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Age-related changes in fear behavior and regional brain monoamines distribution in rats

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Abstract. Differences in fear level assessment based on the time of motionless in the illuminated compartment, time spent in light compartment, number of head dipping from dark to the illuminated compartment and number of returns from dark to the illuminated compartment registered in light/dark transitions test and brain monoamines (NA, DA, 5-HT) and their metabolites (MHPG, DOPAC, 5-HIAA) in the hypothalamus, midbrain, amygdala, hippocampus and pons were examined in 3, 12 and 24 months old Wistar rats. The lowest level of fear was registered in 12 months old rats, a slightly higher level in 3 months old rats and the highest in 24 months old rats. Locomotion activity showed a decreasing tendency within age according to a linear dependence in 3, 12 and 24 months old rats. Neurochemical data showed the decreased activity of NA system and increased activity of DA system in most structures already occurred in 12 months old rats. It remained at the same level in aged rats. The correlation analysis between the behavioral markers of fear level and distribution of monoamines in young, mature and aged rats showed diversified data, only some of them being consistent with the "serotonergic hypothesis" of fear/anxiety. Therefore, we cannot conclude what neurochemical background of fear is.

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Key words: aging, fear, light/dark transitions test, Etho-Vision, brain monoamines, HPLC, rats

INTRODUCTION

Evidence for age-dependent gradual deterioration of a variety of physiological functions in humans and animals has been indicated in many clinical and experimental reports. It is well documented that during normal aging a continuous decline in learning and memory process (Lamberty and Gower 1992, McEntee and Crook 1992, Miyagawa et al. 1998, Rasmussen et al. 1996, Van der Wolf and Baker 1986), in motor activity (Chen et al. 1994, Erim et al. 1999, Fozard et al. 1994, Kish et al. 1992) and endocrinology mechanisms (for reviews see De Kosky and Palmer 1994, Rehman and Masson 2001) occurs.

Many reports indicate that during normal aging of mammals prominent alterations occur in various neurotransmitter systems in the brain, related to reductions in the number of neurons and to a decrease in concentration, synthesis and turnover of neurotransmitters, but literature provides contradictory results on age-related changes in the adrenergic, cholinergic, dopaminergic and serotonergic systems (for reviews see Finch and Roth 1999, Fisher et al. 1992, McEntee and Crook 1991, Morgan and May 1990, Pradham 1980).

In some data concerning the age-related changes in the noradrenergic system the reduction in the NA content in old rats was described in the spinal cord, brainstem and limbic areas (Leslie et al. 1985, Miguez et al. 1999, Ponzio et al. 1982, Roubein et al. 1986, Sirvio et al. 1994), but opposite results, i.e., an increase in the NA content in aged rats were reported in the hypothalamus, striatum and cerebral cortex (Harik and Mc Cracken 1986, Machado et al. 1986, Moretti et al. 1987).

Contrasting results were also obtained for age-related alterations in the serotonergic system. The majority of data indicated age-related decrease in the content, turnover and uptake of 5-HT in the rat limbic areas, striatum, brainstem and frontal cortex (Brunello et al. 1988, Machado et al. 1986, Miguez et al. 1999, Roubien et al. 1986, Strong et al. 1984, Venero et al. 1991). Also evidence for unchanged (Robson et al. 1993) or enhanced (Moretti et al. 1987, Simpkins et al. 1977, Timiras et al. 1982) 5-HT metabolism was observed in the rat hypothalamus, hippocampus and frontal cortex. Moreover, Gozlan et al. (1990) observed age-dependent decreases in 5-HT levels associated with parallel increases in 5-HIAA/5-HT ratio in the hypothalamus, hippocampus, striatum and cerebral cortex, suggesting an accelerated 5-HT turnover in aged rats.

Results of age-related alterations in the dopaminergic system are relatively compatible. During normal aging the following gradual changes have been observed: continuous decline in DA and/or DOPAC levels (Kish et al. 1992, Ma et al. 1999, Machado et al. 1986, Miguez et al. 1999, Moretti et al. 1987, Ponzio et al. 1982, Santiago et al. 1988, Strong et al. 1982, Venero et al. 1991), impairment of DA synthesis and metabolism (Carfagna et al. 1985, Finch 1976, Moretti et al. 1987, Ponzio et al. 1978, Reis et al. 1977, Simpkins et al. 1977, Venero et al. 1991) and a loss of dopaminergic neurons (Brizee et al. 1998, Gerhardt et al. 2002, Siddigi et al. 1999). These alterations occur mainly in the striatum, substantia nigra, putamen, nucleus caudatus, amygdala, hippocampus and hypothalamus.

It is well known from clinical observations that in humans significant alterations occur in the emotional behavior during aging (Duman et al. 1997, Lang et al. 1998, Patel and Hope 1993, Stokes 1996, Weingartner et al. 1981). There are few data referring to age-related changes in emotional behavior in animals what is probably caused by scarcity of respective experimental tests and procedures to investigate such complex brain mechanisms. Most useful in examining of these processes are fear/anxiety and aggressive behavior patterns, especially in rodents because there are numerous well-developed tests based on natural responses of these animals to various stimuli in their environment.

There are very few data referring to aggressive behavior during aging in rodents (Blanchard et al. 1984, 1988). These reports are not unequivocal because some authors (Blanchard et al. 1984, 1988) did not observe any significant age-related changes in such behavior whereas others noticed a decrease in aggressive behavior in old rats (Takahashi and Lore 1982) and in old mice (Engellenner et al. 1986) in comparison with young and mature ones.

However, data referring to age-related changes in fear/anxiety behavior are conforming. In different tests, i.e., open field, plus maze and hole box, an increased level of fear/anxiety in old rats and mice was observed (Blokland and Raaijmakers 1993, Boguszewski and Zagrodzka 2002, Frussa-Filho et al. 1992, Lamberty and Gower 1993, Miyagawa et al. 1998). Research made by Boguszewski and Zagrodzka (2002) provides a penetrating comparative analysis of behavior in 4- and 24-months old rats investigated in the open field with illuminated center, plus maze and social interactions tests. These authors found decrease of motor activity

and higher anxiety level in old rats as compared to young in the open field and in plus maze test, and that the number and time of social interactions did not show age-related differences. Moreover, it was demonstrated that the increased level of anxiety in old rats did not result from their decreased motor activity. It was also found out that in both age groups anxiety and motor activity were independent of each other.

The present study is undertaken to give an answer to the following questions: (i) are there age-related changes in fear behavior evaluated in the light/dark transition test; and (ii) are there differences between the age groups (3, 12 and 24 months old) in the content of monoamines (NA, DA, 5-HT) and their metabolites (MHPG, DOPAC, 5-HIAA) in the hypothalamus, amygdala, hippocampus, midbrain central gray matter and pons – the key areas forming the brain emotional-defensive system? Such an approach should give us comprehensive information on behavioral and neurochemical age-related changes in fear mechanisms.

METHODS

Animals

Male Wistar rats of three different ages, i.e., young – 3 months old ($n = 6$), middle aged, mature – 12 months old ($n = 6$) and 24 months old ($n = 6$) were used in the study. We assumed such a division into the age groups according to the studies of Gozlan et al. (1990) and Nashimura et al. (1998). Rats were bred in the licensed animal husbandry of the Institute of Occupational Medicine in Łódź. The animals were housed in groups, 3-4 per cage, and maintained under controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and on a 12L:12D cycle (light on at 8 h) with food and water available continuously. Experiments were carried out between 9 and 12 a.m. In the experiments rats of normal, without deficits in locomotor activity were used. The experimental procedures have been approved by the Regional Ethics Committee in Łódź for the Use and Care of Laboratory Animals.

Behavioral procedure

In order to measure the reaction of animals to the stressogenic aversive stimulus the modified version of light/dark transitions test (LDT) first described by Crawley and Goodwin (1980) was used. The experimental chamber was divided into two compartments (40 × 40 × 50 cm each) with an opening allowing the animal to change its location. The light conditions were automatically changed at selected order – a bulb (900 lx) located 50 cm above the floor of each compartment brightly illuminated only one compartment at the time, leaving the other one dark and safe. The experiment began after 1 min when the rat was introduced into the experimental chamber. One experimental session consisted of 5 trials with light stimulus. The time duration of the light stimulus was 60 s, and intervals between trials (dark time) were irregular: 120 s between trials 1 and 2, and between 3 and 4, 90 s between trials 2 and 3, and between 4 and 5.

The experimental room was illuminated with a weak red bulb (4 lx). All behavioral experiments were videotaped and next analyzed with the Etho Vision 1.90 software (Noldus, Wageningen, The Netherlands). We measured the following variables of rats' behavior in the experimental chamber: (i) time spent in the illuminated compartment, i.e., time elapsed from the moment of switching on the aversive light stimulus to the moment of the rat's passing to the dark compartment, individualizing here a time of locomotion activity and time of motionless behavior; (ii) time spent in the illuminated compartment after returns from the dark compartment; (iii) time of locomotion activity in the dark compartment; (iv) time of motionless behavior in the dark compartment; (v) number of returns from the dark to the illuminated compartment; (vi) number of head dipping from the dark to the illuminated compartment.

Each animal was individually habituated to the experimental conditions. Rats were introduced into the experimental chamber for 5 min in the absence of any external stimuli. Then the basic experiment was performed.

Biochemical analysis

The concentrations of NA, DA, 5-HT, MHPG, DOPAC and 5-HIAA were determined in the selected brain regions using high-performance liquid chromatography with electrochemical detection (HPLC-ED).

Sample preparation

Five days after the behavioral test all rats from three age-groups were killed by decapitation. Their brains were rapidly removed and kept frozen at -70°C. Next

day the selected brain regions, i.e., hypothalamus (HPT), midbrain central gray matter (MID), amygdala (AMY), hippocampus (HIP) and the pons (PO) were dissected according to the stereotaxic atlas of Paxinos and Watson (1982), placed into the Eppendorf tubes and weighed. Afterwards each brain tissue was homogenized with an ultrasonic cell disrupter (Vibracell 72434, Bioblock, Illkrich-Cedex) in 150 µl 0.1 M perchloric acid containing 0.4 mM sodium metabisulphite. The homogenates were then centrifuged at 10,000 × g for 25 min at 4°C and the supernatants were filtrated through a 0.22 µm filter (Sigma) and frozen at -70°C until analysis. Next 5 µl of filtrates was injected into the HPLC system.

Chromatographic and detection conditions

The HPLC system consisted of a quaternary delivery pump Model HP 1100 (Hewlett-Packard) and an analytical column ODS 2 C18, 4.6 × 250 mm, particle size 5 µm (Hewlett-Packard) protected by guard column (4.6 × 12.5 mm), particle size 5 µm (Hewlett-Packard). The electrochemical detector HP 1049A (Hewlett-Packard) with glassy carbon working electrode was used at a voltage setting of +0.65 V for monoamines and their metabolites vs. an Ag/AgCl reference electrode. Data acquisition was performed using an Agilent ChemStation for LC 3D Systems. The concentration of monoamines and their metabolites were expressed as ng/g wet tissue.

Monoamines and their metabolites determination

The mobile phase comprised a 0.15 M sodium dihydrogen phosphate, 0.1 mM EDTA, 0.5 mM sodium octanesulphonic acid, 10-12% methanol (v/v) and 5 mM lithium chloride. The mobile phase was adjusted to pH 3.4 with ortophosphoric acid and filtrated through 0.22 µm filter (Sigma). Flow rate 1.4 ml/min and a column temperature 32°C were used.

Chemicals

Methanol was purchased from Merck. Other chemicals for HPLC were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistics

The behavioral data were analyzed by the Kruskal-Wallis ANOVA followed by the Mann-Whitney U test, and biochemical results by the two-way ANOVA followed by the planned contrast test. Moreover, correlation between the most indicative markers of the fear level, i.e., time of motionless behavior (TML) in the illuminated compartment, number of head dipping (HDD) from the dark to the illuminated compartment, number of returns (RET) from the dark to the illuminated compartment, time spent in the illuminated compartment (TSL) and changes in content of

Table I

Behavioral events registered during light-dark transitions test in rats of different age

Behavioral events	Group				
	Young	Mature		Aged	
1. Time spent in light (s)	30.0 ± 2.8	12.1 ± 0.6	(a) $P<0.003$	40.1 ± 5.6	(b) $P<0.003$
time of locomotion (s)	24.7 ± 2.4	11.6 ± 0.6	(a) $P<0.003$	25.5 ± 3.7	(b) $P<0.003$
time of motionless (s)	5.3 ± 2.5	0.5 ± 0.1	(a) $P<0.004$	14.6 ± 4.8	(b) $P<0.004$
2. Time spent in light after returns from dark (s)	2.0 ± 1.3	2.3 ± 1.1		0	
3. Time of locomotion in dark (s)	25.9 ± 3.0	36.3 ± 3.7	(a) $P<0.03$	13.7 ± 3.9	(b) $P<0.006$
4. Time of motionless in dark (s)	3.9 ± 1.8	11.5 ± 3.4		6.2 ± 3.2	
5. Number of returns from dark to light	0.1 ± 0.08	0.2 ± 0.08		0	
6. Number of head dipping from dark to light	1.0 ± 0.3	1.2 ± 0.2		0.4 ± 0.1	

Values are mean ± SEM, $n = 6$ for each group. Statistical significance: Mann-Whitney U test; (a) compared to young; (b) compared to mature.

monoamines (NA, DA, 5-HT), their metabolites (MHPG, DOPAC, 5-HIAA), ratio of metabolites to their parent amines in the selected brain regions (HPT, MID, AMY, HIP, PO) were analyzed using Pearson's linear correlation coefficient.

RESULTS

Behavioral data

Behavioral events registered during light/dark transitions test in rats of different age are presented in details in Table I.

In the illuminated compartment of the experimental chamber there was a decrease by 53.1% (P<0.003) in the time of locomotion activity in mature vs. young rats, and an increase by 119.8% (P<0.003) in aged vs. mature rats (the Mann-Whitney U test). Simultaneously, a decrease by 90.6% (P<0.004) in the time of motionless behavior in the illuminated compartment was registered in mature vs. young rats, and an increase by 192% (P<0.004) in aged vs. mature rats. The total time spent in the illuminated compartment (time of locomotion activity + time of motionless behavior) decreased by 59.7% (P<0.003) in mature vs. young rats, and increased by 33.6% (P<0.003) in aged vs. mature rats. In the dark compartment there was an increase by 40.1% (P<0.03) in the time of locomotion activity in mature vs. young rats, and a decrease by 62.3% (P< 0.006) in aged vs. mature rats (the Mann-Whitney U test). No significant changes were observed between different age groups in the time spent in the illuminated compartment after returns from dark to the illuminated compartment, in the time of motionless behavior in the dark compartment, in the number of returns from dark to the illuminated compartment, and in the number of head dipping from dark to the illuminated compartment.

Biochemical data

Regional brain concentrations of monoamines, their metabolites and the ratio of metabolites to their parent amines are demonstrated in detail in Tables II, III and IV.

Table II

Regional brain concentrations (ng/g wet tissue) of NA,DA and 5-HT in rats of different age

Group	Brain region	NA		DA		5-HT	
Young	HPT	1765.8 ± 275.0		824.7 ± 388.2		728.8 ± 130.1	
Mature		6446.8 ± 847.5	(a)****	1632.0 ± 192.0		854.6 ± 67.4	
Age		2483.2 ± 141.2	(b)****	286.0 ± 23.9	(b)***	1035.1 ± 23.2	
Young	MID	1403.1 ± 101.7		337.9 ± 66.9		1330.5 ± 92.6	
Mature		2900.2 ± 183.0	(a)****	894.1 ± 83.3		1457.5 ± 123.9	
Age		1100.7 ± 112.1	(b)****	191.4 ± 12.3		1246.5 ± 107.3	
Young	AMY	877.2 ± 123.5		4080.3 ± 735.6		1259.6 ± 85.1	
Mature		3086.5 ± 520.3	(a)****	6865.4 ± 507.2	(a)****	1600.5 ± 134.1	(a)*
Age		1094.9 ± 78.4	(b)****	2441.6 ± 334.9	(a)****	837.0 ± 86.7	(a)***
					(b)****		(b)****
Young	HIP	225.0 ± 31.1		1056.9 ± 375.8		697.0 ± 61.3	
Mature		2406.4 ± 399.1	(a)****	3170.9 ± 753.4	(a)****	973.1 ± 113.2	
Age		750.0 ± 40.4	(b)****	215.7 ± 30.6	(b)****	407.3 ± 21.3	(b)****
Young	PO	1422.7 ± 201.4		210.7 ± 24.6		1367.7 ± 84.8	
Mature		4018.0 ± 385.3	(a)****	503.6 ± 30.9		1289.5 ± 240.4	
Age		1613.1 ± 170.7	(b)****	199.2 ± 19.0		1118.9 ± 179.2	

Values are mean ± SEM, n = 6 for each group. Statistical significance: two-way ANOVA followed by planned contrast test; (*) P<0.05; (**) P<0.02; (***) P<0.01; (****) P<0.001; (a) compared to young; (b) compared to mature.

NA, MHPG concentrations and MHPG/NA ratio

We have found significant differences between the age groups in the contents of NA ($F_{2,75}$=102.04, P<0.001), of MHPG ($F_{2,75}$=8.35, P<0.001) and of MHPG/NA ratio ($F_{2,75}$=14.17, P<0.001) (ANOVA). Further analysis by means of planned contrast test showed that the concentration of NA was higher in HPT by 265%, in MID by 106.7%, in AMY by 251.8%, in HIP by 969.5% and in PO by 182.4% in mature vs. young rats. The NA concentration was lower in HPT by 61.5%, in MID by 62.1%, in AMY by 64.5%, in HIP by 68.8% and in PO by 59.9% in aged vs. mature rats. No significant differences occurred in the concentration of NA in any brain area in aged vs. young rats. The concentration of MHPG was lower only in MID by 94.3% in aged vs. young rats, and lower only in HPT by 97.7% in aged vs. mature rats. No significant differences occurred in the concentration of MHPG in mature vs. young rats in any brain area. The MHPG/NA ratio was lower in AMY by 88% and in HIP by 90.4% in mature vs. young rats, and lower in MID by 94.6%, in AMY by 78.5% and in HIP by 80.7% in aged vs. young rats. No significant differences occurred in the MHPG/NA ratio in any brain area in aged vs. mature rats.

DA, DOPAC concentrations and DOPAC/DA ratio

There were significant differences between the age groups in the contents of DA ($F_{2,75}$=40.56, P<0.001), DOPAC ($F_{2,75}$=53.30, P<0.001) and DOPAC/DA ratio ($F_{2,75}$=19.38, P<0.001) (ANOVA). Further analysis by means of planned contrast test showed that the concentration of DA was higher in AMY by 68.2% and in HIP by 200% in mature vs. young rats. The DA concentration was lower only in AMY by 40.2% in aged vs. young rats, and lower in HPT by 82.5%, in AMY by 64.5% and in HIP by 93.2% in aged vs. mature rats. The DOPAC concentration was higher in HPT by 176.4%, in MID by 517.7%, in AMY by 136% and in HIP by 183.6% in mature vs. young rats. The concentration of DOPAC was

Table III

Regional brain concentrations (ng/g wet tissue) of MHPG, DOPAC and 5-HIAA in rats of different age

Group	Brain region	MHPG		DOPAC		5-HIAA	
Young	HPT	73.9 ± 22.7		111.1 ± 52.7		556.6 ± 96.6	
Mature		86.9 ± 38.8		307.1 ± 37.5	(a)***	676.7 ± 102.0	
Age		2.0 ± 1.4		67.3 ± 6.4	(b)****	655.0 ± 47.6	
Young	MID	151.3 ± 52.6		31.0 ± 7.8		908.8 ± 23.4	
Mature		87.1 ± 37.9		191.5 ± 28.9	(a)***	1321.1 ± 139.2	(a)**
Age		8.7 ± 4.6	(a)***	41.0 ± 4.0	(b)**	1000.3 ± 152.1	
Young	AMY	102.8 ± 39.3		403.7 ± 63.7		653.4 ± 86.7	
Mature		46.6 ± 21.2		952.9 ± 111.4	(a)****	824.0 ± 92.2	
Age		32.2 ± 29.4		276.9 ± 41.9	(b)****	415.9 ± 29.7	(b)**
Young	HIP	42.7 ± 20.2		120.8 ± 61.3		506.8 ± 17.3	
Mature		28.0 ± 11.4		342.6 ± 75.5	(a)****	600.1 ± 101.6	
Age		26.0 ± 13.7		23.2 ± 6.8	(b)****	364.9 ± 21.8	
Young	PO	79.2 ± 39.9		20.0 ± 7.0		1157.4 ± 134.2	
Mature		134.3 ± 48.1		135.5 ± 22.8		1176.2 ± 249.0	
Age		13.1 ± 4.1	(b)**	85.9 ± 23.2		861.2 ± 265.1	

Values are mean ± SEM, n = 6 for each group. Statistical significance: two-way ANOVA followed by planned contrast test; (*) P<0.05; (**) P<0.02; (***) P<0.01; (****) P<0.001; (a) compared to young; (b) compared to mature.

lower in HPT by 78.1%, in MID by 78.6%, in AMY by 71% and in HIP by 93.2% in aged vs. mature rats. No significant differences occurred in the DOPAC concentration in any brain area in aged vs. young rats. The DOPAC/DA ratio was higher in MID by 125.8% and in PO by 203.4% in mature vs. young rats, it was also higher in HPT by 93.4% in MID by 129% and in PO by 372.7% in aged vs. young rats, and also higher only in PO by 55.8% in aged vs. mature rats.

5-HT, 5-HIAA concentrations and 5-HIAA/5-HT ratio

We have found significant differences between the age groups in the contents of 5-HT ($F_{2,75}$=8.59, P<0.001), of 5-HIAA ($F_{2,75}$=5.32, P<0.01) and 5-HIAA/5-HT ratio ($F_{2,75}$=10.10, P<0.001) (ANOVA). Further analysis by means of planned contrast test showed that the 5-HT concentration was higher only in AMY by 27% in mature vs. young rats, and was lower only in AMY by 33.6% in aged vs. young rats, and was lower in AMY by 47.7% and in HIP by 58.2% in aged vs. mature rats. The concentration of 5-HIAA was higher in MID by 45.3% in mature vs. young rats, and lower only in AMY by 49.5% in aged vs. mature rats. No significant differences occurred in the concentration of 5-HIAA in any brain area in aged vs. young rats. The 5-HIAA/5-HT ratio was higher only in MID by 29% in mature vs. young rats, higher in HIP by 47.4% and lower in PO by 20.5% in aged vs. mature rats. No significant differences occurred in the 5-HIAA/5-HT ratio in any brain area in aged vs. young rats.

Correlation between the behavioral markers of fear level and changes in the regional brain distribution of monoamines

In young rats (3-months old) the time of motionless behavior (TML) in illuminated compartment was positively correlated with NA level in the HPT (r_p=0.93,

Table IV

Ratio of metabolites to their parent amines in regional brain areas in rats of different age

Group	Brain region	MHPG/NA		DOPAC/DA		5-HIAA/5-HT	
Young	HPT	0.056 ± 0.022		0.122 ± 0.026		0.777 ± 0.043	
Mature		0.016 ± 0.008		0.201 ± 0.028		0.771 ± 0.064	
Age		0.0008 ± 0.0004		0.236 ± 0.018	(a)***	0.634 ± 0.047	
Young	MID	0.110 ± 0.041		0.093 ± 0.015		0.699 ± 0.051	
Mature		0.032 ± 0.013		0.210 ± 0.017	(a)***	0.902 ± 0.041	(a)**
Age		0.006 ± 0.003	(a)***	0.213 ± 0.012	(a)***	0.796 ± 0.068	
Young	AMY	0.116 ± 0.044		0.106 ± 0.014		0.519 ± 0.061	
Mature		0.014 ± 0.006	(a)***	0.136 ± 0.007		0.512 ± 0.023	
Age		0.025 ± 0.021	(a)**	0.116 ± 0.012		0.507 ± 0.026	
Young	HIP	0.176 ± 0.079		0.092 ± 0.016		0.747 ± 0.054	
Mature		0.017 ± 0.007	(a)****	0.114 ± 0.006		0.609 ± 0.061	
Age		0.034 ± 0.017	(a)****	0.115 ± 0.034		0.898 ± 0.035	(b)***
Young	PO	0.058 ± 0.030		0.088 ± 0.027		0.869 ± 0.134	
Mature		0.034 ± 0.013		0.267 ± 0.041	(a)****	0.901 ± 0.051	
Age		0.009 ± 0.003		0.416 ± 0.086	(a)**** (b)****	0.717 ± 0.135	(b)*

Values are mean ± SEM, n = 6 for each group. Statistical significance: two-way ANOVA followed by planned contrast test; (*) P<0.05; (**) P<0.02; (***) P<0.01; (****) P<0.001; (a) compared to young; (b) compared to mature.

$P<0.007$), with 5-HT level in the HPT ($r_p=0.85$, $P<0.03$), with MHPG level in the HPT ($r_p=0.93$, $P<0.007$) and in the AMY ($r_p=0.86$, $P<0.03$), and with 5-HIAA level in the HPT ($r_p=0.89$, $P<0.02$). The number of head dipping (HDD) from the dark to the illuminated compartment was negatively correlated with NA level in the MID ($r_p=-0.85$, $P<0.03$), with 5-HIAA level in the AMY ($r_p=-0.87$, $P<0.02$) and with DOPAC/DA ratio in the AMY ($r_p=-0.83$, $P<0.04$). The time spent in the illuminated compartment (TSL) was positively correlated with DOPAC level in the PO ($r_p=0.84$, $P<0.03$) and with 5-HIAA level in the HIP ($r_p=0.91$, $P<0.01$). There were no correlation between the number of returns (RET) from the dark to the illuminated compartment and any neurochemical parameters.

In mature rats (12-months-old) the TML was negatively correlated with NA level in the MID ($r_p=-0.89$, $P<0.02$) and with 5-HT level in the HIP ($r_p= -0.85$, $P<0.03$). The HDD was positively correlated with NA level in the HPT ($r_p=0.94$, $P<0.004$) with 5-HT level in the HPT ($r_p=0.89$, $P<0.02$) and with 5-HIAA level in the HPT ($r_p=0.89$, $P<0.02$). The TSL was positively correlated with MHPG level in the HIP ($r_p=0.85$, $P<0.03$) and negatively correlated with DA level in the HIP ($r_p=-0.87$, $P<0.02$). There were no correlation between the RET and any neurochemical parameters.

In the aged rats (24-months-old) the TML was positively correlated with DA level in the MID ($r_p=0.88$, $P<0.02$), with 5-HIAA level in the MID ($r_p=0.84$, $P<0.04$) and negatively correlated with 5-HT level in the AMY ($r_p=-0.88$, $P<0.02$). The HDD was positively correlated with MHPG/NA ratio in the MID ($r_p=0.91$, $P<0.01$) and negatively correlated with 5-HIAA/5-HT ratio in the HIP ($r_p=-0.90$, $P<0.01$). The TSL was positively correlated with 5-HIAA/5-HT ratio in the PO ($r_p=0.85$, $P<0.03$), with 5-HIAA level in the PO ($r_p=0.86$, $P<0.03$) and negatively correlated with 5-HT level in the AMY ($r_p=-0.92$, $P<0.009$) and with 5-HIAA level in the AMY ($r_p=-0.92$, $P<0.008$). There were no correlation between the RET and any neurochemical parameters.

DISCUSSION

In our light/dark transitions test (LDT) three of all registered behavioral events were crucial for the assessment of fear level. These were: a number of returns (RET) from the dark to the illuminated part of the experimental chamber, a number of head dipping (HDD) from the dark to the illuminated part of the chamber and the time of motionless behavior (TML) in the illuminated part of the chamber. Our previous research with use of LDT test (Koprowska et al. 2002, Romaniuk et al. 2001) provided information that the larger number of RET and HDD with TML being shorter at the same time, the lower a fear level.

In the present study the fear level assessment based on RET, HDD and TML shows that 12 months old rats (mature) had a lower fear level because RET was 0.2, HDD – 1.2 and TML – only 4% of the value of total time spent in the illuminated part of the chamber. Three months old rats (young) showed a slightly higher fear level with their RET being 0.1, HDD – 1.0 and TML – 18% of the value of total time spent in the illuminated part of the chamber. Twenty four months old rats showed the highest level of fear, which was demonstrated by lack of RET, trace HDD number and TML as high as 36% of the value of total time spent in the illuminated compartment of the chamber. These data are compatible with the results of other authors (Boguszewski and Zagrodzka 2002, Frussa-Filho et al. 1992, Miyagawa et al. 1998) who compared only two age groups and applied different tests, i.e., open–field and plus–maze demonstrating a higher fear level and decreased motor activity in 24 months old rats as compared to 4 months old ones. However, the results we obtained by comparing three age groups provided interesting information. It appeared that though the fear level was diversified depending on age no straight linear dependence of "the older, the higher fear level" occurs because scheduling of age groups according to fear level was as follows: 12, 3 and 24 months old rats. Our results also pointed to the age-related decreasing locomotion activity because the ratio of the time of locomotion activity to the time of motionless behavior in the dark compartment in 3, 12 and 24 months old rats was 6.5, 3.1 and 2.2, respectively. And here a straight linear dependence occurred of "the older, the lower locomotion activity". Certainly, this analysis referred only to the data registered in the dark compartment where no stressogenic stimulus was present.

Neurochemical tests under the present study were conducted to obtain information about differences between young, mature and aged rats in the NA, DA and 5-HT neurotransmitter system activity in the specific areas forming the brain emotional-defensive system. We expected to obtain data allowing to demonstrate neurochemical background of different fear levels in

correlation with the aging process since the data exist indicating that rats characterized with a "low-anxiety behavior" had lower 5-HT tissue level in the brain and diminished 5-HT release as compared to "high-anxiety rats" (Bert et al. 2001, Rex et al. 1999). Our results are not so unequivocal because in aged rats (of the highest fear level) as compared to mature rats (of the lowest fear level) the level of 5-HT was lower in AMY and HIP, the level of 5-HIAA was lower in AMY, and the 5-HIAA/5-HT ratio was lower in PO but higher in HIP. The diminished concentration of 5-HT in HIP associated with a parallel increase in 5-HIAA/5-HT ratio in this structure may indicate an accelerated turnover of 5-HT in aged rats, which speaks for an increase in serotonergic system activity in HIP, which in consequence may condition a higher fear level. However, the fact that an increase in 5-HT, 5-HIAA and 5-HIAA/5-HT ratio in MID was observed in mature rats with a slightly lower level of fear than in young rats may speak against such interpretation. A slight difference in the content of 5-HT between aged and young rats coming down to just a lower level of 5-HT in AMY in aged rats, does not indicate any relation between the fear level and neurochemical activity of serotonergic system. Simultaneously, we did not observe differences in the activity of serotonergic system between aged and young rats, which is strongly supported by the absence of differences in 5-HIAA/5-HT ratio. It was an unexpected but not surprising result because earlier studies showed that the content and turnover of 5-HT in the brain areas containing serotonergic terminals were unchanged with aging (Maretti et al. 1987, Ponzio et al. 1982, Pradham 1980, Robson et al. 1993). Whereas many data indicate age-related decrease in the content and turnover of 5-HT in different brain areas (Brunello et al. 1988, Machado et al. 1986, Miguez et al. 1999, Roubien et al. 1986, Strong et al. 1984, Venero et al. 1991).

The results we obtained on age-related changes in the noradrenergic system activity are based primarily on the analysis of MHPG/NA ratio. We show unequivocally that in most structures investigated an attenuation of NA metabolism occurred in rats at age of 12 and 24 months as compared to 3 months old ones. Since there is no difference in the activity of NA system between 12 and 24 months old rats, it means that the process of attenuated noradrenergic system activity is already present in mature rats (12 months old) and remains at the same level in aged rats (24 months old). The analysis of DOPAC/DA ratio demonstrated that in most structures investigated an increase in dopaminergic system activity occurred both in 12 and 24-months old rats as compared to 3 months old ones. In 24 months old rats vs.12 months old ones an increase in the system activity was recorded only in the pons, whereas in other structures a decrease in DA and DOPAC level occurred. Thus it appeared that a change in the activity of dopaminergic system occurred already in mature rats and remained at a similar level in aged rats. This age-related increase in dopaminergic system activity may be explained based on the hypothesis suggested by Miguez et al. (1999) providing that while a number of neurons is subject to reduction during aging, other neurons are subject to increased neurotransmitter metabolism, which is to provide for functional efficiency of the system. But a question arises why this compensatory mechanism occurred only in dopaminergic system. And the answer might certainly be that it is because during normal aging no uniform process of atrophy in various types of neurons and attenuation of metabolism in particular neurotransmission systems takes place (De Kosky and Palmer 1994, Finch and Roth 1999).

"Serotonergic hypothesis" of fear/anxiety behavior proposes that in stressogenic or threatening situations the serotonergic system activity increases whereas the reduction of serotonergic system activity exerts anxiolytic-like effects (for review see Graeff 1990). The correlation analysis we carried on between the most indicative markers of the fear level and changes in a distribution of monoamines, their metabolites, and ratio of metabolites to their parent amines in the key areas forming the brain emotional-defensive system in young, mature and aged rats showed very diversified data. Some of them were consistent and some not with the "serotonergic hypothesis" of fear/anxiety behavior. And thus, for example, TML in young rats was positively correlated with 5-HT and 5-HIAA level in the HPT, but in mature rats it was negatively correlated with 5-HT level in the HIP. Also in aged rats TML was negatively correlated with 5-HT level in the AMY. HDD also shows discrepancies. In young rats HDD was negatively correlated with 5-HIAA level in the AMY, also in aged rats HDD was negatively correlated with 5-HIAA/5-HT ratio in the HIP, but in mature rats HDD was positively correlated with 5-HT and 5-HIAA level in the HPT. Also the NA and DA systems show a series of discrepancies. In such a situation we cannot draw conclusions on neurochemical background of fear.

CONCLUSIONS

The results obtained showed that the fear level was diversified depending on the rat age. In contrast to the locomotor activity that showed a decreasing tendency with age, the fear level dependence was not linear because the lowest level of fear was observed in 12 months rats and the highest level was in 24 months old ones. The process of decreasing noradrenergic system activity and increasing dopaminergic system activity in most emotional-defensive structures was already observed in mature (12 months old) rats and remained at the same level in aged rats (24 months old). The correlation analysis between the most indicative markers of the fear level and changes in distribution of monoamines, their metabolites, ratio metabolites to their parent amines in the selected brain areas in young, mature and aged rats revealed that only some data are consistent with the "serotonergic hypothesis" of fear/anxiety. Based on our results we cannot conclude on the neurochemical background of various fear levels.

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