Expression of Ndrg2 in the rat frontal cortex after antidepressant and electroconvulsive treatment

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Abstract

Although the therapeutic action of antidepressants most likely involves the regulation of serotonergic and noradrenergic signal transduction, no consensus has been reached concerning their precise molecular or cellular mechanisms of action. In the present study, we demonstrated that chronic treatment with a tricyclic antidepressant (imipramine) and a selective serotonin reuptake inhibitor (sertraline) reduced the expression of NDRG2 mRNA and protein in the rat frontal cortex. NDRG2 is a member of the N-Myc downstream-regulated genes. Interestingly, repeated ECT also significantly decreased NDRG2 expression in this region of the brain. These data suggest that NDRG2 may be a common functional molecule that is decreased after antidepressant treatment and ECT. Although, the functional role of NDRG2 in the central nervous system remains unclear, our findings suggest that NDRG2 may be associated with treatment-induced adaptive neural plasticity in the brain, a chronic target of antidepressant action. In conclusion, we have identified NDRG2 as a candidate target molecule of antidepressants and ECT.

Introduction

Antidepressants are very effective agents for preventing and treating depression and have been used clinically for more than 50 yr. Typical antidepressants significantly increase the synaptic concentration of norepinephrine and/or serotonin. However, a latency period of several weeks generally elapses before the therapeutic effects of antidepressants are observed. This delayed therapeutic action could result from either the indirect regulation of other neuronal signal transduction systems or the regulation of gene transcription following chronic treatment. Indeed, antidepressants have been shown to affect the expression of immediate early genes and transcription factors, including c-fos, FosB, junB, NGF1-A, and CREB (see review by Yamada and Higuchi, 2002). These regulatory proteins activate or repress genes that encode specific proteins, and may be involved in critical steps that mediate treatment-induced alterations of central nervous system function. We recently performed expressed-sequence tag (EST) analyses to identify some biological changes observed in rat brain after chronic treatment with antidepressants (Yamada et al., 2001). We developed our original ADRG microarray for high-throughput secondary screening of these candidate genes (Yamada et al., 2000). To date, we have cloned several cDNA candidates as ESTs from the rat brain and have named these antidepressant-related genes (ADRGs).

While antidepressant pharmaceuticals have been shown to be an effective treatment, another important therapy that is widely used for treating depression is repeated electroconvulsive treatment (ECT). Because of its safety, high efficacy, and rapid onset of action, ECT is well-suited for treating patients with severe psychotic depression, severe depression with suicidal ideation, drug-resistant depression, and for treating...
geriatric patients and others with medical illnesses that contraindicate the use of antidepressants. Although ECT is an effective treatment for depression, the basis for its therapeutic mechanism remains unknown. An increasingly popular working hypothesis is that both antidepressants and ECT have therapeutic effects because they share some final common pathway regulating transcription of the same set of downstream genes. Indeed, we have recently reported that VAMP2 (Yamada et al., 2002) and kf-1 (Nishioka et al., 2003; Yamada et al., 2000) are expressed both after chronic antidepressant drug treatment and repeated ECT.

In the present study, we identified ADRG123 as rat Ndrg2 (Swiss-Prot/TrEMBL accession numbers Q8VBU2, Q8VI01). Ndrg2 is highly related to N-Myc downstream-regulated protein 1 (Ndrg1), which has been linked to stress responses, cell proliferation, and differentiation, although Ndrg2 itself is not repressed by N-Myc (Okuda and Kondoh, 1999). Thus far, four different isoforms of rat Ndrg2 have been identified (Figure 1; Boulkroun et al., 2002). The 5'-UTR for Ndrg2a1/Ndrg2b1 is 87 bp, whereas the 5'-UTR for Ndrg2b1/Ndrg2b2 is 50 bp. In the translated region, Ndrg2a1/Ndrg2b1 has an additional 42 bp insertion compared to Ndrg2a2/Ndrg2b2. Here, we denote Ndrg2a1/Ndrg2b1 and Ndrg2a2/Ndrg2b2 to represent Ndrg2L and Ndrg2S respectively. Comparison and alignment of amino-acid sequences indicated that Ndrg2L is longer than Ndrg2S by 14 amino acids and that both isoforms share the characteristic Ndrg family sequence. Here, we provide the first report that chronic antidepressant drug treatment and repeated ECT decreases the expression of Ndrg2 mRNA and protein in the rat frontal cortex.

Materials and methods

Experimental animals and treatments

Male Sprague–Dawley rats (age 7–10 wk, Sankyo Labo Service Co., Tokyo, Japan) were housed in a temperature-controlled environment with a 12 h light/12 h dark cycle and were given free access to food and water. Rats were randomly separated into control and treated groups. Imipramine (Sigma-Aldrich, Inc., St Louis, MO, USA) and sertraline (Pfizer Pharmaceuticals Inc., New York, NY, USA) were dissolved in 1.5% Tween-80. For the chronic antidepressant-treatment group, rats received daily intraperitoneal injections of vehicle, 10 mg/kg of imipramine, or 10 mg/kg sertraline for 21 d. For the ECT group, rats were anaesthetized with sevoflurane, then given either a single electric shock (90 mA, 1.0-s duration) via ear-clip electrodes (single-dose ECT group) or electric shocks (90 mA, 1.0-s duration) every other day for 14 d (repeated ECT group). ECT was delivered with a Ugo Basile Model 7801 unipolar square-wave electroconvulsive stimulation pulse generator (Stoelting Co., Wood Dale, IL, USA). Control rats were treated exactly like the ECT-treated rats but did not receive any electric current.

Twenty-four hours after the final antidepressant or ECT treatment, animals were euthanized by...
decapitation. The brain was quickly removed, dissected, and immediately frozen in liquid nitrogen and stored at −80 °C until later use. All animal studies were carried out in accordance with National Institutes of Health guidelines in line with the OPRR Public Health Service Policy on Humane Care and Use of Laboratory Animals.

**Identification of Ndrg2 by ADRG microarray**

Fabrication of the ADRG microarray and fluorescence image analysis was done as described previously (Yamada et al., 2000). Briefly, each of the ADRG cDNA inserts was amplified by vector primers and negative controls, and 10 different kinds of housekeeping genes were spotted in duplicate onto glass slides with a GMS417 Arrayer (Affymetrix Inc., Santa Clara, CA, USA). Hybridization of fluorescent probes to the microarray was done competitively and in duplicate. After hybridization and washing, each slide was scanned with a GMS418 Array Scanner (Affymetrix Inc.). Gene expression levels were quantified and analysed with ImaGene software (Bio-Discovery Ltd, Swansea, UK). Preliminary assessment of the arrays (data not shown) indicated that the differences in fluorescence intensities (±2-fold) were significant. Sequence analysis of ADRG123 was performed by dideoxy sequencing methods. Homology search and sequence alignment was done using the FASTA search servers at the National Center for Biotechnology Information.

**Expression analysis by real-time quantitative PCR**

As described above, rat Ndrg2 protein consists of two splice variants, Ndrg2S and Ndrg2L. However, we previously demonstrated using conventional RT–PCR analyses that transcript processing into long and short forms of Ndrg2 does not appear to be significantly regulated after antidepressant treatments (data not shown). Therefore, we performed mRNA expression analysis of Ndrg2 with real-time quantitative PCR; total levels of Ndrg2S and Ndrg2L mRNA were examined in the present study.

Total RNA was extracted from samples using Isogen reagent (Nippon Gene Co., Tokyo, Japan) according to the manufacturer’s instructions. Total RNA samples treated with RNase-free DNase I were used to synthesize the first strand cDNA via reverse transcriptase and oligo-dT primer. We quantified Ndrg2 expression in the rat frontal cortex with real-time quantitative PCR using an ABI PRISM 7000 instrument (Applied Biosystems, Foster City, CA, USA). PCR primers were designed using Primer Express Software (Applied Biosystems). A quantity of cDNA corresponding to 20 pg of total RNA was amplified by PCR in duplicate. For each sample, three distinct amplifications were carried out in parallel. The following primers were used for rat Ndrg2 (5'-AACCTTGGACGGAGTGTTGAGA-3' and 5'-ATTCCACCCAGGCATCTCTCA-3') and β-actin (5'-TGCGTGACAGGATGCAAGG-3' and 5'-GCCAGGATA-GACCCACCAAT-3'). The SYBR® Green PCR Core Reagents kit (Applied Biosystems) was utilized for fluorescence detection of cDNA. For quantification, we used the Standard Curve Method (User Bulletin, ABI PRISM 7000 Sequence Detection System). Briefly, for rat Ndrg2 and β-actin, an absolute standard curve was obtained by plotting the threshold cycle following PCR amplification of serial dilutions of control cDNA template.

**Expression analysis by Western blotting**

Anti-rat-Ndrg2 antiserum was prepared as follows. Synthetic rat Ndrg2 peptides (CSLTSAASSIDGSR, RDLNFERGEMTLKC, and CEVQITEEKPLLPGQ) were coupled to activated keyhole limpet haemocyanin using N-maleimidobenzoyl-N-hydroxysuccinimide ester, then injected into Hartley guinea pigs (Takara Tokyo, Japan). Immune serum was then collected and used for Western blot analysis and immunohistochemistry.

Frontal cortices from control and treated rats were homogenized in ice-cold sucrose–Tris buffer (250 mM sucrose, 50 mM Tris–HCl, 5 mM EDTA, 10 mM EGTA, 0.3% mercaptoethanol; pH 7.4). Three rats were used for each treatment group. The protein concentration was determined by the Bradford method and a Bio-Rad protein assay kit. Each fraction (20 μg protein) was separated by 7.5% SDS–PAGE after solubilization and boiling in Laemmli buffer. Electrophoretically separated proteins were transferred from gels onto nitrocellulose membranes via standard techniques. To examine the expression of Ndrg2 in HEK293 cells overexpressing rat Ndrg2S and Ndrg2L respectively, Western blot analyses were performed on protein extracts derived from the transfected cells. Pre-immune serum was used as negative control.

Non-specific immunostaining was blocked by incubating the membranes in blocking buffer comprised of 5% skim milk. The membranes were sequentially incubated in blocking buffer with anti-rat-Ndrg2 antiserum (1:500), followed by HRP-conjugated goat anti-guinea pig antibody (1:2000; ICN Biomedicals Inc., Irvine, CA, USA). Immunoreactive bands were visualized on film via the ECL system. To ensure the fidelity of this analysis, we assayed only film exposed
in the linear range. The optical density of the digitized bands was quantified using NIH Image. NIH Image is a public domain program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Phosphatase digestion

For the phosphatase digestion study, a protein sample from the rat frontal cortex was incubated with lambda protein phosphatase, a Mn\(^{2+}\)-dependent protein phosphatase that acts on phosphorylated serine, threonine, and tyrosine residues. The protein aliquot was incubated for 1 h at 30 °C in 50 μl of lambda-protein phosphatase reaction buffer [50 mm Tris–HCl (pH 7.5), 5 mm dithiothreitol, 0.1 mm Na\(_2\)EDTA, 0.01% Brij 35, and 2 mm MnCl\(_2\)] with or without 1 μl lambda-protein phosphatase (4 000 000 U/ml; New England Biolabs Inc., Beverly, MA, USA). The proteins were then analysed by Western blot together with an identically treated aliquot incubated without phosphatase.

Cell culture and transfection of Ndrg2S and Ndrg2L in HEK293 cells

HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal calf serum, 0.1 mM MEM non-essential amino-acid solution (Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen) at 37 °C in a humidified atmosphere comprised of 5% CO\(_2\).

The coding regions for Ndrg2S and Ndrg2L were obtained by RT–PCR of rat brain mRNA with the following set of primers: 5’-CTCGAGGCCCACATGCGAGACGACAGGCTACCCCATGGTG-3’, 5’-GAATTCTCTCTCAACAGGAGAC-3’ and high fidelity Platinum pfx DNA polymerase (Invitrogen). These primers contain XhoI or EcoRI sites (underlined) to facilitate subcloning. Each of the PCR products were then ligated into pCR II-TOPO vectors (Invitrogen) and transformed into competent DH5α E. coli cells. The resulting plasmid vectors were subcloned into pIRES-EGFP (Clontech, Palo Alto, CA, USA) for transfection. In this study, we used the pIRES-EGFP vector, which can express GFP and target molecules separately. HEK293 cells were then transfected with 3 μg of recombinant plasmid in serum-free medium using 4 μl Lipofectamine reagent (Invitrogen) according to the manufacturer’s instructions.

Immunohistochemistry

Rats were anaesthetized with sodium pentobarbital and transcardially perfused with 4% paraformaldehyde (Sigma-Aldrich) in 0.1 m phosphate buffer (pH 7.4). The brains were then cryoprotected and quickly frozen. The brain was sectioned (40 μm) using a cryostat CM-501 (Sakura, Tokyo, Japan), and floating sections were further fixed with 4% paraformaldehyde overnight. Sections were boiled in phosphate buffer containing 0.9% NaCl (PBS) for 1 h, permeabilized with 0.1% Triton X-100 in PBS (PBST) for 20 min, and then blocked with PBS containing 1.5% normal goat serum for 20 min. Sections were incubated with anti-rat-Ndrg2 antiserum (1:500) in PBST for 24 h at 4 °C, washed three times with PBST, and incubated with biotinylated anti-guinea pig antibody (1:250, Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature. Sections were washed three times with PBST, treated with 0.3% hydrogen peroxide for 30 min, washed three times with PBST again, and incubated with avidin-biotin peroxidase complex (Vector Laboratories) for 30 min. Visualization of the peroxidase was performed with 0.01% hydrogen peroxide and 0.01% diaminobenzidine as a chromogen. The slides were counterstained with haematoxylin and analysed with an Olympus BX-60 light microscope (Olympus Optical, Tokyo, Japan).

Statistical analysis

Data are presented as means ± S.E.M. for each group. For antidepressant or ECT experiments, differences were assessed using analysis of variance (ANOVA) followed by the Dunnett’s test. A value of p < 0.05 was regarded as significant.

Results

Identification of Ndrg2 as ADRG123

Figure 1 shows a pseudo-colour image of the ADRG microarray after hybridization with frontal cortex samples obtained from sertraline- or ECT-treated rats. As expected, we obtained low background and consistent results in duplicate experiments. After normalization of the signals with both negative and positive controls, fluorescence intensities representing ADRG123 decreased 0.63-fold in the sertraline group and 0.49-fold in the ECT group. These data were reproducible and inter-assay variability was negligible. As shown in Figure 2, the ADRG123 fragment obtained from the initial EST analysis was 230 bp (starting at the 3’-end containing poly-A\(^+\) sequences). Homology search of the EMBL/GeneBank database revealed that ADRG123 perfectly matches the full-length cDNA sequence of the rat Ndrg2 gene (SwissProt/TrEMBL accession numbers Q8VBU2, Q8VI01).
Table 1. Real-time RT–PCR analysis of Ndrg2 mRNA expression in the rat frontal cortex after antidepressant treatment or ECT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ndrg2 ( Arbitrary units )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single antidepressant treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100±2.3</td>
</tr>
<tr>
<td>Imipramine</td>
<td>101±13.2</td>
</tr>
<tr>
<td>Sertraline</td>
<td>86.7±2.7</td>
</tr>
<tr>
<td><strong>Chronic antidepressant treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100±7.9</td>
</tr>
<tr>
<td>Imipramine</td>
<td>65.3±2.6*</td>
</tr>
<tr>
<td>Sertraline</td>
<td>65.3±13.2*</td>
</tr>
<tr>
<td><strong>ECT</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100±6.1</td>
</tr>
<tr>
<td>Single-dose ECT</td>
<td>71.5±9.3*</td>
</tr>
<tr>
<td>Chronic ECT</td>
<td>47.2±6.8**</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E.M. *p < 0.05, **p < 0.01, ANOVA followed by Dunnett’s test.

Figure 2. Schematic representations of rat Ndrg2. Rat Ndrg2 consists of four isoforms: Ndrg2a1, Ndrg2a2, Ndrg2b1, and Ndrg2b2. The 5'-UTR for Ndrg2a1/Ndrg2a2 was 87 bp, whereas the 5'-UTR for Ndrg2b1/Ndrg2b2 was 50 bp. In the translated region, Ndrg2a1/Ndrg2b1 has an additional 42-bp insertion compared to Ndrg2a2/Ndrg2b2; both isoforms contained the characteristic Ndrg family sequence in the middle of their sequences. In this study, Ndrg2S (upper) and Ndrg2L (lower) correspond to Ndrg2a2/Ndrg2b2 and Ndrg2a1/Ndrg2b1 respectively. The ADRG123 fragment obtained from the initial EST analysis was part of rat Ndrg2 (230 bp, starting at the 3'-end containing poly-A+ sequences). UTR, untranslated region.

**Messenger RNA expression analysis by real-time quantitative PCR**

Using real-time quantitative RT–PCR, we confirmed the significantly decreased expression of total Ndrg2 mRNA in the frontal cortex that resulted from chronic treatment with either imipramine or sertraline (65.3±2.6% or 65.3±13.2%, Table 1). On the other hand, single-dose treatments of either antidepressant failed to affect the expression of total Ndrg2 mRNA (101±13.2% or 86.7±2.7%). Interestingly, as shown in Table 1, not only repeated ECT but also single-dose ECT significantly decreased total Ndrg2 mRNA expression in rat frontal cortex (71.5±9.3% or 47.2±6.8%).

**Expression analysis of Ndrg2S- and Ndrg2L-protein by Western blot analysis**

Immunoblotting of protein extracts from control frontal cortex demonstrated two Ndrg2-immunoreactive ~39.3 and ~40.8 kDa bands (Figure 3). To examine the specificity of the anti-rat-Ndrg2 antiserum, we immunostained HEK293 cells overexpressing rat Ndrg2S and Ndrg2L. As expected, immunoblotting of protein extracts from HEK293 cells showed a single band corresponding to rat Ndrg2S and Ndrg2L proteins, while pre-immune serum (control) showed no bands. The effect of phosphatase digestion on Ndrg2 immunoreactivity in the rat frontal cortex was also examined. Undigested rat frontal cortex showed two major immunoreactive bands when stained with anti-rat-Ndrg2 antiserum [a, right panel]. As expected, immunoblotting of protein extracts from HEK293 cells showed a single band corresponding to rat Ndrg2S and Ndrg2L proteins, while pre-immune serum (control) showed no bands. The effect of phosphatase digestion on Ndrg2 immunoreactivity in the rat frontal cortex also demonstrated [b, lane 2].

The double bands persisted, even after phosphatase digestion, and did not show a mobility shift in a gel [b, lane 2].

To determine whether the antidepressant-associated decrease of Ndrg2S and Ndrg2L mRNAs also affected protein levels, we examined Ndrg2S and Ndrg2L protein expression in the rat frontal cortex with Western blot analysis. As expected (Figure 4), chronic treatment with either imipramine or sertraline decreased Ndrg2S (82.9±14.1% or 60.2±5.7%) and Ndrg2L (80.1±18.5% or 59.8±5.5%) immunoreactivity. In contrast, single-dose treatments with either antidepressant failed to affect Ndrg2S and Ndrg2L immunoreactivity (Table 2, Figure 4). Moreover, both single-dose and repeated ECT significantly decreased Ndrg2S (57.3±14.3% or 60.2±12.2%)
and Ndrg2L (55.0 ± 18.5% or 53.6 ± 3.1%) immunoreactivity (Table 2, Figure 4).

**Phosphatase digestion**

The insulin-dependent phosphorylation of Ndrg2 has been reported to occur in skeletal muscle of Wistar rats as well as in mouse C2C12 skeletal muscle cells (Burchfield et al., 2004). These findings prompted us to determine whether Ndrg2 is also phosphorylated in the central nervous system. As described above immunoblotting of undigested frontal cortex with anti-rat-Ndrg2 antiserum revealed two major immunoreactive bands (Figure 3b, lane 1). In these experiments, these two bands remained immunoreactive even after phosphatase digestion; moreover, they did not shift in mobility in a gel (Figure 3b, lane 2). Taken together, these findings indicate that these bands do not represent phosphorylated forms of Ndrg2S or Ndrg2L.

**Immunohistochemical localization of Ndrg2 in the rat frontal cortex**

To confirm Ndrg2 protein expression in the central nervous system, we examined anti-rat-Ndrg2 immunostaining in the rat frontal cortex. We observed Ndrg2-immunoreactivity throughout the frontal cortex. Figure 4 presents a typical image of Ndrg2-immunoreactive cells found in the external pyramidal layer (layer III). Interestingly, we also observed small Ndrg2-immunoreactive astrocyte-like cells (Fig. 5). Their entire soma and proximal processes were immunostained.

**Discussion**

We identified an EST, ADRG123, the expression of which decreased after chronic antidepressant treatment and repeated ECT. Sequence and homology comparisons using the EMBL/GeneBank database showed that ADRG123 perfectly matches rat Ndrg2. Ndrg2 is a member of the Ndrg family; thus far, four members of this family, Ndrg1-4, have been identified (Zhou et al., 2001). Although Ndrg members do not possess a clear functional motif, they do share a high level of sequence homology. Phylogenetic analysis of Ndrg1-4 revealed that Ndrg1 and Ndrg3 belong to one subfamily, while Ndrg2 and Ndrg4 belong to another (Qu et al., 2002). In the present study, we demonstrated that chronic treatment with the tricyclic antidepressant imipramine and the selective serotonin reuptake inhibitor sertraline reduced Ndrg2S and Ndrg2L immunoreactivity. This figure represents typical results from three independent experiments.
electroencephalograph activity are altered in the frontal cortices of depressed patients (Drevets et al., 1992). It is reasonable, therefore, to hypothesize that alterations of mood, neurovegetative signs, or even social behaviour of depressed patients may reflect changes in physiological functions within this important brain region. In addition, repeated ECT treatment also decreased Ndrg2 mRNA expression. Although single-dose ECT treatments also significantly decreased Ndrg2 expression, single-dose antidepressant treatments failed to do so. The relatively rapid effect of ECT on Ndrg2 expression may explain the rapid onset of its antidepressant effects in clinical settings. The detailed mechanisms underlying antidepressant-induced adaptive changes are as of yet unknown. However, our findings may suggest that Ndrg2 expression-dependent alterations of the frontal cortex may be an important component of the pharmacological action of antidepressants and ECT.

Phosphorylation of Ndrg proteins has been studied very little, although protein kinase A-dependent phosphorylation of Ndrg1 has been described previously (Agarwala et al., 2000). In addition, Ndrg1 is a multiphosphorylated protein in mast cells, and the kinetics of increased Ndrg1 phosphorylation has been shown to parallel signalling events leading to exocytosis (Sugiki et al., 2004). More recently, it was reported that insulin-dependent phosphorylation of Ndrg2 occurs in skeletal muscle of Wistar rats and in mouse C2C12 skeletal muscle cells (Burchfield et al., 2004). However, in the present study, we demonstrated that two Ndrg2-immunoreactive bands found in the rat frontal cortex remained immunoreactive even after phosphatase digestion; moreover, they did not shift in mobility in a gel. These findings indicate that these bands do not represent phosphorylated forms of Ndrg2S or Ndrg2L, suggesting possible differential regulation of Ndrg2 phosphorylation in the central nervous system.

Ndrg family members may be intimately involved in cellular differentiation and development. Indeed, Ndrg1 expression is induced by hypoxia and has been implicated in cell growth regulation and Schwann cell signalling for axonal survival (Kalaydjieva et al., 2000; Piquemal et al., 1999; Salnikow et al., 2002; Zhou et al., 1998). In human leukaemia cells, Ndrg1 expression is up-regulated by differentiation-related retinoids and vitamin D3 (Piquemal et al., 1999). Suppression of Ndrg4 expression by Ndrg4 antisense transfection inhibits neurite outgrowth in PC12 cells (Ohki et al., 2002). Stable expression of human Ndrg2 in glioblastoma cell lines decreases cell growth rates (Deng et al., 2003). More recently, Ndrg2 mRNA and protein has been shown to be up-regulated in Alzheimer’s disease brains (Mitchelmore et al., 2004). Taken together, these findings indicate that Ndrg’s may be critically involved in developmental processes, and Ndrg2 in particular, may be involved in neural and/or glial development and plasticity. Interestingly, in the present study, we observed Ndrg2 immunoreactivity in small astrocyte-like cells in the rat frontal cortex.

| Table 2. Ndrg2 immunoreactivity in the rat frontal cortex after antidepressant treatment and ECT analysed by Western blot analysis |
|-----------------|-----------------|-----------------|
|                 | Ndrg2S          | Ndrg2L          |
| Single antidepressant treatment |                 |                 |
| Control         | 100 ± 7.2       | 100 ± 13.2      |
| Imipramine      | 104 ± 6.0       | 90.6 ± 12.0     |
| Sertraline      | 107 ± 27.7      | 80.9 ± 7.5      |
| Chronic antidepressant treatment |                 |                 |
| Control         | 100 ± 10.9      | 100 ± 8.4       |
| Imipramine      | 82.9 ± 14.1     | 80.1 ± 18.5     |
| Sertraline      | 60.2 ± 5.7*     | 59.8 ± 5.5*     |
| ECT             |                 |                 |
| Control         | 100 ± 6.0       | 100 ± 11.3      |
| Single-dose ECT | 57.3 ± 14.3*    | 55.0 ± 18.5*    |
| Chronic ECT     | 60.2 ± 12.2*    | 53.6 ± 3.1*     |

Data are expressed as means ± S.E.M. *p < 0.05, ANOVA followed by Dunnett’s test.
There have now been reports showing that glial cell density is reduced in the prefrontal cortex of patients with major depressive disorders (see review by Cotter et al., 2001). These findings suggest that, in addition to examining neuronal or glial pathology, neuronal–glial interactions associated with the pathophysiology of depression also requires in-depth study.

In conclusion, we have identified Ndrg2 as a novel candidate target molecule of antidepressants and ECT in the rat frontal cortex. Although, the functional role of Ndrg2 in the central nervous system remains unclear, our findings suggest that Ndrg2 expression-dependent alterations of the frontal cortex may be an important component of the pharmacological action of antidepressants and ECT. Additional work is necessary to test this hypothesis.

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Statement of Interest

None.

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