

# Validation of dopamine receptor DRD1 and DRD2 antibodies using receptor deficient mice

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Received: 27 February 2017 / Accepted: 2 March 2017 / Published online: 18 March 2017  
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**Abstract** Dopamine receptors 1 and 2 (DRD1, DRD2) are essential for signaling in the brain for a multitude of brain functions. Previous work using several antibodies against these receptors is abundant but only the minority of antibodies used have been validated and, therefore, the results of these studies remain uncertain. Herein, antibodies against DRD1 (Merck Millipore AB1765P, Santa Cruz Biotechnology sc-14001, Sigma Aldrich D2944, Alomone Labs ADR-001) and DRD2 (Abcam ab21218, Merck Millipore AB5084P, Santa Cruz Biotechnology sc-5303) have been tested using western blotting and immunohistochemistry on mouse striatum (wild type and corresponding knock-out mice) and when specific, they were further evaluated on rat and human striatum. Moreover, a DRD1 antibody and a DRD2 antibody that were found specific in our tests were used for immunoprecipitation with subsequent

mass spectrometrical identification of the immunoprecipitate. Two out of nine antibodies (anti DRD1 Sigma Aldrich D2944 and anti DRD2 Merck Millipore AB5084P) against the abovementioned dopamine receptors were specific for DRD1 and DRD2 as evaluated by western blotting and immunohistochemistry and the immunoprecipitate indeed contained DRD1 and DRD2 as revealed by mass spectrometry. The observed findings may question the use of so far non-validated antibodies against the abovementioned dopamine receptors. Own observations may be valuable for the interpretation of previous results and the design of future studies using dopamine receptors DRD1 or DRD2.

**Keywords** Dopamine receptor · Antibody · Immunohistochemistry · Western blotting · Validation

**Electronic supplementary material** The online version of this article (doi:[10.1007/s00726-017-2408-3](https://doi.org/10.1007/s00726-017-2408-3)) contains supplementary material, which is available to authorized users.

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## Introduction

Dopamine plays an important role in the central nervous system where it exerts a multitude of functions including the control of locomotion, neuroendocrine secretion, motivation, affective behavior, emotions, working memory, learning and cognition per se (Jaber et al. 1996; Schultz 2002; Alcaro et al. 2007; Barnes et al. 2011; Puig et al. 2014). The dopaminergic system has been implicated in various neurological and psychiatric disorders such as Parkinson's disease (Al-Khaled et al. 2015), schizophrenia (Davis et al. 1991) and addiction (Keiflin and Janak 2015). Therefore, information on structure, function, localization and distribution of dopaminergic receptors is of pivotal importance.

Several companies offer antibodies against dopamine DRD1 and DRD2, however, mainly without prior validation. Jensen et al. (2009) tested ten commercially available

antibodies against alpha-1-adrenergic receptor subtypes and reported the absence of specificity. Likewise, Jositsch et al. (2009) challenged the suitability of muscarinic acetylcholine receptors using tissue sections of receptor gene-deficient mice. The authors stated that the corresponding antibodies were not specific in immunohistochemical approaches. Bordeaux et al. (2010) have postulated that to validate an antibody, its specificity, selectivity and reproducibility have to be shown in the context for which it is to be used. In a clear review, the authors highlight the common pitfalls when working with antibodies, common practices for validating antibodies and levels of commercial antibody validation for several vendors. A clear and informative proposal for validation of antibodies was published recently by Uhlen and co-workers (2016) convening an ad hoc International Working Group for Antibody Validation to formulate the best approaches for validating antibodies used in common research applications and provide basic guidelines recommending five conceptual “pillars” for antibody validation.

It was, therefore, the aim of the study to validate commercially available antibodies against DRD1 and DRD2 using western blotting (WB), fluorescent immunohistochemistry (IF) and a mass spectrometrical (MS) approach. And indeed, out of seven studied antibodies, two antibodies, DRD1 D2944 (Sigma Aldrich, MO, USA) and DRD2 AB5084P (Merck Millipore, Darmstadt, Germany), were shown to be specific for DRD1 and DRD2.

## Materials and methods

### Brain tissues

Dopamine receptor DRD1 and DRD2 gene-deficient (−/−) mice were bought from and developed by Motoya Katsuki, National Institutes of Natural Sciences (Medical Institute of Bioregulation, Kyushu University, Japan) and their characterization has been described in detail elsewhere (Nguyen et al. 2014; Hu et al. 2015; Yamaguchi et al. 1996). Sprague–Dawley rats and wild type (WT) mouse strain C57BL/6j were provided by the Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics (Medical University of Vienna, Austria). The human brain sample of basal ganglia was provided by the KIN-Biobank, Institute of Neurology (Medical University of Vienna, Austria).

### Antibodies

Primary antibodies used are listed in Table 1a. Details of secondary antibodies are provided in Table 1b.

### Immunoblotting

Brains were removed rapidly from mouse and rat skulls while deeply anesthetized with isoflurane, cleaned of blood and frozen in liquid nitrogen. Whole right brain hemispheres were used for protein extraction from animal brain, while for post-mortem human brain striatum was used exclusively. Brains were further homogenized in SYNER Synaptic Protein Extraction Reagent (87793, Thermo Fisher Scientific) and EDTA-free Protease Inhibitor Cocktail (PIC, Roche Molecular Biochemicals, Mannheim, Germany), and centrifuged for 10 min at 1200×g (Eppendorf Centrifuge 5702 R). Supernatants were further centrifuged for 20 min at 15,000×g (Eppendorf Centrifuge 5415 R). Synaptosomes were solubilized in SDS buffer with EDTA-free Protease Inhibitor Cocktail (Roche Molecular Biochemicals), 50 mM Tris pH 8.0 and 150 mM sodium chloride. Synaptosomes (40 µg of proteins from each mouse and 20 µg of proteins from rat and human striatum) were electrophoresed on a 10% SDS-polyacrylamide gel and transferred to PVDF membranes (GE Healthcare, Uppsala, Sweden). The membranes were blocked in 5% milk (T145.4, Carl Roth, Karlsruhe, Germany) and incubated overnight in primary antibodies diluted in 5% milk. Only for rat samples, the primary anti-DRD1 antibody (D2944, Sigma Aldrich) was preincubated for 1 h at 22–24 °C with AffiniPure Fab Fragment Rabbit Anti-Rat IgG (H + L) (312-006-045, Jackson Immuno Research Lab) and detected with HRP-conjugated anti-rabbit secondary antibody to avoid cross-reactivity with rat IgG in rat samples.

Blots were developed using the ECL reagent (1705061, Bio-Rad). All experiments were done in triplicates after optimal working conditions were determined.

### Immunohistochemistry

Male mice 8 weeks of age and male rats 12 weeks of age were deeply anesthetized with isoflurane and transcardially perfused with 4% paraformaldehyde solution in 0.1 M sodium phosphate buffered (pH 7.4). Animal brains and a post-mortem human basal ganglia were gently removed and post-fixed in the same fixative solution for 17–20 h at 4 °C. Each brain tissue was rinsed well with PBS to remove formaldehyde and immersed in sterilized PBS. Each brain was incubated overnight in 30% sucrose with 0.02% NaN<sub>3</sub> at 4 °C. Immediately prior to cutting, brains were embedded in O.C.T. Tissue-Tek compound (Sakura Finetek, USA) and deep frozen in liquid nitrogen. Tissue was sectioned at 20 µm (mice) and 30 µm (rat and human) with a Leica CM3050 cryostat (Leica, Nussloch, Germany). Sections were blocked in 5% normal donkey serum (NDS) (D9663, Sigma Aldrich), 0.3% Triton X-100 (T8787, Sigma Aldrich), 2% BSA (23208, Thermo Fisher

**Table 1** Tabular presentation of antibodies used for the study

Target	Catalog number	Company	Epitope/isotope	Source	Apparent MW proposed by company	Applications proposed by company	Highest dilution for use on WB	Highest dilution for use on IF
<b>a. Tabular presentation of primary antibodies used for the study</b>								
DRD1	AB1765P	Merck Millipore	Rat (403-415)	Rabbit pAb	50, 75	WB, IHC, ELISA	1:250	1:50
DRD1	sc-14001	Santa Cruz Bio.	Human C-terminus	Rabbit pAb	74	WB, IP, IHC, ELISA	1:500	1:50
DRD1	D2944	Sigma Aldrich	Human C-terminus	Rat mAb	50	WB, IHC, ICC	1:1000	1:500
DRD1	ADR-001	Alomone Labs	Rat C-terminus	Rabbit pAb	100	WB, IHC	1:250	1:100
DRD2	ab21218	abcam	Human (272-282)	Rabbit pAb	48, 51	WB, IP, ICC, ELISA	1:2000	1:50
DRD2	AB5084P	Merck Millipore	Human cytoplasmic loop #3	Rabbit pAb	50, 68, 100	WB, IP, IHC, ELISA	1:250	1:50
DRD2	sc-7523	Santa Cruz Bio.	Human (near N-terminus)	Goat pAb	48/51	WB, IP, IHC, ELISA	1:500	1:50
<b>b. Tabular presentation of secondary antibodies and Fab fragments used for the study</b>								
<b>HRP secondary</b>								
Goat IgG	sc-2768	Santa Cruz Bio.	IgG	Rabbit	53	WB	1:5000	-
Rabbit IgG	ab97080	Abcam	IgG	Rabbit pAb	70, 75, 130	WB, ICC, IHC-P, ELISA	1:20000	-
Rabbit IgG	ab191866	Abcam	IgG	Rec. E. coli. mAb	49	WB, IHC-P, ELISA	1:15000	-
Rat IgG	712-035-150	Jackson Immuno Research Lab	IgG	Donkey pAb	160	WB, IHC, ELISA	1:10000	-
<b>IF secondary</b>								
Goat IgG DyLight 630	SA5-10082	Thermo Scientific	IgG	Rabbit pAb	-	WB, IP, IHC, IF, ICC, FC	-	1:1000
Rabbit IgG Alexa Fluor 488	4412S	Cell Signaling Technology	IgG	Goat	-	IF, FC	-	1:1000
Rat IgG Alexa Fluor 647	4418S	Cell Signaling Technology	IgG	Goat	-	IF, FC	-	1:1000
<b>Fab fragment</b>								
Rat IgG	312-006-045	Jackson Immuno Research Lab	IgG	Rabbit pAb	-	WB, ICC, FC, ELISA	1:700	-

pAb polyclonal antibody, mAb monoclonal antibody, IgG immunoglobulin, WB western blotting, IP immunoprecipitation, IHC immunohistochemistry, PFA paraformaldehyde fixation, ICC immunocytochemistry, IF immunofluorescence, FC flow cytometry

Scientific) in 0.1 M PB 7.4 for 2 h at 22–24 °C and incubated for 48 h with primary antibody at 4 °C in 0.1 M PB, 1% NDS, 0.1% Triton X-100, 0.1% BSA with continuous stirring. After three washing steps in 0.1 M PB, sections were incubated with corresponding secondary antibodies for 2 h at 22–24 °C in 2% BSA. DAPI staining was used to assess the gross cell morphology (D1306, Thermo Fisher Scientific). Primary and secondary antibodies used in this study are summarized in Table 1. All sections were mounted with fluorescence mounting medium (DAKO, Agilent Technologies, Santa Clara, CA) and were imaged using a Zeiss LSM780 confocal microscope (Zeiss, Jena, Germany).

### Immunoprecipitation followed by mass spectrometry

#### *Enrichment of plasma membrane fraction*

Rat striatum tissue samples were homogenized in cold homogenization buffer containing 100 mM HEPES (pH 7.7), 300 mM sucrose supplemented with PIC (Roche Molecular Biochemicals) using an Ultra-Turrax® (IKA, Staufen, Germany). The homogenate was centrifuged at 1000×g for 10 min at 4 °C. The supernatant was collected and the homogenization step was repeated. Supernatants were pooled and centrifuged at 50,000×g for 30 min at 4 °C (Beckman Coulter Optima® L-90K). Pellets were re-suspended in washing buffer (homogenization buffer without sucrose), followed by incubation on ice for 15 min and centrifugation at 50,000×g for 30 min at 4 °C. The membrane pellets were stored at –20 °C until use.

#### *Immunoprecipitation*

The frozen membrane pellets were re-suspended in solubilization buffer containing 1% DDM, 150 mM NaCl, 5% glycerol, 10 mM phosphate buffer (pH 7.4) supplemented with PIC. Samples were incubated on ice for 1 h and vortexed every 10 min. Samples were further centrifuged for 1 h at 15,000×g at 4 °C to remove non-solubilized material. The protein concentration was estimated by the BCA protein assay kit (Pierce, Rockford, IL, USA). Solubilized membrane fractions (2 mg of proteins) were incubated with 10 µg of DRD1 antibody D2944 (Sigma Aldrich) or DRD2 antibody AB5084 (Merck Millipore) for 48 h at 4 °C, followed by incubation with protein G-Sepharose beads (GE Healthcare) on a rotating wheel at 4 °C for 4 h. Bound proteins were washed five times with washing buffer (solubilization buffer without DDM) and eluted with 0.2 M glycine, pH 2.5. Proteins were acetone precipitated overnight. Negative controls were prepared analogously but in the absence of antibodies.

#### *Liquid chromatography tandem mass spectrometry*

Samples were injected onto a Dionex Ultimate 3000 system (Thermo Fisher Scientific) coupled to a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific, Germany). Software versions used for the data acquisition and operation of the Q-Exactive were Tune 2.8.1.2806 and Xcalibur 4. HPLC solvents were as follows: solvent A consisted of 0.1% formic acid in water and solvent B consisted of 0.1% formic acid in 80% acetonitrile. From a thermostated autosampler, 10 µL that corresponds to 1 µg of the peptide mixture was automatically loaded onto a trap column (PM100-C18 3 µm, 75 µm × 20 mm, ThermoFisher Scientific, Austria) with a binary pump at a flow rate of 5 µL/min using 2% acetonitrile in 0.1% TFA for loading and washing the pre-column. After washing, the peptides were eluted by forward flushing onto a 50 cm analytical column with an inner diameter of 75 µm packed with 2 µm-C18 reversed-phase material (PepMap-C18 2 µm, 75 µm × 500 mm, ThermoFisher Scientific, Austria). Peptides were eluted from the analytical column with a 120 min gradient ranging from 10 to 37.5% solvent B, followed by a 10 min gradient from 37.5 to 50% solvent B and, finally, to 90% solvent B for 5 min before re-equilibration to 5% solvent B at a constant flow rate of 300 nL/min.

The LTQ Velos ESI-positive ion calibration solution (Pierce, IL, USA) was used to externally calibrate the instrument prior to sample analysis and an internal calibration was performed on the polysiloxane ion signal at  $m/z$  445.120024 from ambient air (Olsen et al. 2005). MS1 scans were performed from  $m/z$  400–2000 at a resolution of 70,000. Using a data-dependent acquisition mode, the 20 most intense precursor ions of all precursor ions with +2 to +6 charge were isolated (within a 1.6  $m/z$  window) and fragmented to obtain the corresponding MS2 spectra. The fragment ions were generated in a higher energy collisional dissociation (HCD) cell at an NCE of 27% with a fixed first mass fixed automatically and were detected in an Orbitrap mass analyser at a resolution of 17,500. The dynamic exclusion for the selected ions was 20 s. Maximal ion accumulation time allowed in MS and MS2 mode was 30 and 50 ms, respectively. Automatic gain control was used to prevent overfilling of the ion trap and was set to  $1 \times 10^6$  ions and  $5 \times 10^4$  ions for a full Fourier transform MS and MS2 scan, respectively.

#### *Proteolytic digestion of proteins*

Pellets were reconstituted with urea buffer consisting of 7 M urea, 2 M thiourea, 4% CHAPS, 100 mM DTT and 50 mM TEAB with EDTA-free Protease Inhibitor Cocktail (Roche Molecular Biochemicals). Bead controls were prepared by a similar procedure but in the absence of antibodies. Protein amounts were estimated using the Pierce 660 protein assay (Pierce, Rockford, IL, USA). 20 µg of

samples ( $2 \times 20 \mu\text{g}$  each) was digested with trypsin (1:100 w/w) using the filter-aided sample preparation (FASP) as previously described with minor modifications (Wisniewski et al. 2009). All digests for label-free analyses were desalted and concentrated with customized reversed-phase C18 tips. Lyophilized peptides were reconstituted in 5% formic acid and analyzed by LCMS.

#### *Protein identification and label-free quantitation*

The acquired raw MS data files were processed in MaxQuant 1.5.3.30 (Cox et al. 2014) and searched against the rat Swiss-Prot protein database version v 2015.11.11 (9626 sequences, including isoforms). The search parameters were as follows: two tryptic missed cleavage sites, mass tolerances of 5 and 20 ppm for the precursor and fragment ions, respectively. Oxidation of methionine and N-terminal protein acetylation were set as variable modification, while carbamidomethylation of cysteine residues was set as fixed modifications. The data were also matched against a decoy reverse database. Peptides and protein identifications with 1% FDR are reported. Protein identifications requiring a minimum of two peptides sequences were reported.

Protein identifications and LFQ intensities from MaxQuant were analyzed using Perseus statistical package (version 1.5.1.6) (Tyanova et al. 2016). The intensity values were log-transformed and zero-intensities were imputed-replaced by normal distribution. Statistical significance of differences in protein levels between the corresponding antibody-enriched (anti-DRD1 and anti-DRD2) and the bead controls was evaluated using a two-sided *t* test with  $p < 0.05$  (applying permutation-based FDR for truncation).

## Results

### Western blotting

Seven commercially available antibodies (Table 1) against DRD1 or DRD2 were tested for specificity on western blots using brains of knock-out mice with genetic deletion of either DRD1 or DRD2 and wild type in triplicate. Representative results are shown in Fig. 1.

Seven antibodies which were raised either in rabbit, rat or goat, DRD1 ab85608 (Abcam), DRD1 AB1765P (Merck Millipore), DRD1 sc-14001 (Santa Cruz Biotechnology), DRD1 D2944 (Sigma Aldrich), DRD1 ADR-001 (Alomone Labs, Jerusalem, Israel), DRD2 ab21218 (Abcam), DRD2 AB5084P (Merck Millipore) and DRD2 sc-7523 (Santa Cruz Biotechnology) were examined by immunoblotting.

Out of seven tested antibodies, two were confirmed to be specific by western blot analysis, detecting bands in lanes loaded with WT mouse brain proteins but not in lanes

loaded with corresponding KO brain proteins (Fig. 1). The DRD1 antibody D2944 (Sigma Aldrich) showed a major bands at ~95 kDa and a minor band at ~100 kDa in wild type mice but no signal was detected in the DRD1-KO mouse (Fig. 1a). Antibody reactivity was further tested on rat and human striatum. Bands of comparable molecular weight, a major band at ~95 kDa and a minor band at ~100 kDa were detected in rat samples, while in the human sample a single band at ~95 kDa was detected (Fig. 1a). All the other antibodies against DRD1 used in this study detected bands in both WT and DRD1-KO mice at ~50 or ~100 kDa (Fig. 1b).

WBs using the DRD2 antibody AB5084 (Merck Millipore) revealed that DRD2 protein was completely absent from DRD2-KO mice and only in WT three bands was detected between ~50 and ~100 kDa (Fig. 1c). Comparable bands were detected in rat brain, while in human brain three bands between 70 and ~100 kDa (Fig. 1c) were observed. None of the other two antibodies against DRD2 used in this study was considered to be specific. Both detected a single band in WT and DRD2-KO mice at ~100 kDa (Fig. 1d).

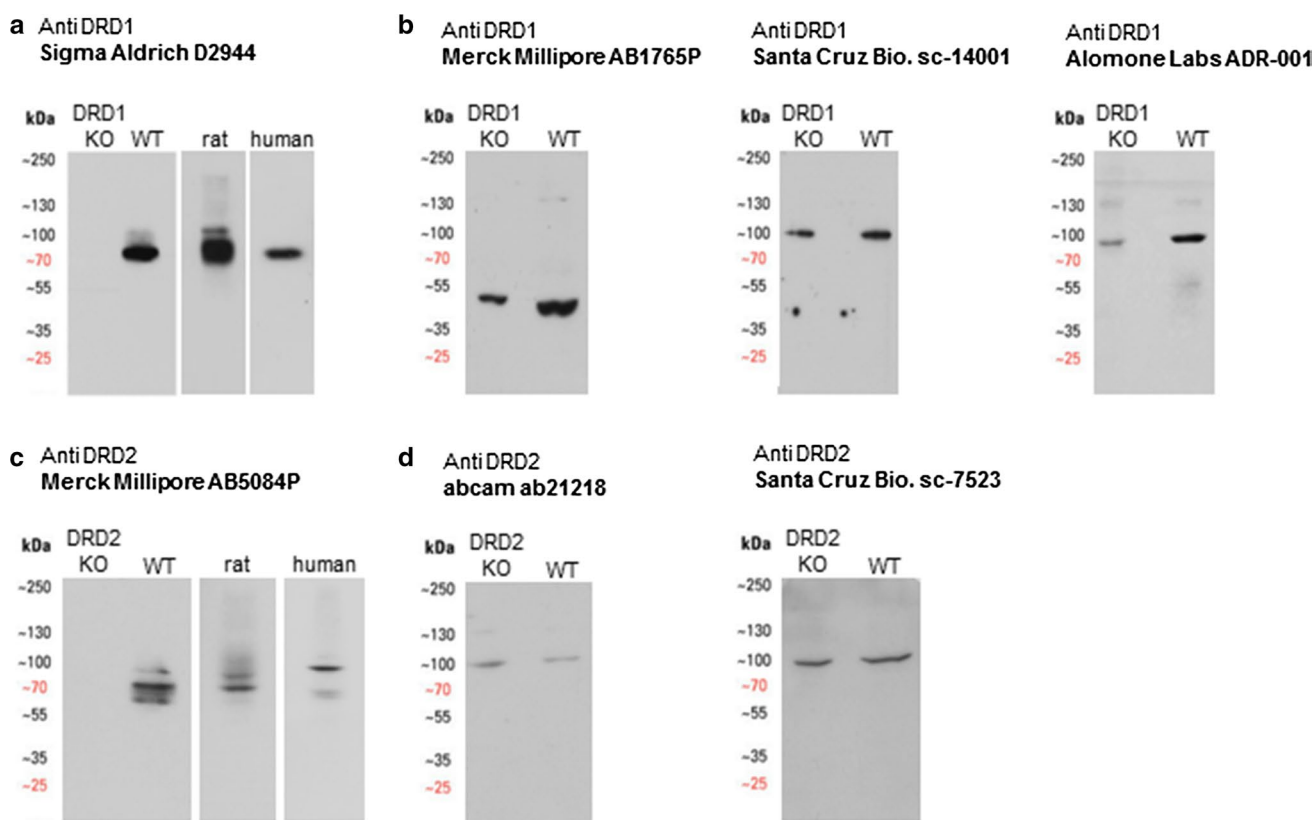
### Immunohistochemistry

The DRD1 antibody D2944 (Sigma Aldrich) was highly specific on IF analysis (Fig. 2a–d). No signal was detected in DRD1-KO mouse striatal tissue (Fig. 2a) as compared to highly specific DRD1 detection in corresponding WT (Fig. 2b). The antibody was reacting with rat and human striatum as well, where it was clearly confined to the striatal matrix which is known to be rich in dopamine receptors and showed only minimal immunoreactivity in surrounding areas (Fig. 2c, d).

The antibody against DRD2 AB5084 (Merck Millipore) was further evaluated on IF, where it showed no specific immunoreactivity in striatum of DRD2-KO mice (Fig. 2e) as compared to the corresponding area of WT mice (Fig. 2f), indicating specific protein recognition on IF analysis. The antibody reacted with rat and human striatum as well, where it was confined to the striatal matrix with only minimal immunoreactivity in surrounding areas (Fig. 2g, h). None of the other two anti-DRD2 antibodies used in this study was considered to be specific as strong immunoreactivity was observed in WT and KO mice.

### Immunoprecipitation followed by mass spectrometry

Immunoprecipitation in combination with mass spectrometry was performed to verify that the two specific antibodies interact specifically with the intended protein target (DRD1 or DRD2). Membrane fractions from rat striatum were immunoprecipitated, trypsin digested and analyzed by quantitative LC–MS/MS. Mass spectrometry



**Fig. 1** Western blot analysis using antibodies against DRD1 and DRD2. Western blots showing expression of the dopamine DRD1 and DRD2 protein in the brains of DRD1-KO, DRD2-KO, WT mice, rats and human. Molecular weight markers (kDa) were used to determine the apparent molecular weight. **a** Western blot using DRD1 D2944 (Sigma Aldrich) revealed a major band at ~95 kDa and minor band at ~100 kDa in tissue extracts from WT mouse brain, but not in corresponding DRD1-KO mouse brain. A major band at ~95 kDa and a minor band at ~100 kDa were detected in rat test samples as well, while in the human sample a single band at ~95 kDa was detected. **b** Antibodies against DRD1 AB1765P (Merck Millipore), sc-14001

(Santa Cruz Biotechnology) and ADR-001 (Alomone Labs) detected bands in both, WT and DRD1-KO mice at ~50 or ~100 kDa. **c** Western blot using DRD2 AB5084P (Merck Millipore) revealed that DRD2 protein was completely absent from DRD2-KO mice and only in WT three bands was observed between ~50 kDa and ~100 kDa. Comparable bands were detected in rat brain, while in human brain three bands between ~70 kDa and ~100 kDa were detected. **d** Antibodies against DRD2 ab21218 (abcam) and sc-7523 (Santa Cruz Biotechnology) detected a single band in both WT and DRD2-KO mice at ~100 kDa. *DRD1-KO* dopamine receptor 1 knock-out mouse, *DRD2-KO* dopamine receptor 2 knock-out mouse, *WT* wild type

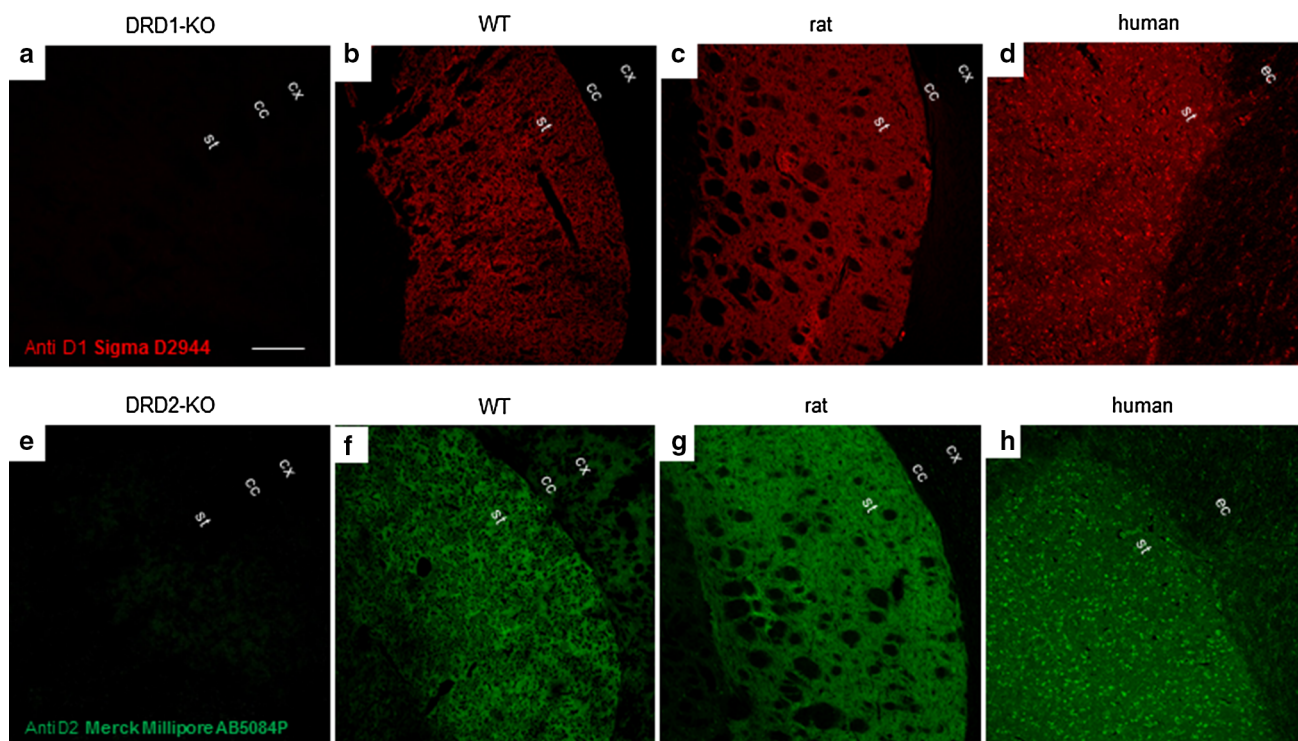
directly identifies peptide sequences and abundance (signal intensities) from the proteins of the immunoprecipitated sample. The differences in abundance of each of the protein identified from both the tested antibody and bead control for unspecific binding were determined.

In Fig. 3a, the enrichment of the target DRD1 protein using the antibody against DRD1 D2944 (Sigma Aldrich) was apparent. In addition, other proteins co-immunoprecipitated and are listed in Supplementary Table 1 (63 for DRD1 and DRD2). These proteins represent known and potential interaction partners or cross-reactivity targets. Notably, the DRD1 D2944 (Sigma Aldrich) antibody showed the highest enrichment with the target DRD1 protein as compared to the rest of the proteins (Fig. 3b). DRD1, together with the immunoglobulins IGG-C and IGG-2A from the antibody utilized, comprise the top three proteins enriched.

Similarly, DRD2 was highly enriched with the DRD2 AB5084P (Merck Millipore) antibody in comparison to the other proteins (Fig. 3a, c). Proline-rich transmembrane protein 1, PRRT1 (a component of the outer core of AMPAR complex), and tubulin TUBB2A were also highly enriched with this antibody. GAPDH, a ubiquitous protein, was present in both immunoprecipitated samples and bead controls.

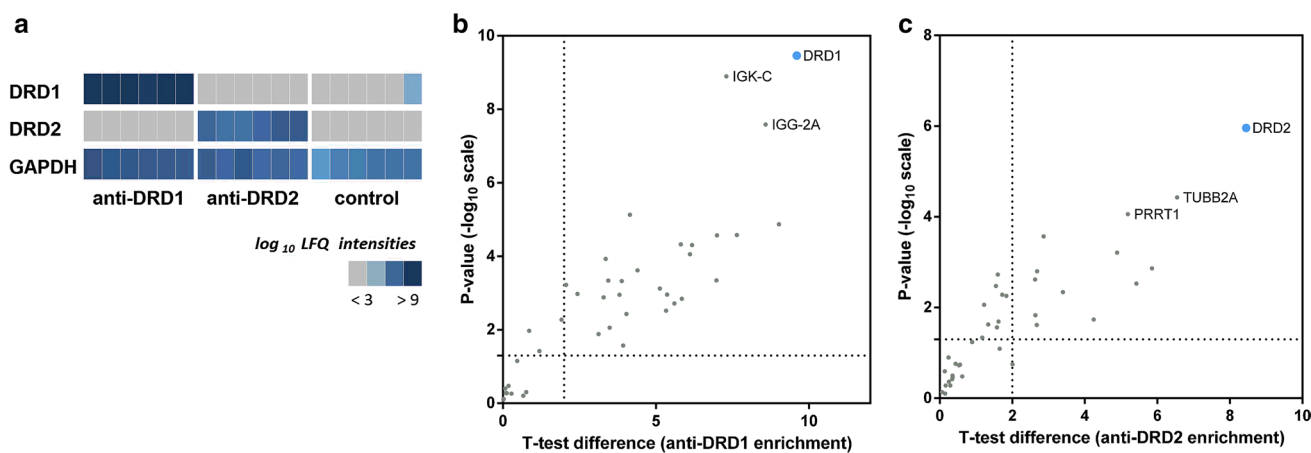
## Discussion

Given the importance of dopamine receptors as key signaling elements in the brain, DRD1 and DRD2 antibody specificity were tested and indeed, two antibodies were identified as valuable for neuroscience research in immunoblotting and on immunohistochemistry. The application



**Fig. 2** Immunohistochemical analysis using antibodies against DRD1 and DRD2. Representative images of DRD1 D2944 (Sigma Aldrich) immunolabeling in DRD1-KO mouse (a), WT mouse (b), rat (c) and human (d) brain. Representative images of DRD2 AB5084P (Merck Millipore) immunolabeling in DRD2-KO mouse

(e), WT mouse (f), rat (g) and human (h) brain. *DRD1-KO* dopamine receptor 1 knock-out mouse, *DRD2-KO* dopamine receptor 2 knock-out mouse, *WT* wild type, *cc* corpus callosum, *cx* cerebral cortex, *ec* external capsule, *st* striatum



**Fig. 3** IP-MS of antibodies against DRD1 and DRD2. Membrane fractions from rat striatum were immunoprecipitated using the two specific antibodies against DRD1 and DRD2, trypsin-digested and analyzed by quantitative LCMS. **a** Immunoprecipitated samples showed enrichment of DRD1 protein using an antibody against DRD1 D2944 (Sigma Aldrich) and DRD2 protein using an antibody

against DRD2 AB5084P (Merck Millipore). The ubiquitous GAPDH is present in both immunoprecipitated samples and bead controls. **b** The DRD1 protein showed the highest enrichment with DRD1 D2944 (Sigma Aldrich) antibody as compared to the other 27 proteins. **c** Similarly, DRD2 is highly enriched with the DRD2 AB5084P (Merck Millipore) antibody relative to the bead controls

of immunoprecipitation of mass spectrometry has clearly shown that the abovementioned antibodies recognized the two receptors. The current work also shows that both

antibodies can be used for studies on the rat and human brain. Moreover, previous work from Levey et al. (1993) on the DRD1 antibody was re-validated. Unfortunately, this

type of antibody is no longer commercially available from Sigma.

As to the individual apparent molecular weights observed in the case of DRD1, posttranslational modifications including glycosylation and lipidation may account for different apparent molecular weights observed in mouse (Fig. 1a; uniprot.org/uniprot/Q61616) and rat [uniprot.org/uniprot/P18901; (Bermak et al. 2001)].

Individual differences in apparent molecular weight of the DRD2 may be not only due to posttranslational modifications (mouse and rat, glycosylation; uniprot.org/uniprot/P61168 and P61169) but also due to the presence of (tissue-specific) splice variants (Monsma et al. 1989; Giros et al. 1989; Khan et al. 1998). Species, tissue and area specificity is a major issue in neuroscience and is reflected by the different apparent molecular weights on immunoblotting using the specific antibody against DRD2 used herein. Zhang and co-workers (Zhang et al. 2015) showed area-specific protein levels using the identical antibody revealing signals at different apparent molecular weights, although evaluation of this publication is hampered by the fact that bands were cut out and did not show the full images.

As a technical remark, the above-mentioned specific antibody against the recombinant fusion protein containing the C-terminal 97 amino acid of human D1 dopamine receptor produced in rats was used to detect DRD1 also in rat striatum and cross-reactivity with a secondary antibody had to be overcome. This problem was solved using a sandwich method incubating and applying AffiniPure Fab Fragment Rabbit Anti-Rat IgG along with the primary antibody against DRD1 with subsequent detection by a polyclonal HRP-conjugated anti-Rabbit IgG.

Taken together, evidence for antibody specificity against DRD1 and DRD2 for both applications, immunoblotting and immunohistochemistry, is provided and this forms a valuable tool for studying the dopaminergic system in the brain. We furthermore propose the use of mass spectrometry for the validation of antibodies in particular, as the availability of knock-out animals is limited.

**Acknowledgements** We are highly indebted to Prof. Tibor Harkany for supporting the study.

**Compliance with ethical standards**

**Conflict of interest** The authors report no conflict of interest.

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