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# D1 dopamine receptors distribution following photothrombotic stroke in rat cerebral cortex

**Karolina Rogozinska** and **Jolanta Skangiel-Kramska**

Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Short communication

**Abstract.** The effect of focal photothrombotic stroke on the distribution of D1 dopamine receptor (D1R) sites was examined in different cortical areas of rat brain with quantitative receptor autoradiography using [$^3$H]SCH23390 as a ligand. Unilateral cortical stroke was located in the primary somatosensory cortex. After different survival times (1, 7 and 28 days) D1R binding levels were determined in the lesion core, penumbra, frontoparietal motor (FrPaM) and somatosensory (FrPaSS) areas as well as in homotopic regions in the contralateral hemisphere. One day after stroke, D1R density decreased by 36% ($P$<0.01) in the lesion core relative to sham-operated controls. At 7th day binding density was further reduced by 56% ($P$<0.002). Twenty-eight days after infarction, D1R binding returned to control level. No alterations in D1R binding levels were found in penumbra and other investigated regions. We suggest that the return of D1R binding to control level in the area initially corresponding to the infarct results from the shrinkage of the lesion volume.

The correspondence should be addressed to J. Skangiel-Kramska, Email: j.kramska@nencki.gov.pl

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Many studies demonstrate that excitatory and inhibitory neurotransmission is impaired after lesion induction in different experimental models of stroke. Data obtained in photothrombotic and permanent middle cerebral artery (MCA) occlusion models show that [$^3$H]MK801 binding to N-methyl-D-aspartate (NMDA) receptors is increased and the [$^3$H] muscimol binding to γ-amino butyric acid $(GABA)_A$ receptors is decreased in widespread regions both ipsilaterally and contralaterally to the cortical lesions (Que et al. 1999a,b, Qü et al. 1998a,b, Schiene et al. 1996). Complex and long-lasting decreases in messenger RNA expression of NMDA receptor subunit NR1 were shown by *in situ* hybridization (Friedman et al. 2000). Dysregulation of GABAergic system (downregulation of α1, α2, α5, γ2 subunits and upregulation of α3 subunit) was found using immunohistochemistry (Neumann-Haefelin et al. 1998, Redecker et al. 1999). Also electrophysiological research brings evidence that excitability changes appear both in the periinfarct and remote areas after transient MCA occlusion (Neumann-Haefelin and Witte 2000) and photothrombotic lesion (Buchkremer-Ratzmann et al. 1996). Efforts to redress the balance between inhibitory and excitatory systems using compounds affecting them directly (for example using antagonists of NMDA receptors or enhancing the effect of GABA at the $GABA_A$-receptors) failed in clinical trials (Davis et al. 1997, Wahlgren et al. 1999). However, alterations in levels and distribution patterns of different neurotransmitter receptors after stroke suggest that some compensatory and adaptive mechanisms might develop (Que et al. 1999b). Worthy of examination are modulatory receptors changes after ischemic damage. Modulatory systems may play a role in the lesion-induced reorganization that serves to regain function lost by the injury. Some promising data indicate that the administration of compounds affecting catecholaminergic systems, *d*-amphetamine (Hurwitz et al. 1991, Stroemer et al. 1998) and levodopa (Scheidtmann et al. 2001), improve behavioral recovery after various cortical lesions including stroke. These reports focused our attention on the dopaminergic system operating within the cerebral cortex. Although cortical dopaminergic innervation, which originates from the mesencephalon, is scarce, it exerts a potent influence on behavior and on a wide range of cognitive functions (Granon et al. 2000, Sawaguchi and Goldman-Rakic 1991, Williams and Goldman-Rakic 1995). Dopamine (DA) can selectively modulate the excitatory and inhibitory microcircuits in the prefrontal cortex (Gao and Goldman-Rakic 2003). Five different dopamine receptors are involved in DA action. Among them D1Rs, that belong to the G-protein-coupled receptors family and are positively linked to adenylate cyclase, appear to be most abundant in the cerebral cortex (Gaspar et al. 1995, Zilles et al. 1991). D1R sites are unevenly distributed throughout different cortical regions and show interlaminar differences with the deeper layers containing higher densities of receptors than superficial layers (Dawson et al. 1986, Gaspar et al. 1995). Recently, it appeared that cortical D1Rs are both post- and presynaptic and they are also present outside the synaptic cleft (Levey et al. 1993, Paspalas and Goldman-Rakic 2005). Pharmacological and genetic data provide evidence for the role of D1Rs in the modulation of synaptic plasticity in the prefrontal cortex (Huang et al. 2004). They have shown that D1R agonist selectively facilitates, while D1R antagonist selectively depresses, the maintenance of long-term synaptic potentiation (LTP). In heterozygous D1R knockout mice the dopaminergic modulation of both LTP and LTD is disrupted (Huang et al. 2004). Other studies indicated that activation of D1Rs is required for NMDA receptor-dependent LTP at hippocampal-prefrontal cortex synapses (Gurden et al. 2000, Wirkner et al. 2004). Several studies accentuated that the interaction between dopamine and glutamate receptors may be critical for correct work of the prefrontal cortex and suggest that synergistic action of D1 and NMDA receptors on pyramidal cell excitability may facilitate the mechanisms of cortical plasticity (Jay 2003, Tseng and O'Donnell 2004).

The present study was undertaken to examine the distribution and level of D1R sites following small focal stroke of the somatosensory cortex in rats after different survival times. For this purpose a quantitative receptor autoradiography was used with [$^3$H]SCH23390 as a ligand. Photothrombotic strokes were targeted to the vicinity of the barrel cortex, a cortical representation of facial vibrissae. This location was chosen because this part of the sensory cortex was reported to undergo functional reorganization and to develop new horizontal connections by axonal sprouting after MCA occlusion (Carmichael et al. 2001).

Male Wistar rats ($n$=28) weighting 250–280 g were used. All experiments were carried out in accordance with the European Committee Council Directive of 24

November 1986 (86/609/EEC) and approved by the Local Ethics Committee. The animals were subjected to the unilateral cortical lesions induced photochemically (Watson et al. 1985). For surgery, animals were anesthetized with isoflurane. During the operation the temperature of the body was kept at ~37°C using a self regulating heating pad. The femoral vein of the rat was prepared for the insertion of the catheter. Then, the rat was placed in a stereotaxic frame. A fibre-optic bundle (aperture 1.5 mm), mounted onto a cold light source (KL 1500 LCD, Germany), was placed straight on the skull surface on the right hemisphere with a position 4.5 mm posterior to bregma and 4 mm lateral to the midline. The illumination (2750 K) lasted 20 min. Following the onset of illumination 0.4 ml of the photosensitive dye Rose Bengal (Sigma Aldrich Chemie, 10 mg/ml) was infused through a catheter into the femoral vein, thus causing a lesion in the irradiated area. After the induction of thrombosis the catheter was removed and the wounds were sutured. After the operation the animals were allowed to awake from anesthesia and returned to their cages with free access to rat pellets and water. As controls we used sham-operated (that received saline and light or only the dye) ($n$=9) and naive ($n$=6) weight-matched rats. At various time after surgery (1, 7 and 28 days) the rats ($n$=4–5 for each time point) were deeply anesthetized by nembutal (100 mg/kg i.p.) before decapitation. The brains were removed and immediately frozen by immersion in heptane (-65°C) and stored at -76°C until sectioning. Coronal cryostat sections (20 μm) were cut at -17°C and then mounted on poly-L-lysine-coated slides.

Quantitative *in vitro* D1R autoradiography was performed using [3H]SCH23390 (SA 85.0 Ci/mmol; NEN – Boston, MA, USA) as a ligand. The sections were preincubated at room temperature for 10 min in 50 mM Tris-HCl buffer (pH 7.4) containing 154 mM NaCl, 1 mM EDTA and 0.1% BSA, and then incubated at room temperature for 100 min in the same buffer with a saturated concentration (1 nM) of radioactive ligand (Dawson et al. 1986). After incubation, the sections were washed twice for 10 min in ice-cold buffer and rinsed three times in ice-cold distilled water. Non-specific binding was established by incubating the adjacent sections with 10 μM (+) butaclamol. After drying, the sections were exposed to radiation sensitive films (Kodak BioMaxMR), together with calibrated [$^3$H] microscales (Amersham, UK) in X-ray cassettes, for 11 weeks. The films were developed, washed and fixed for 3 minutes at room temperature (Kodak dental X-ray developer and fixer). After exposure, the sections were stained with cresyl violet for histological examination.

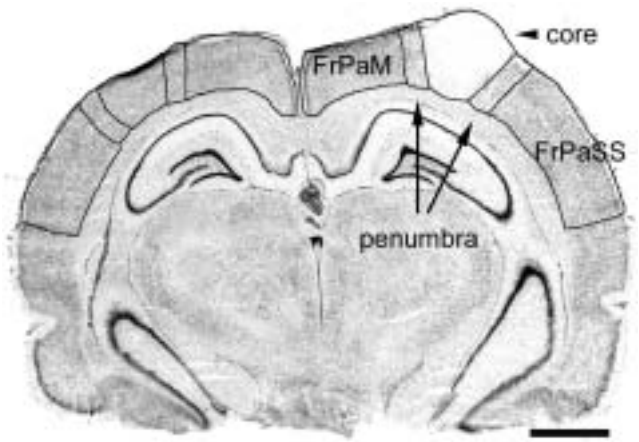


Fig. 1. Nissl stained coronal section from the rat brain 1 day after stroke with schematic drawing of the position of cortical areas analyzed quantitatively. The lesion was induced in the right hemisphere. Note the edema in lesioned cortex. (FrPaM) frontoparietal motor area; (FrPaSS) frontoparietal somatosensory area. Scale bar: 2 mm.

To quantify D1R sites the autoradiograms were digitized with a computer assisted image analysis system (MCID, Imaging Research Inc., St-Catherine, Ontario, Canada). The grey value images of the co-exposed plastic standards were used as a calibration curve, which defined the relationship between grey values in the autoradiograms and concentrations of radioactivity. Using manual-outlining mode, the optic density measurements were taken from cortical regions of the lesion core, penumbra, FrPaM and FrPaSS both in the left and right hemisphere separately (Fig. 1). Cortical areas were identified according to Paxinos and Watson (1982). This model of microcirculatory occlusion results in a small focal cortical infarction with a diameter of 2 mm (range 1.8–2.4 mm) (Que et al. 1999a) and extension through all cortical layers, while leaving white matter intact. Since the size of the lesion core changed with post-stroke survival time (Shanina et al. 2005), a template, corresponding to the area of the lesion determined 1 day after stroke, was prepared to make the comparison of binding after 7 and 28 days possible. Usually eight sections from the level of infarct were analyzed. The mean value obtained for a

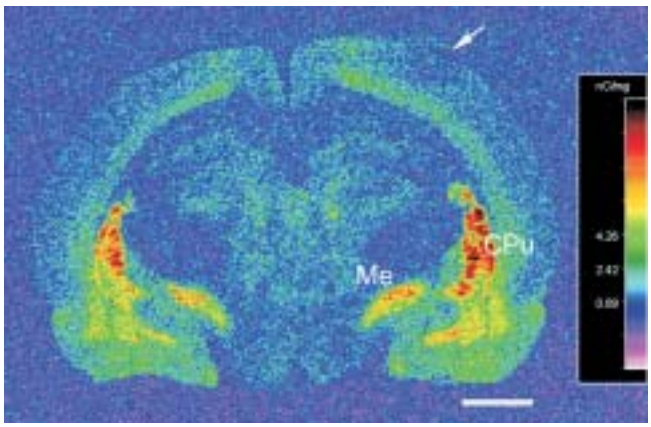


Fig. 2. Representative autoradiogram (in pseudo-colors) showing [$^3$H]SCH23390 binding sites distribution in frontal section from rat brain 1 day after stroke. Note a decrease of D1Rs in the lesioned area (arrow) and a very high concentration of D1Rs in (CPu) caudate putamen and (Me) medial amygdaloid nucleus. Color scale shows the density of labeling (nCi/mg wt). Scale bar: 2 mm.

particular cortical area from one hemisphere was considered as one observation.

Data were analyzed using one-way analysis of variance (ANOVA) with repeated measures, followed by *post hoc* tests. Student's *t*-tests for independent samples were used to compare binding levels between sham-operated and naive rats. Statistically significant differences were accepted at $P<0.05$. All analyses were done using STATISTICA 6.0 program.

Regional and laminar distribution pattern of [$^3$H]SCH23390 binding sites at the level of lesion area are shown in Figure 2. D1R binding density in the cortex was very low compared to the caudate putamen and amygdala. Infragranular layers expressed higher densities of D1Rs than supragranular ones. No differences in D1R densities were observed between naive and sham-operated animals (unpaired *t*-tests).

To examine the effect of stroke, values of D1R binding levels determined at different time points in the regions of ipsilateral and contralateral sides of lesioned brains were compared with respective values obtained from sham-operated animals. A significant effect of time was found exclusively in the lesion core ($F_{3,9}=11.54$, $P<0.002$). *Post hoc* tests revealed that in this area 1 and 7 days after stroke D1R level was significantly lower than in the sham-operated control (Fig. 3). One day after stroke binding density decreased by 36% ($P<0.01$). After 7 days the decrease was further reduced by 56% ($P<0.002$) relative to control. Twenty-eight days after stroke D1R binding returned to the control level (Fig. 3). In penumbra, FrPaM and FrPaSS no alterations in [$^3$H]SCH23390 binding sites were found at any examined time points. This was also true in the homotopic regions in the contralateral hemisphere.

The pattern of distribution of D1R binding sites observed by us fully confirmed that previously reported by other authors (Boyson et al. 1986, Dawson et al. 1986, Mansour et al. 1992, Ritchfield et al. 1989). We found that focal unilateral photothrombotic stroke affected D1R binding only in the infarct area. No changes in binding values were registered in other investigated cortical regions both in the lesioned and contralateral intact hemisphere. The decrease of binding in the lesion core 1 and 7 days after stroke is apparently related to cell death in this area. However, about 40% of binding sites are still present. Most probably they represent receptor sites in preserved tissue in the border of the infarct. Twenty-eight days after stroke D1R binding returned to the control level. The question arises, what is a cause of this effect. One possible reason may be a glial scar formation and the presence of D1R sites on glial cells. The presence of D1Rs was recently reported in astrocytes from rat basal ganglia and cortical astroglial cells (Miyazaki et al. 2004, Reuss et al. 2001). However, we did not observe any alteration of D1R binding in penumbra, in spite of the

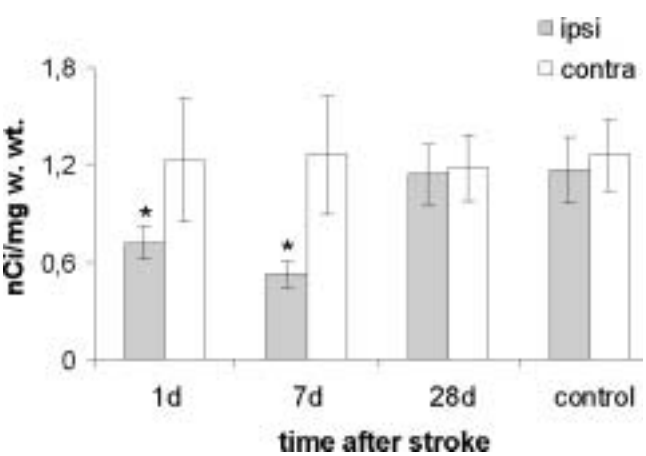


Fig. 3. [$^3$H]SCH23390 binding sites in the lesion core after unilateral cortical stroke. Data represent mean ± SD from 4–5 rats for each survival time. *$P<0.01$, as compared to control values obtained from sham-operated animals (one-way ANOVA).

presence of the cohort of glial fibrillary acidic protein (GFAP) immunoreactive astrocytes in this area after 28 days (data not shown). We cannot exclude that D1Rs are expressed on microglia, but to our knowledge there are no such reports. Another possibility is that near the lesioned area a supersensitivity of D1Rs develops as a result of denervation. For example, in rat cerebral cortex an increase of D1R binding was found following ventral tegmental area denervation and considered as a compensatory process (Pioli et al. 2004). However, in view of the lack of an increase of D1R sites in penumbra, the development of supersensitivity seems hardly probable. Even less likely is the possibility that the return of D1R level to the control value was due to sprouting phenomena, especially in view of a subcortical origin of dopaminergic innervation of the cortex. It should be stressed that one month after a photothrombotic stroke the lesioned area shrinks considerably (Shanina et al. 2005), while the area of the lesion core measured by us was independent of survival time. Therefore, the return of binding values to the control level in the lesion core may result from changes in the infarct volume. Thus, it seems that D1Rs are not involved in the reorganization processes in the cortex occurring after photothrombotic stroke.

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Boyson SJ, McGonicle P, Molinoff PB (1986) Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. J Neurosci 6: 3177–3188.

Buchkremer-Ratzmann I, August M, Hagemann G, Witte OW (1996) Electrophysiological transcortical diaschisis after cortical photothrombosis in rat brain. Stroke 27: 1105–1109.

Carmichael ST, Wei L, Rovainen CM, Woolsey TA (2001) New patterns of intracortical projections after focal cortical stroke. Neurobiol Dis 8: 910–922.

Davis SM, Albers GW, Diener HC, Lees KR, Norris J (1997) Termination of acute stroke studies involving selfotel treatment. ASSIST steering committed. Lancet 349: 32.

Dawson TM, Gehlert DR, McCabe RT, Barnett A, Wamsley JK (1986) D1 dopamine receptors in the rat brain: a quantitative autoradiographic analysis. J Neurosci 6: 2352–2365.

Friedman LK, Belayev L, Alfonso OF, Ginsberg MD (2000) Distribution of glutamate and preproenkephalin messenger RNAs following transient focal cerebral ischemia. Neuroscience 95: 841–857.

Gao W-J, Goldman-Rakic PS (2003) Selective modulation of excitatory and inhibitory microcircuits by dopamine. Proc Natl Acad Sci U S A 100: 2836–2841.

Gaspar P, Bloch B, Le Moine C (1995) D1 and D2 receptor gene expression in the rat frontal cortex: Cellular localization in different classes of efferent neurons. Eur J Neurosci 7: 1050–1063.

Granon S, Passetti F, Thomas K, Dalley J, Everitt B, Robbins T (2000) Enhanced and impaired attentional performance after infusion of D1 dopaminergic agents into the prefrontal cortex. J Neurosci 20: 1208–1215.

Gurden H, Tahita M, Jay TM (2000) Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses in vivo. J Neurosci 20: RC106.

Huang Y-Y, Simpson E, Kellendonk C, Kandel ER (2004) Genetic evidence or the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. Proc Natl Acad Sci U S A 101: 2836–2841.

Hurwitz BE, Dietrich WD, McCabe PM, Alonso O, Watson BD, Ginsberg MD, Schneiderman N (1991) Amphetamine promotes recovery from sensory-motor integration deficit after thrombotic infarction of the primary somatosensory rat cortex. Stroke 22: 648–654.

Jay TM (2003) Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Prog Neurobiol 69: 375–390.

Levey AI, Hersch SM, Rye DB, Sunahara RK, Niznik HB, Kitt CA, Price DL, Maggio R, Brann MR, Ciliax BJ, et al. (1993) Localization of D1 and D2 dopamine receptors in rat brain with subtype-specific antibodies. Proc Natl Acad Sci U S A 90: 8861–8865.

Mansour A, Meador-Woodruff JH, Zhou Q, Civelli O, Akil H, Watson SJ (1992) A comparison of D1 receptor binding and mRNA in rat brain using receptor autoradiographic and in situ hybridization techniques. Neuroscience 46: 959–971.

Miyazaki I, Asanuma M, Diaz-Corrales FJ, Miyoshi K, Ogawa N (2004) Direct evidence for expression of dopamine receptors In astrocytes from basal ganglia. Brain Res. 1029: 120–123.

Neumann-Haefelin T, Staiger JF, Redecker C, Zilles K, Fritschy JM, Mohler H, Witte OW (1998) Immunohistochemical evidence for dysregulation of the GABAergic system ipsilateral to photochemically induced cortical infarcts in rats. Neuroscience 87: 871–879.

Neumann-Haefelin T, Witte OT (2000) Periinfarct and remote excitability changes after transient middle cerebral artery occlusion. J Cereb Blood Flow Metab 20: 45–52.

Paspalas CD, Goldman-Rakic PS (2005) Presynaptic D1 dopamine receptors in primate prefrontal cortex: Target-specific expression in the glutamatergic synapse. J Neurosci 25: 1260–1267.

Paxinos G, Watson C (1982) The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pioli E, Reinhard S, Meissner W, Barthe N, Gross CE, Bezard E, Bioulac BH (2004) Partial bilateral mesencephalic lesions affect D1 but not D2 binding in both the striatum and cortex. Neurochem Int. 45: 995–1004.

Que M, Schiene K, Witte OW, Zilles K (1999a) Widespread up-regulation of N-methyl-D-aspartate receptors after focal photothrombotic lesion in rat brain. Neurosci Lett 273: 77–80.

Que M, Witte OW, Neumann-Haefelin T, Schiene K, Schroeter M, Zilles K (1999b) Changes in GABAA and GABAB receptor binding following cortical photothrombosis: A quantitative receptor autoradiographic study. Neuroscience 93: 1233–1240.

Qü M, Buchkremer-Ratzmann I, Schiene K, Schroeter M, Witte OW, Zilles K (1998a) Bihemispheric reduction of GABAA receptor binding following focal cortical photothrombotic lesions in the rat brain. Brain Res 813: 374–380.

Qü M, Mittmann T, Luhmann HJ, Schleicher A, Zilles K (1998b) Long-term changes of ionotropic glutamate and GABA receptors after unilateral permanent focal cerebral ischemia in the mouse brain. Neuroscience 85: 29–43.

Redecker C, Wang W, Fritschy J-M, Witte OW (1999) Widespread and long-lasting alterations in GABAA -receptor subtypes after focal cortical infarcts in rats: Mediation by NMDA-dependent processes. J Cereb Blood Flow Metab 22: 1463–1475.

Reuss B, Lorenzen A, Unsicker K (2001) Dopamine and epinephrine, but not serotonin, downregulate dopamine sensitivity in cultured cortical and striatal astroglial cells. Receptors Channels. 7: 441–451.

Ritchfield EK, Young AB, Penney JB (1989) Comparative distributions of dopamine D1 and D2 receptors in the cerebral cortex of rats, cats and monkeys. J Comp Neurol. 286: 409–426.

Sawaguchi T, Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: Involvement in working memory. Science 251: 947–950.

Scheidtmann K, Fries W, Müller F, Koenig E (2001) Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: A prospective, randomized, double-blind study. Lancet 358: 787–790.

Schiene K, Bruehl C, Zilles K, Qü M, Hagemann G, Kraemer M, Witte OW (1996) Neuronal hyperexcitability and reduction of GABAA -receptor expression in the surround of cerebral photothrombosis. J Cereb Blood Flow Metab 16: 906–914.

Shanina EV, Redecker C, Reinecke S, Schallert T, Witte OW (2005) Long-term effects of sequential cortical infarcts on scar size, brain volume and cognitive function. Behavioral Brain Res 158: 69–77.

Stroemer RP, Kent TA, Hulsebosch CE (1998) Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with d-amphetamine therapy after neocortical infarction in rats. Stroke 29: 2381–2395.

Tseng KY, O'Donnell P (2004) Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. J Neurosci 24: 5131–5139.

Wahlgren NG, Ranasinha KW, Rosolacci T, Franke CL, van Erven PM, Ashwood T, Claesson L (1999) Clomethiazole acute stroke study (CLASS): Results of a randomized, controlled trial of clomethiazole versus placebo in 1360 acute stroke patients. Stroke 30: 21–28.

Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD (1985) Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 17: 497–504.

Williams GV, Goldman-Rakic PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376: 572–575.

Wirkner K, Krause T, Köles L, Thümmler S, Al-Khrasani M, Illes P (2004) D1 but not D2 dopamine receptors or adrenoceptors mediate dopamine-induced potentiation of N-methyl-D-aspartate currents in the rat prefrontal cortex. Neurosci Lett 372: 89–93.

Zilles K, Werner L, Qü M, Schleicher A, Gross G (1991) Quantitative autoradiography of 11 different transmitter binding sites in the basal forebrain region of the rat – evidence of heterogeneity in distribution patterns. Neuroscience 42: 473–481.

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