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Regional Brain Patterns of Dopamine, Metabolites and D_2 Receptors in Memory

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GALKINA, O. V. AND E. K. PODGORNAYA. *Regional brain patterns of dopamine, metabolites and D_2 receptors in memory.* PHARMACOL BIOCHEM BEHAV 54(2) 453-460 1996.—Patterns of dopamine (DA), 3,4-dihydroxyphenyl acetic (DOPAC), and homovanillic (HVA) acids, para-tyramine (p-TA), and D_2 receptors for eleven structures of forebrain and midbrain were examined at 1 and 6 days after one trial passive avoidance training of rats, at 1 day after "psychogenic" amnesia production, immediately following training, and at the last day after foot shock of rats with previous "psychogenic" amnesia administration. Essential dopaminergic differences have been found between the groups studied. More significant neurochemical changes were observed in rats from trained and retrieval training groups. Regional DA changes were accompanied by a diminution of DA metabolism, an increase of D_2 receptor density and p-TA. These neurochemical alterations differentially characterized the regional neurochemical patterns found in rat's exhibition nonretention (trained), forgetting, and amnesia. It is suggested that the present data reflect the existence of quantitative relationships between D_2 receptors, DA, and p-TA, which are probably important in modulation of memory.

Training with retention Forgetting "Psychogenic" amnesia Training without retention Retrieval training D_2 receptor density p-Tyramine Dopamine Dopamine metabolites Regional neurochemical brain patterns

THERE is evidence of dopaminergic system involvement in the modulation of memory. On the one hand, this evidence is based on studies of facilitation of memory retrieval under a number of dopaminergic stimulating agents (1,6); on the other hand, on the conclusion that the disruption of DA neurotransmission after the injection of 6-hydroxydopamine causes the deficits in the performance of shock-avoidance tasks (37,38). As well as the blockade of DA-receptors by chlorpromazine, haloperidol leads to impairment of memory performance (2,12,22). However, we do not know enough about the dopaminergic system's mode of action. One may think that the dopaminergic mechanism of memory modulation is reflected in the biochemical indices of change. There is experimental evidence of training-induced dopaminergic neurochemical changes; alterations in prefrontal D_2 receptors, an increase in density, and decrease of affinity were observed 24 h after the foot shock stress in rats (27). A DA level decrease in the neostriatum and an increase in the neocortex and brainstem were obtained at 10 min after training in a one-trial passive avoidance task (10). The changes of DA turnover or metabolism in the structures of mesolimbic or nigrostriatal systems were examined in behavioral tasks (11,18,20). In our previous studies of neurochemical changes in trained animals, it was demonstrated that one-trial passive avoidance training at 24-h resulted in both DA increases (frontal cortex, hypothalamus, amygdala) and decreases (striatum) without DOPAC increases. These alterations were accompanied by increasing D_2 receptor density and appearance of significant amounts of p-TA (32). Such regional patterns of DA metabolites and D_2 receptors were obtained by high performance liquid chromatography and radioligand binding in the same brain tissue. We have associated the obtained data with high activity of DA and D_2 receptor functioning because p-TA was formed from exogenic DA bound by D_2 receptors of synaptic membranes of trained rats in a model system that was exposed to the influence of anodal microdischarges as the generators of active particles: electrons, OH radicals (31,33). Thus, our previous data can support the conclusion that catecholamine func-

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tional availability in brain, rather than its overall level, is important in the memory process (30). It's clear that such experimental data are mainly dopaminergic neurochemical correlates of training. The addition of neurochemical correlates of amnesia, forgetting, and untraining to neurochemical correlates of training is necessary to determine, at least partly, the dopaminergic mechanism of memory modulation. Thus, to obtain and examine data associated with the dopaminergic mechanism of memory modulation training-, amnesia-, forgetting-, and untraining-related profiles of DA, metabolite concentrations and density of D_2 receptors were studied in the present work. Patterns of DA, metabolites, and D_2 receptors were determined by using our methodical approach, mentioned above, for eleven brain regions, including structures that play an important role in cognitive processes and behavior.

METHODS

Animals

The animals used in these studies were male Wistar rats (160–180 g) obtained from Rupple colony (Sant-Petersburg). The animals were housed in groups of 4 in standard hanging wire cages with food and water available ad lib and maintained for the course of the experiment on a 12-h light-dark cycle (8 a.m. on, 8 p.m. off). All animals were allowed at least 1 day to adapt to laboratory conditions prior to training.

Behavioral

The apparatus used in these experiments was a long alleyway divided into 2 compartments by a sliding door: well-lit start compartment (24 × 14 × 12 cm) and a dimly lit black shock compartment (37 × 14 × 12 cm) containing a stainless-steel grid floor.

Each rat was handled 1 day prior to training. Passive avoidance training based on the method of Jarvik and Kopp (14) consisted of placing a rat into the well-lit start compartment facing away from the door. As the rat turned around, the door was opened and the latency to step-through into the dimly lit black shock compartment was recorded. As soon as the rat stepped through the door, it was closed, and the rat was given a 1 mA foot shock for 6 s.

The second group of animals did not receive foot shock training; after passing into the second compartment, the animal was immediately returned to its cage. They formed the control group for all experimental groups. One day after foot shock, the rat was again placed in the illuminated compartment for the retention test. Testing consisted of measuring step-through latency for a maximum of 180 s. These latency data were used as the index of retention performance. Long latency (180 s) was interpreted as reflecting good retention. The animals with good retention formed the Trained, with retention group. Short latency step-through (less than 25 s) was interpreted as reflecting poor retention. The animals with poor retention formed the Trained, without retention group.

Some of the animals from the Trained group were tested once more at 6 days after foot shock training. The animals with poor retention formed the Forgetting group. "Psychogenic" amnesia based on the procedure of Robustelli and Jarvik (34) was administered immediately after passive avoidance training. After foot-shock training, the rat was detained in the darkened section for 5 min and then moved to its home cage. Twenty-four hours later, the animal was placed in a start compartment, as in the training session, and the step-through latency (maximum of 180 s) was recorded. Short latencies (less than 25 s) were taken to indicate poor retention. The rats with poor retention formed the Amnesia group. Immediately after the retention test, some of the rats from the Amnesia group were moved to another room for reminder treatment. Each rat was placed in a dark cage and given a single 5 mA foot shock for 2 s. Response latencies were based on a single retention test trial given 24-h later after foot-shock stimulation. Long latency (180 s) was interpreted as reflecting good retention. The animals with good retention formed the Retrieval training group.

In all experiments, the rats from the Control group were tested as the experimental animals.

Neurochemistry

Immediately following testing, the rats were decapitated with a guillotine. The brains were quickly removed, placed on ice, and stored for 2–3 hours at the temperature of −20°C. The frozen brain was dissected on ice into the frontal cortex, hypothalamus, hippocampus, baso-lateral and cortico-medial amygdalas, caudate nucleus, nucleus accumbens, A9, A10, A11, (B7 + B8) groups (19). Tissue samples were prepared for radioligand assay and high-performance liquid chromatography (HPLC) by homogenization on ice in 9 vol. of cold 0.32 M sucrose in 10 mM Tris-HCl containing 2 mM EDTA (pH 7.4 at 4°C) using glass-glass grinders and centrifugation of homogenates at 50,000 g for 20 min at 4°C (32). The supernatants were removed and used for HPLC catecholamine measuring (see below).

Tissue pellets were used for binding assay of [^{3}H]-DA according to modifications of the method of Morroi and Hsu (29) made by Podgornaya, Galkina and Ilyuchenok (32). A pellet was resuspended in a 0.2 M K-phosphate buffer supplemented with 1 mM EDTA, 1 mM ditiotreitol (DTT), 1 μM pargyline and 0.2% ascorbic acid (pH 7.4 at 4°C). In homogenates with synaptic membranes the specific binding of [^{3}H]-DA (0.2–2.4 nM in saturation experiments) to approximately 500 μg of protein per sample was defined using 1 mM unlabeled DA. The homogenates were incubated in triplicate (kinetic experiments) with ligand in the absence or presence of 1 mM unlabeled DA for nonspecific binding. The incubation time equaled 17 h at 3°C. The final reaction volume equaled 0.5 ml The incubation of synaptic membranes was terminated by rapid filtration through whatman GF/B (2.4 cm) glass microfiber filters by suction. The filters retaining tissue samples were quickly rinsed once in 5 ml of an ice-incubation buffer. The radioactivity in each vial was measured with a Delta-30 (Belgium) liquid scintillation counter. A specific DA binding was determined using the difference between the radioactivity in the absence and presence of 1 mM cold DA. Protein in the tissue pellet was estimated using the method of Lowry (24). The bound of [^{3}H]-DA was expressed as fmol/mg protein. The K_d (the equilibrium binding constant) and B_{max} (maximum number of binding sites) were determined by Scatchard transform of saturation analysis and linear regression.

The concentrations of catechols were quantified by means of the HPLC procedure with electrochemical detection (ED) according to modifications of the method of Sundberg, Bennet and Morris (40) made by Podgornaya, Galkina and Ilyuchenok (32). Quantitative evaluations were made using amperometric detection of the column effluent with a potential of +0.65 V vs. a Ag/AgCl reference electrode of electrochemical detector Model 2143 (LKB, Sweden). Chromatographic separations were made using a stainless-steel column (250 × 4.6

mm i.d.) packed with RP18, 5 μm particle size (LKB, Sweden). The mobile phase was 0.1 M NaH_2PO_4 containing 1 mM EDTA, 10 μM NaCl, 5 mg/l of octyl sodium sulfate and 10% methanol (pH 4.0). It was pumped through the column at a rate of 1.0 ml/min using a double piston pump Model 2150 (LKB, Sweden) equipped with an injector (Rheo-Dyne Inc., USA) and a 50 μl sample loop. The catechols in supernatant of homogenate with adding 10–50 ng of the internal standard (3,4-dihydroxybenzilamine) were extracted by adsorption and desorption on alumina with 100–500 μl of 0.1 N HCl and chromatographed. The sample concentrations were calculated using the peak height for each analyte relative to the internal standard. With these procedures, recovery of DA, DOPAC, HVA, p-TA ranged from 90–95%. Biogenic amine contents were expressed as pmol/mg protein.

Statistical Analyses

The general approach to statistical analyses of the research data was by one-way ANOVA for analyses of differences among all groups studied. When significant effects were found amoung groups, ANOVAs were supplemented by Scheffe post hoc contrasts. Significance is defined in terms of the 0.01 level of confidence, with exact p values noted in the text.

RESULTS

Results of the training, "psychogenic" amnesia, trained without retention, retrieval training, and forgetting on the concentrations of DA, its metabolities and characteristics for $[^3H]$-dopamine binding in different brain regions are summarized in Table 1. One-way ANOVAs of these data indicated that, among group levels of DA, its metabolites and means of B_{max} for D_2 receptors were significantly different for each brain region studied (Table 2). Only in the hypothalamus overall ANOVA did not reveal a significant effect of processes of memory on HVA level: $F(5, 54) = 0.2, p = 0.96$.

It is necessary to subdivide the result section according to the five groups of animals used in the experiment. We will begin with a description of the results for the trained animals.

Effects of Training in Trained Animals

The trained animals demonstrated long retention latencies (180 s) on the 1st and 6th days after foot shock training. Concentrations of DA, DOPAC, and HVA in various brain regions of the rats of this group are shown in Table 1 (Trained, with retention group). In trained animals, the frontal cortex DA increased by 238%, hypothalamus DA by 87%, A9 group DA by 7%, A10 group DA by 8%, A11 group DA by 6%, and DA has decreased by 73% in the nucleus accumbens, 61% in caudate nucleus, and 76% in baso-lateral amygdala, 16% in cortico-medial amygdala, 14% in hippocampus, and 14% in (B7 + B8) group compared to the control DA levels.

The DOPAC levels were considerably lower in the nucleus accumbens (73%), caudatus nucleus (51%), baso-lateral amygdala (75%), and considerably less in the frontal cortex (40%), hypothalamus (25%), and A9 group (9%). The HVA concentrations decreased in the nucleus accumbens (64%), caudate nucleus (36%), and frontal cortex (28%). The observed alterations of DA concentrations were accompanied by the appearance of significant levels of p-TA in the brain tissue: both the nucleus accumbens and caudate nucleus p-TA levels were 400% and 300% greater than the control p-TA levels (Table 1). In the frontal cortex, hypothalamus, baso-lateral amygdala, and A10 group p-TA was measured only after passive avoidance training (Table 1). Considerable increases in density of D_2 receptors in the frontal cortex (167%), hypothalamus (162%), nucleus accumbens (150%), caudate nucleus (100%), baso-lateral amygdala (100%), cortico-medial amygdala (95%), hippocampus (100%), and A10 cells (100%) were observed.

Scheffe comparisons demonstrated that the training produced regional neurochemical patterns, which differed significantly from those of the control, amnesia, trained without retention, and forgetting groups but to a lesser extent relative to the retrieval training group (Table 1).

Effects of Forgetting in Trained Animals

Animals from the forgetting group demonstrated long retention latencies (180 s) 24 h after training and short ones (below 20 s) on the 6th day before decapitation. In contrast to the trained with retention group, in the forgetting group there were no significant changes in catechol concentration and density of D_2 receptors in all the brain regions studied as compared with control levels (Table 1). Only the amygdala basolateral DA decreased by 15% compared with the control level.

Scheffe contrasts of obtained data confirmed that forgetting produced regional neurochemical patterns that significantly differed from those of the trained with retention, amnesia, trained without retention, and retrieval training groups, but not to a significant extent relative to control groups.

Effects of Posttraining "Psychogenic" Amnesia Production in Animals

Twenty four hours after "psychogenic" amnesia production, rats (the amnesia group) demonstrated poor retention latencies (below 20 s).

Similar neurochemical changes were observed in the rats of this group compared to the trained animals. However, alterations of catechol concentrations and values of B_{max} for D_2 receptors were not so significant in comparison with those of the trained group (Table 1). The DA level declined in the frontal cortex (42%) and hypothalamus (28%), and increased in the nucleus accumbens (120%), caudate nucleus (57%), and baso-lateral amygdala (113%) compared to the animals from the trained group. DOPAC increased in the frontal cortex (52%), hypothalamus (21%), nucleus accumbens (129%), caudate nucleus (55%), and baso-lateral amygdala (129%), and HVA increased in the nucleus accumbens (87%) and caudatus nucleus (32%). Animals of the amnesia group also had a decrease of p-TA in the frontal cortex (58%), hypothalamus (58%), nucleus accumbens (62%), nucleus caudatus (32%), baso-lateral amygdala (51%), and A10 cells (43%) as well as a decline in density of D_2 receptors in the frontal cortex (39%), hypothalamus (29%), nucleus accumbens (42%), caudate nucleus (32%), baso-lateral amygdala (30%), cortico-medial amygdala (22%), and hippocampus (30%) in comparison with the trained with retention rats. On the other hand, the neurochemical profile of rats of the amnesia group essentially differ from those of the control group: there were changes in DA concentrations, the appearance of p-TA in the frontal cortex, hypothalamus, amygdala baso-lateral, and A10 cells and its increases in the caudate nucleus and nucleus accumbens; also, D_2 receptor density was higher than control values of B_{max} (Table 1).

It is very interesting that, in some cases, amnesia production results in more significant increases of DA concentrations in the frontal cortex and hypothalamus than in the trained with retention animals (data not shown).

TABLE 1

MEAN CONCENTRATIONS (±SD) OF CATECHOLS AND A SUMMARY OF BINDING CHARACTERISTICS FOR [^{3}H]-DOPAMINE BINDING IN DIFFERENT BRAIN REGIONS OF RATS FROM THE CONTROL (C), TRAINED WITH RETENTION (T), AMNESIA (A), TRAINED WITHOUT RETENTION (U), RETRIEVAL TRAINING (R), AND FORGETTING (F) GROUPS

	Group Concentration (PMol/mg protein ± SD)				D2 Receptor Binding	
Group	DA	DOPAC	HVA	p-TA	B_{max} (FMol/mg) protein (± SD)	K_d (MN ± SD)
Frontal Cortex:						
C	4,16 ± 0,53	1,00 ± 0,14†	1,23 ± 0,19	ND	21 ± 2†	1,0 ± 0.1
T	14,05 ± 2,28*	0,60 ± 0,11*	0,88 ± 0,26*	14,09 ± 3,05*	56 ± 3*	1,0 ± 0,1
A	8,21 ± 1,30	0,91 ± 0,16†	0,88 ± 0,32*	5,93 ± 1,86	34 ± 4	0,9 ± 0,1
U	19,00 ± 3,19	0,91 ± 0,23†	1,09 ± 0,26	1,77 ± 0,84	28 ± 3	0,9 ± 0,1
R	12,93 ± 2,14*	0,63 ± 0,10*	0,95 ± 0,19†	15,63 ± 3,39*	56 ± 3*	1,0 ± 0,1
F	4,72 ± 0,60	0,91 ± 0,23†	0,95 ± 0,32†	ND	21 ± 2†	1,0 ± 0,1
Nucleus Accumbens:						
C	96,50 ± 10,79†	10,93 ± 1,19†	5,37 ± 0,63†	11,37 ± 1,60†	22 ± 2†	1,0 ± 0.1
T	26,30 ± 5,33*	2,93 ± 0,63	1,91 ± 0,69	56,86 ± 9,16*	55 ± 3*	1.0 ± 0.1
A	57,91 ± 6,39	6,72 ± 0,84	3,58 ± 0,88	21,72 ± 3,56	32 ± 3	0,9 ± 0,1
U	76,14 ± 9,58	9,81 ± 1,46	5,63 ± 0,88	13,74 ± 3,05	27 ± 3	0,9 ± 0,1
R	29,19 ± 6,23*	4,91 ± 0,91	2,42 ± 0,88	59,58 ± 9,51*	55 ± 3*	1,0 ± 0,1
F	102,14 ± 12,60†	10,09 ± 1,05†	5,23 ± 0,77†	11,02 ± 1,35†	22 ± 2†	1,0 ± 0,1
Amygdala Baso-Lateral:						
C	5,16 ± 0,53	1,95 ± 0,23	ND	ND	20 ± 2†	0,9 ± 0,1
T	1,21 ± 0,23	0,49 ± 0,14	ND	6,28 ± 1,35*	40 ± 3*	1,0 ± 0,1
A	2,58 ± 0,60	1,09 ± 0,30	ND	3,05 ± 0,77	28 ± 2	0,9 ± 0,1
U	4,72 ± 0,67	1,53 ± 0,42	ND	ND	24 ± 3	0,9 ± 0,1
R	1,44 ± 0,23	0,67 ± 0,14	ND	6,53 ± 1,53*	40 ± 3*	1,0 ± 0,1
F	4,39 ± 0,98	1,88 ± 0,28	ND	ND	20 ± 2†	0,9 ± 0,1
Amygdala Cortico-Medial:						
C	6,84 ± 0,84†	2,23 ± 0,26*	ND	ND	21 ± 2†	0,9 ± 0,1
T	5,77 ± 0,60*	2,19 ± 0,49*	ND	ND	41 ± 4*	1,0 ± 0,1
A	5,32 ± 1,07*	1,95 ± 0,53	ND	ND	32 ± 3	0,9 ± 0,1
U	6,53 ± 0,77†	2,23 ± 0,28*	ND	ND	24 ± 3	0,9 ± 0,1
R	6,77 ± 0,91†	2,30 ± 0,28*	ND	ND	41 ± 4*	1,0 ± 0,1
F	6,23 ± 0,98	2,16 ± 0,28*	ND	ND	21 ± 2†	0,9 ± 0,1
A 10 Cells:						
C	32,98 ± 4,09†	6,02 ± 0,70	ND	ND	5 ± 1†	0,8 ± 0,1
T	35,56 ± 5,33*	6,72 ± 1,05*	ND	24,28 ± 5,93*	10 ± 2*	0,9 ± 0,1
A	34,19 ± 5,60*†	6,51 ± 1,12*	ND	13,74 ± 2,88	7 ± 1	0,9 ± 0,1
U	33,21 ± 4,32†	6,93 ± 0,77*	ND	ND	6 ± 1	0,9 ± 0,1
R	32,53 ± 3,49†	6,16 ± 0,98	ND	25,63 ± 6,53*	10 ± 2*	0,9 ± 0,1
F	31,30 ± 4,26†	5,81 ± 0,77	ND	ND	5 ± 1†	0,9 ± 0,1
A 9 Cells:						
C	31,91 ± 6,53†	21,02 ± 3,79†	ND	ND	3 ± 1†	0,8 ± 0,1
T	34,21 ± 6,84*	19,19 ± 4,77*	ND	ND	5 ± 1*	0,9 ± 0,1
A	37,83 ± 5,93	21,30 ± 4,49†	ND	ND	3 ± 1†	0,9 ± 0,1
U	31,60 ± 6,53†	21,58 ± 3,93†	ND	ND	3 ± 1†	0,9 ± 0,1
R	34,65 ± 6,28*	20,09 ± 4,07*	ND	ND	4 ± 1*	0,9 ± 0,1
F	35,42 ± 6,53	20,60 ± 3,79*†	ND	ND	3 ± 1†	0,8 ± 0,1
Caudatus Nucleus:						
C	102,60 ± 9,37†	12,32 ± 1,46†	7,79 ± 1,21†	12,72 ± 6,44†	25 ± 2†	1,0 ± 0.1
T	40,28 ± 4,72	6,02 ± 1,21*	4,08 ± 0,88	50,07 ± 6,85*	50 ± 2*	1,0 ± 0,1
A	63,07 ± 6,53	9,35 ± 1,26	6,58 ± 1,09	25,63 ± 3,56	34 ± 2	1,0 ± 0,1
U	79,79 ± 10,86	10,93 ± 1,60	7,42 ± 1,39†	16,62 ± 5,42	30 ± 3	1,0 ± 0,1
R	38,60 ± 5,77	6,30 ± 1,19*	5,23 ± 1,02	47,19 ± 9,00*	47 ± 3	1,0 ± 0,1
F	95,91 ± 10,32†	12,00 ± 1,67†	7,53 ± 1,09†	12,39 ± 3,56†	25 ± 2†	1,0 ± 0,1

(continued)

TABLE 1

(continued)

Group	Group Concentration (PMol/mg protein ± SD)				D2 Receptor Binding	
	DA	DOPAC	HVA	p-TA	B_{max} (FMol/mg) protein (± SD)	K_d (MN ± SD)
Hippocampus:						
C	1,67 ± 0,21	ND	ND	ND	23 ± 3†	0,9 ± 0,1
T	1,44 ± 0,23*	ND	ND	ND	46 ± 3*	1,0 ± 0,1
A	1,51 ± 0,19†	ND	ND	ND	32 ± 3	0,9 ± 0,1
U	1,60 ± 0,28	ND	ND	ND	29 ± 3	0,9 ± 0,1
R	1,49 ± 0,26†	ND	ND	ND	47 ± 3*	1,0 ± 0,1
F	1,47 ± 0,28*†	ND	ND	ND	24 ± 2†	0,9 ± 0,1
Hypothalamus:						
C	5,53 ± 0,47†	2,00 ± 0,21†	0,25 ± 0,03†	ND	16 ± 2†	0,9 ± 0,10
T	10,33 ± 1,14	1,49 ± 0,30	0,20 ± 0,04	4,42 ± 1,35	42 ± 4	1,0 ± 0,10
A	7,44 ± 0,77	1,81 ± 0,26†	0,27 ± 0,03†	1,86 ± 0,77	30 ± 2	0,8 ± 0,10
U	10,79 ± 1,30	1,67 ± 0,32†	0,23 ± 0,03†	0,93 ± 0,53	26 ± 2	0,9 ± 0,05
R	10,12 ± 1,07	1,60 ± 0,30	0,26 ± 0,04†	4,07 ± 1,28	39 ± 2	1,0 ± 0,10
F	5,70 ± 0,60†	1,97 ± 0,26†	0,25 ± 0,04†	ND	16 ± 2†	0,9 ± 0,05
A 11 Cells:						
C	32,37 ± 3,65†	ND	ND	ND	ND	ND
T	34,21 ± 4,02*	ND	ND	ND	ND	ND
A	35,12 ± 4,25*	ND	ND	ND	ND	ND
U	33,14 ± 3,79†	ND	ND	ND	ND	ND
R	33,58 ± 4,09†	ND	ND	ND	ND	ND
F	34,81 ± 3,49*	ND	ND	ND	ND	ND
(B7 + B8) Cells:						
C	23,72 ± 2,58†	ND	ND	ND	ND	ND
T	20,37 ± 3,19*	ND	ND	ND	ND	ND
A	22,18 ± 2,88*	ND	ND	ND	ND	ND
U	24,58 ± 2,65	ND	ND	ND	ND	ND
R	23,60 ± 3,02†	ND	ND	ND	ND	ND
F	22,95 ± 2,81*	ND	ND	ND	ND	ND

Note: n = 10–15 for all groups.
ND, not detected.
* or †, no statistically significant differences were found between groups with identical symbols; all remaining pair comparisons have demonstrated significant differences, Scheffe post hoc contrasts ($p < 0.01$).

Scheffe comparisons have shown that "psychogenic" amnesia produced other regional neurochemical patterns in comparison with those of control, trained with retention, trained without retention, forgetting, and retrieval training groups (Table 1)

Effects of Foot-Shock Training in Nonretention but Trained Rats

Twenty-four hours after foot-shock training, the trained without retention animals demonstrated short retention latencies (below 20 s).

Substantially different neurochemical changes were observed in the trained, without retention rats (Table 1) as compared with animals from the other groups. This conclusion was supported by Scheffe contrasts of all obtained neurochemical data. There were extreme increases of DA in the frontal cortex (357%) and hypothalamus (95%). However, only very small amounts of p-TA and insignificant increases in density of D_2 receptors were observed in the frontal cortex (33%) and hypothalamus (62%) of the nonretention but trained rats compared to the control ones.

Effects of Reminder Treatment in Animals After Amnesia Production

Twenty-four hours after reminder treatment, the animals of the amnesia group demonstrated long retention latencies (180 s) and 24 h after reminder treatment neurochemical changes in the structures of the dopaminergic mesocorticolimbic and nigrostriatal systems of rats with retrieval retention performance were similar to those in the trained with retention rats (Table 1, the trained and retrieval training groups). As mentioned above, Scheffe comparisons have shown only some differences (mainly in DA level) between the regional neurochemical patterns of rats of the retrieval training group and trained with retention group, but significant differences relative to the other groups (Table 1).

DISCUSSION

The passive avoidance task performance and brain neurochemical correlates presented in this study are dealing with a basis for memory as opposed to motor activity in rats, because similar step-through latency data were shown by poor avoiders

TABLE 2

ANALYSES OF VARIANCE OF EFFECTS OF PROCESSES OF MEMORY ON THE REGIONAL LEVELS OF DOPAMINE, ITS METABOLITES, AND D_2 RECEPTOR DENSITY (ANOVA)*

Brain region	df	F Value	Probability
Dopamine			
Frontal cortex	5	274	0,00000
Nucleus accumbens	5	249	0,00000
Amygdala baso-lateral	5	218	0,00000
Amygdala cortico-medial	5	31	0,00000
Caudatus nucleus	5	2007	0,00000
Hippocampus	5	107	0,00000
Hypothalamus	5	575	0,00000
A9 Group	5	24	0,00000
A10 Group	5	7	0,00012
A11 Group	5	8	0,00000
(B7 + B8) Group	5	11	0,00000
Dopac			
Frontal cortex	5	20	0,00000
Nucleus accumbens	5	146	0,00000
Amygdala baso-lateral	5	233	0,00000
Amygdala cortico-medial	5	2	0,04908
Caudatus nucleus	5	462	0,00000
Hippocampus	–	–	–
Hypothalamus	5	51	0,00000
A9 Group	5	4	0,00263
A10 Group	5	37	0,00000
A11 Group	–	–	–
(B7 + B8) Group	–	–	–
HVA			
Frontal cortex	5	91	0,00000
Nucleus accumbens	5	228	0,00000
Amygdala baso-lateral	–	–	–
Amygdala cortico-medial	–	–	–
Caudatus nucleus	5	1152	0,00000
Hippocampus	–	–	–
Hypothalamus	5	0.2	0,96259
A9 Group	–	–	–
A10 Group	–	–	–
A11 Group	–	–	–
(B7 + B8) Group	–	–	–
para-Tyramine			
Frontal cortex	3	150	0,00000
Nucleus accumbens	5	215	0,00000
Amygdala baso-lateral	2	197	0,00000
Amygdala cortico-medial	–	–	–
Caudatus nucleus	5	224	0,00000
Hippocampus	–	–	–
Hypothalamus	3	378	0,00000
A9 Group	–	–	–
A10 Group	2	184	0,00000
A11 Group	–	–	–
(B7 + B8) Group	–	–	–
B_{max} of D_2 Receptors			
Frontal cortex	5	1097	0,00000
Nucleus accumbens	5	1465	0,00000
Amygdala baso-lateral	5	236	0,00000
Amygdala cortico-medial	5	224	0,00000
Caudatus nucleus	5	744	0,00000
Hippocampus	5	2332	0,00000
Hypothalamus	5	871	0,00000
A9 Group	5	20	0,00000
A10 Group	5	75	0,00000
A11 Group	–	–	–
(B7 + B8) Group	–	–	–

*Source of variance is overall model.

and controls with different neurochemical brain patterns (Table 1, Trained without retention, Forgetting, Amnesia and Control groups).

Despite the existing considerable data on the role of the dopaminergic system in memory modulation and learning processes, there are few regional brain studies with biochemical indices of system functioning in normal nondrug-treated animals. It was indicated that high DA cortical concentration facilitated learning of an alternation task (36). The decrease of DA level in the neostriatum and its increase in neocortex and brainstem were observed 10 min after training in a one-trial passive avoidance task (10). Recent studies have shown that changes in prefrontal D_2 receptors, an increase in density, and a decrease in affinity, are observed about one day following foot-shock stress in rats (26). Our previous studies demonstrated that one-trial passive avoidance training resulted after 24 h in DA and DOPAC decreases, as well as in increases of p-TA and D_2 receptor density in the striatum, increases of DA content, D_2 receptor density, and the appearance of significant amounts of p-TA in the hypothalamus, amygdala, and frontal cortex of trained rats (32).

In the present study, the characteristics of specific [^{3}H]-DA binding to synaptic membrane receptors as well as DA, DOPAC, HVA, and p-TA contents have been determined in the structures of mesocorticolimbic, nigrostriatal systems and the hypothalamus under conditions in which the response output was observed or not (Table 1). The results obtained here are consistent with the ones mentioned above. Neurochemical profiles presented are not associated with foot-shock stress. Foot-shock stress itself appears to increase the level of DOPAC in the mesocorticolimbic system (5,11,35), whereas a marked decrease of DOPAC content in the frontal cortex, nucleus accumbens, caudatus nucleus, and baso-lateral amygdala was observed in the present series of experiments (Table 1). A

decrease of DOPAC/dopamine ratio in the nucleus accumbens and medial prefrontal cortex of trained "learner" rats was observed (9). These changes of DOPAC level may be caused by the diminution of deaminating system activity because, according to recent data, decreasing monoamine oxidase (MAO) activity in the frontal cortex (the lowering of V_{max} for DA deamination) and striatum (the lowering of MAO affinity for DA) of trained rats may be observed about one day following training foot shock (28). Our findings support the reciprocal relationship between dopamine metabolism and p-tyramine concentration (15,16). The reduction of dopamine metabolism in the structures of the mesocorticolimbic and nigrostriatal systems one day and later after foot-shock training was accompanied by the appearance of p-TA in the frontal cortex, baso-lateral amygdala, A10 group, hypothalamus, and its increase in the nucleus accumbens and caudatus nucleus (Table 1). We associate the phenomenon of the appearance of marked amounts of p-TA and a significant increase in D_2 receptor density in the brain tissue with D_2 receptor functioning after training (31). P-TA was shown to be produced from DA bound by D_2 receptors of the synaptic membranes of the trained rats in the electrolyte solution that was exposed to the influence of microdischarge electroradiolysis in vitro (31,33). We suppose that both the lower p-TA level (or its absence) and low D_2 receptor density in the structures of the mesocorticolimbic, nigrostriatal systems, and hypothalamus of the rats from the amnesia and untrained groups possibly reflect a significantly low level of the activation and functioning of D_2 receptors compared with those of the trained rats (Table 1). Facilitation of memory retrieval by administered (+)- and (−)-3-PPP as well as quinpirole (16) indirectly supports our conclusion because (+)- and (−)-3-PPP and quinpirole as dopamine agonists produce changes in the pre- or postsynaptic dopamine receptors.

Neurochemical brain patterns of rats of all the groups studied clearly show that both DA, p-TA concentrations and D_2 receptor density play a very important role in dopaminergic system activation that increases the probability and vigor of response. The mode of p-TA action in the neurochemical mechanisms of memory is not determined, but the some biochemical significance of this monoamine is known. There are data on p-TA-induced DA releasing and its ability to inhibit DA uptake (8); p-TA seems to be a reversible, endogenously produced, high-affinity marker of the vesicular carrier for DA and there are data supporting the putative functional involvement of p-TA sites in the vesicular transport of DA (41).

Antagonists of dopamine metabolism (reserpine, α-methyl-p-tyrosine, chlorpromazine, haloperidol) are known to interfere with learning and memory, whereas agonists—dopamine-stimulating agents (amphetamine, lisuride, pergolide, apomorphine, nomifensine, bupropion, amfonelic acid, amantadine) can improve these processes (1,6,7,26). However, the contrary effects may also occur. Antagonists such as reserpine (25) can facilitate the performance of learning in the rat as well as α-amphetamine, an agonist, can impair its retention (17). The results of the present experiments permit an interpretation of such paradoxical effects, because both higher and lower DA levels alone do not change the response output; however, in addition, a high level of activation of D_2 receptors and production of p-TA are necessary for probable response output and its conservation (Table 1).

Considering the experimental data presented here and the role of the dopaminergic system in memory and learning processes, we suggest the existence of a mechanism connecting DA, p-TA concentrations, and D_2 receptor density; this mechanism is the base of dopaminergic system functioning. Its initiation leads to response output (the trained and retrieval training groups, Table 1) and its natural reverse leads to forgetting (the forgetting group, Table 1). Its disruption results in amnesia or poor retention (the amnesia and trained, without retention groups, Table 1). The proposed mechanism is reflected in variance of quantitative relationships between DA, D_2 receptors and p-TA. Such relationships are region-specific due to anatomical, biochemical, and functional differences between the brain areas. In summary, the neurochemical database has been obtained for further studies of the subtle mechanisms of dopaminergic functioning in cognitive processes and behavioral response expression.

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