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The Effects of Prenatal Exposure to Hypoxia on the Behavior of Rats During Their Life Span

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JÄNICKE, B. AND H. COPER. *The effects of prenatal exposure to hypoxia on the behavior of rats during their life span.* PHARMACOL BIOCHEM BEHAV **48**(4) 863–873, 1994. – The aim of this study was to investigate the influence of moderate prenatal damage on adaptability during the juvenile, adult, and senile phases. Pregnant rats were exposed to a 12% normobaric hypoxia from day 1 to 17 postconception. Pregnancy was normal in both the treated animals and the controls. Erythrocytes, hemoglobin, and hematocrit did not increase in the treated pregnant animals. During the first 3 weeks, the F1 generation showed developmental deviations in physiological characteristics. Throughout subsequent ontogeny, motor performance, cognitive ability, and adaptability to physical stress were determined with a test battery of varying demands. Some of the differences (e.g., locomotor activity, learning ability) between juvenile untreated and treated rats disappeared during the adult phase. Motor and coordinative abilities, however, remained partially impaired in the old rats, especially under high demands. This study, and previous findings with alcohol (37), indicate that prenatal exposure to a noxa may result in a highly differentiated brain injury pattern. Depending on the different functions, damage may intensify age-dependent adaptive disorders or provoke impairment without influencing the course of development.

Prenatal hypoxia exposure Life span study

A MAIN characteristic of aging in multicellular organisms is the progressive reduction of the ability to adapt to endogenous and exogenous stimuli, which leads to limited capacity, stability, and reserves to maintain normal homeostasis and to respond to exceptional requirements (13). This reduction in adaptability occurs gradually depending on the function and degree of complexity of the specific demand and is differentiated along a time course. It has been shown that basic abilities are relatively stable compared to more complex ones: the more complex the demand, the earlier the critical load of performance is reached (33). This hypothesis of decreasing adaptability during aging has repeatedly been verified in cross-sectional analyses of many different species (12,31,33,63). However, as the time at which the symptoms of old age become manifest and the extent of age-typical changes depend on the biography of the individual, it was necessary to conduct long-term studies of the entire ontogeny. A study has already been performed on this basis to examine the effects of prenatal alcohol exposure on locomotor, motor-coordinative, cognitive, and physiological functions in view of the onset and extent of old age phenomena (37).

In the present long-term study, the hypothesis was tested that prenatally hypoxia-induced damage modifies the rats' adaptability throughout the different phases of life. For this purpose, pregnant rats were kept in a reduced oxygen atmosphere (12% O_2). Their offsprings' adaptability regarding the above functions was investigated in time and extent throughout their life span. The changes in performance were measured using a test battery of demands differing in complexity [principle: testing the limits (3)], which is a proven method of determining performance limits during ontogeny. We postulated in the present study that in the juvenile phase, only small or transient effects occur, whereas the adult phase is characterized by a relatively stabile capability to adapt. In the late adult and senile phases, which are the focus of this study, limitations in stability, capacity, and reserves manifest themselves sooner and are more pronounced compared to untreated aging rats. Hypoxia was chosen because of available

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findings from previous cross-sectional studies in adult and senile rats concerning the effects of a continuous reduction of a certain percentage of the oxygen supply on physiological and behavioral performance (51). Additionally, it has been established that prenatal hypoxia causes only subtle psychomotor and psychosensory behavioral disorders under the present conditions (19,20,45). Severe oxygen deprivation (< 12% O_2), however, would have caused a higher mortality rate, whereas a much higher oxygen content would not have impeded normal development (23,26,46,59). Thus, the prenatal burden in the present study was not intended to limit the vital abilities of the animals and, therefore, neither a high mortality rate nor a shortening of the lifespan was expected to be found. To our knowledge, this experimental approach, involving the F1 generation up to its natural death, has hardly ever been applied. Therefore, a comparison with findings of similar investigations is not, or only partially, appropriate because the nature and period of oxygen reduction during pregnancy differed (8,20,38,59,65).

METHOD

The parent animals were 27 nulliparous female (200 g) and 27 male (200 g) Wistar rats (SPF, Hagemann/Lippe, INOVA), unrelated to one another. Fifteen female and 15 male rats were selected for hypoxia treatment. Following adaptation to an oxygen-reduced (15% O_2) normobaric atmosphere for 14 days, the animals were mated. Successful mating was ascertained by the presence of sperm in the vaginal smear or by means of a sperm plug. All the 15 pregnant rats were exposed to 12% O_2 hypoxia from day 1 to 17 postconception. Macrolon cages (type III), each containing two rats, were placed in test chambers (14). Room temperature was kept at 23 ± 0.5°C and relative humidity was 60%. The cages were illuminated 12 h daily (dark phase 1900–0700 h). From day 18 postconception, they were kept individually under normoxic conditions (20.9% O_2). Food (Altromin 1320 food pellets) and tap water were ad lib. During acclimatization and pregnancy, body weight, and food and fluid consumption were recorded every day. To document the adaptation to the hypoxic conditions, an analysis of blood components (erythrocytes, hemoglobin, and hematocrit) was carried out on day 17 postconception. For this purpose, eight out of the 15 dams were briefly restrained in special tubes to obtain blood samples from the tail vein. Comparatively, the blood of eight controls was analyzed. Moreover, to consider the physiological status of pregnancy, a blood analysis of nonpregnant rats under normoxic (20.9% O_2) and hypoxic conditions was performed (n = 8 per group). The 12 pregnant control animals (likewise two per Macrolon cage, type III) were housed at 20.9% O_2 at the same room temperature, humidity, light/dark cycle, as well as food and water supply.

After birth, the total offspring of the treated rats were randomly assigned to 15 new (fostered) groups, each consisting of four females and four males. In each group, the eight animals were unrelated to one another. The pups were weighed, inspected for any obvious structural abnormalities, and marked for identification. The same procedure was followed concerning the controls.

The body weight of all the pups was measured daily until day 21, when they were weaned and kept in groups of four per Macrolon cage (type IV) with same-sexed conspecifics. After that, their weight and state of health were monitored weekly, up to their natural death. To determine serious developmental disorders, the young animals were tested for the following characteristics that are generally employed to define physiological maturity: time of incisor eruption (appearance of both the upper and lower incisors through the gums), opening of the eyes (both eyes open), testes descent (complete descent of both testes), vaginal opening (complete vaginal opening), and the acoustic startle reflex (a visible flinch elicited with a 4-kHz tone applied near the pup's head) (2).

TABLE 1

SUMMARY OF TESTS TO INVESTIGATE FUNCTIONS AND BEHAVIORAL PERFORMANCE OF RATS AT DIFFERENT AGES

Function	Test	Age
	Juvenile Phase	Days
Reflexes and locomotor behavior	righting reflex (2)*	2, 7
	swimming (49)	6
	olfactory test (2)	7–11
	water maze (1)	21
	water escape test (66)	26
	open field (4)	48
Coordination	negative geotaxis (2)	4, 8
	chimney test (9)	21
	rotorod (33)	36
	Adult and Senile Phases	Months
Reflexes and locomotor behavior	holeboard	4, 8
Coordination	rotorod	5
	rotorod (long-term test)	5–27
	running belt	19
	traction test	21, 28
	negative geotaxis	27
Cognitive abilities	FR 1 : 10/DRL	11
	active avoidance	10, 20
	passive avoidance	10, 20
Adaptation	cold stress	10
	hypoxia stress (10% O_2)	19

*Numbers in parentheses are references.

Behavioral performance was investigated using the principle "testing the limits" with various tests that are comparable to test batteries used by other researchers (30–32,58,63,64) and that have proven worthwhile in previous cross-sectional (33,52) and long-term studies (37). Table 1 lists the tests conducted in the juvenile (0–3 months), adult (4–27 months), and senile (older than 27 months) life phases. For the tests carried out in the juvenile phase, references are provided under the Results section because these tests are well known and the focus of this study is the late-adult (19–27 months) and senile phases. In each test, the number of male and female rats was equally distributed. To avoid performance interference, the individual rats were examined only once per functional area test (e.g., coordination, cognitive abilities). For each test, the rats were randomly chosen from all fostered groups.

TABLE 2

BLOOD ANALYSIS OF FEMALE RATS (ON DAY 17 POSTCONCEPTION)

	Erythrocytes (millions/μl)	Hemoglobin (g/dl)	Hematocrit (%)
	Normoxia (20.9% O_2)		
Nonpregnant	7.11 ± 4.89	15.20 ± 0.40	44.0 ± 0.63
Pregnant	7.33 ± 5.10	15.52 ± 0.93	43.0 ± 4.65
	Hypoxia (12% O_2)		
Nonpregnant	8.87 ± 6.75	19.10 ± 0.72	52.7 ± 1.53
Pregnant	7.03 ± 1.03	15.60 ± 0.15*	43.7 ± 2.50*

Values are the mean ± SD.
*$p < 0.05$ different from nonpregnant rats.

Motor Activity and Coordination

Holeboard test. The rat was placed in a corner of a walled-in wooden area (1 m^2) with a double floor. The area was subdivided into 16 white-lined squares; in five squares there was a hole with a diameter of 3.5 cm. According to a modified method, the number of crossings, rearings, groomings, and head dips was monitored over a period of 10 min (16).

Negative geotaxis. For this test, the rats had to maintain their balance on a rough surface (41 × 27 cm) that was tilted from 0–90° within 3–4 s. The angle at which the animals could no longer avoid sliding off the board was measured. The largest angle of three attempts was the basis for the statistical analysis (33).

Traction test. The animals were hung by their front paws from the middle section of a metal rod (ϕ 0.4 mm, 80 cm long). The duration (max. 60 s) of hanging by two or four extremities was measured. Of three collected values per animal, the longest duration was the value used for evaluation. A resting phase of approximately 5 min was allowed between consecutive attempts.

Running belt test. In previous experiments with 19-month-old rats under normoxia, results failed to reveal an impaired running performance. Therefore, the running belt test was carried out under the increased demand of a normobaric hypoxia. This is in line with the principle of "testing the limits." To raise the demands for the running belt test, the rats were first adapted to the conditions of an oxygen-reduced atmosphere (10% O_2) for a period of 7 days (51). On day 8, the animals had to learn to run on the running belt (30 × 10 cm) in the opposite direction. To prevent the animals' escaping from the running belt, the floor of the test chamber was equipped with electrified bars (2 mA, scrambled shock; Rhema-Labortechnik). After an acclimatization phase of 3 min (belt speed: 3.6 m/min), the speed was increased to the maximum value of 12 m/min within 5 s. The test was discontinued whenever the rat did not run forwards anymore, when it left the running belt and did not jump back immediately, or when the stipulated running time of 10 min had been completed.

Rotorod test. Five-month-old animals had to run on a rotating rod (hard plastic, ϕ 5 cm for females, ϕ 7 cm for males) in the opposite direction of its rotation (33). The rats had to keep their balance for a certain time at different speeds that were increased on 3 consecutive days, beginning at 10 rpm: females—2 min (10 rpm), 2 min (20 rpm); males—1 min (10 rpm), 2 min (10 rpm), 1 min (20 rpm). The number of starts for each speed level and the running time until the animal fell off were recorded. There was a 3–5-min break between two starts. The number of starts per day was limited to 10. As soon as 60% of a group achieved the intended running time,

TABLE 3

PHYSIOLOGICAL CHARACTERISTICS

	Treated Rats		Controls	
	Male	Female	Male	Female
Mortality (%) (pre-/postnatal, up to 2nd day p.p.)	2%	5%	4%	3%
Number of rats per litter	5.1 ± 0.6	4.0 ± 0.5	5.5 ± 0.6	5.1 ± 0.5
Birth weight (g)	5.5 ± 0.6*	5.1 ± 0.6*	6.5 ± 0.4	6.2 ± 0.6
Auditory startle response (days 12 and 13 p.p.)	positive	positive	positive	positive
Incisor eruption (days p.p.)	12.9 ± 0.3*	13.2 ± 0.2*	14.2 ± 0.1	14.2 ± 0.1
Opening of the eyes (days p.p.)	15.0 ± 0.1*	14.9 ± 0.1	14.4 ± 0.1	14.4 ± 0.1
Testes descent (days p.p.)	22.8 ± 0.4*		24.1 ± 0.2	
Vaginal opening (days p.p.)		31.3 ± 0.3*		29.8 ± 0.3

Values are the mean ± SEM.
*$p < 0.05$ different from controls.

TABLE 4
ASSESSMENTS IN THE JUVENILE PHASE

Test Procedure	Measured Parameter	Age (days)	Treated Rats		Controls	
Righting reflex	reaction time (s)	2	12.1 ± 0.9*		7.2 ± 1.1	
(n = 85 each)	"	7	3.8 ± 0.7*		1.6 ± 0.1	
Swimming	(in arbitrary units)					
(n = 50 each)	use of limbs	6	1.0 ± 0.1		1.0 ± 0.1	
	direction		2.2 ± 0.1		2.1 ± 0.1	
	angle of body axis in water		1.6 ± 0.1*		2.6 ± 0.1	
Negative geotaxis	time to turn (s)	4	55.6 ± 2.1*		33.8 ± 2.6	
(n = 96 each)	"	8	26.4 ± 2.3		27.5 ± 2.2	
Olfactory test	time (s) to reach home	7	44.7 ± 8.2		43.1 ± 6.7	
(n = 20 each)	shaving					
	"	11	48.8 ± 4.6*		27.8 ± 1.8	
Chimney test	climbing time (s)	21	40.5 ± 2.6*		28.5 ± 2.8	
(n = 30 each)						
Water maze	time (s) to reach goal	21	35.9 ± 2.5		37.1 ± 1.8	
(n = 20 each)	"	23	13.0 ± 1.3		21.1 ± 1.0	
	successful rats (%)	21	30†		75	
	"	23	95		86	
Water escape test	swimming time (s)	26	4.7 ± 0.3*		2.6 ± 0.1	
(n = 32 each)						
Rotorod	running time (s) at:					
(n = 20 each)	10 rpm	36	48.8 ± 4.2		50.7 ± 3.8	
	20 rpm		54.1 ± 2.8		58.7 ± 1.2	
	30 rpm		55.1 ± 2.0		49.0 ± 3.3	
			male	female	male	female
Open field	frequency (1/10 min) of:					
(n = 10 each)	squares crossed	48	83.1 ± 5.9*	126.8 ± 7.3*	51.6 ± 3.7	58.2 ± 4.3
	rearing		20.0 ± 2.9	27.6 ± 4.3	18.2 ± 2.5	20.0 ± 3.0
	grooming		1.8 ± 0.6†	3.4 ± 1.7	4.0 ± 0.8	3.5 ± 1.3

Values are the mean ± SEM.
*$p < 0.01$, †$p < 0.05$, different from controls.

the test was terminated. Pretesting in a group of untreated rats showed that the criterion of 60% successful animals was a suitable measure of age-related changes in performance. After the 5-month-old rats had concluded the series of tests with different rotation speeds, they were subjected to long-term training at 14-day intervals, up to the age of 27 months. The different tasks for male and female rats (1st day—males: 2 min at 10 rpm, females: 2 min at 20 rpm; 2nd day—males: 1 min at 20 rpm, females: no test) were necessary because, in pretrials, even untreated and task-inexperienced male rats could not complete the running time of 2 min at 20 rpm.

Cognitive Abilities

Active avoidance test. In this test, 10-month-old rats had to learn to avoid an electric foot shock (male: 1.8 mA; female: 2 mA for 4.3 s) in a two-compartment shuttle-box (Rhema-Labortechnik 337 500) by changing compartments. A light signal lasting for 3.8 s given shortly before, announced the punishment as a "conditioned stimulus." Following a 5-day acquisition phase (50 tests per day), the extent to which the animals could reproduce the reaction learned was tested 7 days later. The following parameters were recorded: frequencies of missed avoidance reactions and of changing from one compartment to the other between the separate tests, and the period of latency until avoidance. Two weeks later, an extinction test was conducted to establish whether the treated rats differed from the controls in adaptability to an altered situation in which no punishment was used. At the age of 20 months, they were reexamined under the same conditions.

Passive avoidance test. One part of a two-compartment box (Rhema-Labortechnik 337 500) was kept dark and the other was illuminated (40 W). An animal was placed in the lit compartment daily on 3 consecutive days. Compartment changes within a period of 3 min were counted to measure the activity level. On day 4, the rats were again placed in the lit compartment, and the period of latency until entering the dark one was recorded. There, however, the animal received an unavoidable foot shock (male: 1.8 mA; female: 2 mA for 4.3 s). Twenty-four hours later, the period of latency was remeasured for a period of 3 min. The rats' avoidance reaction in this period consisted in remaining passive. The test was first

TABLE 5
ASSESSMENTS IN THE ADULT AND SENILE PHASES

Test Procedure	Measured Parameter	Age (months)	Treated Rats		Controls	
			Male	Female	Male	Female
Holeboard	frequency (1/10 min) of:					
(n = 10 each)	squares crossed	4	47.8 ± 12.5	75.6 ± 16.2	44.8 ± 16.2	66.1 ± 18.5
	rearing		6.2 ± 1.2*	9.5 ± 1.8†	17.4 ± 2.8	24.9 ± 2.9
	grooming		0.7 ± 0.3*	1.7 ± 0.6	5.2 ± 1.4	2.4 ± 0.7
	head dips		3.2 ± 0.6	3.3 ± 0.5	3.3 ± 0.8	6.2 ± 0.8
Rotorod	number of starts at:					
(n = 20 each)	10 rpm	5	10.0 ± 0.7	4.0 ± 0.8	6.0 ± 0.9	3.0 ± 0.5
	20 rpm		4.0 ± 0.8	3.0 ± 1.2	3.0 ± 0.5	3.0 ± 0.4
Active avoidance	failure to avoid (day 1)	10	3.2 ± 1.0†	6.4 ± 1.2	6.4 ± 1.0	6.9 ± 0.8
(n = 20 each)	failure to avoid (day 5)		1.0 ± 1.2	1.4 ± 0.6	3.0 ± 1.0	2.4 ± 0.7
	latency to avoid (s) (day 1)		3.1 ± 0.5†	4.1 ± 0.5	4.8 ± 0.6	4.5 ± 0.4
	latency to avoid (s) (day 5)		2.4 ± 0.2	2.6 ± 0.4	2.9 ± 0.5	2.4 ± 0.4
Passive avoidance	latency to enter (s):					
(n = 12 each)	acquisition (day 1)	10	30.3 ± 2.0		22.0 ± 2.1	
	acquisition (day 4)		7.2 ± 2.3		8.4 ± 2.2	
	retention		114.4 ± 10.1†		164.2 ± 8.4	
FR/DRL relearning	days until success:					
(n = 12 each)	FR 1 : 10	11	2.7 ± 1.4		3.0 ± 0.8	
	DRL		5.6 ± 0.9†		4.6 ± 1.7	
Traction test	traction time (s)	21	10.7 ± 1.8		10.8 ± 1.6	
(n = 25 each)	traction time (s)	28	3.0 ± 0.5†		5.0 ± 1.0	
Negative geotaxis (n = 20 each)	sliding (deg.)	27	49.7 ± 0.1		51.3 ± 0.1	

Values are the mean ± SEM.
*$p < 0.01$, †$p < 0.05$, different from controls.

performed when the rats were 10 months old. At the age of 20 months, retention was retested.

Fixed ratio 1 : 10/differential reinforcement of low rates (FR 1 : 10/DRL). The experiment consisted of two phases. In phase I, the rats were trained to perform a FR-10 program in a Skinner box (10 responses/reinforcement). The criterion was an average of 90 reinforcements/30 min (0.045-g Noyes Pellets) on 2 consecutive days. Phase II involved switching to the DRL program in which the animals needed to pause for 10 s between successive rewards. If the lever was activated during this time, the unrewarded phase was reset again to 10 s. A light signal marked the end of the pause, and thus, the possibility of obtaining a pellet. The criterion for phase II was also an average of 90 pellets/30 min on 2 consecutive days. The efficiency (reinforcement^2/effort) was calculated from the squared reinforcement rate and the number of lever pressings (36).

Adaptation

Adaptation of physiological parameters at 10% O_2 atmosphere. To measure the adaptability of physiological functions, the rats were exposed to a 10% O_2 normobaric atmosphere. The animals were kept individually in air-tight chambers (60 × 40 × 40 cm) with transparent walls. Food and water consumption (ad lib) and body weight were recorded daily during the 1-week test phase. The ambient temperature was 22°C, and the day/night phases alternated in a 12-h rhythm (51).

Regulation of the body temperature during cold stress. Without any acclimatization phase, the animals were kept individually in Macrolon cages (type III) at a room temperature of −20°C for 20 min. Body temperature was measured rectally (Digimed H II) before and after exposure to cold until it normalized.

Statistical Analysis

All the data were processed on a computer (HP 9816) using the SAS system (Statistical Analysis System, Cary, NC). For the statistical comparison of the experimental results between the prenatally treated and untreated animals (test of body temperature regulation during cold stress), a multiple factorial analysis of variance (ANOVA) was performed (the saturated models—main effects: treatment, age, sex, and their interactions—were used). In the event of no interaction of sex, males and females were pooled. A nonparametric test (Mann–Whitney U-test) was chosen to compare two independent random samples for the variables: birth weight; peri- and postnatal mortality; physiological development; blood analysis; individual results in motor tests (negative geotaxis, traction, running belt, holeboard tests), learning tests, and in testing the adaptation of physiological parameters at 10% O_2 atmosphere. Concerning the body weight development, the curve fit was performed according to nonlinear regression [model: weight $= a \times (1 - e^{-b \times t})$; computational method: Gauss-Newton; SAS-Statistics]. In the long-term rotorod test, the curve of the

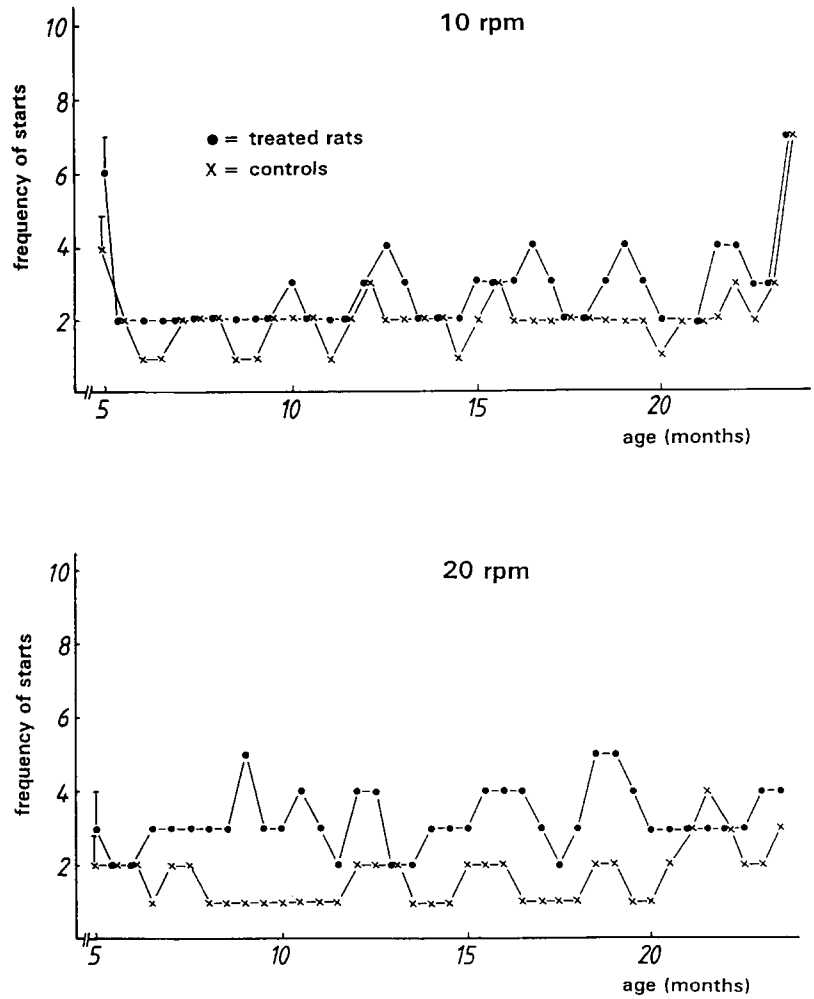

FIG. 1. Long-term training of male rats, prenatally exposed to hypoxia (12% O_2), in the rotorod test in comparison to controls. Median ± SD, n = 10 per group.

starting frequency of the control and experimental animals was divided into two time sequences (first to 19th, and 20th to 38th measurements), and the data were pooled. A t-test for independent random samples was conducted for the variable number of starts, after classifying them according to sex and rotation speed. The areas under the curves (running time at the first start on each test day in relation to age) were integrated to test the difference between the control and treated groups in running time. The Student's t-test was employed to compare the two groups with respect to this variable. The mean values are principally presented with the SEM, if not indicated differently. The level of significance for all the tests was set at $p < 0.05$.

RESULTS

Treatment of the Pregnant Rats

Body weight, and food and fluid consumption. The female rats that had first been adapted to a 15% O_2 atmosphere for 14 days, and after successful mating were kept in a 12% O_2 atmosphere, had a gestation period similar to that of the control animals (22 ± 0.3 vs. 21 ± 0.4 days). Food consumption was unchanged during the gestation period; however, fluid consumption decreased and body weight remained 25 g under that of the pregnant rats under normoxic conditions.

Blood analysis. Under the 12% O_2 hypoxia, hemoglobin content and hematocrit were statistically significantly lower in the pregnant rats than in the nonpregnant animals on day 17 postconception (Table 2). In contrast, no difference concerning the erythrocytes, hemoglobin content, and hematocrit could be proven between nonpregnant and pregnant rats in a normoxic atmosphere.

Juvenile Phase

Physiological characteristics. No difference between untreated vs. treated rats could be found concerning the mortality rate or number of rats per litter. However, the birth weight of the treated rats was considerably lower ($p < 0.05$, Table 3). During the growth phase, the weight curve differences between the treated and control animals of both sexes were statistically significant ($p < 0.05$). The body weight of the prenatally influenced rats increased faster. However, at the end of the growth phase, a distinctly higher body weight was found only in the male rats compared to the controls ($p <$

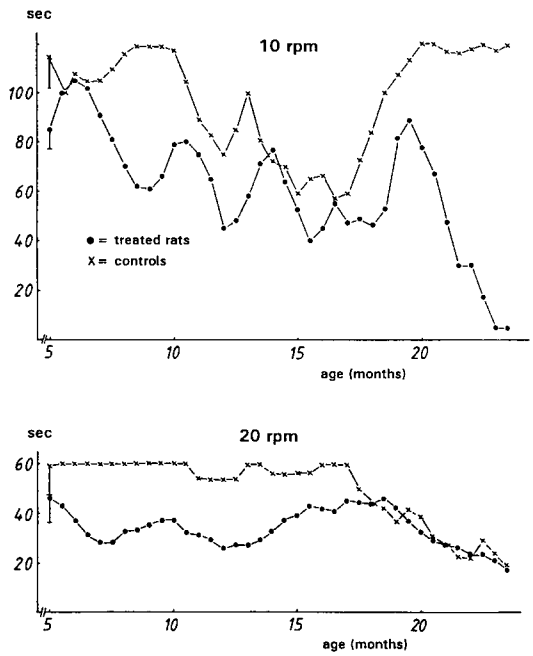

FIG. 2. Long-term training of male rats, prenatally exposed to hypoxia (12% O_2), in the rotorod test in comparison to controls. The running time for the first start in each test is given. Median ± SD, n = 10 per group.

0.05). The curve fit after Gauss-Newton shows that the curves of the treated and untreated animals of either sex are typical growth curves. In further physiological maturation indices, hypoxic effects were either premature or delayed (Table 3).

Behavioral tests. In the juvenile phase (0–3 months), the rats prenatally exposed to hypoxia showed a delayed development in tasks that required balance, coordination, and/or orientation, as in the righting reflex (2) and in the negative geotaxis [(2); Table 4). In addition, it led to reduced effectiveness in the swimming movements (49) and a longer climbing time ($p < 0.05$) in the chimney test (9); however, no difference compared to the controls could be detected concerning the running time on the rotating rod (37). A diminished spatial orientation in the water escape test (66) and marked hyperactivity in the open field test (4) were recorded. In cognitive performance, a clear delay in the development of the prenatally treated animals could be observed, in that they oriented themselves less successfully in the olfactory test (2) and water maze [modified method (1)].

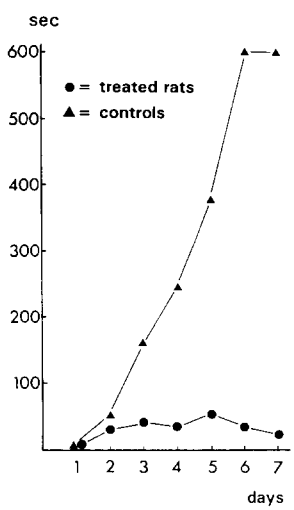

FIG. 3. Running time (s) on a belt under oxygen reduced air (10% O_2) of 19-month-old rats that had been prenatally exposed to a normobaric hypoxia (n = 10) in comparison to controls (n = 11), median.

Adult Phase

Motor activity and coordination.

Holeboard test. The prenatally treated rats (aged 4 months) showed a lower frequency of rearings than the controls ($p < 0.05$, Table 5), which is interpreted as a reduced exploratory activity. At the age of 8 months, no difference could be observed.

Rotorod test. At the beginning of the rotorod test with 10 rpm, the 5-month-old prenatally treated males needed more starts to fulfill the test criterion compared to the controls. With practice, however, there were only slight deviations. After an increase of the rotation speed to 20 rpm, a statistically significant difference between the compared groups could be ascertained (overall $p < 0.05$, Fig. 1). The treated males, especially at the first start, remained on the rod for a markedly shorter time than the controls ($p < 0.05$, Fig. 2). This weakness led to an overall poorer performance throughout the adult phase. The performance of the female rats showed no difference.

Cognitive abilities.

Active avoidance test. In the active avoidance test, the 10-month-old rats prenatally exposed to hypoxia learned to avoid the foot shock by changing compartments just as effectively as the controls (Table 5). The male rats were even more successful than their untreated conspecifics at leaving the compartment at the appropriate time to avoid the adverse stimulus ($p < 0.05$). The females did not show any statistically significant difference. In the retest, performed a week later, all the rats avoided the foot shock. In the extinction test, experimental and control groups no longer showed any avoidance behavior.

Passive avoidance test. In the passive avoidance test, the period of latency until entering the dark compartment decreased continuously in all the rats during the 4 test days (Table 5). Twenty-four hours after receiving the punishment on the fourth test day, however, 45% of the treated rats vs. 80% of the controls ($p < 0.05$) avoided the dark compartment during the stipulated time of 180 s in the retest. Thus, retention in treated animals was notably shorter ($p < 0.05$). There was no sex-specific difference in the treated vs. untreated rats.

FR 1 : 10/DRL. At the age of 11 months, all the rats learned the basic schedule of the operant conditioning task. On average, the task was accomplished within the same period of time in both groups (FR 1 : 10, Table 5). After changing the learning program to DRL, the untreated animals fulfilled the test criterion of 90 pellets each on 2 consecutive days distinctly faster than the treated rats ($p < 0.05$).

Adaptation.

Regulation of the body temperature during cold stress. At the age of 10 months, the regulation of the body temperature during cold stress was equally pronounced in all the rats. The core temperature (38°C at 21°C room temperature) of these

rats increased at −20°C by approximately 1.1 ± 0.2°C within 20 min. Also, the subsequent acclimatization period at room temperature was the same in all the rats.

Late Adult/Senile Phase

Motor activity and coordination.

Negative geotaxis. The results showed no difference between the experimental and control groups of either sex concerning compensational reactions on the tilted surface (Table 5).

Traction test. In this test, the average traction duration was similar in 21-month-old prenatally treated rats and their untreated conspecifics (Table 5). However, at the age of 28 months, the traction performance of the controls was distinctly better ($p < 0.05$).

Running belt test. After the physiological adaptation to the oxygen-reduced atmosphere, the animals' performance on a running belt was tested. All the rats improved their running performance over the 7 test days. However, there was a statistically significant difference between the prenatally treated animals and the controls concerning the running time during the test period from day 1 to 7 (Fig. 3, $p < 0.05$). Thus, although the prenatally damaged rats were capable of adapting to the hypoxic atmosphere, as demonstrated by their food and fluid consumption, their limited capacity became obvious when running on the belt; their performance gain remained low.

Rotorod test. In the coordinative capabilities that had already been lower in the adult phase, no further decrement could be observed regarding the frequencies of starts up to the age of 24 months (Fig. 1). Concerning the running time, however, there was a loss of performance at 10 rpm, whereas at 20 rpm the normal age-related decrease in running time was not exceeded (Fig. 2). The test had to be discontinued prematurely because the treated rats no longer showed any statistically evaluable performance.

Cognitive abilities.

Active avoidance test. At the age of 10 months, all the rats had learned in the extinction test that foot shocks no longer had to be avoided and again switched equally often between the two compartments. No difference between the behavior of the untreated and that of the treated rats could be perceived on testing under the same conditions at the age of 20 months.

Passive avoidance test. At the age of 10 months, learning impairments could be clearly recognized in the passive avoidance test. At the age of 20 months, no difference in remembering the punishment in the dark compartment could be determined between the two groups. An equal number of rats in both groups entered into the dark compartment or avoided it.

Adaptation.

Adaptation of physiological parameters at 10% O_2 atmosphere. During the 5-day acclimatization phase, the degree of adaptability of the physiological functions such as body weight, and food and water consumption of the 19-month-old treated rats was the same as that of the controls. In all the rats, the body weight decreased by approximately 10%; food and fluid consumption normalized to the same extent and with the same growth rate.

DISCUSSION

Life is considered to be a continuous process of adaptation. This ability to adapt to endogenous and exogenous stimuli changes during ontogeny. The aging process is primarily associated with a reduction in adaptability (17,55). This decrement is characterized by limited capacity, impaired stability, and diminished reserves of various functions (13). The capacity to compensate is defined as a dynamic range in which a variable can oscillate without deviating from the homeostasis. Stability can be quantified by the degree of intensity of a disturbance. Performance, achieved by practice or during short-term overtaxation, mirrors the reserves of an organism. Employing the approach of studying long-term effects after prenatal treatment with hypoxia, the hypothesis of age-dependent changes has been supported in principle. The onset, intensity, and timing of the changes, however, were modified by the influence of hypoxia, resulting in a differentiated performance profile. Therefore, the same range of changes was not expected to be found in all the tests. In a previous study using the same method, rats were prenatally treated with alcohol (6 g/kg/day, orally) (37). Also, the results of this study showed a characteristic course of changes compared to untreated rats. Physiological development and performance tests during the juvenile phase hardly revealed any differences vs. control rats. In adult rats, impairment of motor and cognitive abilities vs. untreated animals could only be determined under increased demand. With advancing age, the differences, in relation to the complexity of the task, gradually became more pronounced. Even if prenatal alcohol vs. hypoxia exposure results in a different course of changes, some parallel findings have been established. This especially refers to long-term compensation during the adult phase regarding impairment observed during the juvenile phase.

Juvenile Phase/Adult Phase

Comparable findings concerning the prenatal phase have been described by other authors (20). They exposed pregnant Long-Evans rats to a 13% oxygen atmosphere for 2 months and likewise failed to find any deviations regarding body weight and food consumption. Gestational periods were of normal duration. Even the slight differences in litter size and sex ratio corresponded to our results. In another study, rats were subjected to a 4% O_2 hypoxia for 5 min daily during pregnancy (8). They found surprisingly few divergences in litter size, mortality rate, and body weight. Thus, in accordance with the few comparable studies, the experimental conditions employed in the present trial have proven appropriate for inducing moderate damage to prenatal development. This is important, as numerous investigations indicate how limited adaptability is under oxygen reduction. Obviously, if in addition to oxygen reduction, air humidity, temperature, or food supply sinks below normal values, serious physiologically disturbed maturation has to be expected (38,59,65). Concerning physiological landmarks, our results show that the induced disorders were manifestations of an untimely development (i.e., either premature or delayed). The body weight increase beyond the control level, especially in prenatally treated males, can be interpreted as compensation for the lower birth weight. This is supported by other research (5,38,54,59). Regarding the behavioral tests, a delayed performance was noticeable in the righting reflex, the negative geotaxis, and the chimney tests, which conforms to other findings (54). Clear coordinational disorders were perceived under raised requirements, especially in the water escape and swimming tests. Notably higher locomotor activity (open field test) in 48-day-old experimental rats was confirmed by another study (22). The observation that heightened activity is transitory and therefore not exhibited by adult rats has been proven by other authors (45,54). The decline in hyperactivity has been related to a delayed maturation of inhibiting neuronal structures in the hippocampus, which have been shown to be sensitive to hy-

poxia (21,27). The spatial orientation and learning ability (olfactory test, water maze) of young hypoxia-exposed rats were impaired compared to the controls. With practice, these deficits were eliminated in the experimental rats, especially in the water maze. Similar results have been found in Wistar rats concerning maze learning (45).

In the active avoidance test, the shorter latency period of the 10-month-old male animals, prenatally exposed to hypoxia, was remarkable. However, it is obvious that this faster avoidance reaction was not caused by increased locomotor activity, as other tests (e.g., holeboard test) yielded no indication in this direction for the adult rats. The deviating behavior of the treated rats might be better accounted for as heightened sensomotoric sensitivity. This explanation also substantiates the fast extinction of the learned behavior after discontinuing the aversive stimulus. Also, in the passive avoidance test, increased locomotor activity can be excluded as a reason for the deviating behavior of the prenatally treated animals. At the beginning of the test, there was no difference in the crossing rate—as criterion for locomotor activity—between the examined groups. The higher score of errors in the hypoxic group could be interpreted either as deficient inhibition of spontaneous reaction (11,50) or as disturbed retention. In comparison to these results, the deficits in the FR/DRL test might have a different cause. One possibility is that the prenatally hypoxic rats' emotionality was affected. During the test phase DRL, in which a strict time schedule is required to get a reward, high emotionality could have compromised the animals' attention, resulting in lower efficacy. This is in line with other results (46,62). Alternatively, the poorer performance could be due to a deficient relearning ability (27). The inadequate modification of operant behavior under changed conditions can be viewed as rigidity normally found only in aged rats (7,40). In the rotorod long-term test at 10 rpm, the adult experimental rats performed as well as the controls. At 20 rpm, however, they needed considerably more practice throughout the adult phase. These data constitute evidence of a relatively stable adult phase, in that impairment only becomes apparent under high demands.

Late Adult/Senile Phase

In the motor-coordinative field (rotorod long-term test), there was no further evident performance impairment in the experimental rats at either rotation speeds (10 rpm and 20 rpm), up to about the 22nd month. Older rats, however, showed a further decrease in performance that paralleled the age-related changes of the controls. This performance profile may be characteristic of hypoxia-induced damage, if compared to secondary damage caused by other agents. For example, after prenatal alcohol exposure, the running performance of the adult rats, even under higher demands (20 rpm), equalled that of the controls until the age of 18 months (37). Only in older rats could a marked difference be statistically verified between the alcohol-exposed rats and the controls. Thus, it can be assumed that the reduced running performance after prenatal exposure to hypoxia might have had other causes. In the running belt test, other investigators found an age-related decline in performance that was reduced by approximately 85% within the months 10–30 (29). The 19-month-old controls of the present study achieved similar values even under the 10% O_2 hypoxia. In contrast, the poor running performance of the hypoxic rats is presumably noxae-characteristic; this decrease could be indicative of premature aging. In the cognitive field (passive avoidance test), the hypoxia-exposed animals too showed learning deficits during the adult phase. However, at 20 months, they did not differ from the untreated rats. A superimposition of the treatment effect upon the normal course of age-dependent decline could offer a possible explanation. It is generally accepted that learning impairment can be observed at the age of 20 months, particularly under raised demands (10,18,25,28,34,36,41).

The prenatal treatment did not affect any vital functions beyond the impaired adaptability, which is concomitant with advancing age concerning physiological parameters. Despite prenatal hypoxia treatment, regulation of body temperature remained remarkably stable in adult rats, even under a drastic reduction of the surrounding temperature. This is backed up by other findings that show body temperature regulation only becomes labile in old age (35,43). Similar to this, 19-month-old animals, prenatally exposed to hypoxia, compensated for a burden of 10% O_2 atmosphere as slowly as the controls. Food and water consumption as well as body weight normalized after a delay. Thus, the ability to adapt to a reduced oxygen supply exhibits an age-related decline (51) with no further impairment due to the effect of the prenatal treatment.

Numerous investigators have examined the effects resulting from exposure during the pre-, peri-, or neonatal phase to a mild or moderate hypoxia in different species (39,48,60). The provoked defects ranged from sensomotoric coordination disturbances to impaired learning behavior (26,42,45). It has repeatedly been shown that most of the defects were no longer discernible after some further development (23,54). This finding is related to the fact that the immature brain is less vulnerable to oxygen reduction than the mature one (15,60). The extent of long-term changes in the brain is not only highly dependent on the timing of the exposure but also on the duration and degree of oxygen reduction. Moreover, compensatory processes during the posthypoxic phase play an important role. There seems to be a certain degree of hypoxia that, because of compensatory circulating and metabolic mechanisms known as ontogenetic adaptations (24), does not result in central nervous system injury and subsequent motor and cognitive deficits. The complex mechanisms responsible for reversible or irreversible injuries in neurons are still largely unknown. After hypoxia, disturbances of monoamine metabolism and changes in neurotransmitter levels (catecholaminergic, indoleaminergic, cholinergic, and glutaminergic systems) have been found. These effects are above all ascribed to a lower activity of oxygen-dependent enzymes in the biosynthetic pathways. This could be proven for certain brain areas such as the cerebral cortex, hippocampus, limbic circuits, corpus striatum, hypothalamus, cerebellum, and medulla oblongata (21,44,53,56). Disturbances of cell migration, as well as injury, atrophy, or loss of neuronal cells or impaired reorganization of damaged neuronal connectivity, may also contribute to long-term damage (6,23,27,56,61). Repeatedly, the discrepancy between conspicuous histopathological findings and/or neurobiochemical alterations on the one hand, and comparable deficits in behavioral performance on the other hand, has been recorded (26,42,45,47). This complicates comprehension of the specific means and extent of hypoxia-related effects on behavioral development. Moreover, it is difficult to predict how performance might be influenced by moderate prenatal exposure to this neurotoxicant during the senile phase.

An evaluation of all the above results allows the conclusion that prenatal exposure to hypoxia provokes a characteristic pattern of damage throughout the different life phases. Basic performance (e.g., negative geotaxis, traction test, regulation of body temperature) changed only slightly or not at all com-

pared to unexposed aged rats. However, in complex functions (e.g., running belt, rotorod tests, FR 1 : 10/DRL) premature and/or more pronounced impairment was found vs. untreated individuals. This performance profile reflects a modified course of decreasing adaptability that might indicate an earlier onset of the aging process. The hypothesis of decreasing adaptability during aging is also supported by another long-term study using the noxa diazepam. In this investigation, rats were prenatally treated with 20 mg diazepam/kg/day via food from day 1 to 17 postconception (unpublished). The findings demonstrated only minor changes compared to controls; that is, they deviated from the present results as well as from those found in the alcohol study. The physiological maturation of the young animals was not affected. Only in individual cases were developmental delays observed in motor function tests, such as the righting reflex, swimming, and chimney tests. A noticeable influence could not be established in the late adult and senile phases.

In conclusion, all the data from these three life span studies confirm the principle that centrally controlled adaptive abilities can, depending on the noxa used, be differentially and irreversibly impaired in range and intensity. They contribute to the understanding of the interrelationship between prenatally induced long-term influences and the extent of adaptive counterbalance.

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