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Abstract: D2 and D4 dopamine receptors play an important role in cognitive functions in the prefrontal cortex and they are involved in the pathophysiology of neuropsychiatric disorders such as schizophrenia. The eventual effect of dopamine upon pyramidal neurons in the prefrontal cortex depends on which receptors are expressed in the different neuronal populations. Parvalbumin and calbindin mark two subpopulations of cortical GABAergic interneurons that differently innervate pyramidal cells. Recent hypotheses about schizophrenia hold that the root of the illness is a dysfunction of parvalbumin chandelier cells that produces disinhibition of pyramidal cells. In the present work we report double in situ hybridization histochemistry experiments to determine the prevalence of D2 receptor mRNA and D4 receptor mRNA in glutamatergic neurons, GABAergic interneurons and both parvalbumin and calbindin GABAergic subpopulations in monkey prefrontal cortex layer V. We found that around 54% of glutamatergic neurons express D2 mRNA and 75% express D4 mRNA, while GAD-positive interneurons express around 34% and 47% respectively. Parvalbumin cells mainly expressed D4 mRNA (65%) and less D2 mRNA (15-20%). Finally, calbindin cells expressed both receptors in similar proportions (37%). We hypothesized that D4 receptor could be a complementary target in designing new antipsychotics, mainly because of its predominance in parvalbumin interneurons.

D2 and D4 dopamine receptor mRNA distribution in pyramidal neurons and GABAergic subpopulations in monkey prefrontal cortex: implications for schizophrenia treatment

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Abstract

D2 and D4 dopamine receptors play an important role in cognitive functions in the prefrontal cortex and they are involved in the pathophysiology of neuropsychiatric disorders such as schizophrenia. The eventual effect of dopamine upon pyramidal neurons in the prefrontal cortex depends on which receptors are expressed in the different neuronal populations. Parvalbumin and calbindin mark two subpopulations of cortical GABAergic interneurons that differently innervate pyramidal cells. Recent hypotheses about schizophrenia hold that the root of the illness is a dysfunction of parvalbumin chandelier cells that produces disinhibition of pyramidal cells. In the present work we report double *in* situ hybridization histochemistry experiments to determine the prevalence of D2 receptor mRNA and D4 receptor mRNA in glutamatergic neurons, GABAergic interneurons and both parvalbumin and calbindin GABAergic subpopulations in monkey prefrontal cortex layer V. We found that around 54% of glutamatergic neurons express D2 mRNA and 75% express D4 mRNA, while GAD-positive interneurons express around 34% and 47% respectively. Parvalbumin cells mainly expressed D4 mRNA (65%) and less D2 mRNA (15-20%). Finally, calbindin cells expressed both receptors in similar proportions (37%). We hypothesized that D4 receptor could be a complementary target in designing new antipsychotics, mainly because of its predominance in parvalbumin interneurons.

Keywords: calbindin, co-localization, glutamatergic neurons, in situ hybridization, parvalbumin.

Running title: Dopamine receptors in monkey prefrontal cortex.

Dopaminergic afferents in the prefrontal cortex (PFC) play an important role in normal cognitive functions and neuropsychiatric pathophysiology (Seeman et al., 1995; Williams and Goldman-Rakic, 1995). Dopaminergic inputs regulate different aspects of working memory, planning, attention, and malfunction could help explain some positive, negative and cognitive symptoms observed in schizophrenia.

The majority of studies into the etiology and treatment of schizophrenia focus on dopamine D2 receptors, due to the affinity most antipsychotic drugs show for them. However, some antipsychotics such as clozapine show a high affinity for D4 receptors (Van Tol et al., 1991; Kapur and Remington, 2001). Furthermore, D4 receptors are predominant in monkey PFC (Lidow et al., 1998; Mulcrone and Kerwin, 1997; Staley et al., 2000). Taken together, this could mean that part of the therapeutic effect of some antipsychotics attributed to D2 receptors could be due to the action of these drugs on D4 receptors (Seeman et al., 1997; Kapur and Remington, 2001).

Dopaminergic axons innervate PFC pyramidal neurons and GABAergic interneurons (Goldman-Rakic et al., 1989; 1998; Sesack et al., 1995), and D2-like receptors are present in both populations (Vincent et al., 1993; Khan et al., 1998; Santana et al., 2009; Mrzljak et al., 1996; Wedzony et al., 2000). However, there is no precise and accurate quantification of dopamine receptors in PFC cellular populations in primates.

There are several types of GABAergic neurons in the PFC. Two important subpopulations are those that express parvalbumin (PV) and those that express calbindin (CB). The former have the morphology of chandelier cells or large basket cells, while the majority of the latter have the morphology of double-bouquet cells (Conde et al., 1994; DeFelipe, 1997; Zaitsev et al., 2005). Changes in these cell populations have been described in schizophrenia, (Daviss and Lewis, 1995; Hashimoto et al., 2003; Beasley et

al., 2002), bipolar disorder (Sakai et al., 2008) and major depressive disorder (Rajkowska et al., 2007). Recent hypotheses about the pathophysiology of schizophrenia maintain that there is a dysfunction of PV+ chandelier cells that results in a disinhibition of pyramidal cells. The functional deficit of PV+ interneurons could produce hyperactivity in basal conditions, saturating the capacity of pyramidal neurons, preventing normal PFC and hippocampus recruitment during cognitive tasks, and this could contribute to working memory dysfunction in subjects with schizophrenia (Lewis et al., 2005). This suggest that the main effect of most antipsychotics is the inhibition of the inhibitory activity of D2-like receptors localized in PV+ GABAergic interneurons, which increases their excitability and consequently reduces pyramidal neuron hyperactivity (Lewis and Gonzalez-Burgos, 2006; Lisman et al., 2008).

Our aim in the present work is to contribute to the study of the cellular localization and distribution of both D2 and D4 receptor mRNA in identified neuronal populations of monkey PFC. To this end we use dual-label *in situ* hybridization histochemistry with specific oligonucleotides for these receptors and for the cellular markers. We quantify the proportion of glutamatergic and of GABAergic neurons (as well as PV+ and CB+ GABAergic subpopulations) that express D2 and D4 dopamine receptors in monkey PFC.

Materials and Methods

Specimens and tissue preparation

Three monkey brains (*Macaca fascicularis*, aged 2 years and 2 months) were used. The animals were administered an overdose of sodium pentobarbital (60 mg/kg, i.v.). All procedures followed European Union regulations (O.J. of E.C. L358/1 18/12/1986). Upon removal of the brain from the skull, tissue blocks about 1 cm thick were dissected,

immediately frozen and kept at - 20ºC until used. Coronal tissue sections 20 μm thick were cut from the frozen blocks using a microtome-cryostat (Microm HM500 OM, Walldorf, Germany), thaw-mounted on slides coated with APTS (3-aminopropyltriethoxysilane, Sigma, St Louis, MO, USA) slides and kept at - 20ºC until used.

Hybridization probes

Different oligonucleotides complementary to the mRNA coding for monkey D2 and D4 receptors were used: three oligonucleotides for D2 mRNA complementary to bases 52-101, 1315-1365, and 1355-1400 (GenBank accession number M29066); two oligonucleotides for D4 mRNA, complementary to bases 66-113 and 1093-1135 (GenBank accession number NM_000797). Each region was chosen because it shares no similarity with the other dopamine receptor subtypes. The results shown here for each receptor subtype were obtained by simultaneously using all the radioactively labeled oligonucleotides for the corresponding receptor as hybridization probes.

Glutamatergic cells were identified by the vesicular glutamate transporter vGluT1 mRNA with two oligonucleotides complementary to bases 26-67 and 1626-1670 (GenBank acc no NM_020309). GABAergic cells were identified by the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), which in adult brain has two major isoforms, GAD65 and GAD67. Three oligonucleotides for each mRNA isoform were made: bases 237-281, 496-540 and 736-780 (M81882) and bases 811-855, 1059-1103 and 2267-2311 (M81883). PV cells were identified by hybridization with oligonucleotides complementary to: 19-63, 70-111, 173-215, 335-379 bp of human PV mRNA (X63070). CB cells were identified by hybridization with oligonucleotides complementary to: 183-227, 448-492, 512-556, 809-853, 887-931, 938-982 bp of

human CB mRNA (NM_004929). All the oligonucleotides were synthesized and HPLC purified by Isogen Bioscience BV (Maarsden, The Netherlands).

Each dopamine receptor oligonucleotide was individually labeled at its 3'-end with terminal deoxynucleotidyltransferase (TdT, Oncogene Research Products, San Diego, CA, USA) and [^{33}P] α-dATP (3000 Ci mmol^{-1}, New England Nuclear, Boston, MA, USA). Labeled probes were purified through ProbeQuant G-50 microcolumns (GE Healthcare, Little Chalfont, UK). All GAD, vGluT1, PV and CB oligonucleotides (100 pmol) were individually labeled with TdT and Dig-11-dUTP (Boehringer Mannheim, Germany), in line with a procedure described elsewhere (Schmitz et al., 1991).

In situ hybridization histochemistry

The protocols for single- and double-label *in situ* hybridization histochemistry were based on previously described procedures (Tomiyama et al., 1997; Landry et al., 2000) and have already been published (Serrats et al., 2003). Frozen tissues were brought to room temperature (22 ± 2 ºC), air-dried and fixed for 20 min in 4% paraformaldehyde in phosphate-buffered saline (PBS: 2.6 mM KCl, 1.4 mM KH_2PO_4, 136 mM NaCl, 8 mM Na_2HPO_4). They were then washed once in 3xPBS, twice in 1xPBS, 5 min each, and incubated in a freshly prepared solution of predigested pronase (incubated at 37ºC, 4 hr, and kept frozen in aliquots) (Calbiochem, San Diego, CA, USA) at a final concentration of 12 U/mL in 50 mM Tris-HCl pH 7.5 and 5 mM EDTA for 2 min at room temperature (22 ± 2 ºC). Proteolytic activity was stopped by immersion for 30 sec in 2 mg/mL glycine in PBS. Tissues were rinsed in PBS and dehydrated in 70% and 100% ethanol for 2 min each. For hybridization, the radioactive and non-radioactive labeled probes were appropriately combined and diluted in hybridization buffer (50% formamide, 4xSSC, 1x Denhardt's solution, 1% sarkosyl, 10% dextran sulfate, 20 mM phosphate buffer, pH 7, 250 μg /mL yeast tRNA and 500 μg/mL salmon sperm DNA) at approximately 1-2 x

10^4cpm/μL. For single *in situ* hybridization, all the oligonucleotides used were independently labeled with radioactivity (a total of 4 different oligonucleotides for dopamine receptor mRNA, 2 oligonucleotides for vGluT1 mRNA and 1 each for GAD65 and GAD67 mRNA). For the double *in situ* hybridization experiments, all dopamine receptor probes (labeled with ^{33}P) and all available probes for vGluT1, GAD65/67, PV or CB (labeled with digoxigenin) were combined and diluted to a final concentration of approximately 2 nM. Tissues were covered with 100μL of the hybridization solution and overlaid with Nescofilm (Bando Chemical Ind, Kobe, Japan) coverslips to prevent evaporation. Tissues were incubated in humid boxes overnight at 42ºC and then washed 4 times (45 min each) in 600 mM NaCl, 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA at 60ºC. Hybridized sections were exposed to Biomax-MR (Kodak, Rochester, NY, USA) films for 3-5 weeks at - 70ºC with intensifying screens.

Development of radioactive and non-radioactive hybridization signal

After washing, double *in situ* hybridized sections were immersed for 30 min in a buffer containing 0.1 M Tris-HCl pH 7.5, 1 M NaCl, 2 mM $MgCl_2$ and 0.5% bovine serum albumin (Sigma) and incubated overnight at 4ºC in the same solution with alkaline-phosphate-conjugated anti-digoxigenin-F(ab) fragments (1:5000; Boehringer Mannheim). Afterwards, they were washed three times (10 min each) in the same buffer (without antibody), and twice in an alkaline buffer containing 0.1 M Tris-HCl pH 9.5, 0.1 M NaCl and 5 mM $MgCl_2$. Alkaline phosphatase activity was developed by incubating the sections with 3.3 mg nitroblue tetrazolium and 1.65 mg bromochloroindolyl phosphate (Gibco BRL, Gaithersburg, MD, USA) diluted in 10 mL of alkaline buffer. The enzymatic reaction was blocked by extensive rinsing in the alkaline buffer containing 1 mM EDTA. The sections were then briefly dipped in 70% and 100% ethanol, air-dried and dipped into Ilford K5 nuclear emulsion (Ilford,

Mobberly, Cheshire, UK) diluted 1:1 with distilled water. They were exposed in the dark at 4°C for 6 weeks, developed in Kodak D19 (Kodak) for 5 min, and fixed in Ilford Hypam fixer (Ilford).

Specificity of the probes

The specificity of the autoradiographic signal obtained in the *in situ* hybridization histochemistry experiments was confirmed by performing a series of routine controls (Pompeiano et al., 1992). Briefly, for each mRNA under study, several oligonucleotide probes complementary to different regions of the same mRNA were used independently as hybridization probes in consecutive tissue sections showing identical pattern hybridization. For a given oligonucleotide probe, addition to the hybridization solution of an excess of the same unlabeled oligonucleotide resulted in the complete abolition of the specific hybridization signal. The remaining autoradiographic signal was considered background. If the unlabeled oligonucleotide included in the hybridization was a different oligonucleotide, then the hybridization signal was not affected (data not shown). The thermal stability of the hybrids was examined by washing at increasing temperatures: a sharp decrease in the hybridization signal was observed at a temperature consistent with the T_m of the hybrids. To confirm the specificity of the non-radioactive hybridization signal, we compared the results obtained with the same probe labeled radioactively (data not shown).

Analysis of results

Tissue sections were examined and cells quantified with an Olympus BX51 Stereo Microscope (Olympus, Tokyo, Japan). Visiopharm Integrator System software (Olympus) was used to draw contours around the zone of interest at low magnification. We used the optical dissector of the software to randomly sample cells in the region of interest with a 100x oil immersion objective.

Glutamatergic, GAD-positive, PV and CB cells were identified as cellular profiles exhibiting a dark precipitate (alkaline phosphatase reaction product) surrounding or covering the nucleus. Dopamine receptor mRNA hybridization signal was considered positive when accumulation of silver grains over the stained cellular profiles was greater than four times that of the background. Cells were counted using Visiopharm Integrator System software.

Cell counting for dorsolateral PFC (DLPFC) was performed in areas 9 and 46, and for orbitofrontal cortex (OFC) in area 11; see Figure 1. Quantification was performed in layer V, where both types of dopamine receptor mRNA is consistently present. The percentage of cells expressing dopamine receptors was determined from an average of 40.2 cells per cortical layer from each area and each case examined, with a total of 1931 cells counted. Cortical layer V was identified in cresyl violet-stained sections.Analysis of variance (ANOVA) and Bonferroni post-tests were performed using GraphPad Prism software (GraphPad Software, San Diego, CA). $P < 0.05$ was considered statistically significant.

Preparation of figures

Photographs of the film autoradiograms of the hybridized tissue sections were taken with a Wild 420 Leica macroscope equipped with a digital camera (DXM1200 F, Nikon, Tokyo, Japan) and ACT-1 Nikon Software. Microphotography of the hybridized tissue slides was performed using a Nikon Eclipse E1000 microscope equipped with a digital camera (DXM1200, Nikon) and analySIS Software (Soft Imaging System, Münster, Germany). Figures were prepared for publication using Adobe Photoshop software (Adobe Software, San Jose, CA, USA). The contrast and brightness of the images were the only variables we adjusted digitally.

Results

Distribution of D2 and D4 receptor mRNA in glutamatergic and GABAergic cortical neurons

Previous studies have described the presence of D2 and D4 receptor mRNA mainly in layer V of primate PFC (Lidow et al., 1998), and in both glutamatergic and GABAergic neurons (2001; Khan et al., 1998; Paspalas and Goldman-Rakic, 2004). In good agreement with these findings, we found that D2 receptor is mainly expressed in layer V cells, whereas D4 receptor is expressed in all layers of the PFC except layer I, and its highest levels are in layer V (Fig. 1). As expected, D2 (Fig. 2a, 2c) and D4 (Fig. 2b, 2d) receptor mRNAs co-localized with the cellular markers vGluT1 (Fig. 2a, 2b) and GAD 65/67 (Fig. 2c, 2d). D2 receptor mRNA is expressed in about 55% of glutamatergic neurons and in about 35% of GAD-positive interneurons. The presence of D4 receptor was significantly higher than that of D2 receptor in both neuronal populations ($p<0.05$). About 75% of glutamate neurons and nearly half of the GAD-positive interneurons express D4 receptor (Fig. 4). Thus, the data extends previous findings by showing a more widespread distribution of D4 receptors than D2 receptors both in pyramidal neurons and GABAergic interneurons across the monkey dorsolateral PFC and OFC.

D2 and D4 receptor expression in PFC interneurons

A few reports based on immunohistochemistry describe the presence of D2 receptor and D4 receptors in primate PFC PV+ interneurons (Khan et al., 2001; Mrzljak et al., 1996). However, and despite the relevance of PV+ and CB+ interneuron subpopulations in schizophrenia, bipolar disorder and depression, there are no quantitative studies assessing the relative abundance of D2 and D4 receptors in PV+ and CB+ interneurons. Here we quantified D2 and D4 mRNA expression in layer V, where both types of dopamine receptor mRNA are consistently present.

Double in situ hybridization shows that CB and PV mRNAs can co-localize with D2 and D4 mRNAs (Fig. 3). Both D2 and D4 receptors were expressed in 40% of CB+ neurons. However, a high proportion of PV+ neurons expressed D4 receptor (65-66%) and a relatively low percentage expressed D2 (15-20%) (Fig. 4). There were no significant differences between OFC and DLPFC in any of the cases studied (Fig. 4). Thus, there is a heterogeneous distribution of dopamine receptors in cortical interneuron populations, where PV+ interneurons are enriched in D4 receptors.

Discussion

The present work establishes the expression of D2 and D4 receptor mRNA in different cellular populations of monkey PFC. Our main findings are: 1) D4 receptor mRNA has a more widespread laminar distribution than D2 receptor, which is expressed almost exclusively in neurons in layer V; 2) both receptors are expressed in a large percentage of glutamatergic and GAD-positive neurons in layer V, but D4 receptor is present in a greater proportion of both neuronal populations than is D2 receptor; 3) most (about 65%) PV+ perisomal interneurons express D4 receptors while only a minority (15-20%) express D2 receptors.

Schizophrenia and the distribution of D2 and D4 receptors in the PFC

Irregularities in PFC inhibitory neurotransmission in schizophrenia, bipolar disorder and major depressive disorder seem to be mostly restricted to alterations in two subpopulations of GABAergic neurons; CB and PV interneurons (Daviss and Lewis, 1995; Rajkowska et al., 2007; Sakai et al., 2008; Hashimoto et al., 2003; Beasley et al., 2002). The most recent hypotheses on the pathophysiology of schizophrenia suggest that there is a hypofunction of the chandelier cells (PV+ GABAergic interneurons) due to a hypofunction of NMDA receptors (see (Lewis and Gonzalez-Burgos, 2006; Lisman et al., 2008) for a review). In primate PFC, these perisomal interneurons are activated by axon collaterals of pyramidal neurons, and they provide a potent inhibitory feedback by acting over the axon cone (Melchitzky and Lewis, 2003). The model suggests that a functional deficit of PV+ interneurons would diminish the effectiveness of the inhibiting loop, resulting in disinhibition of the pyramidal neurons. In this way, the functional deficit of PV+ interneurons causes hyperactivity in basal conditions, saturating the capacity of pyramidal neurons and preventing normal recruitment of the PFC and

hippocampus during cognitive tasks (Lewis et al., 2005). Our results show that when analyzing the population of GABAergic interneurons as a whole, the proportion of interneurons expressing D2 receptor (35%) did not differ greatly from the proportion of those expressing D4 receptor (47%) (Fig. 4). Nevertheless, when considering only the perisomal PV+ interneurons, the majority of them (65%) express D4 receptors, while and only 15-20% express D2 receptors. Because of the predominance of D4 receptor in parvalbumin interneurons, we hypothesived that D4 receptor could be a complementary target in designing new antipsychotics. The effect of those antipsychotics could be the inhibition of the activity of the inhibitory D4 receptors located in the inhibitory PV+ interneurons. This would increase the activity of PV cells and thereby diminish pyramidal neuron hyperactivity.

D4 activation has been shown to hamper synaptic excitation in GABAergic interneurons that would lead to decreased GABAergic inhibition in the PFC circuit (Yuen and Yan, 2009). Moreover, the main problem with classic antipsychotics are extra-pyramidal effects, partly as a result of the occupation of D2 receptors located in the caudate-putamen nuclei. Studies to determine the presence and densities of D4 receptors in the striatum have produced controversial results (from high to very low levels) by using either immunohistochemistry with different antibodies (Defagot et al., 1997; Mauger et al., 1998; Rivera et al., 2002; Mrzljak et al., 1996; Ariano et al., 1997), or receptor autoradiography (Defagot et al., 2000; Defagot and Antonelli, 1997; De La and Madras, 2000; Primus et al., 1997; Murray et al., 1995). *In situ* hybridization describes D4 mRNA levels in the caudate-putamen as ranging from low to very low (Lidow et al., 1998) (de Almeida and Mengod, unpublished observations). This is consistent with the low to very low levels resulting from rat and human RT-PCR experiments (Suzuki et al., 1995; Van Tol et al., 1991; Matsumoto et al., 1995;

Matsumoto et al., 1996), and also with single-cell RT-PCR from human striatal neurons (Surmeier et al., 1996). All this indicates that the presence of D4 receptors in the striatum is still a subject of debate, but if there are indeed there, it is in much smaller quantities than D2 receptors. This suggests that the replacement of part of D2 antagonism by D4 antagonism in the development of antipsychotics could reduce the unwanted side effects of these drugs.

In conclusion, both D2 and D4 receptors are found in different neuronal populations in the PFC of primates. D4 receptor is more ubiquitous in both laminar and cellular distributions. This is important when assessing the performance of these receptors in the dopaminergic modulation of the PFC through receptors from the D2 family, and also their link with schizophrenia and its therapies. For a better understanding of dopamine modulation in the PFC, D1-like dopamine receptors should also be studied in different neural populations. Knowledge of the differential distribution of dopamine receptors in pyramidal neurons and interneurons in primate PFC could elucidate the specific functional contribution of each receptor to inherent PFC functions, such as working memory. Furthermore, this knowledge could pinpoint the role of alterations in the abundance of these receptors or in their cellular location on diseases such as Parkinson's or schizophrenia.

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Figure Legends

Figure 1. Autoradiographic localization of dopamine D2 (A) and D4 (B) receptor mRNA in monkey prefrontal cortex. Both mRNA transcripts were visualized by *in situ* hybridization with oligonucleotides labeled with ^{33}P. Panel A shows the areas where cells were quantified. (DLPFC: dorsolateral prefrontal cortex, OFC: orbitofrontal cortex). Bar: 5 mm.

Figure 2. Localization of D2 and D4 receptor mRNA in glutamatergic and GABAergic cells in primate prefrontal cortex. High-magnification, bright-field microphotographs of emulsion-dipped sections simultaneously showing by double *in situ* hybridization the presence of D2 (a, c) and D4 (b, d) dopamine receptor mRNA in glutamatergic (a, b) and GABAergic (c, d) layer V neurons of monkey prefrontal cortex. The oligonucleotides complementary to the receptor mRNA were radiolabeled and are observed as clusters of dark silver grains. The oligonucleotides complementary to the mRNA of the glutamatergic marker vGluT1 and GABAergic marker GAD65/67 were labeled with digoxigenin and are observed as heavily purple stained cells. Bar: 20 μm.

Figure 3. Localization of D2 and D4 receptors mRNA in parvalbumin (PV) and calbindin (CB) cell populations in monkey prefrontal cortex. High-magnification bright-field microphotographs of emulsion-dipped sections of layer V monkey dorsolateral prefrontal cortex, simultaneously showing the different mRNA visualized by double *in situ* hybridization using ^{33}P-labeled oligonucleotides complementary to the mRNA coding for D2 (a, c) and D4 (b, d) dopamine receptors (clusters of dark silver grains), with DIG-labeled oligonucleotides (dark precipitate) for PV mRNA, panels a and b, or for CB mRNA, panels c and d. Bar: 20 μm.

Figure 4. Bar graph showing the distribution of D2 and D4 dopamine receptors in different neuronal populations of primate prefrontal cortex (vGluT1: vesicular glutamate transporter, GAD: gamma-aminobutiric acid decarboxylase, PV: parvalbumin, CB: calbindin, DLPFC: dorsolateral prefrontal cortex, OFC: orbitofrontal cortex). Data are the mean and SEM of three monkeys and represent the percentage of counted cells expressing D2 and D4 dopamine receptor mRNA in different neuronal populations. Each percentage was determined from an average of 40.2 cells (1931 cells counted).

Figure

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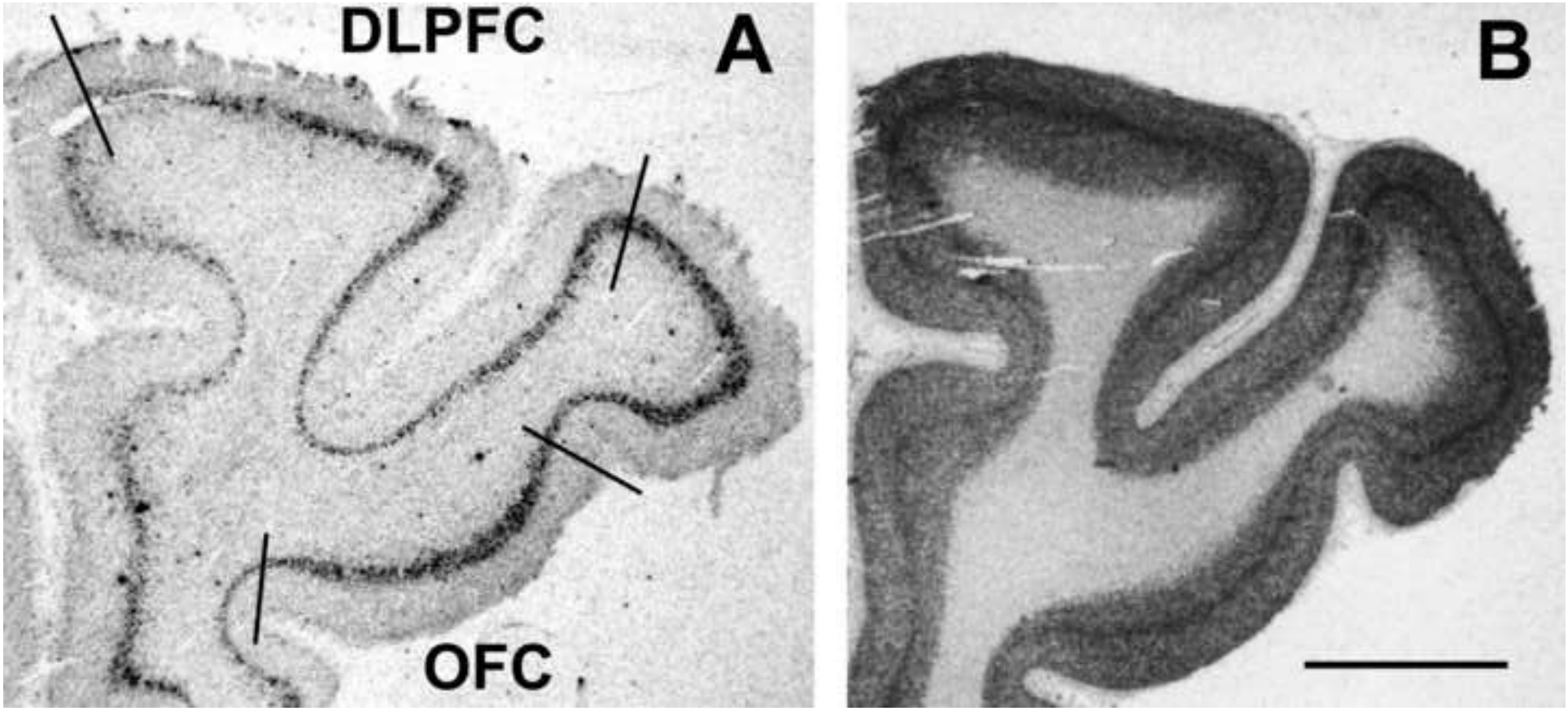

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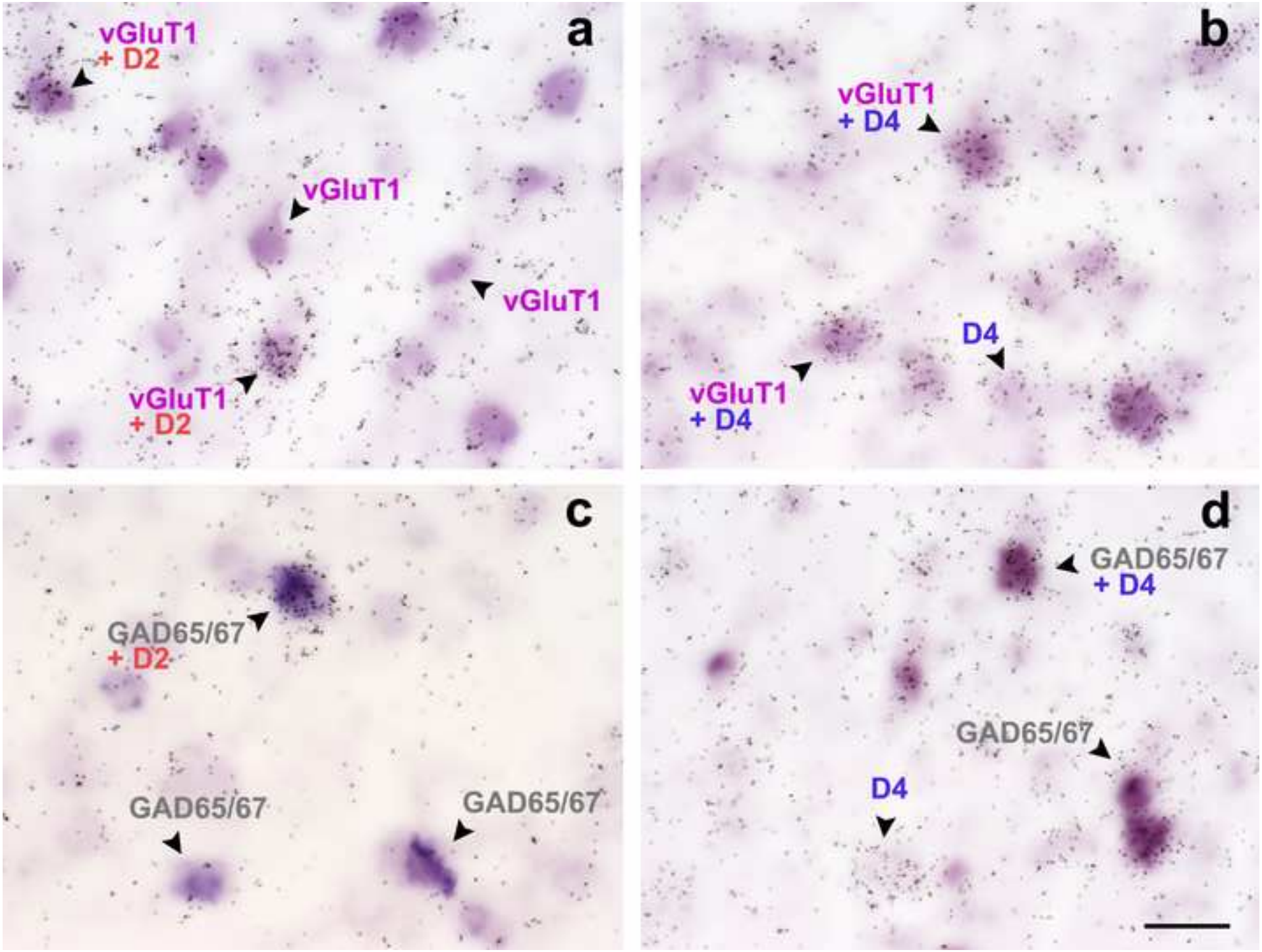

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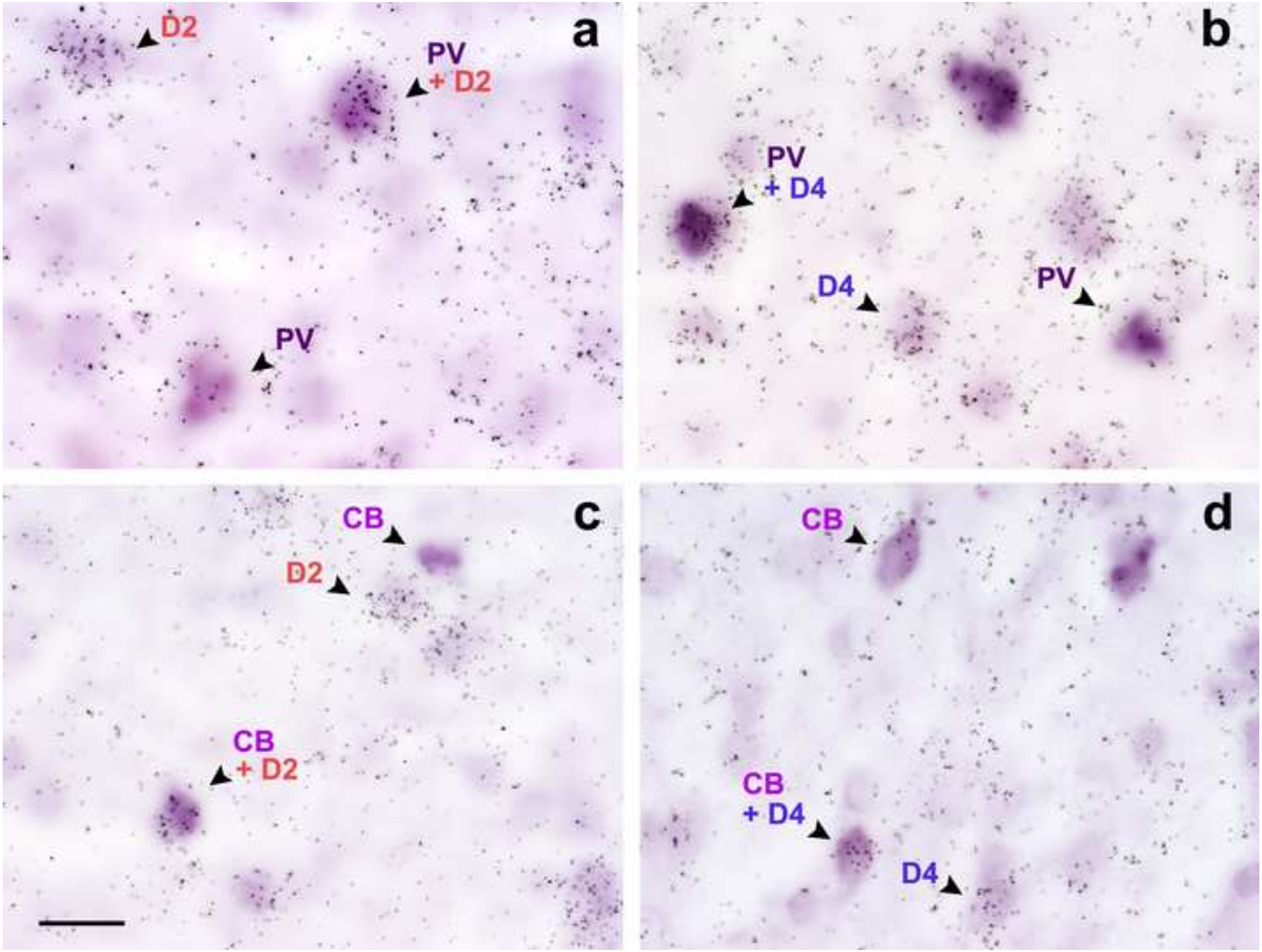

Figure 4

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