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Effect of Chronic Estradiol Treatment on Brain Dopamine Receptor Reappearance after Irreversible Blockade: An Autoradiographic Study

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SUMMARY

Quantitative autoradiography was used to investigate dopamine receptor repopulation kinetics after irreversible dopamine receptor inactivation with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). The striatum and substantia nigra of two groups of ovariectomized female rats were compared. One group of rats was pretreated with estradiol (10 μg, twice daily, for 2 weeks), and another group received the vehicle. Striatal D1 dopamine receptors had larger degradation and production rate constants, compared with D2 receptors. The D2 receptor degradation rate constant increased rostro-caudally in the striatum of vehicle-treated rats, whereas this was not observed for estradiol-treated animals. A trend similar to that for D2 receptors was observed for the D1 receptor degradation rate constant in the striatum of vehicle-treated rats, whereas in estradiol-treated animals this constant decreased rostro-caudally. In the anterior and the middle parts of the striatum D2 receptor recovery parameters were not affected by chronic estradiol treatment, but in the posterior part estradiol-treated rats had lower receptor degradation and production rate constants. In the anterior part of the striatum, chronic estradiol treatment did not affect the recovery parameters of D1 receptors, whereas lowered receptor degradation and production rate constants were observed in the middle and posterior parts. D1 receptor recovery parameters in the substantia nigra were not affected by chronic estradiol treatment. After EEDQ administration to vehicle-treated rats, striatal dopamine levels decreased gradually, to reach a minimum 4 days later, and returned to control values after 7 days. In estradiol-treated rats, however, dopamine levels increased 2 days after EEDQ. Levels of the dopamine metabolites dihydroxyphenylacetic acid and homovanillic acid increased in the striatum after EEDQ administration in vehicle-treated rats. Even greater increases that lasted longer were observed in estradiol-treated rats after EEDQ. Striatal levels of serotonin and its metabolite 5-hydroxyindoleacetic acid were not significantly affected by EEDQ or estradiol administration. In summary, estradiol decreased striatal D1 and D2 receptor degradation rate constants, with the greatest effect being observed in the caudal part of the striatum. EEDQ dopamine receptor inactivation also revealed an increase of dopamine and its metabolites in the striatum after estradiol treatment.

Estradiol is known to affect dopaminergic neurotransmission in the central nervous system of mammals at the DA receptor level. Chronic estradiol treatment increases the density of rat striatal D1 (1, 2) and D2 (3–7) receptors. These increases have been correlated functionally with increased behaviors (such as stereotypies induced by DA agonists) (8). Behavioral and biochemical studies have revealed a functional heterogeneity of the striatum. Joyce *et al.* (9) observed that the lateral part of the striatum is associated with sensorimotor functions, whereas the medial part is related to associative functions. In addition, a rostro-caudal gradient in the densities of both D1 and D2 receptors (9, 10) and a marked lateral to medial gradient of D2 receptors in the rat striatum (9–11) have been observed. The effect of estradiol on rat striatal D2 receptors is also heterogeneous, with the lateral part being more susceptible than the medial part (11). In addition, Neve *et al.* (12) reported that DA denervation leads to an increase in the density of D2 sites in the lateral striatum weeks earlier than in the medial striatum. Furthermore, Savasta *et al.* (13) observed denervation supersensitivity of striatal D2 receptors restricted to the ventro- and dorsolateral regions of the striatum.

EEDQ is an irreversible blocker of several receptors, including monoaminergic (14), cholinergic (15), and serotonergic (16) receptors, due to its ability to bind covalently to these receptors, thereby inactivating them. In rats, EEDQ produces a profound and irreversible inactivation of both D1 and D2 receptors, to the same extent (17, 18). Peripheral administration of EEDQ

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ABBREVIATIONS: DA, dopamine; DL, dorsolateral; DM, dorsomedial; DOPAC, dihydroxyphenylacetic acid; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; OVX, ovariectomized; SN, substantia nigra; VL, ventrolateral; VM, ventromedial; G protein, guanine nucleotide-binding protein; HPLC, high performance liquid chromatography.

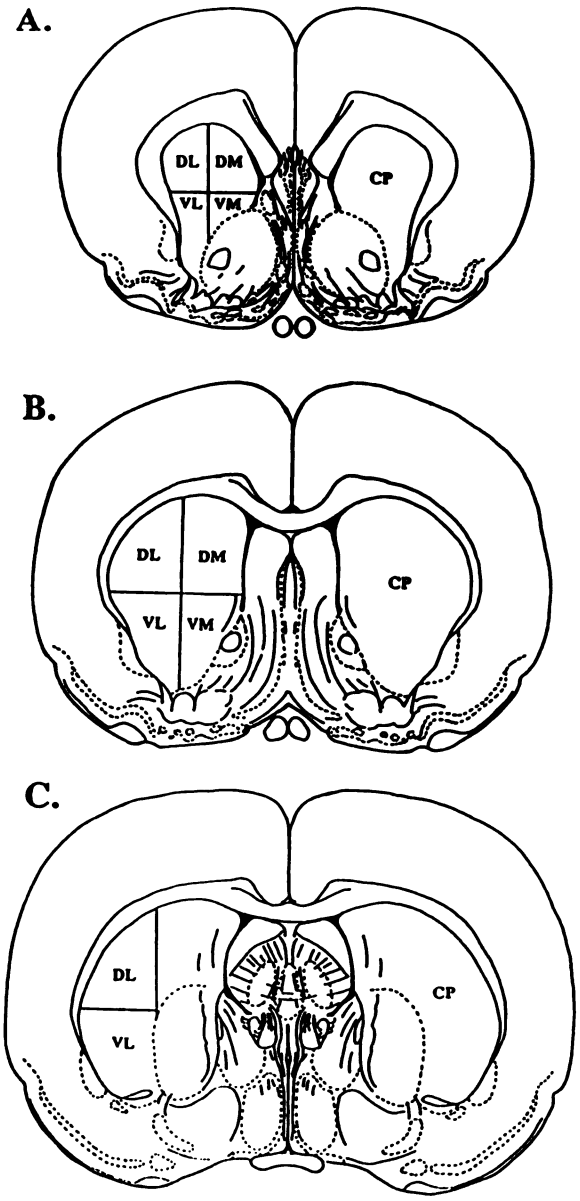


Fig. 1. Diagram of coronal sections of the rat brain, showing the anterior (A9140–A8920) (A), middle (A8380–A7890) (B), and posterior (A6790–A6570) (C) parts of the striatum (CP), according to the atlas of the rat brain by König and Klippel (32). For each coronal section, the left and right anterior and middle striata were divided into four parts (DM, DL, VM, and VL), whereas the posterior part was divided into two parts (DL and VL).

markedly reduces rat striatal D1 and D2 receptor binding, but no functional modification of either the G protein or the catalytic subunit of striatal adenylate cyclase is observed (19, 20). In addition, peripheral administration of EEDQ produces long term catalepsy (14) and inhibits both amphetamine- and apomorphine-induced stereotypies (17), as well as conditioned avoidance behavior (21). Therefore, EEDQ provides an appropriate tool for the *in vivo* study of DA receptor turnover and occupancy (22–24) and for the investigation of the mechanism involved in DA receptor changes observed after denervation (25), neuroleptic treatment (26), or reserpine treatment (27).

In vivo, EEDQ treatment in combination with quantitative receptor autoradiography has been used to monitor the recovery of DA receptors in discrete brain areas (28, 29). This experimental procedure provides a direct localization of DA receptor inactivation and an evaluation of alterations in the rate of receptor production and/or degradation. In order to evaluate the possible regional effects of estrogen on DA receptor kinetic parameters, we used quantitative receptor autoradiography to study striatal DA receptor recovery at various time intervals after peripheral EEDQ administration in rats chronically treated with 17β-estradiol or its vehicle. Striatal biogenic amines were also measured for correlation with receptor changes.

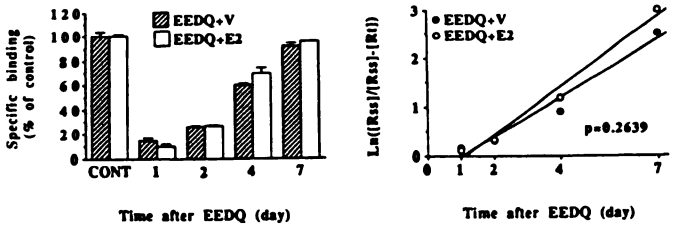


Fig. 2. *Left*, histograms. Recovery of nigral D1 receptors after EEDQ administration in vehicle- (*EEDQ+V*) and estradiol-treated (*EEDQ+E2*) OVX female rats. Values are expressed as percentage of their respective control groups (*CONT*) and represent the means ± standard errors obtained from three animals assayed individually. The 100% value for vehicle-treated rats was 873 ± 63 fmol/mg of tissue. For estradiol-treated rats, the 100% value was 791 ± 10 fmol/mg of tissue. *Right*, linear regressions. Corresponding semilogarithmic plot of time course of striatal D1 receptor recovery in vehicle- and estradiol-treated OVX female rats. [R_{ss}], respective steady state receptor levels; [R_t], receptor concentrations at various time intervals after EEDQ blockade. The slope values, which represent the receptor degradation rate constant, k, were equal to 0.0166 ± 0.0020 and 0.0206 ± 0.0020 hr^{-1} for vehicle- and estradiol-treated rats, respectively. Corresponding receptor production rates, r, were 14.5 and 16.3 fmol/mg of tissue/hr, and half-times of the receptor repopulation, $t_{1/2}$ were 41.7 and 33.6 hr (obtained by substitution of k into the equation $t_{1/2} = 0.693/k$).

Materials and Methods

Adult Sprague-Dawley female rats were purchased from Charles River Canada Inc. (St. Constant, Québec). Female rats, weighing 250–350 g, were bilaterally OVX under ether anesthesia, housed two/cage, and maintained at 22–23° on a 14/10-hr light-dark cycle (lights on from 5:00 a.m. to 7:00 p.m.). They received rat chow and water *ad libitum*. Beginning the day after ovariectomy, animals were injected with 10 μg of 17β-estradiol (Sigma) in 0.2 ml, subcutaneously, twice each day, for 2 weeks, whereas a second group received injections of the vehicle (0.3% gelatin in saline solution). Estradiol was initially dissolved in a minimum of ethanol and then diluted to the appropriate concentration in the vehicle. The final volume of ethanol never exceeded 0.01% of the volume injected (0.2 ml). EEDQ (Aldrich Chemical Co.), freshly dissolved in ethanol/water (1:1, v/v), was injected intraperitoneally at a dose of 10 mg/kg, on a 0.5 ml/kg basis, after chronic estradiol or vehicle treatment. Two groups of rats, chronically treated with estradiol or the vehicle, each received the ethanol/water (1:1) mixture as controls. All animals were sacrificed by decapitation, in the morning (between 9:00 and 11:00 a.m.), 1, 2, 4, or 7 days after EEDQ injection. Only rats demonstrating intense catalepsy, with a marked decrease of DA receptor density, 24 hr after administration of EEDQ were included in the study. Estradiol was also injected during recovery times after EEDQ, to allow constant exposure to the hormone. The effect of chronic estradiol treatment on striatal D2 receptors was previously shown to be similar in rat treated for 1 compared with up to 4 weeks (6, 30). Control groups (vehicle- and estradiol-treated animals that did not receive EEDQ) were sacrificed at the same time as the last recovery time group (seventh day). A group of these rats were used for homogenate assays of DA receptors, to study the effect of estradiol on DA receptor turnover in the total striatum and the anterior pituitary (31).

TABLE 1

Recovery of striatal D1 receptors after EEDQ administration in chronically vehicle- or estradiol-treated OVX female rats

OVX Sprague-Dawley female rats were treated with either vehicle (0.3% gelatin in saline solution) or 17β-estradiol (10 μg, twice each day) for 2 weeks before EEDQ administration (10 mg/kg, intraperitoneally). They were sacrificed by decapitation, and caudate-putamen was rapidly dissected. Binding of [^{3}H]SCH-23390 (1 nM) to 10-μm sections of anterior, middle, and posterior caudate-putamen was evaluated by autoradiography, as described in Materials and Methods. Values are expressed as percentage of their respective control groups and represent the means ± standard errors from three animals assayed individually.

Time after EEDQ	D1 receptor density									
	Anterior striatum				Middle striatum				Posterior striatum	
	DM	DL	VM	VL	DM	DL	VM	VL	DL	VL
days	*% of control*									
Vehicle-treated rats										
Control	100 ± 17	100 ± 19	100 ± 20	100 ± 21	100 ± 5	100 ± 2	100 ± 5	100 ± 1	100 ± 3	100 ± 2
1	20 ± 1	21 ± 3	21 ± 3	19 ± 3	22 ± 4	21 ± 2	21 ± 3	19 ± 2	27 ± 9	22 ± 8
2	36 ± 4	37 ± 6	37 ± 6	36 ± 6	38 ± 1	35 ± 1	39 ± 4	36 ± 2	37 ± 4	39 ± 2
4	63 ± 6	67 ± 6	65 ± 8	66 ± 5	62 ± 3	62 ± 7	64 ± 4	67 ± 7	66 ± 3	72 ± 7
7	109 ± 3[b]	112 ± 5[b]	106 ± 4[b]	105 ± 1[b]	91 ± 7	86 ± 9	87 ± 6	83 ± 7	92 ± 9	94 ± 4
100% values[a]	995 ± 178	1016 ± 192	1098 ± 219	1185 ± 248	1008 ± 46	1075 ± 25	1041 ± 54	1249 ± 17	545 ± 18	788 ± 17
Estradiol-treated rats										
Control	100 ± 22	100 ± 22	100 ± 24	100 ± 22	100 ± 2	100 ± 2	100 ± 7	100 ± 4	100 ± 1	100 ± 5
1	19 ± 1	19 ± 1	19 ± 1	18 ± 1	18 ± 2	17 ± 1	17 ± 1	17 ± 1	14 ± 3	13 ± 3
2	36 ± 1	37 ± 1	37 ± 2	38 ± 2	34 ± 1	30 ± 1	31 ± 3	31 ± 1	40 ± 1	39 ± 2
4	66 ± 5	65 ± 4	65 ± 5	67 ± 4	63 ± 1	57 ± 1	59 ± 3	60 ± 3	59 ± 7	58 ± 6
7	110 ± 7[b]	105 ± 8[b]	107 ± 9[b]	103 ± 9[b]	84 ± 8	75 ± 8	82 ± 8	81 ± 7	74 ± 9	71 ± 7
100% values[a]	1122 ± 252	1210 ± 273	1241 ± 294	1366 ± 302	1017 ± 16	1129 ± 25	1120 ± 73	1273 ± 58	592 ± 1	830 ± 43

[a] fmol/mg of tissue.
[b] These time points were not included in the repopulation curves, because 100% recovery had already been reached at the earlier recovery interval.

TABLE 2

Recovery of striatal D2 receptors after EEDQ administration in chronically vehicle- or estradiol-treated OVX female rats

OVX Sprague-Dawley female rats were treated with either vehicle (0.3% gelatin in saline solution) or 17β-estradiol (10 μg, twice each day) for 2 weeks before EEDQ administration (10 mg/kg, intraperitoneally). They were sacrificed by decapitation, and caudate-putamen was rapidly dissected. Binding of [^{3}H]spiperone (1 nM) to 10-μm sections of anterior, middle, and posterior caudate-putamen was evaluated by autoradiography, as described in Materials and Methods. Values are expressed as percentage of their respective control groups and represent the means ± standard errors from three animals assayed individually.

Time after EEDQ	D2 receptor density									
	Anterior striatum				Middle striatum				Posterior striatum	
	DM	DL	VM	VL	DM	DL	VM	VL	DL	VL
days	*% of control*									
Vehicle-treated rats										
Control	100 ± 15	100 ± 16	100 ± 18	100 ± 22	100 ± 2	100 ± 4	100 ± 4	100 ± 2	100 ± 3	100 ± 1
1	19 ± 2	18 ± 1	28 ± 1	27 ± 1	11 ± 3	12 ± 3	13 ± 4	11 ± 1	19 ± 5	14 ± 4
2	31 ± 5	31 ± 5	39 ± 6	35 ± 3	20 ± 2	25 ± 3	29 ± 3	28 ± 2	32 ± 12	33 ± 9
4	49 ± 5	51 ± 7	60 ± 5	62 ± 7	50 ± 3	56 ± 8	57 ± 8	57 ± 6	64 ± 10	64 ± 10
7	70 ± 5	71 ± 3	83 ± 3	84 ± 4	84 ± 9	84 ± 2	80 ± 11	74 ± 4	88 ± 3	86 ± 2
100% values[a]	373 ± 57	451 ± 70	333 ± 59	369 ± 81	291 ± 6	413 ± 17	323 ± 13	497 ± 10	165 ± 6	270 ± 4
Estradiol-treated rats										
Control	100 ± 22	100 ± 13	100 ± 19	100 ± 13	100 ± 9	100 ± 4	100 ± 1	100 ± 2	100 ± 17	100 ± 10
1	16 ± 2	14 ± 1	21 ± 4	17 ± 1	12 ± 3	15 ± 5	18 ± 6	14 ± 5	12 ± 1	15 ± 1
2	40 ± 11	35 ± 8	42 ± 10	43 ± 9	43 ± 6	39 ± 6	47 ± 5	41 ± 5	34 ± 7	36 ± 7
4	62 ± 4	63 ± 9	69 ± 6	64 ± 6	60 ± 9	58 ± 2	63 ± 7	61 ± 8	49 ± 4	46 ± 3
7	68 ± 4	68 ± 9	70 ± 1	69 ± 7	84 ± 4	78 ± 1	86 ± 5	81 ± 2	73 ± 6	69 ± 3
100% values[a]	352 ± 76	419 ± 56	344 ± 66	387 ± 50	270 ± 9	371 ± 15	287 ± 23	440 ± 10	197 ± 12	297 ± 10

[a] fmol/mg of tissue.

Brains were immediately removed, frozen in isopentane (−30°), and kept at −70° until assayed. The brains were cut into coronal sections of 10 μm, on a cryostat (−18°), and were thaw-mounted onto gelatin-coated slides. The adjacent coronal sections were from the anterior (A9140–A8920; 5–10 slices), middle (A8380-A7890; 5–10 slices), and posterior (A6790–A6570; 5–10 slices) striatum and from SN (A2180–A1610; 5–10 slices), according to the system of König and Klippel (32) (Fig. 1). Autoradiography assays, as well as film standards and analyses, were performed as previously described (33), using 1 nM [^{3}H]spiperone (D2 antagonist; 70–110 Ci/mmol) or 1 nM [^{3}H]SCH 23390 (D1 antagonist; 75–85 Ci/mmol), with 1 μM (+)-butaclamol (D2) or 1 μM SCH 23390 (D1) to estimate nonspecific binding. In the D2 receptor assay, ketanserin (50 nM) was added to the incubation medium to block 5-HT_2 serotonin receptors. For each coronal section, the left and right striata were divided into four parts (DM, DL, VM, and VL), except for the posterior part, which was divided into two parts (DL and VL) (Fig. 1). The total SN was assayed. Three rats in each treatment group were analyzed for each time interval after EEDQ, giving a total of 30 rats.

The mean of the data from each of the 5–10 brain coronal sections for each rat was computed. This mean was then used to calculate the mean value of the group. Using the same approach reported by most investigators (22, 25–27, 31), the mean value of each group was used to calculate the kinetic parameters by regression analysis. According to the method described by Neter and Wasserman (34), in which β_3 terms of multiple regression curve equations are tested for zero equality, statistical comparison between slopes of respective treated groups was

TABLE 3

Effect of chronic estradiol treatment on regional kinetic recovery parameters of striatal D1 and D2 receptors in OVX female rats after EEDQ administration (10 mg/kg)

Values were obtained from semilogarithmic plots of the time course of striatal D1 and D2 receptor recovery, which were obtained from data in Tables 1 and 2. The slope values of the semilogarithmic plots, which represent the receptor degradation rate constant, k, are expressed as means ± standard errors, determined according to simple linear regression. Corresponding receptor production rates, r, and half-times of receptor repopulation, $t_{½}$, are also shown.

	Anterior striatum				Middle striatum				Posterior striatum	
	DM	DL	VM	VL	DM	DL	VM	VL	DL	VL
D1 receptor										
Vehicle										
k (hr^{-1})	0.0109 ± 0.0010	0.0124 ± 0.0010	0.0116 ± 0.0010	0.0121 ± 0.0010	0.0148 ± 0.0020	0.0123 ± 0.0010	0.0127 ± 0.0010	0.0112 ± 0.0010	0.0155 ± 0.0020	0.0182 ± 0.0010
$t_{½}$ (hr)	63.6	55.9	59.7	57.3	46.8	56.3	54.6	61.9	44.7	38.1
r (fmol/mg of tissue/hr)	10.8	12.6	12.7	14.3	14.9	13.2	13.2	14.0	8.5	14.3
Estradiol										
k (hr^{-1})	0.0121 ± 0.0010	0.0118 ± 0.0003	0.0118 ± 0.0004	0.0128 ± 0.0010	0.0115 ± 0.0003	0.0086 ± 0.0004	0.0108 ± 0.0004	0.0104 ± 0.0030	0.0079 ± 0.0010	0.0073 ± 0.0010
$t_{½}$ (hr)	57.3	58.7	58.7	54.1	60.3	80.6	64.2	66.6	87.7	94.9
r (fmol/mg of tissue/hr)	13.6	14.3	14.6	17.5	11.7	9.7	12.1	13.2	4.7	6.1
D2 receptor										
Vehicle										
k (hr^{-1})	0.0069 ± 0.0023	0.0072 ± 0.0001	0.0102 ± 0.0010	0.0108 ± 0.0010	0.0120 ± 0.0010	0.0120 ± 0.0010	0.0104 ± 0.0003	0.0088 ± 0.0010	0.0133 ± 0.0010	0.0128 ± 0.0003
$t_{½}$ (hr)	100.4	96.3	67.9	64.1	57.8	57.8	66.6	78.8	52.1	54.1
r (fmol/mg of tissue/hr)	2.6	3.2	3.4	4.0	3.5	4.9	3.4	4.4	2.2	3.5
Estradiol										
k (hr^{-1})	0.0065 ± 0.0020	0.0069 ± 0.0020	0.0066 ± 0.0002	0.0065 ± 0.0020	0.0115 ± 0.0010	0.0090 ± 0.0010	0.0116 ± 0.0010	0.0102 ± 0.0010	0.0079 ± 0.0010	0.0067 ± 0.0010
$t_{½}$ (hr)	106.6	100.4	105.0	106.6	60.3	77.0	59.7	67.9	87.7	103.4
r (fmol/mg of tissue/hr)	2.3	2.9	2.3	2.5	3.1	3.3	3.3	4.5	1.2	2.0

TABLE 4

Regional kinetic parameters of striatal D1 and D2 receptor recovery of vehicle- or estradiol-treated OVX female rats after EEDQ administration (10 mg/kg)

Values were obtained from semilogarithmic plots of the time course of striatal D1 and D2 DA receptor recovery, which were obtained from data in Fig. 2. Receptor degradation rate constants, k, are expressed as means ± standard errors, determined according to simple linear regression. Corresponding receptor production rates, r, and half-times of receptor repopulation, $t_{½}$, are also shown.

	Autoradiography			Homogenates[a]
	Anterior striatum	Middle striatum	Posterior striatum	
D1 receptor				
Vehicle				
k (hr^{-1})[b]	0.0117 ± 0.0010	0.0126 ± 0.0010	0.0168 ± 0.0020	0.0127 ± 0.0010
$t_{½}$ (hr)	59.2	55.0	41.3	54.6
r (fmol/mg of tissue/hr)	12.6	13.8	11.2	10.2
Estradiol				
k (hr^{-1})[c]	0.0122 ± 0.0010	0.0100 ± 0.0002	0.0076 ± 0.0010	0.0065 ± 0.0002
$t_{½}$ (hr)	56.8	69.3	91.2	106.6
r (fmol/mg of tissue/hr)	15.1	11.3	5.4	6.4
D2 receptor				
Vehicle				
k (hr^{-1})[d]	0.0085 ± 0.0003	0.0107 ± 0.0010	0.0122 ± 0.0003	0.0066 ± 0.0010
$t_{½}$ (hr)	81.5	64.8	56.8	105.0
r (fmol/mg of tissue/hr)	3.2	4.1	2.7	1.6
Estradiol				
k (hr^{-1})[e]	0.0066 ± 0.0020	0.0105 ± 0.0010	0.0073 ± 0.0010	0.0073 ± 0.0010
$t_{½}$ (hr)	105.0	66.0	94.9	94.9
r (fmol/mg of tissue/hr)	2.5	3.6	1.8	2.0

[a] From Ref. 29.

[b–e] Assuming homogeneity of variances, a rostro-caudal gradient was tested for [b]D1 receptor, vehicle ($p = 0.086$), [c]D1 receptor, estradiol ($p = 0.062$), [d]D2 receptor, vehicle ($p = 0.002$), and [e]D2 receptor, estradiol ($p = 0.115$).

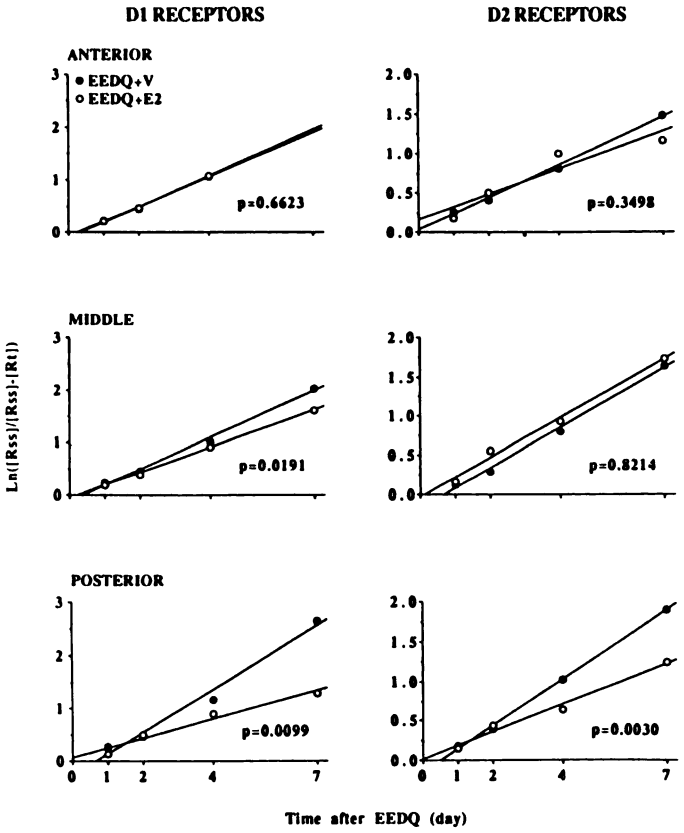
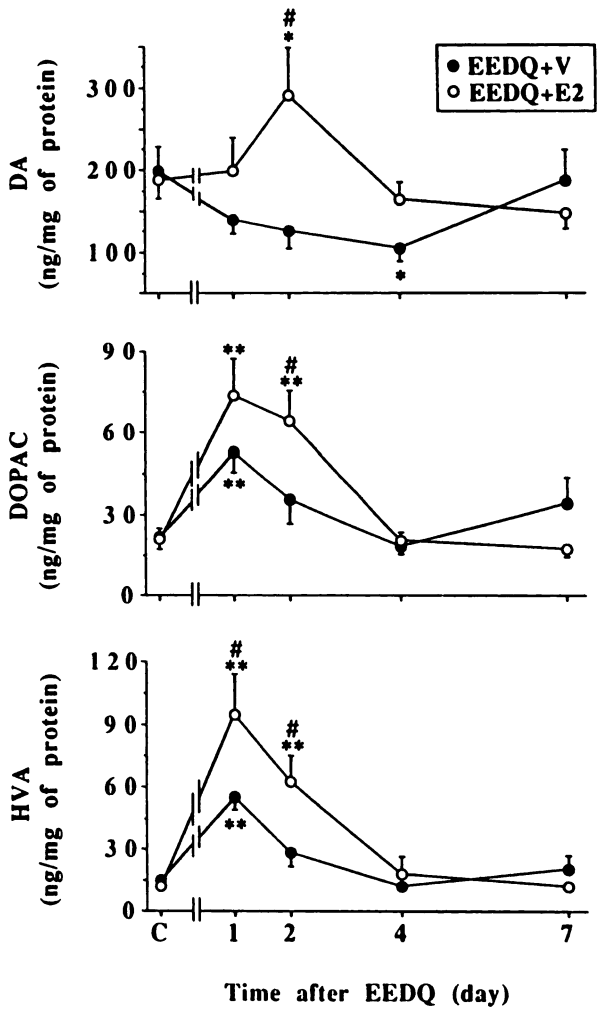


Fig. 3. Semilogarithmic plot of the time course of striatal D1 and D2 receptor recovery of vehicle- (*EEDQ+V*) and estradiol-treated (*EEDQ+E2*) OVX female rats. Recovery data are for the whole striatum in its anterior, middle, and posterior parts, for which detailed evaluation is shown in Tables 1 and 2, for D1 and D2 receptors, respectively. [R_{ss}], respective steady state receptor levels; [R_t], receptor concentrations at various time intervals after EEDQ blockade. For anterior, middle, and posterior striatum, the slope values, which represent the receptor degradation rate constant, k, and the corresponding receptor production rates, r, and half-times of the receptor repopulation, $t_{1/2}$, are shown in Table 4.



*** p < 0.05, ** p < 0.01 vs respective control values.**

 # p < 0.01 vs respective EEDQ+V values

Fig. 4. Time course of the change of striatal DA, DOPAC, and HVA levels after vehicle (C) EEDQ administration in vehicle- (*EEDQ+V*) and estradiol-treated (*EEDQ+E2*) OVX female rats. Each *curve* represents data (mean ± standard error) of seven to nine determinations, obtained by dissection of two 20-μm serial sections of anterior, middle, and posterior striatum. These sections were from the same brains used for the autoradiography of DA receptors.

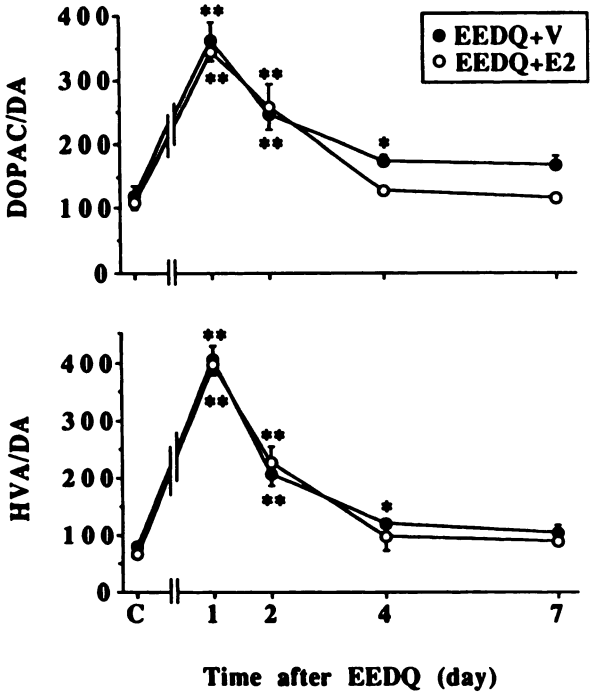
performed by assuming homogeneity of variances. Data were obtained from the same rat for both D1 and D2 receptors, thereby allowing a good comparison between the two receptors.

The concentrations of biogenic amines and their metabolites were measured by HPLC with electrochemical detection. The striatum was dissected (−18°) from two 20-μm serial coronal sections of each anterior, middle, and posterior part. These sections were cut from the same rat brains used for the autoradiography of DA receptors. Striatal tissue preparation and HPLC assays were done as previously described (35). Biogenic amines were measured in anterior, middle, and posterior parts of the striatum in individual animals, and the results for each area were pooled. Statistical significance of the catecholamine results was calculated by analysis of variance, followed by pairwise comparisons with the Duncan-Kramer multiple range test (36).

Results

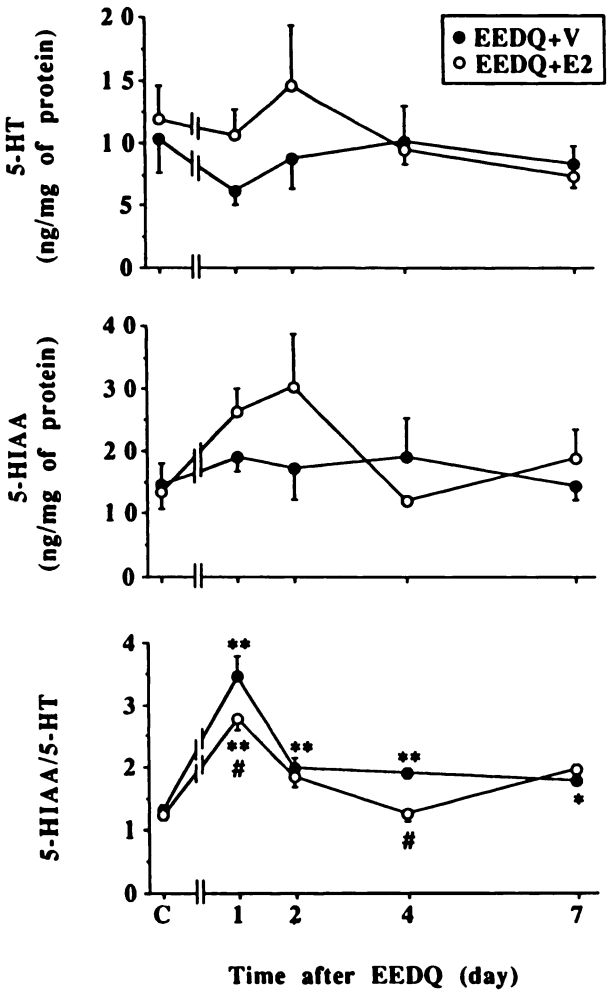
Striatal DA receptor gradients. A rostral to caudal gradient in the striatal densities of D1 receptors was observed in vehicle- and estradiol-treated rats, with higher densities being seen rostrally (anterior and middle part) (middle versus posterior: vehicle, $p = 0.0009$; estradiol, $p = 0.003$) (Table 1). Both lateral to medial and ventral to dorsal gradients in the densities of D2 receptors were observed in the middle part of the striatum in vehicle-treated (lateral versus medial: $p = 0.0005$; ventral versus dorsal: $p = 0.003$) and estradiol-treated (lateral versus medial: $p = 0.0009$; ventral versus dorsal: $p = 0.02$) rats, with higher densities being seen in the lateral and ventral parts (Table 2). Higher densities of D2 receptors were also observed in vehicle- and estradiol-treated rats in the rostral striatum (anterior and middle regions), compared with the posterior striatum (middle versus posterior: vehicle, $p = 0.0006$; estradiol, $p = 0.0005$) (Table 2).

Effect of EEDQ on DA receptor binding. In the anterior, middle, and posterior parts of the striatum, specific binding of [^{3}H]spiperone and [^{3}H]SCH 23390 showed a marked decline (80–90%) 1 day after EEDQ administration (Tables 1 and 2). In the SN, 90% of [^{3}H]SCH 23390 binding was depleted 1 day after EEDQ administration (Fig. 2). Seven days after EEDQ administration, 50–70% of D1 and D2 receptors had reappeared in the anterior, middle, and posterior parts of the striatum in vehicle- and estradiol-treated animals (Table 1 and 2), except for the anterior part of the striatum, where D1 receptors had recovered >100% (Table 1). In the SN, 80% of D1 receptors



* p < 0.05, ** p < 0.01 vs respective control values.

Fig. 5. Time course of the change of striatal DOPAC/DA and HVA/DA ratios after vehicle (C) or EEDQ administration in vehicle- (*EEDQ+V*) and estradiol-treated (*EEDQ+E2*) OVX female rats. Each *curve* represents data (mean ± standard error) of seven to nine determinations, obtained by dissection of two 20-μm serial sections of anterior, middle, and posterior striatum. These sections were from the same brains used for the autoradiography of DA receptors.



* p < 0.05, ** p < 0.01 vs respective control values.

\# p < 0.01 vs respective EEDQ+V values

Fig. 6. Time course of the change of striatal 5-HT and 5-HIAA levels, as well as the 5-HIAA/5-HT ratio, after vehicle (c) or EEDQ administration in vehicle- (*EEDQ+V*) and estradiol-treated (*EEDQ+E2*) OVX female rats. Each *curve* represents data (mean ± standard error) of seven to nine determinations, obtained by dissection of two 20-μm serial sections of anterior, middle, and posterior striatum. These sections were from the same brains used for the autoradiography of DA receptors.

had recovered 7 days after EEDQ administration (Fig. 2). In vehicle- and estradiol-treated rats, no significant difference was seen in the recoveries of D1 or D2 receptors between subregions (DL, DM, VL, and VM) in the anterior, middle, and posterior striatum (Tables 1 and 2). Recoveries of D1 or D2 receptors were generally similar in vehicle- and estradiol-treated rats (Tables 1 and 2) for each subregion tested, although these recoveries tended to be lower in the posterior striatum of steroid-treated animals.

Receptor recovery kinetic model. According to Mauger *et al.* (37), if the receptor production rate is constant (zero order) and receptor degradation is dependent on receptor concentration (first order), then repopulation kinetics after irreversible blockade of receptors may be described by the equation

$$[R_t] = (r/k)(1 - e^{-kt}) \quad (1)$$

where $[R_t]$ is the receptor concentration at time t, r is the receptor production rate, and k is the rate constant for receptor degradation. When t tends to infinity, $[R_t]$ approaches r/k, which is equal to $[R_{ss}]$, the concentration of receptors at steady state (B_{max}); therefore, $[R_{ss}] = r/k$. Logarithmic transformation of eq. 1 gives $\ln[R_{ss}]/[R_{ss}] - [R_t] = kt$.

Kinetic recovery parameters. Experimental repopulation curves (not shown), calculated according to the equation $\ln[R_{ss}]/[R_{ss}] - [R_t] = kt$, of the receptor recovery data detailed in Tables 1 and 2 revealed that repopulation was a monoexponential process. Slopes of the plots, which are equal to the rate constant of receptor degradation k, as well as receptor repopulation half-times and receptor production rates, are shown in Table 3. In Fig. 3, the means of each subregion (DM, DL, VM, and VL) of anterior, middle, and posterior parts of the striatum, shown in Tables 1 and 2, were used to generate plots of receptor recovery. Recovery of nigral D1 receptors after EEDQ administration is shown in Fig. 2.

Chronic estradiol treatment did not affect D1 receptor recovery parameters in the anterior striatum, whereas these parameters were reduced in the middle and posterior parts (Fig. 3). In the posterior striatum, estradiol treatment doubled the half-time for receptor repopulation of D1 receptors (Table 3). In the anterior and middle parts of the striatum, D2 receptor recovery parameters were not affected by chronic estradiol treatment, but they were reduced in the posterior part (Fig. 3; Table 3). Nigral D1 receptor recovery parameters were not affected by chronic estradiol treatment (Fig. 2).

Dorsal-ventral and lateral-medial comparisons of kinetic recovery parameters. Within the anterior, middle, and posterior parts of the striatum, no difference was observed between the degradation rate constants for D1 receptors in the DM, DL, VM, and VL parts for vehicle-treated rats (Table 3).

The degradation rate constant for D2 receptors in the anterior striatum of control rats was smaller in the dorsal, compared with the ventral, striatum ($p = 0.0059$). This was also observed in the DM versus the VM, as well as in the DL versus the VL, subregions (Table 3). In the middle striatum, a slightly larger D2 receptor degradation rate constant was observed in the DL, compared with the VL, subregion ($p = 0.0450$). In the posterior striatum, the degradation rate constant of D2 receptors was the same in the DL, compared with the VL, part for vehicle-treated rats. Estradiol treatment did not change the D1 or the D2 degradation rate constants in the DM, DL, VM, or VL parts of the anterior and middle striatum, except for a slight decrease in the DL part of the middle striatum. Estradiol decreased the degradation rate constant for D1 and D2 receptors in both parts (DL and VL) of the posterior striatum assayed.

Rostro-caudal comparisons of kinetic recovery parameters. A rostral to caudal gradient was observed in the receptor degradation rate constants for D2 receptors in striatum of vehicle-treated rats. The largest receptor degradation rate constant was in the caudal region. No gradient was observed in estradiol-treated rats (Table 4). A similar trend was observed for the D1 receptor degradation rate constant in the striatum of vehicle-treated rats, whereas an opposite trend was observed in estradiol-treated animals, where this constant decreased rostro-caudally.

Biogenic amine results. Similar catecholamine changes were obtained in the anterior, middle, and posterior striatum after EEDQ administration in vehicle- and estradiol-treated rats. Results from the striatal subregions were, therefore, pooled. After EEDQ administration, striatal DA levels in vehicle-treated rats decreased gradually by half, to reach a minimum 4 days later, and returned to control values after 7 days, whereas in estradiol-treated rats DA levels increased 2 days after EEDQ (+54% versus respective control) (Fig. 4). Striatal DOPAC levels doubled and HVA levels tripled 1 day after EEDQ administration in vehicle-treated rats. Even greater increases were observed 1 and 2 days after EEDQ in estradiol-treated rats; DOPAC levels tripled and HVA levels increased 7-fold (Fig. 4). In vehicle- and estradiol-treated animals, the DOPAC/DA and HVA/DA ratios peaked 1 day after EEDQ and decreased gradually, to reach respective control values after 7 days in vehicle-treated animals and after 4 days in estradiol-treated animals (Fig. 5). No change was seen in the striatal levels of 5-HT or its metabolite 5-HIAA, after EEDQ administration, in vehicle- or estradiol-treated rats (Fig. 6). In contrast, the striatal 5-HIAA/5-HT ratio was increased 1–7 days after EEDQ in vehicle-treated rats, whereas it increased to a lesser extent and for only 1 day after EEDQ in estradiol-treated rats (Fig. 6).

Discussion

Striatal DA receptor gradients. The distribution of striatal D1 receptors was generally observed to be more uniform than that of D2 receptors. D1 receptors displayed a decrease rostro-caudally in the striatum. This decrease was also observed for D2 receptors, but with a higher density in the lateral and ventral parts of this brain area. These results are in agreement with previous reports (9–11).

Effect of EEDQ on DA receptor binding and DA receptor recovery parameters. The present study confirms previous reports (26–28, 31, 38) that EEDQ administration produced a marked decline of both striatal D1 and D2 receptors. The decline of both DA receptors was similar 24 hr after EEDQ in vehicle- and estradiol-treated rats, suggesting that brain EEDQ levels reached similar levels and were not influenced by estradiol. In the striatum of vehicle-treated rats, we observed larger degradation and production rate constants for D1 receptors than for D2 receptors, confirming previous studies (22, 24–26, 31, 39). Several studies have demonstrated that the striatum is heterogeneous in structure and function (9–11, 39, 40). This is also reflected in the present study by the differences in DA receptor recovery kinetics across the striatum. Indeed, the striatal D2 receptor degradation rate constant increased rostro-caudally. A similar trend was also observed for the D1 receptor degradation rate constant, which increased rostro-caudally in the striatum, with similar values in the posterior striatum and the SN. Similar to the more uniform distribution of striatal D1 receptors, compared with D2 receptors, we also observed that the degradation rate constant of D1 receptors was the same in the dorsal versus ventral and lateral versus medial regions of the striatum of vehicle-treated rats. D2 receptors recovery parameters were more heterogeneous, with a dorsal-ventral gradient. D1 receptor recovery parameters in the posterior striatum were similar to those observed in the SN. The striatal recovery kinetic parameters measured here by autoradiography are consistent with our previous report (31), where these parameters were evaluated in striatal homogenates, and with reports by other groups (22, 26–28).

Effect of estradiol on DA receptor recovery parameters. The effect of chronic estradiol treatment on D1 receptor recovery kinetics was heterogeneous across the striatum, with the posterior part being more affected. The kinetics of D2 receptor repopulation in the rostral part of the striatum (anterior and middle) were not affected by estradiol treatment. As observed for D1 receptors, the D2 receptor degradation and production rate constants were decreased by estradiol treatment in the posterior region of the striatum, a region with a lower density of receptors.

We have previously shown (31), using receptor assays in homogenates of the total striatum, that EEDQ and estradiol affect mainly the density (B_{max}) and leave the affinity (K_d) of D1 and D2 receptors unchanged. Therefore, the changes in specific binding reported in the present autoradiographic study most likely reflect changes in the density of receptors. Under the experimental conditions of the present study, we have shown (31) in a larger group of rats that estradiol increases the density of D1 and D2 receptors. In fact, our group (2–4, 11, 30, 31) and several other investigators (1, 5–8) have previously reported that chronic estradiol treatment increases the density of striatal D1 and D2 receptors.

The present results, as well as results from our previous study (29), show that estradiol increases the density of D1 and D2 receptors. This increase is associated with a decrease in the production and degradation rate constants of these receptors. This is similar to the effect of chronic neuroleptic treatment (26), where haloperidol increases the density of D2 receptors and also decreases the production and degradation rate constants of these receptors, as evaluated from the recovery after EEDQ. In contrast to haloperidol, estradiol does not act on DA recognition sites (30).

Estradiol can affect *in vitro* and *in vivo* striatal D2 agonist binding sites at the level of G proteins associated with this receptor (41). Modification of the striatal membrane cholesterol/phospholipid ratio has been shown to influence mem-

brane fluidity, as well as D1 receptors (42). Membranous methyltransferases are enzymes responsible for phospholipid methylation, a process that can affect membrane fluidity (43). The activity of these enzymes was shown to vary during the estrous cycle in the rat pituitary (44). The decrease in receptor degradation and production rate constants observed in the present study could be a consequence of a membrane effect of estradiol on coupling of the receptor to G proteins and/or on the cholesterol/phospholipid ratio and methyltransferase activity, leading to changes in membrane fluidity. This could interfere and slow the internalization and degradation of receptors. It is also possible that the effect of estradiol on D1 and D2 receptor recovery parameters involves first only one DA receptor subtype and the other is subsequently affected through D1/D2 receptor interactions. Cameron and Crocker (45) previously observed such a D1/D2 receptor interaction in EEDQ experiments, where one DA receptor was blocked and this blockade influenced the recovery of the other receptor.

The receptor production rate constant, *r*, describes a multistep process that includes both the synthesis and insertion of receptors into the membrane. Cycloheximide administration before EEDQ inhibits the receptor recovery rate, indicating that repopulation of receptors after irreversible blockade is dependent on protein synthesis of *de novo* receptor (22). Our results suggest that the internalization and degradation processes, as well as protein synthesis and insertion of D1 and D2 receptors, are regulated differentially in the rat striatum. The degradation and production processes for DA receptors are faster in the posterior part than in the rostral part. Furthermore, these processes are slowed in the caudal region by estradiol treatment. EEDQ affects several receptors in the central nervous system. Other transmitter systems may, therefore, have contributed to the effect observed on the recovery parameters of striatal DA receptors. In addition, an action of estradiol on other transmitters may have contributed to its effects on DA receptor recovery parameters.

Biogenic amine results. As previously observed (46) in vehicle-treated rats, EEDQ administration decreased striatal DA concentrations and increased the DA metabolites DOPAC and HVA. The opposite was observed in estradiol-treated rats, where striatal DA levels were increased after EEDQ. In addition, the increase in DOPAC and HVA levels in the striatum was greater for rats receiving steroid treatment in addition to EEDQ. These differences in DA, DOPAC, and HVA levels between steroid-treated and untreated rats were observed only from 1 to 4 days after EEDQ. Rapid (after 1 and 2 days) increases in striatal DOPAC and HVA levels after EEDQ may be explained by increased turnover of DA, in order to compensate for hampered DA transmission produced by EEDQ DA receptor inactivation. This activated system may later become depleted of DA, as observed in the decrease of DA 4 days after EEDQ. Acute estradiol treatment has been shown to increase striatal DOPAC and HVA levels, as well as to shift the D2 receptor from its high to its low affinity agonist state (35). These effects of estradiol, in combination with those of EEDQ, may explain the greater increases in striatal DOPAC and HVA levels for rats receiving the steroid treatment and an EEDQ injection. The increase in striatal DA levels in estradiol-treated rats after EEDQ, in contrast to the depletion observed after EEDQ alone, may be because of the increase in the DA uptake sites (47), as we have previously documented after an acute injection of estradiol, and/or an increase of release and synthesis of DA.

Correlation between DA concentration and DA receptor changes. Although the estradiol effect on DA receptor repopulation kinetics is heterogeneously observed in the striatum, the effect of this steroid on levels of DA and its metabolites is similar rostro-caudally in this brain area. This suggests that these two effects are not causally related. This is also supported by the observation of an increase in striatal D2 receptor density in 6-hydroxydopamine-lesioned rats after chronic estradiol treatment (5), indicating that this receptor increase is not a consequence of changes in DA and its metabolite levels.

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