCs, the interaction of CREB with the *Bdnf* promoter IV is enhanced by KCl stimulation (K25), whereas it is not enhanced by NMDA treatment (supplemental Fig. 1). Thus, we can conclude that in CGCs, NMDAR stimulation triggers CREB-dependent *Nurr1* activation that results in a BDNF up-regulation. In this context, we have observed *in vivo* that Nurr1 and mature BDNF levels increase from P9 to P21, suggesting Nurr1 as a mediator of survival of CGCs during cerebellum development. Additionally, our immunohistochemistry analyses of Nurr1 localization during cerebellum development report for the first time a lobe-specific expression of Nurr1.

In summary, we have identified by whole genome expression profiling which neuronal genes change their expression level when survival of CGCs is triggered by NMDAR stimulation. We have characterized Nurr1 as a key mediator factor in NMDA-dependent survival of CGCs. *Nurr1* is a downstream target of CREB activation and is responsible of the

increase in mature BDNF expression linked to NMDA prosurvival effect. Moreover, the histological analysis that revealed that Nurr1 restricted expression in the nodulus during cerebellum development opens a possibility to consider Nurr1 as a regulator of cerebellar lobe functional definition.

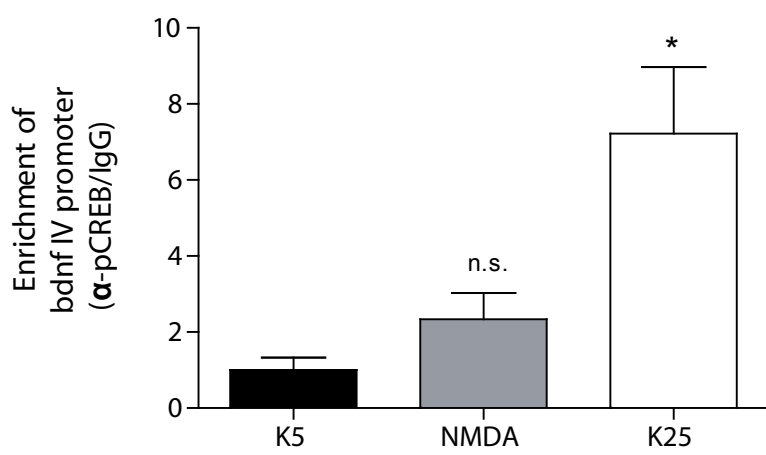
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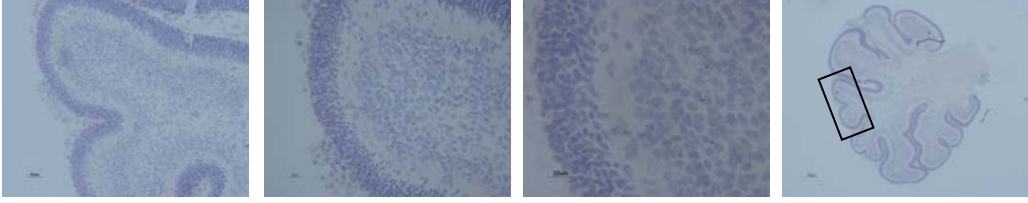
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Nurr1 Mediates CGCs Survival by NMDA

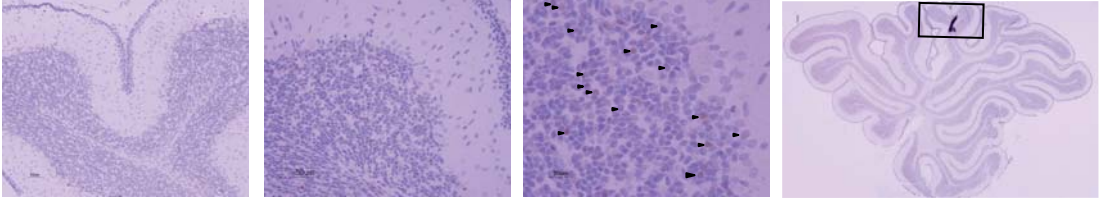
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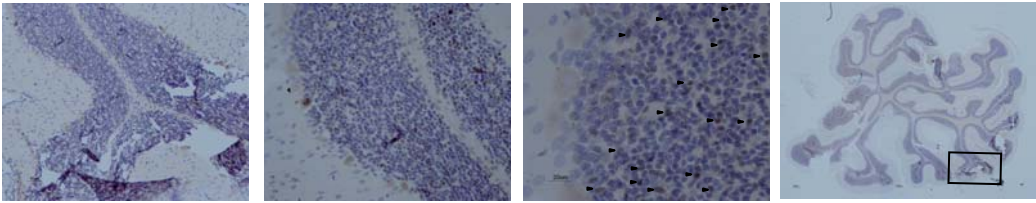
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Supplemental Table I. Up- and down-regulated genes by NMDA at 3 hours.

<i>RefSeq</i>	<i>Gene Title</i>	<i>Gene Symbol</i>	<i>FC</i>	<i>adj.P.Val</i>
NM_012603	myelocytomatosis viral oncogene homolog (avian)	Myc	↑	8,6E-02
NM_030997	VGF nerve growth factor inducible	Vgf	↑↑	2,3E-02
NM_022236	phosphodiesterase 10A	Pde10a	↑↑↑	1,1E-01
NM_053727	nuclear factor, interleukin 3 regulated	Nfil3	↑	1,9E-01
NM_017110	cocaine and amphetamine regulated transcript	Cart	↑	1,9E-01
NM_021693	SNF1-like kinase	Snf1lk	↑	1,9E-01
NM_012513	brain derived neurotrophic factor	Bdnf	↑↑	6,5E-02
NM_019328	nuclear receptor subfamily 4, group A, member 2	Nr4a2	↑↑↑	1,0E-02
NM_017352	nuclear receptor subfamily 4, group A, member 3	Nr4a3	↑↑↑	7,6E-02
NM_131905	claudin 16	Cldn16	↑	1,6E-01
NM_012912	activating transcription factor 3	Atf3	↑↑	1,4E-01
NM_022211	fibroblast growth factor 5	Fgf5	↓	1,8E-01
NM_022685	rad and gem related GTP binding protein 2	Rem2	↑	1,9E-01
NM_013086	cAMP responsive element modulator	Crem	↑↑	3,5E-02
XM_001061568	Rho GDP dissociation inhibitor (GDI) gamma (predicted)	Arhgdig_predicted	↑	5,0E-02
NM_053514	lin-7 homolog a (C. elegans)	Lin7a	↑	1,9E-01
NM_138506	adrenergic receptor, alpha 2c	Adra2c	↓	1,1E-01
NM_031707	homer homolog 1 (Drosophila)	Homer1	↑↑	8,6E-02
NM_001024371	neurocalcin delta	Ncald	↑	7,3E-02
XM_001071842	TBC1 domain family, member 1 (predicted)	Tbc1d1_predicted	↑	1,6E-01
XM_001065223	DiGeorge syndrome critical region gene 6 (predicted)	Dgcr6_predicted	↑	1,9E-01
XM_001068984	similar to KIAA0999 protein	LOC684112	↑	1,9E-01
XM_001063425	PHD finger protein 17 (predicted)	Phf17_predicted	↑	1,8E-01
NM_022197	FBJ murine osteosarcoma viral oncogene homolog	Fos	↑↑↑	2,9E-02
XM_001062276	similar to mKIAA1924 protein (predicted)	RGD1561785_predicted	↑	9,6E-02
XM_001064150	hypothetical protein LOC683022	LOC683022	↓	1,9E-01
XM_001061674	Embryonic ectoderm development (predicted)	Eed_predicted	↑↑	1,6E-01
XM_001057421	prickle-like 1 (Drosophila)	Prickle1	↑	1,6E-01
XM_001061749	Eomesodermin	LOC316052	↑↑	5,2E-02
XM_001077526	similar to Protein UNQ9166/PRO28631 precursor (predicted)	RGD1563996_predicted	↓	5,0E-02
NM_001024360	NOD3-like protein	nod3l	↑	1,8E-01
NM_012993	Nardilysin, N-arginine dibasic convertase 1	Nrd1	↑	1,6E-01
NM_031559	Carnitine palmitoyltransferase 1a, liver	Cpt1a	↑	1,8E-01
NM_001033079	solute carrier family 6 (neurotransmitter transporter), member 17	Slc6a17	↑	1,5E-01
NM_199253	proprotein convertase subtilisin/kexin type 9	Pcsk9	↓	1,1E-01
XM_001080515	atonal homolog 7 (Drosophila) (predicted)	Atoh7_predicted	↑↑	5,0E-02
NM_024388	nuclear receptor subfamily 4, group A, member 1	Nr4a1	↑↑↑	1,0E-02
NM_053346	neuritin	Nrn1	↑	1,9E-01

NM_012760	pleiomorphic adenoma gene-like 1	Plagl1	↑	1,0E-01
NM_017091	proprotein convertase subtilisin/kexin type 1	Pcsk1	↑↑	1,0E-02
NM_001037365	brain expressed X-linked 1	Bex1	↑	7,2E-02
XM_001079173	Similar to Cysteine-rich protein 1 (Cysteine-rich intestinal protein) (CRIP)	LOC691657	↑	1,9E-01
NM_001033951	nuclear protein UKp68	Npuk68	↑	1,9E-01
XM_001072139	Eukaryotic translation initiation factor 4 gamma, 2	Eif4g2	↑	1,7E-01
NM_017086	Early growth response 3	Egr3	↑	1,8E-01
XM_001080220	Hypothetical protein LOC691938	LOC691938	↓	1,6E-01
NM_031123	Stanniocalcin 1	Stc1	↑	1,6E-01
XM_001064328	Kinesin family member 5C (predicted)	Kif5c_predicted	↑	9,6E-02
NM_012576	Nuclear receptor subfamily 3, group C, member 1	Nr3c1	↑	1,9E-01
NM_012836	Carboxypeptidase D	Cpd	↑	3,5E-02
XM_001064743	abhydrolase domain containing 8 (predicted)	Abhd8_predicted	↓	1,8E-01
NM_001033984	Ankyrin 3, epithelial	Ank3	↑	1,7E-01

Supplemental Table II. Up- and down-regulated genes by NMDA at 8 hours.

RefSeq ID	Gene Title	Gene Symbol	FC	adj.P.Val	3h
NM_017091	proprotein convertase subtilisin/kexin type 1	Pcsk1	↑↑↑	2.4E-03	*
NM_001037220	synaptotagmin V	Syt5	↑↑	5.1E-03	
NM_019328	nuclear receptor subfamily 4, group A, member 2	Nr4a2	↑↑↑	8.1E-03	*
NM_024388	nuclear receptor subfamily 4, group A, member 1	Nr4a1	↑↑↑	8.1E-03	*
NM_030997	VGF nerve growth factor inducible	Vgf	↑↑↑	8.1E-03	*
NM_017092	TYRO3 protein tyrosine kinase 3	Tyro3	↑↑	1.1E-02	
NM_017214	regulator of G-protein signaling 4	Rgs4	↑↑	1.1E-02	
NM_001024371	neurocalcin delta	Ncald	↑	1.1E-02	*
NM_022197	FBJ murine osteosarcoma viral oncogene homolog	Fos	↑↑↑	1.1E-02	*
NM_012513	brain derived neurotrophic factor	Bdnf	↑↑↑	1.1E-02	*
NM_001024360	NOD3-like protein	nod3l	↑↑	1.1E-02	*
NM_001033079	solute carrier family 6 (neurotransmitter transporter), member 17	Slc6a17	↑↑	1.1E-02	*
NM_001024980	similar to hypothetical protein MGC46719	RGD1308412	↑	1.1E-02	
XM_001062276	similar to mKIAA1924 protein (predicted)	RGD1561785_predicted	↑	1.1E-02	*
NM_017352	nuclear receptor subfamily 4, group A, member 3	Nr4a3	↑↑↑	1.1E-02	
NM_184051	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit	Prkg2	↑↑↑	1.1E-02	
XM_001073000	Similar to Bcl2-associated athanogene 2	LOC690038	↑↑	1.1E-02	
XM_001060664	hypothetical protein LOC682248	LOC682248	↑	1.1E-02	
NM_199253	proprotein convertase subtilisin/kexin type 9	Pcsk9	↓	1.1E-02	
NM_012760	pleiomorphic adenoma gene-like 1	Plagl1	↑	1.3E-02	
NM_012746	proprotein convertase subtilisin/kexin type 2	Pcsk2	↑	1.3E-02	
NM_001037365	brain expressed X-linked 1	Bex1	↑	1.3E-02	*
NM_031123	Stanniocalcin 1	Stc1	↑↑	1.4E-02	*
XM_001065204	similar to heterogeneous nuclear ribonucleoprotein methyltransferase-like 4	LOC683275	↑↑	1.4E-02	
NM_017102	solute carrier family 2 (facilitated glucose transporter), member 3	Slc2a3	↑	1.4E-02	
NM_133568	RASD family, member 2	Rasd2	↑	2.0E-02	
NM_022236	phosphodiesterase 10A	Pde10a	↑↑↑	2.1E-02	*
NM_053881	protein tyrosine phosphatase, receptor type, N	Ptpn	↑	3.0E-02	
XM_001065526	G protein-coupled receptor 68 (predicted)	Gpr68_predicted	↑	3.1E-02	
NM_012971	potassium voltage-gated channel, shaker-related subfamily, member 4	Kcna4	↑↑	3.7E-02	
XM_001060522	similar to mKIAA0774 protein (predicted)	RGD1562533_predicted	↓	3.9E-02	
XM_001072382	hypothetical LOC314467 (predicted)	RGD1305627_predicted	↑	3.9E-02	
NM_001044276	candidate mediator of the p53-dependent G2 arrest	Rprm	↑↑	3.9E-02	
XM_001072774	transferrin receptor	Tfrc	↑	4.4E-02	
NM_053727	nuclear factor, interleukin 3 regulated	Nfil3	↑	4.4E-02	*
XM_001064328	Kinesin family member 5C (predicted)	Kif5c_predicted	↑	4.4E-02	*
NM_012836	Carboxypeptidase D	Cpd	↑	4.4E-02	*
NM_031707	homer homolog 1 (Drosophila)	Homer1	↑	4.7E-02	

XM_001081119	testis expressed gene 14 (predicted)	Tex14_predicted	↓	4.7E-02	
NM_019348	somatostatin receptor 2	Sstr2	↓	4.7E-02	
XM_001063271	Septin 6 (predicted)	Sept6_predicted	↑	5.0E-02	
NM_024162	fatty acid binding protein 3	Fabp3	↑	5.0E-02	
NM_001014149	similar to RIKEN cDNA 4631403P03	RGD1305276	↑	5.1E-02	
XM_001065359	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like (predicted)	Mthfd11_predicted	↑	5.1E-02	
XM_001057421	prickle-like 1 (Drosophila)	Prickle1	↑	5.1E-02	
XM_001059193	Myosin regulatory light chain interacting protein (predicted)	Mylip_predicted	↑	5.1E-02	
NM_012993	Nardilysin, N-arginine dibasic convertase 1	Nrd1	↑	5.1E-02	
NM_053391	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	Hs3st1	↑	5.1E-02	
XM_001080527	ryanodine receptor 3	Ryr3	↑	5.2E-02	
NM_031559	Non-coding RNA expressed in the brain, repeat sequence, clone 3	Cpt1a	↑	5.2E-02	
XM_001063425	PHD finger protein 17 (predicted)	Phf17_predicted	↑	5.2E-02	
XM_001064150	hypothetical protein LOC683022	LOC683022	↓	5.2E-02	
XM_001063356	similar to hypothetical protein FLJ20037 (predicted)	RGD1311381_predicted	↑	5.4E-02	
XM_001070067	arrestin domain containing 2	Arrdc2	↓	5.4E-02	
NM_012769	guanylate cyclase 1, soluble, beta 3	Gucy1b3	↑	5.5E-02	
NM_012576	Nuclear receptor subfamily 3, group C, member 1	Nr3c1	↑	5.5E-02	*
NM_022685	Rad and gem related GTP binding protein 2	Rem2	↑	5.5E-02	*
XM_001058806	similar to RIKEN cDNA 9330161F08 (predicted)	RGD1564315_predicted	↓	5.5E-02	
XM_001080220	Hypothetical protein LOC691938	LOC691938	↓	5.5E-02	
XM_001077526	similar to Protein UNQ9166/PRO28631 precursor (predicted)	RGD1563996_predicted	↓	5.5E-02	*
XM_001064796	similar to Microtubule-associated serine/threonine-protein kinase 3	LOC684053	↓	5.5E-02	
XM_001077761	similar to septin 6	LOC691335	↑	5.9E-02	
NM_017029	neurofilament 3, medium	Nef3	↑	6.1E-02	
XM_001081348	SH3 and cysteine rich domain 2 (predicted)	Stac2_predicted	↑	6.2E-02	
NM_022669	secretogranin 2	Scg2	↑↑	6.4E-02	
XM_001065637	peroxisome proliferative activated receptor, gamma, coactivator-related 1 (predicted)	Pprc1_predicted	↑	6.4E-02	
NM_001017503	transcription factor Pur-beta	pur-beta	↓	6.4E-02	
NM_001011966	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	Smarcd3	↓	6.6E-02	
NM_001014065	zinc finger, CCHC domain containing 12	Zcchc12	↑	6.6E-02	
NM_024399	aspartoacylase	Aspa	↓	6.7E-02	
NM_019343	regulator of G-protein signaling 7	Rgs7	↑	6.8E-02	
NM_172321	solute carrier family 6 (neurotransmitter transporter), member 15	Slc6a15	↑	7.2E-02	
XM_001061674	Embryonic ectoderm development (predicted)	Eed_predicted	↑↑	7.2E-02	*
NM_013086	CAMP responsive element modulator	Crem	↑↑	7.3E-02	*
XM_001061749	Eomesodermin	LOC316052	↑	7.3E-02	*
NM_182816	adhesion molecule with Ig like domain 2	Amigo2	↑	7.3E-02	
XM_001077893	similar to RIKEN cDNA 2310042D19	RGD1304931	↑	7.7E-02	
NM_001012042	X Kell blood group precursor related family	Xkr6	↑	7.7E-02	

	member 6 homolog			
NM_178097	Src-like adaptor	Sla	↓	7.7E-02
NM_001009619	Nucleotide binding protein 1	Nubp1	↓	7.7E-02
NM_031559	Carnitine palmitoyltransferase 1a, liver	Cpt1a	↑	7.8E-02
NM_001007684	Kruppel-like factor 2 (lung) (predicted)	Klf2_predicted	↑	7.8E-02
XM_001081777	similar to ataxin 2-binding protein 1 isoform 2 (predicted)	RGD1560070_predicted	↓	7.8E-02
NM_181629	small glutamine-rich tetratricopeptide repeat (TPR)-containing, beta	Sgtb	↑	7.9E-02
XM_001080229	retinol binding protein 4, plasma	Rbp4	↑	7.9E-02
XM_001058142	Similar to TAF4A protein	LOC680647	↑	8.0E-02
NM_031971 /// NM_212504 NM_199402	heat shock 70kD protein 1A /// heat shock 70kD protein 1B (mapped) spermatogenesis associated 20	Hspa1a /// Hspa1b Spata20	↑ ↓	8.0E-02 8.1E-02
NM_031242	CDP-diacylglycerol synthase 1	Cds1	↑	8.3E-02
NM_030987	Guanine nucleotide binding protein, beta 1	Gnb1	↑	8.4E-02
NM_198750	cryptochrome 1 (photolyase-like)	Cry1	↑	8.4E-02
XM_001065852	chordin-like 2 (predicted)	Chrdl2_predicted	↑	8.4E-02
XM_001053462	similar to TAF4A5 (predicted)	RGD1562115_predicted	↓	8.8E-02
NM_001047916	Synaptotagmin binding, cytoplasmic RNA interacting protein	Syncrip	↑	8.8E-02
XM_001055358	similar to glyoxylate reductase/hydroxypyruvate reductase	LOC680021	↓	8.9E-02
XM_001072139	Eukaryotic translation initiation factor 4 gamma, 2	Eif4g2	↑	8.9E-02
NM_053360	SH3-domain kinase binding protein 1	Sh3kbp1	↓	8.9E-02
NM_022209	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta isoform	Ppp2r2b	↓	8.9E-02
NM_024398	Aconitase 2, mitochondrial	Aco2	↑	9.0E-02
XM_001079868	zinc finger protein 537 (predicted)	Zfp537_predicted	↓	9.0E-02
NM_001017453	similar to HGFL protein	RGD1311203	↓	9.0E-02
XM_001078877	similar to genetic suppressor element 1 (predicted)	RGD1562686_predicted	↓	9.0E-02
NM_017110	cocaine and amphetamine regulated transcript	Cart	↑	9.0E-02
XM_001061568	Rho GDP dissociation inhibitor (GDI) gamma (predicted)	Arhgdig_predicted	↑	9.0E-02
XM_216568	similar to immunoglobulin superfamily, member 21	LOC684776	↓	9.0E-02
NM_012731	neurotrophic tyrosine kinase, receptor, type 2	Ntrk2	↓	9.0E-02
XM_001055675	similar to TBC1 domain family, member 8	LOC680133	↑	9.1E-02
XM_001077434	suppression of tumorigenicity 5 (predicted)	St5_predicted	↑	9.2E-02
XM_001065263	24-dehydrocholesterol reductase	Dhcr24	↓	9.2E-02
NM_053888	myelin transcription factor 1-like	Myt1l	↓	9.2E-02
XM_001060956	Rap guanine nucleotide exchange factor (GEF) 4	Rapgef4	↓	9.2E-02
NM_031783	neurofilament, light polypeptide	Nefl	↑	9.3E-02
NM_019242	interferon-related developmental regulator 1	Ifrd1	↑	9.3E-02
XM_001072206	Solute carrier family 35, member F2 (predicted)	Slc35f2_predicted	↑	9.3E-02
NM_012603	myelocytomatosis viral oncogene homolog (avian)	Myc	↑	9.4E-02
NM_031112	ribosomal protein S24	Rps24	↑	9.5E-02
NM_022211	fibroblast growth factor 5	Fgf5	↓	9.6E-02

XM_001056692	similar to MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C) (predicted)	Mef2c	↑↑	9.7E-02
NM_001024823	GNAS complex locus	Gnas	↑	9.7E-02
NM_001039002	similar to CG8312-PA	RGD1304790	↑	9.7E-02
NM_017025	lactate dehydrogenase A	Ldha	↑	9.7E-02
NM_001013189	PQ loop repeat containing 1	Pqlc1	↑	9.7E-02
NM_001007664	abhydrolase domain containing 14b	Abhd14b	↓	9.7E-02
NM_133411	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	Abcc4	↓	9.7E-02
NM_001039325	zinc finger, DHHC-type containing 22	Zdhhc22	↓	9.7E-02
NM_013074	hypocretin (orexin) receptor 2	Hcrtr2	↑	9.9E-02
NM_172041	potassium channel, subfamily K, member 2	Kcnk2	↓	9.9E-02
NM_198783	hormone-regulated proliferation associated protein 20	Hrpap20	↑	9.9E-02
XM_001081727	hypothetical protein LOC688274	LOC688274	↓	9.9E-02
NM_001007004	tubulin, alpha 4	Tuba4	↑	1.0E-01
NM_013192	potassium inwardly-rectifying channel, subfamily J, member 6	Kcnj6	↓	1.1E-01
NM_012856	Potassium voltage gated channel, Shaw-related subfamily, member 1	Kcnc1	↓	1.1E-01
XM_001066887	transducin-like enhancer of split 1, homolog of Drosophila E(spl) (predicted)	Tle1_predicted	↑	1.1E-01
NM_019161	cadherin 22	Cdh22	↑	1.1E-01
NM_030856	leucine rich repeat protein 3, neuronal	Lrrn3	↑	1.1E-01
NM_013113	ATPase, Na ⁺ /K ⁺ transporting, beta 1 polypeptide	Atp1b1	↑	1.1E-01
NM_031063	mevalonate kinase	Mvk	↓	1.1E-01
XM_001066524	similar to RIKEN cDNA B130052G07 (predicted)	RGD1564105_predicted	↓	1.1E-01
NM_053514	lin-7 homolog a (C. elegans)	Lin7a	↑	1.1E-01
XM_001081495	Histone deacetylase 5	Hdac5	↑	1.1E-01
XM_001064836	POU domain, class 6, transcription factor 1	Pou6f1	↑	1.1E-01
XM_001069930	laminin, beta 3	Lamb3	↑	1.1E-01
NM_138708	RAB geranylgeranyl transferase, b subunit	Rabggtb	↑	1.1E-01
NM_053820	early B-cell factor 1	Ebf1	↓	1.1E-01
XM_001073833	spindle assembly 6 homolog (C. elegans) (predicted)	Sass6_predicted	↓	1.1E-01
NM_001037554	brain expressed X-linked 4	Bex4	↑	1.1E-01
NM_012686	Visinin-like 1	Vsnl1	↑	1.1E-01
XM_001054307	Similar to RIKEN cDNA A930008G19 (predicted)	RGD1310799_predicted	↓	1.1E-01
NM_017295	gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 5	Gabra5	↑↑	1.1E-01
XM_001080750	hypothetical protein LOC691995	LOC691995	↓	1.1E-01
NM_138835	synaptotagmin XII	Syt12	↓	1.2E-01
NM_001013185	chaperone, ABC1 activity of bc1 complex like (S. pombe)	Cabc1	↓	1.2E-01
NM_183327	gamma-aminobutyric acid A receptor, gamma 2	Gabrg2	↑	1.2E-01
NM_139097	sodium channel, voltage-gated, type III, beta	Scn3b	↑	1.2E-01
XM_001059403	Eph receptor B3 (predicted)	Ephb3_predicted	↓	1.2E-01
NM_012988	nuclear factor I/A	Nfia	↓	1.2E-01
XM_001073490	similar to dachshund b	LOC686314	↓	1.2E-01

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NM_022666	glutamate receptor, metabotropic 4	Grm4	↑↑	1.2E-01
NM_053500	solute carrier family 25, member 27	Slc25a27	↑	1.2E-01
NM_053585	MAP-kinase activating death domain	Madd	↑	1.2E-01
XM_001062174	Ttk protein kinase (predicted)	Ttk_predicted	↓	1.2E-01
NM_001014231	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 1	Slc9a3r1	↑	1.2E-01
XM_001071422	similar to hypothetical protein	LOC304280	↑	1.3E-01
XM_001062329	similar to sphingosine kinase type 1-interacting protein (predicted)	RGD1311951_predicted	↑	1.3E-01
XM_001080134	transmembrane protein 16C (predicted)	Tmem16c_predicted	↑	1.3E-01
XM_001066073	Similar to sterile alpha motif domain containing 12 (predicted)	RGD1561402_predicted	↑	1.3E-01
NM_001025119	similar to TNF intracellular domain-interacting protein	RGD1311487	↑	1.3E-01
NM_153470	leucine zipper, putative tumor suppressor 1	Lzts1	↑	1.4E-01
XM_001073412	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3 F (predicted)	Sema3f_predicted	↓	1.4E-01
XM_001068286	cell division cycle associated 2	Cdca2	↓	1.4E-01
XM_001071720	insulin-like growth factor binding protein-like 1 (predicted)	Igfbp1_predicted	↓	1.4E-01
NM_031763	platelet-activating factor acetylhydrolase, isoform 1b, alpha subunit 45kDa	Pafah1b1	↑	1.4E-01
NM_022671	one cut domain, family member 1	Onecut1	↓	1.4E-01
XM_001072174	similar to RIKEN cDNA E430013E20 gene (predicted)	RGD1311132_predicted	↓	1.4E-01
NM_130403	protein phosphatase 1, regulatory (inhibitor) subunit 14A	Ppp1r14a	↓	1.4E-01
NM_031821	polo-like kinase 2 (Drosophila)	Plk2	↑	1.4E-01
NM_019344	Regulator of G-protein signaling 8	Rgs8	↑	1.4E-01
NM_017217	solute carrier family 7 (cationic amino acid transporter, y+ system), member 3	Slc7a3	↑	1.4E-01
XR_008359	similar to selenoprotein SelM (predicted)	RGD1565037_predicted	↓	1.4E-01
NM_138519	dickkopf homolog 3 (Xenopus laevis)	Dkk3	↑	1.4E-01
XM_001064688	FERM-domain-containing protein 163SCII	LOC257646	↑	1.4E-01
NM_022282	Discs, large homolog 2 (Drosophila)	Dlgh2	↑	1.4E-01
XM_233830	Similar to CG12467-PA (predicted)	RGD1304935_predicted	↑	1.4E-01
XM_001070073	similar to FAT tumor suppressor homolog 4 (predicted)	RGD1564291_predicted	↑	1.4E-01
XM_001057585	similar to Ankyrin repeat domain protein 28 (predicted)	RGD1559931_predicted	↑	1.4E-01
NM_019238	farnesyl diphosphate farnesyl transferase 1	Fdft1	↓	1.4E-01
XM_001075473	PDZ domain containing RING finger 3 (predicted)	Pdzn3_predicted	↓	1.4E-01
NM_080688	phospholipase C, delta 4	Plcd4	↓	1.4E-01
NM_001024341	similar to hypothetical protein MGC39325-like protein	MGC109194	↓	1.4E-01
NM_130744	cytoglobin	Cygb	↓	1.4E-01
NM_001012061	Cnksr family member 3	Cnksr3	↑	1.4E-01
NM_001007691	protease, serine, 23	Prss23	↑	1.5E-01
NM_080587	gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 4	Gabra4	↑	1.5E-01
NM_021693	SNF1-like kinase	Snf1lk	↑	1.5E-01
XM_001067009	coenzyme Q10 homolog A (yeast) (predicted)	Coq10a_predicted	↑	1.5E-01
NM_133566	cystatin E/M	Cst6	↓	1.5E-01

NM_001007641	Rho family GTPase 3	Rnd3	↓	1.5E-01
NM_053851	calcium channel, voltage-dependent, beta 2 subunit	Cacnb2	↑	1.5E-01
NM_001024991	fumarylacetoacetate hydrolase domain containing 1	Fahd1	↑	1.5E-01
XM_001077662	SLIT and NTRK-like family, member 1 (predicted)	Slitrk1_predicted	↑	1.5E-01
NM_001024309	similar to RIKEN cDNA C430004E15	LOC499749	↓	1.5E-01
NM_013079	asparagine synthetase	Asns	↑	1.5E-01
NM_001013094	cyclin L2	Ccnl2	↑	1.5E-01
XM_001075222	EphA5	Epha5	↑	1.5E-01
NM_031106	ribosomal protein L37	Rpl37	↑	1.5E-01
NM_001014034	glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1	Qrs1	↑	1.5E-01
XM_001053648	cytidine 5'-triphosphate synthase (predicted)	Ctps_predicted	↑	1.5E-01
XM_001057357	similar to Iroquois-class homeodomain protein IRX-1 (Iroquois homeobox protein 1) (Homeodomain protein IRXA1)	Irx1_predicted	↓	1.5E-01
NM_001024966	sterile alpha motif domain containing 14	Samd14	↓	1.5E-01
NM_017012	Glutamate receptor, metabotropic 5	Grm5	↓	1.5E-01
NM_022441	activin A receptor type II-like 1	Acvr1	↓	1.5E-01
XM_001060640	CTF18, chromosome transmission fidelity factor 18 homolog (S. cerevisiae) (predicted)	Chtf18_predicted	↓	1.5E-01
NM_130429	lymphoid enhancer binding factor 1	Lef1	↓	1.6E-01
NM_031610	potassium inwardly-rectifying channel, subfamily J, member 3	Kcnj3	↑	1.6E-01
XM_001081783	similar to KIAA1447 protein (predicted)	RGD1306500_predicted	↓	1.6E-01
NM_012912	activating transcription factor 3	Atf3	↑	1.6E-01
NM_138887	heat shock protein, alpha-crystallin-related, B6	Hspb6	↑	1.6E-01
XM_001073157	hypothetical protein LOC686259	LOC686259	↑	1.6E-01
NM_031730	potassium voltage gated channel, Shal-related family, member 2	Kcnd2	↑	1.6E-01
XM_001076038	phosphoribosyl pyrophosphate amidotransferase	Ppat	↑	1.6E-01
XM_001058624	similar to mKIAA1737 protein (predicted)	RGD1309492_predicted	↓	1.6E-01
XM_001079414	core-binding factor, runt domain, alpha subunit 2; translocated to, 3 (predicted)	Cbfa2t3_predicted	↓	1.6E-01
NM_001004069	Ng23 protein	Ng23	↓	1.6E-01
XM_001056498	Elastin microfibril interfacier 2 (predicted)	Emilin2_predicted	↑	1.6E-01
XM_001077247	Similar to FLJ00414 protein (predicted)	RGD1304675_predicted	↓	1.6E-01
XM_001079197	GTPase activating RANGAP domain-like 1	Garn1	↑	1.6E-01
NM_012893	actin, gamma 2	Actg2	↓	1.6E-01
NM_181823	ras homolog gene family, member T2	Rhot2	↑	1.6E-01
NM_031510	isocitrate dehydrogenase 1 (NADP+), soluble	Idh1	↓	1.6E-01
XM_001076847	Similar to AT motif-binding factor (predicted)	RGD1560268_predicted	↓	1.6E-01
XM_001068102	similar to RIKEN cDNA 1700045I19 (predicted)	RGD1565844_predicted	↑	1.6E-01
XM_001060381	similar to RIKEN cDNA E130201N16 (predicted)	RGD1311589_predicted	↑	1.6E-01
NM_001037653	Far upstream element (FUSE) binding protein 1	Fubp1	↑	1.6E-01
XM_001054203	similar to RIKEN cDNA 4931414P19 (predicted)	RGD1565222_predicted	↓	1.6E-01
NM_053394	Kruppel-like factor 5	Klf5	↑	1.7E-01

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NM_031792	sperm associated antigen 4	Spag4	↑	1.7E-01
NM_053482	sperm autoantigenic protein 17	Spa17	↓	1.7E-01
NM_001006966	peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase	Peci	↓	1.7E-01
NM_153468	Granzyme A	Gzma	↓	1.7E-01
NM_013015	prostaglandin D2 synthase	Ptgds	↓	1.7E-01
XM_001062287	similar to RIKEN cDNA 4631427C17 (predicted)	RGD1564895_predicted	↑	1.7E-01
XM_001070886	similar to enoyl Coenzyme A hydratase domain containing 3	LOC684538	↓	1.7E-01
XM_001060505	Fibronectin type III domain containing 5	Fndc5	↓	1.7E-01
NM_184050	putative aminopeptidase Fxna	Fxna	↓	1.7E-01
NM_053346	neuritin	Nrn1	↑	1.7E-01
XM_001080615	similar to mKIAA0664 protein (predicted)	RGD1307222_predicted	↑	1.7E-01
NM_198760	solute carrier family 16 (monocarboxylic acid transporters), member 6	Slc16a6	↑	1.7E-01
NM_012820	acyl-CoA synthetase long-chain family member 1	Acs1	↑	1.7E-01
NM_017093	thymoma viral proto-oncogene 2	Akt2	↓	1.7E-01
NM_017216	Solute carrier family 3, member 1	Slc3a1	↑	1.7E-01
NM_139086	Syncollin	Sycn	↓	1.7E-01
NM_001033888	Numb-like	Numb1	↑	1.7E-01
NM_206847	phosphofructokinase, platelet	Pfkip	↑	1.7E-01
XM_001076507	Similar to expressed sequence AW121567 (predicted)	RGD1559498_predicted	↑	1.7E-01
XM_001075314	citrate lyase beta like	Clybl	↓	1.7E-01
XM_001059692	similar to RIKEN cDNA 6330406115 (predicted)	RGD1307396_predicted	↓	1.7E-01
NM_053949	potassium voltage-gated channel, subfamily H (eag-related), member 2	Kcnh2	↓	1.7E-01
NM_133603	potassium voltage-gated channel, Isk-related family, member 2	Kcne2	↑	1.7E-01
BC086440	midasin homolog (yeast)	Mdn1	↑	1.7E-01
XM_001079124	phosphatidylinositol transfer protein, membrane-associated 2 (predicted)	Pitpm2_predicted	↑	1.8E-01
NM_031552	adducin 3 (gamma)	Add3	↓	1.8E-01
XM_001080401	glycine decarboxylase (predicted)	Gldc_predicted	↓	1.8E-01
NM_030999	corticotropin releasing hormone receptor 1	Crhr1	↑	1.8E-01
XM_001081500	similar to Dual specificity protein phosphatase 3 (T-DSP11) (predicted)	RGD1560049_predicted	↑	1.8E-01
NM_012703	thyroid hormone responsive protein	Thrsp	↓	1.8E-01
XM_001064743	abhydrolase domain containing 8 (predicted)	Abhd8_predicted	↓	1.8E-01
XM_001068291	leucine rich repeat neuronal 6A	Lrrn6a	↓	1.8E-01
NM_173337	Calcium/calmodulin-dependent protein kinase II inhibitor 1	Camk2n1	↓	1.8E-01
XM_001055504	cadherin 11	Cdh11	↓	1.8E-01
XM_001074044	septin 8 (predicted)	Sept8_predicted	↓	1.8E-01
NM_001034125	period homolog 1 (Drosophila)	Per1	↑	1.8E-01
NM_022005	FXYD domain-containing ion transport regulator 6	Fxyd6	↓	1.8E-01
NM_001013950	limb region 1-like homolog (mouse)	Lmbr1l	↑	1.8E-01
XM_001079732	similar to Pinin	LOC368070	↑	1.8E-01
XM_001080515	atonal homolog 7 (Drosophila) (predicted)	Atoh7_predicted	↑	1.8E-01

NM_001025634	Similar to cDNA sequence BC006662	RGD1304748	↓	1.8E-01
NM_172035	frizzled homolog 2 (Drosophila)	Fzd2	↓	1.8E-01
NM_032462	Calsenilin, presenilin binding protein, EF hand transcription factor	Csen	↓	1.8E-01
XM_001073569	similar to Probable G-protein coupled receptor 62 (hGPCR8) (predicted)	RGD1560166_predicted	↓	1.8E-01
XM_001060304	similar to Galectin-5 (RL-18)	LOC682182	↑	1.8E-01
NM_019357	villin 2	Vil2	↑	1.8E-01
XM_001071064	SNF1-like kinase 2 (predicted)	Snf1lk2_predicted	↑	1.9E-01
XM_001080745	p21 (CDKN1A)-activated kinase 6 (predicted)	Pak6_predicted	↑	1.9E-01
XM_001064835	trichorhinophalangeal syndrome I (predicted)	Trps1_predicted	↓	1.9E-01
XM_001071011	retinol dehydrogenase 13 (all-trans and 9-cis) (predicted)	Rdh13_predicted	↑	1.9E-01
NM_199499	Leucine-rich repeat LGI family, member 4	Lgi4	↓	1.9E-01
NM_033351	Fc receptor, IgG, alpha chain transporter	Fcgrt	↓	1.9E-01
NM_053375	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1	Hcn1	↑	1.9E-01
NM_131905	claudin 16	Cldn16	↑	1.9E-01
XM_001054594	similar to hypothetical protein FLJ14503 (predicted)	RGD1564852_predicted	↑	1.9E-01
NM_022185	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 2	Pik3r2	↓	1.9E-01
NM_001013054	ADP-ribosylhydrolase like 1	Adprh1	↓	1.9E-01
NM_022270	solute carrier family 22 (organic cation transporter), member 4	Slc22a4	↓	1.9E-01
NM_053788	syntaxin 1A (brain)	Stx1a	↑	1.9E-01
NM_134408	latrophilin 2	Lphn2	↑	1.9E-01
XM_001077922	Similar to RIKEN cDNA 2610039E05 (predicted)	RGD1560840_predicted	↑	2.0E-01
XM_001062503	similar to RIKEN cDNA 2610200G18 (predicted)	RGD1561205_predicted	↓	2.0E-01
NM_031569	opioid receptor-like 1	Opr1	↓	2.0E-01
NM_001044278	Similar to ring finger protein 133	LOC681395	↑	2.0E-01
NM_001014036	similar to RIKEN cDNA 1700108L22	RGD1307509	↑	2.0E-01

Supplemental Table III. Functional annotation cluster of up-regulated genes by NMDA at 8 hours.

E. Score: 1.56		Signal Transduction (Cluster 1)	N° genes	EASE	FC
GO_BP		cell communication	47	1,90E-03	1,5
GO_BP		intracellular signaling cascade	24	9,00E-03	1,7
GO_BP		signal transduction	39	1,30E-02	1,4
GO_BP		cell surface receptor linked signal transduction	20	6,00E-02	1,5
GO_MF		molecular transducer activity	22	8,20E-02	1,4
GO_BP		G-protein coupled receptor protein signaling pathway	11	1,50E-01	1,6
E. Score: 1.39		Neurogenesis (Cluster 2)	N° genes	EASE	FC
GO_BP		generation of neurons	10	1,80E-02	2,5
GO_BP		cell development	22	2,10E-02	1,6
GO_BP		neuron differentiation	9	2,30E-02	2,6
GO_BP		neurogenesis	10	3,20E-02	2,2
GO_BP		nervous system development	15	4,20E-02	1,8
GO_BP		system development	26	6,40E-02	1,4
GO_BP		neuron development	5	2,60E-01	1,9
E. Score: 1.35		Ion binding (Cluster 3)	N° genes	EASE	FC
GO_MF		ion binding	41	1,40E-03	1,6
GO_MF		metal ion binding	39	3,00E-03	1,5
GO_MF		cation binding	35	1,20E-02	1,5
GO_MF		zinc ion binding	17	8,00E-02	1,5
KEYWORDS		zinc	13	1,40E-01	1,5
KEYWORDS		metal-binding	18	2,30E-01	1,3
GO_MF		transition metal ion binding	19	2,50E-01	1,2
KEYWORDS		zinc-finger	7	4,80E-01	1,3
E. Score: 1.32		Synaptic transmission (Cluster 4)	N° genes	EASE	FC
GO_BP		synaptic transmission	12	1,30E-02	2,3
GO_BP		transmission of nerve impulse	12	2,90E-02	2,1
GO_CC		synapse	8	3,90E-02	2,5
KEYWORDS		Postsynaptic cell membrane	5	7,00E-02	3,2
KEYWORDS		cell junction	7	1,00E-01	2,1
KEYWORDS		synapse	6	1,10E-01	2,3
GO_BP		cell-cell signaling	15	4,30E-02	1,7
GO_BP		neurological system process	15	6,20E-02	1,7
GO_BP		system process	18	1,10E-01	1,4
GO_BP		regulation of neurotransmitter levels	5	1,10E-01	2,7
E. Score: 1.26		Anti-apoptosis (Cluster 5)	N° genes	EASE	FC
GO_BP		cell development	22	2,10E-02	1,6
GO_BP		negative regulation of apoptosis	8	2,30E-02	2,8
GO_BP		positive regulation of cellular process	18	3,90E-02	1,6
GO_BP		regulation of apoptosis	12	4,00E-02	2
GO_BP		negative regulation of cellular process	18	4,20E-02	1,6
GO_BP		regulation of programmed cell death	12	4,40E-02	1,9

GO_BP	anti-apoptosis	6	4,60E-02	3
GO_BP	negative regulation of biological process	19	4,70E-02	1,6
GO_BP	cell differentiation	25	5,30E-02	1,4
GO_BP	positive regulation of cell proliferation	7	7,30E-02	2,4
GO_BP	regulation of cell proliferation	11	7,60E-02	1,8
GO_BP	positive regulation of biological process	18	8,60E-02	1,5
GO_BP	cell proliferation	14	9,00E-02	1,6
GO_BP	apoptosis	13	9,10E-02	1,6

E. Score: 1.19		Ion metabolism (Cluster 6)	N° genes	EASE	FC
GO_MF		transporter activity	23	9,80E-03	1,7
GO_MF		substrate-specific transmembrane transporter activity	17	1,10E-02	2
GO_MF		substrate-specific transporter activity	19	1,90E-02	1,8
GO_MF		alkali metal ion binding	7	2,10E-02	3,2
GO_MF		transmembrane transporter activity	17	2,20E-02	1,8
GO_MF		ion transmembrane transporter activity	14	3,20E-02	1,9
GO_MF		potassium ion binding	5	4,40E-02	3,7
GO_BP		metal ion transport	10	4,80E-02	2,1
GO_BP		cation transport	11	5,00E-02	2
GO_MF		gated channel activity	8	5,40E-02	2,3
KEYWORDS		voltage-gated channel	6	5,50E-02	2,9
GO_BP		monovalent inorganic cation transport	8	5,60E-02	2,3
GO_MF		voltage-gated ion channel activity	6	6,20E-02	2,8
KEYWORDS		potassium transport	5	6,60E-02	3,3
GO_MF		cation transmembrane transporter activity	11	7,20E-02	1,8
GO_MF		metal ion transmembrane transporter activity	8	7,70E-02	2,1
KEYWORDS		potassium	5	8,50E-02	3
GO_BP		transport	32	9,40E-02	1,3
GO_BP		ion transport	13	9,80E-02	1,6
GO_BP		establishment of localization	33	1,00E-01	1,3
GO_MF		voltage-gated cation channel activity	5	1,00E-01	2,8
GO_MF		ion channel activity	8	1,00E-01	2
KEYWORDS		Ionic channel	8	1,00E-01	2
GO_BP		potassium ion transport	5	1,10E-01	2,7
GO_MF		substrate specific channel activity	8	1,20E-01	1,9
GO_BP		localization	36	1,30E-01	1,2
GO_MF		channel activity	8	1,30E-01	1,9
GO_MF		passive transmembrane transporter activity	8	1,30E-01	1,9
KEYWORDS		ion transport	10	1,50E-01	1,6
KEYWORDS		transport	18	1,60E-01	1,4
GO_MF		cation channel activity	6	2,00E-01	1,9

E. Score: 1.09		Development (Cluster 7)	N° genes	EASE	FC
GO_BP		nervous system development	15	4,20E-02	1,8
GO_BP		developmental process	39	4,20E-02	1,3
GO_BP		system development	26	6,40E-02	1,4

GO_BP	multicellular organismal process	41	7,20E-02	1,2
GO_BP	multicellular organismal development	29	8,40E-02	1,3
GO_BP	anatomical structure development	27	1,70E-01	1,2
GO_BP	organ development	18	2,00E-01	1,3
E. Score: 1.05	Membrane localization (Cluster 8)	N° genes	EASE	FC
GO_MF	substrate-specific transmembrane transporter activity	17	1,10E-02	2
KEYWORDS	membrane	46	1,80E-02	1,3
GO_MF	substrate-specific transporter activity	19	1,90E-02	1,8
GO_MF	transmembrane transporter activity	17	2,20E-02	1,8
GO_MF	ion transmembrane transporter activity	14	3,20E-02	1,9
KEYWORDS	glycoprotein	34	3,50E-02	1,4
GO_CC	plasma membrane part	24	4,70E-02	1,5
KEYWORDS	transmembrane	35	5,60E-02	1,3
GO_CC	intrinsic to plasma membrane	17	8,70E-02	1,5
GO_CC	plasma membrane	27	9,10E-02	1,3
GO_BP	transport	32	9,40E-02	1,3
GO_BP	establishment of localization	33	1,00E-01	1,3
GO_CC	membrane part	45	1,20E-01	1,2
GO_CC	integral to plasma membrane	16	1,30E-01	1,4
GO_CC	integral to membrane	39	1,50E-01	1,2
GO_CC	intrinsic to membrane	39	1,60E-01	1,2
KEYWORDS	transport	18	1,60E-01	1,4
KEYWORDS	transmembrane protein	11	1,90E-01	1,5
GO_CC	membrane	51	3,20E-01	1,1
E. Score: 0.84	Signalling pathways (Kinase regulation, Cluster 9)	N° genes	EASE	FC
GO_BP	MAPKKK cascade	6	5,00E-02	3
GO_BP	protein kinase cascade	9	5,90E-02	2,1
GO_BP	regulation of catalytic activity	9	1,20E-01	1,8
GO_BP	positive regulation of catalytic activity	6	1,60E-01	2,1
GO_BP	regulation of a molecular function	9	1,90E-01	1,6
GO_BP	regulation of protein kinase activity	5	2,50E-01	2
GO_BP	regulation of kinase activity	5	2,60E-01	1,9
GO_BP	regulation of transferase activity	5	2,80E-01	1,8
E. Score: 0.78	Metabolic processes (Cluster 10)	N° genes	EASE	FC
GO_BP	glucose metabolic process	5	6,60E-02	3,2
GO_BP	hexose metabolic process	5	1,30E-01	2,6
GO_BP	monosaccharide metabolic process	5	1,30E-01	2,5
GO_BP	cellular carbohydrate metabolic process	6	2,30E-01	1,8
GO_BP	carbohydrate metabolic process	7	2,70E-01	1,6
GO_BP	alcohol metabolic process	6	2,80E-01	1,7
E. Score: 0.78	Second messengers transduction (Cluster 11)	N° genes	EASE	FC
GO_BP	second-messenger-mediated signaling	8	6,30E-02	2,2
GO_BP	G-protein coupled receptor protein signaling pathway	11	1,50E-01	1,6
KEYWORDS	transducer	6	5,10E-01	1,3

E. Score:0.74 Receptor Activity (Cluster 12)				
		N° genes	EASE	FC
GO_BP	cell surface receptor linked signal transduction	20	6,00E-02	1,5
GO_MF	signal transducer activity	22	8,20E-02	1,4
GO_MF	molecular transducer activity	22	8,20E-02	1,4
PATHWAY	Neuroactive ligand-receptor interaction	6	1,80E-01	1,9
GO_MF	receptor activity	15	3,40E-01	1,2
KEYWORDS	receptor	12	4,60E-01	1,2
GO_MF	transmembrane receptor activity	9	5,50E-01	1,1
E. Score: 0.71 Cell homeostasis (Cluster 13)				
		N° genes	EASE	FC
GO_BP	cellular homeostasis	8	7,60E-02	2,1
GO_BP	chemical homeostasis	7	1,50E-01	1,9
GO_BP	di-, tri-valent inorganic cation homeostasis	5	1,60E-01	2,4
GO_BP	cellular di-, tri-valent inorganic cation homeostasis	5	1,60E-01	2,4
GO_BP	cellular chemical homeostasis	6	1,60E-01	2,1
GO_BP	cellular ion homeostasis	6	1,60E-01	2,1
GO_BP	ion homeostasis	6	2,00E-01	1,9
GO_BP	cation homeostasis	5	2,20E-01	2
GO_BP	cellular cation homeostasis	5	2,20E-01	2
GO_BP	homeostatic process	8	2,60E-01	1,5
GO_BP	regulation of biological quality	10	7,10E-01	1
E. Score: 0.7 Vesicles (Cluster 14)				
		N° genes	EASE	FC
GO_CC	vesicle	8	1,10E-01	1,9
GO_CC	cytoplasmic vesicle	7	1,80E-01	1,8
GO_CC	cytoplasmic membrane-bound vesicle	6	2,80E-01	1,7
GO_CC	membrane-bound vesicle	6	3,00E-01	1,6
E. Score: 0.68 Secretion (Cluster 15)				
		N° genes	EASE	FC
GO_BP	synaptic transmission	12	1,30E-02	2,3
GO_BP	transmission of nerve impulse	12	2,90E-02	2,1
GO_BP	secretion by cell	7	1,70E-01	1,8
GO_BP	secretory pathway	6	2,20E-01	1,8
GO_BP	secretion	7	3,80E-01	1,4
GO_BP	establishment of cellular localization	11	4,90E-01	1,1
GO_BP	cellular localization	11	5,10E-01	1,1
GO_BP	cellular component organization and biogenesis	26	5,70E-01	1
GO_BP	intracellular transport	5	9,40E-01	0,7
E. Score: 0.67 Transcription (Cluster 16)				
		N° genes	EASE	FC
GO_MF	sequence-specific DNA binding	11	2,60E-03	3,1
KEYWORDS	DNA binding	7	8,90E-03	3,9
KEYWORDS	Transcription regulation	12	5,10E-02	1,9
KEYWORDS	Transcription	12	5,30E-02	1,9
GO_BP	transcription from RNA polymerase II promoter	11	5,50E-02	1,9
GO_BP	regulation of transcription, DNA-dependent	19	6,50E-02	1,5
KEYWORDS	dna-binding	12	7,10E-02	1,8
GO_MF	transcription factor activity	12	7,40E-02	1,8

GO_BP	transcription, DNA-dependent	19	9,50E-02	1,4
GO_MF	transcription factor binding	8	9,50E-02	2
GO_BP	RNA biosynthetic process	19	9,70E-02	1,4
GO_MF	transcription regulator activity	16	1,00E-01	1,5
GO_BP	positive regulation of transcription	7	1,00E-01	2,2
GO_BP	biological regulation	48	1,10E-01	1,2
GO_BP	regulation of transcription	19	1,20E-01	1,4
GO_BP	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	7	1,30E-01	2
GO_BP	regulation of gene expression	20	1,40E-01	1,3
GO_BP	regulation of transcription from RNA polymerase II promoter	8	1,70E-01	1,8
GO_BP	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	19	1,70E-01	1,3
GO_BP	transcription	19	1,80E-01	1,3
KEYWORDS	nucleus	21	2,00E-01	1,3
GO_BP	regulation of cellular process	36	2,00E-01	1,2
GO_BP	positive regulation of cellular metabolic process	7	2,40E-01	1,7
GO_BP	regulation of biological process	40	2,60E-01	1,1
GO_BP	positive regulation of transcription, DNA-dependent	5	2,80E-01	1,8
GO_BP	RNA metabolic process	20	2,90E-01	1,2
GO_BP	positive regulation of metabolic process	7	3,00E-01	1,5
GO_BP	regulation of cellular metabolic process	19	3,70E-01	1,2
GO_BP	regulation of metabolic process	20	3,80E-01	1,1
GO_MF	DNA binding	15	4,10E-01	1,2
GO_BP	gene expression	23	4,80E-01	1,1
GO_MF	nucleic acid binding	20	6,70E-01	1
GO_BP	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	23	7,00E-01	1
GO_CC	organelle	53	7,90E-01	0,9
GO_BP	macromolecule metabolic process	45	8,30E-01	0,9
GO_CC	intracellular organelle	52	8,30E-01	0,9
GO_CC	cytoplasm	40	8,40E-01	0,9
GO_CC	intracellular	66	8,60E-01	0,9
GO_CC	cytoplasmic part	29	8,80E-01	0,9
GO_BP	biopolymer metabolic process	29	9,00E-01	0,9
GO_CC	nucleus	23	9,10E-01	0,8
GO_CC	intracellular part	60	9,40E-01	0,9
GO_CC	intracellular membrane-bound organelle	37	1,00E+00	0,8
GO_CC	membrane-bound organelle	37	1,00E+00	0,8

E. Score: 0.56	Cytoskeleton and intracellular organelle (Cluster 17)	N° genes	EASE	FC
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GO_CC	cytoskeleton	13	6,90E-02	1,7
GO_CC	cytoskeletal part	10	7,30E-02	1,9
GO_CC	intracellular non-membrane-bound organelle	17	2,40E-01	1,3
GO_CC	non-membrane-bound organelle	17	2,40E-01	1,3
GO_MF	structural molecule activity	9	2,70E-01	1,4
GO_CC	microtubule cytoskeleton	5	3,30E-01	1,7

GO_CC	intracellular organelle part	23	6,90E-01	1
GO_CC	organelle part	23	7,00E-01	1
KEYWORDS	Coiled coil	6	7,50E-01	1
E. Score: 0.54	Cell adhesion (Cluster 18)	N° genes	EASE	FC
KEYWORDS	cell adhesion	6	5,40E-02	2,9
GO_BP	biological adhesion	6	6,60E-01	1,1
GO_BP	cell adhesion	6	6,60E-01	1,1
E. Score 0.47	Extracellular space (Cluster 19)	N° genes	EASE	FC
KEYWORDS	signal	27	6,50E-02	1,4
GO_CC	extracellular space	21	3,90E-01	1,1
GO_CC	extracellular region	23	4,10E-01	1,1
GO_CC	extracellular region part	21	4,60E-01	1,1
E. Score: 0.43	Organ morphogenesis (Cluster 20)	N° genes	EASE	FC
GO_BP	organ development	18	2,00E-01	1,3
GO_BP	organ morphogenesis	7	3,10E-01	1,5
GO_BP	anatomical structure morphogenesis	10	8,40E-01	0,9
E. Score: 0.38	Nucleus (Cluster 21)	N° genes	EASE	FC
GO_MF	transcription regulator activity	16	1,00E-01	1,5
GO_CC	nucleoplasm part	5	3,70E-01	1,6
GO_CC	membrane-enclosed lumen	8	4,90E-01	1,2
GO_CC	organelle lumen	8	4,90E-01	1,2
GO_CC	nucleoplasm	5	5,00E-01	1,3
GO_CC	nuclear lumen	6	5,30E-01	1,2
GO_CC	nuclear part	6	9,00E-01	0,8
E. Score: 0.33	Cellular processes (Cluster 22)	N° genes	EASE	FC
GO_MF	binding	97	1,80E-02	1,1
GO_MF	protein binding	60	1,10E-01	1,1
GO_CC	cell part	99	3,60E-01	1
GO_CC	cell	99	3,60E-01	1
GO_BP	cellular process	89	7,70E-01	1
GO_BP	macromolecule metabolic process	45	8,30E-01	0,9
GO_CC	intracellular	66	8,60E-01	0,9
GO_BP	biopolymer metabolic process	29	9,00E-01	0,9
GO_CC	intracellular part	60	9,40E-01	0,9
GO_BP	primary metabolic process	51	9,50E-01	0,9
GO_BP	metabolic process	56	9,70E-01	0,9
GO_BP	cellular metabolic process	49	9,80E-01	0,8
E. Score: 0.33	Proteolysis (Cluster 23)	N° genes	EASE	FC
KEYWORDS	protease	6	3,30E-01	1,6
GO_BP	proteolysis	8	4,30E-01	1,3
GO_MF	peptidase activity	7	4,40E-01	1,3
GO_MF	endopeptidase activity	5	5,10E-01	1,3
KEYWORDS	hydrolase	10	7,20E-01	1
E. Score: 0.29	Cell morphogenesis (Cluster 24)	N° genes	EASE	FC

GO_BP	cell projection morphogenesis	5	3,30E-01	1,7
GO_BP	cellular structure morphogenesis	5	8,00E-01	0,9
GO_BP	cell morphogenesis	5	8,00E-01	0,9
GO_BP	anatomical structure morphogenesis	10	8,40E-01	0,9
E. Score: 0.29	Nucleotid binding (Cluster 25)	Nº genes	EASE	FC
GO_MF	GTP binding	6	1,60E-01	2,1
GO_MF	guanyl nucleotide binding	6	1,70E-01	2
GO_MF	purine ribonucleotide binding	14	6,70E-01	1
GO_MF	purine nucleotide binding	14	7,30E-01	0,9
KEYWORDS	nucleotide-binding	10	7,70E-01	0,9
GO_MF	nucleotide binding	15	8,10E-01	0,9
GO_MF	ATP binding	9	8,80E-01	0,8
GO_MF	adenyl ribonucleotide binding	9	9,00E-01	0,8
GO_MF	adenyl nucleotide binding	9	9,30E-01	0,7
E. Score: 0.28	Catabolic processes (Cluster 26)	Nº genes	EASE	FC
GO_BP	macromolecule catabolic process	5	4,80E-01	1,4
GO_BP	catabolic process	8	4,80E-01	1,2
GO_BP	cellular catabolic process	6	6,30E-01	1,1
E. Score: 0.25	Phosphatase and Hydrolase activity (Cluster 27)	Nº genes	EASE	FC
GO_MF	nucleoside-triphosphatase activity	6	5,30E-01	1,2
GO_MF	pyrophosphatase activity	6	5,70E-01	1,2
GO_MF	hydrolase activity, acting on acid anhydrides	6	5,80E-01	1,2
E. Score: 0.21	Acid metabolic processes (Cluster 28)	Nº genes	EASE	FC
GO_BP	monocarboxylic acid metabolic process	5	3,90E-01	1,6
GO_BP	carboxylic acid metabolic process	6	7,60E-01	1
GO_BP	organic acid metabolic process	6	7,60E-01	0,9
E. Score: 0.11	Response to stimulus (Cluster 29)	Nº genes	EASE	FC
GO_BP	response to stimulus	22	7,00E-01	1
GO_BP	response to external stimulus	8	7,10E-01	1
GO_BP	response to wounding	5	8,10E-01	0,9
GO_BP	response to stress	10	8,60E-01	0,8
E. Score: 0.08	Kinase activity (Cluster 30)	Nº genes	EASE	FC
GO_MF	protein serine/threonine kinase activity	5	6,80E-01	1,1
GO_MF	protein kinase activity	6	7,10E-01	1
KEYWORDS	nucleotide-binding	10	7,70E-01	0,9
GO_MF	transferase activity, transferring phosphorus-containing groups	8	8,10E-01	0,9
GO_MF	kinase activity	7	8,20E-01	0,9
GO_MF	phosphotransferase activity, alcohol group as acceptor	6	8,30E-01	0,9
GO_BP	phosphorylation	6	8,60E-01	0,8
GO_BP	phosphate metabolic process	7	8,80E-01	0,8
GO_MF	ATP binding	9	8,80E-01	0,8
GO_MF	adenyl ribonucleotide binding	9	9,00E-01	0,8
KEYWORDS	atp-binding	6	9,20E-01	0,7
GO_MF	adenyl nucleotide binding	9	9,30E-01	0,7

GO_MF

transferase activity

10

9,70E-01

0,7