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THE STRIATAL DOPAMINE DEPENDENCY OF LIFE SPAN IN MALE RATS. LONGEVITY STUDY WITH (−)DEPRENYL

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SUMMARY

Long-term experiments on male rats revealed that better performers in the mating test are better learners in the shuttle box and the more active animals live significantly longer than their less active peers.

It was established by the aid of (−)deprenyl, a highly specific chemical tool, which increases superoxide dismutase activity in the striatum, facilitates the activity of the nigrostriatal dopaminergic neurons with utmost selectivity, and protects these neurons from their age-related decay, that the efficiency of a male rat in behavioral tests, as well as the duration of its life are striatal dopamine dependent functions.

As a measure of striatal function, sexual activity was tested once a week in a group of male rats ($n = 132$) from the 24th month of their life. Because of the age-related decay of this function none of the 2-year-old animals displayed full scale sexual activity. By dividing the group equally the rats were treated with saline (1 ml/kg, s.c.) and deprenyl (0.25 mg/kg, s.c.), respectively, three times a week. In the saline-treated group ($n = 66$) the last signs of sexual activity vanished to the 33rd week of treatment. (−)Deprenyl treatment restored full scale sexual activity in 64 out of 66 rats.

The longest living rat in the saline-treated group lived 164 weeks. The average lifespan of the group was 147.05 ± 0.56 weeks. The shortest living animal in the (−)deprenyl-treated group lived 171 weeks and the longest living rat died during the 226th week of its life. The average lifespan was 197.98 ± 2.36 weeks, i.e. higher than the estimated maximum age of death in the rat (182 weeks). This is the first instance that by the aid of a well-aimed medication members of a species lived beyond the known lifespan maximum.

Key words: Striatal dopamine; Aging of the striatum; (−)Deprenyl; Sexual activity; Learning; Superoxide dismutase activity; Rat lifespan maximum

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is probable that only by increasing life span, or maximum age of death, of ers of a species, will important insight be made into the aging process" [1]. the case of the human race, the lifespan maximum [the human technical n (TLS^h)], most accurately established, according to the Guiness Book of Records, is 115 years. This is at present the longest properly documented a human being. It is probable that tens and even hundreds of millions die ery person actually approaching this limit. However, the mere existence of ossibility is convincing proof that there are opportunities of science to lerably increase the average span of life.

e mainly to revolutionary developments in chemotherapy, immunology, and ıe, life expectancy changed during the 20th century in a striking manner. In veloped countries the proportion of the citizens over 65 years of age is five times higher by now than 100 years ago. According to Fries [2] life tancy from birth in this century in the USA increased 26 years, from 47 to ırs, from age 60 the increase was found to be 5 years, from age 80 only 2.5 The maximum age of death, TLS^h, remained unaltered.

h regard to the rat, the most commonly used experimental animal, the ıum age of death can, in the lack of exact statistics, only be estimated. g a combination of data in the literature and personal experience, 3.5 years weeks) would be a reasonable estimate of the lifespan maximum [rat cal lifespan (TLS^r)].

ɔiogerontological literature different kinds of diets and medications in mice increasing life expectancy, but leaving lifespan maximum unchanged, are bed. An example, closely related in its topic to the present study, is the of Cotzias *et al.* [3], describing that the dopaminergic drug, levodopa, istered to mice in their diet prolonged the span of life by a maximum of As shown in Fig. 1 the longest living mice in the levodopa-treated group efore reaching their 1100th day. None of the several thousands of the Swiss mice used in this experiment approached the lifespan maximum of this (1300 days, as a minimum, would be a reasonable estimate).

only paper found in literature referring to dopaminergic activity and life ancy *in rats* was that of Clemens *et al.* in 1979 [4]. The paper, dealing with ıeuroendocrine aspects of aging mentions as a secondary observation, that , but not male, rats treated with lergotrile mesylate, the dopamine receptor t which was a forerunner of bromocryptine in the clinic and was later awn because of side effects, tended to live longer than the controls. The gures showing the results demonstrate that in two series of experiments a percentage of the females fed lergotrile mesylate in the diet were alive at d of their 2nd year of age as in the control group. The fact that about 40% animals were lost before completing their 2nd year discredits the data. ıgh this study can be taken just as a hint that long-term medication with a

dopamine receptor agonist increases life expectancy in the rat, it essentially supports the finding of Cotzias *et al.* [3].

The development of (−)deprenyl (for review see [5]) offered an approach for studying the relation between brain dopaminergic activity and longevity. This safe and unique molecular tool allowed for the first time to keep members of a species (male rats) alive beyond the maximum age of death. The experimental findings and their theoretical interpretation are now presented in this paper.

THE AGING OF THE STRIATAL DOPAMINERGIC SYSTEM. ITS RELEVANCE TO THE AGE-RELATED DECLINE OF DRIVE-MOTIVATED BEHAVIOR AND THE PHARMACOLOGICAL CONSIDERATIONS TO COUNTER IT

The rapid increase of the proportion of the aging and aged in the total population of the developed countries makes the efforts to counter the age-related decay of mental performances and decrease the incidence of such age-dependent CNS illnesses, like Parkinson's disease, Alzheimer's disease, senile dementia, involutional depression, etc. imperative.

It is too late to fight, as we do in actual practice, against the age-related mental decline and age-dependent CNS diseases after their manifestation. The ideal strategy is to reveal one by one the brain structures the age-related biochemical lesions of which lead to mental impairment and then take our chance to develop that safe and utmost selective drug which will prevent or slow down the progress of culpable changes. To illustrate this new line of research, striatal dopamine and (−)deprenyl served as an experimental model (for review see [6–9]).

The nigrostriatal dopaminergic system seems to be an ideal model for two reasons. On the one hand, striatal dopamine is essential for the formation and upkeep of that specific kind of central excitatory state [10] which under natural conditions enables that the animal will be ready to surmount every obstacle, even if life is in the balance, to seize its food or reach its sexual partner. On the other hand, as far as we now know, the nigrostriatal dopaminergic neurons are the most rapidly aging neurons in the brain.

The dopamine content of the human caudate nucleus decreases by 13% per decade over age 45 [11]; dopamine itself is with high probability the culpable substance [9]. The complex auto-oxidation of the high amounts of dopa and dopamine in the striatum, continuously generating substantial quantities of toxic free radicals and highly reactive quinones, creates a permanent danger for the nigrostriatal dopaminergic neurons, which have to mobilize their natural defensive measures to protect themselves from the deleterious effect of these toxic byproducts. Neuromelanin, which is generated via the polymerization of oxidative products of dopamine with the evident aim of finally depositing waste products, is in the substantia nigra the visible sign of the successful self-defense of the neurons against the free radicals and quinones originating from dopamine

metabolism. The sluggish depositing of neuromelanin in the human substantia nigra [12] is in excellent agreement with this view.

Table I illustrates the age-related changes in the physiological function of the human striatum. We know that parkinsonian symptoms appear if the dopamine content of the caudate sinks below 30% of the normal level. Thus it is understandable that during the normal average life span the age-related decay of the striatal dopaminergic system does not lead to parkinsonian symptoms.

As in Parkinson's disease the dopamine content of the striatum decreases rapidly below the critical level, what essentially happens in this illness is a dramatic dissociation between the chronological and the physiological age of the striatum, i.e. the disease is the premature rapid aging of the striatal dopaminergic system of unknown origin. A 65-year-old patient usually lives with a striatum of a physiological age of 115 years. Remarkably, the low level of dopamine (less than 10%), found characteristically in the striatum of parkinsonian patients post mortem, is theoretically reached in a normal aging brain at the maximum age of death of the human race (see Table I).

Aging of the nigrostriatal dopaminergic neuron is essentially similar in rodents as in humans (for review see [13]), though changes in the aging rodent brain might be smaller than in the human brain (for review see [14]). The most convincing direct biochemical evidence in rats that striatal dopaminergic function declines with age is the unequivocally demonstrated loss of striatal D_2-receptors in the aging brain [15–17].

As an adaptive physiological compensation for the loss of nigrostriatal dopaminergic neuronal activity an upregulation of postsynaptic D_1-receptors in the caudate nucleus was found by Morgan *et al.* [18] in the human brain, but not

TABLE I

ILLUSTRATION OF THE AGE-RELATED CHANGES IN THE PHYSIOLOGICAL FUNCTION OF THE HUMAN STRIATUM

The calculation is based on the fact that the average loss of dopamine per decade in the human caudate over age 45 amounts to 13% [11].

Chronological age	*Physiological status of the striatal function in %*	
45	100	
55	87	
65	74	
75	61	
85	48	
95	35	Critical threshold
105	22	
115	9	TLS^h

Critical threshold: Parkinsonian symptoms appear if the dopamine content sinks below 30% of the normal level. *TLS^h:* Technical lifespan, i.e. maximum age of death in humans (115 years).

corroborated by Bzowej and Seeman [19]. Whereas O'Boyle and Waddingt [20] and Finch and Morgen [21] did not detect any changes in the availability D_1-receptors in senescent rats, Henry *et al.* [22] described an age-relat decrease of D_1-receptors in the striatum of Wistar rats. All things consider there is convincing direct biochemical evidence that the striatal dopaminerg system in the rat brain shows an age-related decline.

As the unavoidable natural aging process of the nigrostriatal dopaminerg neurons is to all probability due to specific endogenous neurotoxins originati from dopamine, it seems reasonable to develop drugs which provide protecti against the self-produced neurotoxins and slow down the age-related changes the striatum. The rat can be used as an appropriate animal for this research.

We developed (−)deprenyl [23], the first selective inhibitor of B-ty monoamine oxidase (MAO) [24] and still the only one in clinical use, a sa substance which when administered in very small doses over a long perio facilitates the activity of the nigrostriatal dopaminergic neurons with high sele tivity [5,25] and protects these neurons from the neurotoxicity of 6-hydrox dopamine (6-OHDA) [26] and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridi (MPTP) [27].

The hypothesis was put forward in 1981 [6,7] that long-term (−)depren treatment may counter the marked age-related decline of the nigrostriat dopaminergic neurons, and sexual performance of male rats, a quantifiab striatal-dopamine dependent function decreasing with age, was selected as experimental model to check the validity of this hypothesis. Using this model were able to demonstrate that the continuous administration of low doses (−)deprenyl restored the lost sexual vigour of aged male rats [6–8,28]. In a pil study of 16 rats also, the extension of lifespan of rats by long-term (−)depren treatment was demonstrated [29].

The aim of this study is to clarify with new experimental data the relationsh between striatal dopamine, sexual activity and lifespan in the male rats.

CONSIDERATIONS ABOUT THE DISSOCIATION BETWEEN 'CHRONOLOGICAL' AN 'PHYSIOLOGICAL' AGE

Aging, the unfortunate common fate of all mature adults, is a physiologic phenomenon. It essentially means the decadence of the quality of life with tl passage of time. The easily recognizable outside appearance of aging (greyi hair, wrinkling skin, need of glasses for reading, etc.) gives some informatic about the chronological age of the person, but these signs are not necesssarily complete harmony with the physiological age of the organ systems, with tl measurable decrements of integrated functions (maximum O_2 capacity, maximu breathing capacity, maximum work rate, etc.) and with the almost unmeasurab mental deterioration.

The exact measurement of the age-related changes in man remains difficul

because the most reliable technique, to follow the changes in the same person over the entire age span is practically unfeasible. The available information about the age-related changes in human population stems from cross sectional studies, i.e. the comparison of differences in performances between different age-groups.

One factor, which explains the extreme difficulties in measuring age-related changes with statistical methods, lies in the non-linear nature of the regression of a number of functions. The main problem, however, is that the individual differences within a particular age-group for any complex and important function are extreme. The reason for this exorbitant variation is the lack of a general factor of physiological age. In cross sectional studies no single age emerges as the point of sharp decline in function. Any individual may show different levels of performance and the careful observer finds many dissociations between 'chronological' and 'physiological' age.

Data with regard to the sexual vigour of the human male is a good example to illustrate how difficult it is to estimate the age-related decline of this function. Sexual activity in the human male is known to be influenced by a number of factors, like good health, stable marriage, satisfactory sexual partner(s), adequate financial and social status, etc. But even in the males who meet all the requirements for retention and maintenance of sexual functioning, there is an age-related decrease in the sexual vigour, the reason of which still remains obscure.

Martin [30] studied coital activity as a function of age, interviewing 628 members of the Baltimore Longitudinal Study of Aging. The subjects were white, married, urban residents in good health from the Washington-Baltimore area, varying from 20 to 95 years of age. According to this study the median coital activity was the highest, 2.1/week, between ages 30 and 34 and decreased progressively with increasing age, sinking to 0.2/week in the 65–69 year age group. Thus the age-related decline of coital activity is unequivocaly detectable by the statistical method.

The data in Martin's study, however, also throw light on the enormous individual variations in sexual vigour. The mean frequency of total sexual activity in 159 males was found to be 520 sexual events per 5 years in the 20–39 year age group, including young males performing below 100 sexual events per 5 years and those with frequencies of total sexual activity over 1000 sexual events per 5 years.

In the 65–79 year age group, the mean frequency of total sexual activity decreased to 75 sexual events per 5 years, but even in this group subjects producing 400–700 sexual events per 5 years were registered. Exactly the same correlations were found in our studies with male rats.

AN EXPERIMENTAL APPROACH TO EXPLAIN THE INDIVIDUAL DIFFERENCES IN BEHAVIORAL PERFORMANCES AND LIFE EXPECTANCY

Table II classifies a huge group ($n = 381$) of healthy, 3–6-month-old, sexually inexperienced male rats according to their sexual performance in four con-

TABLE II

THE INDIVIDUAL DIFFERENCES IN THE COPULATORY ACTIVITY OF MALE RATS

The sexual performance of male CFY rats, 3–6 months of age was tested once a week and the performance was evaluated from four consecutive mating tests. The copulatory patterns of the male (mounting, intromission and ejaculation) in the presence of the receptive female were scored in the light phase between 1100 h and 1430 h. For methodological details see: [28].

Distribution of a group of 381 males according to their performance

Qualification of performance (mark)	*Complete sexual inactivity (1)*	*Mountings only (2)*	*Mountings and intromission ('sluggish') (3)*	*Ejaculation in one test only (4)*	*Repeated ejaculations during testing (5)*
	21	20	140	80	120

secutive weekly mating tests. As expected, the majority of the young adult males (200 out of 381) displayed at least one ejaculation during the four consecutive mating tests. But more than one third of the population (140 rats) showed two of the measured patterns only and a lower percentage of the group was even less active during the tested period.

The first question is of course, why do we have, with regard to such basic physiological activity like sexual performance, so perplexing differences in a healthy young adult male population?

Table III shows the individual differences in learning performance of 138 randomly selected naive male rats trained at 20 trials daily in the shuttle box for 5 days (Series 1). The data are typical. The overwhelming majority of the rats

TABLE III

THE INDIVIDUAL DIFFERENCES IN THE LEARNING ABILITY OF MALE RATS IN THE SHUTTLE BOX (SERIES 1)

Screening in the shuttle box. Each rat was trained at 20 trials daily for 5 days. Unconditioned stimulus (US) = electric shock via the grid of the floor; conditioned stimulus (CS) = buzzer + light. Unconditioned avoidance response: the rat escapes to US within 5 s; conditioned avoidance response (CAR): the rat escapes to CS within 10 s. The rat's performance was rated according to the total number of CARs produced during the 5 days of training as follows: miserable: 0–5; weak: 6–15; medium: 16–30; good: 31–45; excellent: >45.

Distribution of a group of 138 males according to their performance

Qualification of performance (mark)	*Miserable (1)*	*Weak (2)*	*Medium (3)*	*Good (4)*	*Excellent (5)*
	6	21	68	41	2

belong to the medium and good performers, a few are dull and 2 out of the 138 are extremely bright.

Table IV shows the individual differences in learning performance of 75 randomly selected naive male rats trained at 100 trials daily for 5 days in the shuttle box (Series 2). Data are essentially similar to the ones observed in the group of rats trained at only 20 trials daily.

Once more the primitive question arises, what is essentially different between the dull animals and the top performers?

To approach the answer to the two questions, a third question has to be asked. Are the individual performances in the two selected tests, which, represent two unrelated behavioral patterns, completely independent from each other, or the observed great individual differences in the two tests have something common in origin and reflect the individual differences in the state of a system of basic importance for both sexual and learning performances?

To answer the third question, which is decisive for further work, experiments were carried out as follows. According to the data in Table II, 31.8% of the rats displayed full scale sexual activity, whereas 5.7% did not present any of the copulatory patterns. Thus, we selected from a huge population 94 sexually inactive rats and 99 fully active ones. We checked then how these specially chosen two groups of rats perform in the shuttle box. Table V shows the results.

The difference in the learning ability of the sexually inactive rats compared to their sexually active peers is dramatic. Whereas 2 out of the 99 sexually active male rats proved to be miserable performers in the shuttle box, 55 out of the 94 sexually inactive rats turned out to be dull.

Now we can put a further question. There are well known individual differences in the lifespan of otherwise healthy male rats. Is there any relation

TABLE IV

THE INDIVIDUAL DIFFERENCES IN THE LEARNING ABILITY OF MALE RATS IN THE SHUTTLE BOX (SERIES 2)

Screening in the shuttle box. Each rat was trained at 100 trials daily for 5 days. Unconditioned stimulus (US) = electric shock via the grid of the floor; conditioned stimulus (CS) = buzzer + light. Unconditioned avoidance response: the rat escapes to US within 5 s; conditioned avoidance response (CAR): the rat escapes to CS within 10 s. The rat's performance was rated according to the total number of CARs produced during the 5 days of training as follows: miserable: 0–25; weak: 26–100, medium: 101–250; good: 251–400; excellent: >400.

Distribution of a group of 75 males according to their performance

Qualification of performance (mark)	*Miserable (1)*	*Weak (2)*	*Medium (3)*	*Good (4)*	*Excellent (5)*
	9	22	18	18	8

TABLE V

THE OUTSTANDING LEARNING PERFORMANCE OF 8 MONTHS OLD SEXUALLY ACTIVE MALE RATS COMPARED TO THEIR INACTIVE PEERS

For methodological details see Table II.

Distribution of the groups according to their performance in the shuttle box					
Qualification: of performance (mark)	*Miserable (1)*	*Weak (2)*	*Medium (3)*	*Good (4)*	*Excellent (5)*
Sexually active rats ($n = 99$)	2	18	33	42	4
Sexually inactive rats ($n = 94$)	55	21	14	4	0

between the individual differences in behavioral performances and life epectancy?

To approach an answer to this question let us follow the fate of 66 sexually inexperienced, randomly selected, 2-year-old male rats which were first tested for their copulatory activity during the 24th month of their life. Three patterns (mounting, intromission and ejaculation) were scored.

As it will be analyzed in detail later (see Tables X and XI) 2-year-old male rats can be taken from the point of view of coital activity equivalent with at least 85-year-old men. At this age none of the rats displayed ejaculation during the four consecutive mating tests, i.e. none of the animals was sexually fully active. Of the 66 rats 22 proved to be sluggish (showed two of three patterns: mounting and intromission), 21 produced the mounting reaction only and 23 proved to be non-copulators and did not present any of the patterns.

Table VI shows the dying out of the animals in the three groups of different copulatory activity. The non-copulators, i.e. the group of rats without any sign of sexual activity, died out first. The next group displaying at least one of the patterns (mounting), lived longer. The longest living animals were those which performed two of the patterns (mounting and intromission) during the test period ('sluggish' group).

The results can be interpreted as prima facie evidence that the observed individual differences in the two different behavioral tests are somehow related to each other and seem to reflect individual differences in the activity of a system of common importance for both performances and the better performer, i.e. the more active animal, has a better chance of living longer.

Considering the physiological basis of the drives, as described in a previous work [10], and the role of the catecholaminergic system in the general activation of the brain we may assume that the individual differences in the cate-

TABLE VI

THE DYING OUT OF MALE RATS WITH DIFFERENT SEXUAL ACTIVITY

66 sexually inexperienced male rats were checked for copulatory activity in four consecutive weekly mating tests during the 24th month of their life. According to this screening they were divided in three groups: 23 'non-copulators', 21 mounting rats and 22 'sluggish'. After screening they were treated with saline (0.1 ml/100 g, subcutaneously) three times a week and their survival rate is shown below.

Week of saline treatment	*Number of dying animals in*		
	Group 1 ('non-copulators') (n = 23)	*Group 2 (showing mounting reaction only) (n = 21)*	*Group 3 (sluggish rats displaying mounting and intromission) (n = 22)*
36th	2		
37th	4		
38th	8	1	
39th	—	—	
40th	3	—	
41st	5	1	
42nd	1	7	
43rd		6	1
44th		3	4
45th		—	1
46th		2	2
47th		1	5
48th			3
49th			1
50th			2
51st			—
52nd			—
53rd			—
54th			—
55th			—
56th			2
57th			—
58th			—
59th			—
60th			1

cholaminergic tone might be responsible for the observed individual differences in behavioral performances. The postulated hypothesis predicts that by increasing the catecholaminergic tone in the brain over a long period, via appropriate continuous medication, a short living low performer will change to a long living good performer. This is exactly the change we succeeded in achieving in 2-year-old male rats by the continuous administration of (−)deprenyl to the end of their life.

THE PHARMACOLOGICAL PROFILE OF (−)DEPRENYL. ITS UNIQUE SELECTIVITY TO THE NIGROSTRIATAL DOPAMINERGIC NEURONS

The main physiological role of dopamine in the striatum is the continuous inhibition of the release of acetylcholine from the cholinergic interneurons of the caudate nucleus. Regarding the physiological significance of this function, one aspect, its rate-limiting role in the control of motor functions, is firmly established by now (for review see [31]).

According to our studies, striatal dopamine plays a role of basic importance in the maintenance of drives in the rat. The best model for testing this physiological role of striatal dopamine is the analysis of copulatory activity in the male rat, as this kind of drive-motivated behavior can be measured quantitatively on the long run. The age-related decay of this function is clearly related in the male rats to the aging of the nigrostriatal dopaminergic system [6,7,28].

As it was previously mentioned the nigrostriatal dopaminergic neurons are probably the most rapidly aging neurons in the brain. Thus, to check the validity of the thesis, proposed first in 1981 and extended later [6–9,29], that aging of the striatal dopaminergic system might be countered selectively by protecting it from the deleterious effect of its self-produced toxic metabolites via long-term administration of (−)deprenyl, seems to be of great practical importance.

(−)Deprenyl is for the time being the only promising tool for this purpose in humans because of the following reasons:

(a) Given concurrently with levodopa and a peripheral decarboxylase inhibitor (−)deprenyl is now widely used in Parkinson's disease [32], it is an efficient antidepressant [33,34] and its beneficial effect in Alzheimer's disease was recently demonstrated [35]. There is general agreement about its safeness. The small dose administration of (−)deprenyl even for several years was described to be free of significant side effects (for review see [36]).

(b) The supplementation of (−)deprenyl to Madopar ($n = 564$) significantly prolonged the survival of parkinsonian patients as compared to those on Madopar alone ($n = 377$), indicating that (−)deprenyl protected somehow the nigrostriatal dopaminergic neurons in these patients [37].

(c) (−)Deprenyl exerts its specific pharmacological effect in animals with an excellent safety margin [26]. The subcutaneous administration of a very small dose, 0.25 mg/kg per day for 2–4 weeks, is sufficient to observe the highly selective pharmacological spectrum in the rat. In long-term experiments (several months or years) 0.25 mg/kg three times a week is appropriate for maintaining the effect. The relation of the effective doses to the subcutaneous LD_{50} in the rat (205 mg/kg) and the pharmacological selectivity of the compound explain the safeness of the drug.

(d) (−)Deprenyl was the fist described highly selective inhibitor of B-type MAO [24,38] and is still the internationally used reference substance for this purpose and the only one available clinically.

The role of the inhibition of glial MAO-B in the enhancement of the physiological function of the nigrostriatal dopaminergic neuron was analyzed in detail [6,7,31,39]. (−)Deprenyl was demonstrated to exert this effect in the low dose range (0.05–0.25 mg/kg) the long-term administration of which left the activity of MAO-A, in the brain and in the periphery, sufficient for the maintenance of its physiological role in metabolizing the biogenic amines (for review see [5,25,38,40–42]).

(e) (−) Deprenyl turned out to be an efficient inhibitor of the uptake of dopamine in the striatum and this effect was found to play an important role in the facilitation of the activity of the nigrostriatal dopaminergic neurons in animals treated continuously with (−)deprenyl [5,25,39,40].

(f) (−)Deprenyl in striking contrast to the MAO inhibitors used previously, inhibits the noradrenaline-releasing effect of indirectly-acting amines (phenylethylamine, tyramine) in vascular smooth muscle [43]. This latter effect of (−)deprenyl, which is not shared even with other known selective inhibitors of B-type MAO [39,44], is the reason for the safety of (−)deprenyl. It is, at present, the only MAO inhibitor free of the 'cheese effect' (for review see [42]).

(g) (−)Deprenyl protects the nigrostriatal dopaminergic neurons from the neurotoxic effect of 6-hydroxydopamine [5,26,45], and abolishes the dopamine receptor super-sensitivity observed following 6-hydroxydopamine treatment [46].

(h) (−)Deprenyl protects the nigrostriatal dopaminergic neurons from the neurotoxic effect of MPTP in monkeys [27].

(i) (−)Deprenyl enhances the activity of the nigrostriatal dopaminergic neurons at a dose of 0.25 mg/kg daily in the rat, whereas the activity of the limbic dopaminergic system is influenced by high doses (over 10 mg/kg) only [47].

(j) (−)Deprenyl in a daily dose of 0.25 mg/kg inhibits the release of acetylcholine in the caudate nucleus, which is indirect evidence of the enhanced activity of the nigrostriatal dopaminergic neurons [26,45]. The turnover rate of acetylcholine altered in (−)deprenyl-treated rats only in the striatum, but remained unchanged in the hippocampus, proving that the substance has no action on the cholinergic system [46].

(k) The selectivity of (−)deprenyl, in the small dose-range, to the nigrostriatal dopaminergic neuron is also supported by our findings that whereas the turnover rate of dopamine was enhanced in the striatum, a significant decrease of the turnover rate of noradrenaline and unchanged level of this amine in the brain stem were found [48].

(l) (−)Deprenyl has only a very small effect on the activity of the serotonergic system in the brain [46,49].

(m) The release of dopamine from the striatum of rats treated with 0.25 mg/kg per day (−)deprenyl for 3 weeks is increased 7-fold to stimulation (direct measurements made with reverse phase high performance liquid chromatography with electrochemical detection) [8,50].

(n) Previous studies with (−)deprenyl showing the protection of nigrostriatal dopaminergic neurons from the toxic effect of 6-hydroxydopamine led to the assumption that (−)deprenyl enhances scavenger function in these neurons [5]. To find direct evidence, measurements of superoxide dismutase (SOD), catalase and glutathione peroxidase activity have been made in the rat striatum of saline-, deprenyl- and clorgyline-treated rats. These enzymes protect the neurons against endogenous toxic materials. SOD seems to play the key role in the detoxication of free radicals resulting from auto-oxidation of the endogenous metabolites of dopamine.

Daily administration of (−)deprenyl for 3 weeks enhanced the activity of SOD in the striatum in proportion to the dose given (Table VII). Though catalase and glutathione peroxidase activity did not change in a statistically significant manner, the tendency of an increased activity in (−)deprenyl-treated rats is undeni-

TABLE VII

SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN THE STRIATUM OF RATS TREATED WITH (−)DEPRENYL AND CLORGYLINE, RESPECTIVELY

Total SOD activity was determined by the adrenochrom method according to Misra and Fridovich [56]. MAO inhibitors were injected subcutaneously daily for 21 days. A group of ten rats was used for each dose. Enzyme activity was measured 24 h after the last injection.

	SOD activity in the striatum (mean activity: unit/mg protein ± S.E.M.)	
	Male	*Female*
Saline	6.24 ± 0.27	6.42 ± 0.50
Treated with (−)deprenyl (mg/kg/day)		
0.25	9.03 ± 1.00	9.76 ± 1.68
0.50	9.46 ± 1.30	17.43 ± 2.22*
1.00	40.62 ± 3.49**	17.96 ± 2.46**
2.00	67.60 ± 3.25**	26.50 ± 4.62**
Saline	6.24 ± 0.27	6.42 ± 0.5
Treated with clorgyline (mg/kg/day)		
0.1	3.14 ± 0.17	3.06 ± 0.15
0.25	2.31 ± 0.15	3.09 ± 0.22
0.50	3.17 ± 0.13	3.47 ± 0.27
1.00	2.34 ± 0.24*	2.69 ± 0.37*

Significances according to Student's *t*-test for two means: *$P < 0.001$; **$P < 0.0001$.

TABLE VIII

CATALASE AND GLUTATHIONE PEROXIDASE ACTIVITY IN THE STRIATUM OF RATS TREATED WITH (−)DEPRENYL AND CLORGYLINE, RESPECTIVELY

Significances were calculated according to Student's *t*-test for two means. Catalase activity was measured according to Beers and Sizer [57] and expressed in Bergmeyer units. Glutathione peroxidase activity was measured according to Chiu *et al.* [58]. MAO inhibitors were injected subcutaneously daily for 21 days. A group of ten rats were used for each dose. Enzyme activity was measured 24 h after the last injection.

	Catalase activity ($\times 10^{-5}$) (mean activity: unit/mg protein ± S.E.M.)		*Glutathione peroxidase activity($\times 10^{-3}$) (mean activity: unit/mg protein ± S.E.M.)*	
	Male	*Female*	*Male*	*Female*
Saline	18.53 ± 0.54	15.60 ± 0.40	34.67 ± 2.03	29.67 ± 0.33
Treated with (−)deprenyl mg/kg/day				
0.25	23.60 ± 0.55	21.60 ± 0.46	40.33 ± 0.33	38.33 ± 0.67
0.50	25.80 ± 0.40	23.73 ± 0.52	45.00 ± 0.58	36.00 ± 0.58
1.00	26.70 ± 1.00	23.60 ± 0.57	49.00 ± 1.00	42.33 ± 1.86
2.00	26.97 ± 0.64	23.90 ± 0.69	47.00 ± 1.15	43.23 ± 0.88
Treated with clorgyline mg/kg/day				
0.10	18.23 ± 0.93	21.80 ± 1.10	22.07 ± 0.82	18.93 ± 0.35
0.25	15.00 ± 0.92	16.60 ± 1.19	20.33 ± 0.47	23.27 ± 2.12
0.50	12.20 ± 1.07	13.40 ± 0.67	17.70 ± 0.55	21.87 ± 0.34
1.00	12.00 ± 0.68	13.37 ± 0.62	16.77 ± 0.62	16.17 ± 0.35

able (Table VIII). To check the role of MAO inhibition in the enhancement of SOD activity by (−)deprenyl, we measured the effect of clorgyline, one of the most potent MAO inhibitors, on scavenger function in parallel experiments. The data in Tables VII and VIII clearly show that the enhancement of the scavanger function in the rat striatum by (−)deprenyl is unrelated to the MAO inhibitory potency of this drug as the administration of clorgyline for 21 days, up to the 1 mg/kg dose which inhibits both types of MAO, results in a decrease of SOD activity in the rat striatum and also catalase and glutathione peroxidase activity shows, in contrast to the (−)deprenyl-treated rats, a tendency of being inhibited.

All in all, (−)deprenyl is a unique experimental tool for increasing the activity of the nigrostriatal dopaminergic neurons with utmost selectivity and for protecting these neurons from their endogenous toxic metabolites.

THE EXTENSION OF LIFESPAN OF MALE RATS BEYOND THE MAXIMUM AGE OF DEATH BY THE CONTINUOUS ADMINISTRATION OF (−)DEPRENYL

Based on its peculiar pharmacological spectrum we used (−)deprenyl as a sophisticated and highly selective tool for increasing the activity of the nigrostriatal dopaminergic neurons. The measure of the effectiveness of (−)deprenyl-treatment was the change in copulatory activity, a quantifiable striatal dopamine-dependent function in the male rat.

In excellent agreement with the findings in the human male the age-related decline in copulatory activity of male rats was previously demonstrated [5,6,7,28,42]. Table IX shows the copulatory activity of three age cohorts of male rats. Whereas over 50% of 3–6-month-old rats show ejaculation when tested, none of the animals completing their 2nd year display full scale sexual activity. The age-related decline of coital activity and the individual differences within the age cohorts are essentially similar in the rat (Table X) and in humans (Table XI). We have to consider, however, by judging the data in the two tables that the male rat is sexually mature much earlier than the human male. A 2-month old, sexually mature male rat equals by age a 5-year old boy. The highest average coital activity was found in the 3–6-month old age cohort of the male rats (correlating to 6–12-year-old human males), whereas 30–35-year-old males showed peak coital activity [30]. On the other hand, compared to human males the sexual activity of male rats show a more rapid decline. Collating the human data of Martin [30] with our rat data, 2-year-old male rats (equivalent by age to 50-year-old men) equal by their coital activity to at least 85-year-old human males.

The 132 two-year-old rats figured in Table IX were checked first in their life for sexual activity during the 24th month of age. They were classified according to their performance in four consecutive weekly mating tests. As seen in Table

TABLE IX

THE AGE-RELATED DECLINE OF THE SEXUAL ACTIVITY IN THE MALE RAT

For methodological details see Table II.

Age of animals (months)	*No. of animals*	*Number of males showing*			
		Complete sexual inactivity	*Mountings only*	*Mounting and intromission ('sluggish' males)*	*Ejaculation*
3–6	381	21	20	140	200
12–18	137	27	27	76	8
24	132	46	42	44	0

TABLE X

THE PERCENTAGES OF STRIKING INDIVIDUAL DIFFERENCES FROM AVERAGE COPULATORY BEHAVIOR IN DIFFERENT AGE COHORTS OF MALE RATS

Age (months)	*Comparable to the human male*		*Average copulatory behavior*	*Percentage of striking differences between physiological and chronological age*
	In chronological age (years)	*In sexual activity (years)*		
Young adult males				
3–6	6–12	20–39	>50% full scale copulatory activity (3 patterns)	6% non-copulators
8	16	40–49	>50% full scale activity	8% non-copulators
Aging and aged				
12–18	24–36	65–80	>50% 'sluggish' (2 patterns)	5% full scale activity
24	48	>85	>70% one pattern only or none	—

IX about one third of the rats (46 rats), displayed none of the three patterns (mounting, intromission and ejaculation) scored by us ('non-copulator' group). An almost similar number of the rats (42 rats) showed mounting only during testing ('mounting' group) and 44 rats presented intromission also ('sluggish'

TABLE XI

THE PERCENTAGES OF STRIKING INDIVIDUAL DIFFERENCES FROM AVERAGE COPULATORY BEHAVIOR IN DIFFERENT AGE COHORTS OF HUMAN MALES

Based on data of Martin [30].

Age category	*Chronological age (years)*	*Average performance (Sexual events/5 years)*	*Percentage of striking differences between physiological and chronological age*
Young adult males	20–39 (30–34)	≈500 (≈600)	≈4% below 100
Mature adult males	40–49 50–64	300 slowly down to 100	≈8% below 100 ≈15% over 400 ≈8% over 600
Aging and aged	65–80	below 100	≈3% over 400 ≈1.5% over 600

group). None of the animals displayed a single ejaculation during the four consecutive mating tests (for methodological details see [28,51]).

We divided each of the three categories into equal groups which were then treated subcutaneously with saline (0.1 ml/100 g) or (−)deprenyl (0.25 mg/kg), respectively, three times a week.

Tables XII, XIII and XIV show the copulatory activity of the rats from the beginning of treatment until death. (−)Deprenyl, in excellent agreement with our previous findings [6,7,28], restored lost copulatory activity in the senescent male rats. As shown in Table VI the lifespan in the control group ($n = 66$) was found to be closely related to the initial sexual potency of the animals and this relation was detected in an even more explicit manner in the (−)deprenyl-treated group of rats.

TABLE XII

SEXUAL ACTIVITY OF RATS TREATED WITH SALINE ($n = 23$) OR (−)DEPRENYL ($n = 23$), RESPECTIVELY, BELONGING TO THE 'NON-COPULATOR' GROUP BEFORE TREATMENT

Rats were tested once a week until death. For methodological details see: [28].

No. of animal	*Number of tests in which the animal displayed mounting (M), intromission (I) and ejaculation (E)*					
	Saline-treated			*Deprenyl-treated*		
	M	*I*	*E*	*M*	*I*	*E*
1	0	0	0	29	10	4
2	0	0	0	59	25	9
3	1	0	0	52	18	1
4	2	0	0	24	8	2
5	0	0	0	24	13	5
6	2	0	0	20	9	5
7	5	0	0	20	11	6
8	9	0	0	79	55	19
9	5	0	0	30	13	6
10	0	0	0	78	39	15
11	0	0	0	44	24	1
12	7	0	0	21	11	6
13	0	0	0	61	28	9
14	0	0	0	53	18	0
15	0	0	0	23	12	6
16	0	0	0	44	31	13
17	0	0	0	15	2	1
18	0	0	0	65	37	21
19	0	0	0	39	22	0
20	3	0	0	59	27	11
21	0	0	0	79	53	19
22	0	0	0	79	51	27
23	0	0	0	50	25	4

TABLE XIII

SEXUAL ACTIVITY OF RATS TREATED WITH SALINE ($n = 21$) OR (−)DEPRENYL ($n = 21$), RESPECTIVELY, BELONGING TO THE 'MOUNTING' GROUP BEFORE TREATMENT

Rats were tested once a week until death. For methodological details see: [28].

No. of animal	*Number of tests in which the animal displayed mounting (M), intromission (I) and ejaculation (E)*					
	Saline-treated			*(−)Deprenyl-treated*		
	M	*I*	*E*	*M*	*I*	*E*
1	22	4	0	47	24	5
2	21	1	0	55	36	9
3	22	1	0	55	37	9
4	25	1	0	64	40	11
5	25	0	0	51	37	13
6	0	0	0	54	33	9
7	25	0	0	53	40	10
8	25	1	0	35	15	7
9	23	0	0	52	21	3
10	24	0	0	46	19	2
11	23	1	0	50	23	7
12	14	0	0	50	29	6
13	14	0	0	71	51	20
14	15	2	0	39	16	3
15	19	3	0	49	22	7
16	23	4	0	61	30	8
17	23	12	0	61	43	8
18	24	9	0	53	33	9
19	26	10	0	57	30	7
20	15	2	0	64	41	12
21	16	3	0	62	40	7

Figure 1 shows the dying out of the 46 non-copulator rats. Out of the 23 saline-treated rats we lost the first two animals during the 140th week of their age and the longest living saline-treated male lived 146 weeks. In the (−)deprenyl-treated group ($n = 23$), however, the first rat died during the 171st week of its life and the longest living animal lived 216 weeks. This means that (−)deprenyl extended the lifespan of this group in an extraordinary manner. The shortest living (−)deprenyl-treated rat approached the maximum age of death.

The real extension of lifespan by (−)deprenyl treatment is shown by the fact that even in the group of non-copulators, which was the shortest living group, 12 rats lived beyond the TLS^r and 4 rats lived longer than 4 years.

Figure 2 shows the death rate of the 42 rats which displayed one pattern (mounting) during the first testing period. The 21 rats of this group treated with saline lived longer than their non-copulator peers. The (−)deprenyl-treated rats of this group lived longer than their (−)deprenyl-treated peers in the non-

TABLE XIV

SEXUAL ACTIVITY OF RATS TREATED WITH SALINE ($n = 22$) OR (−)DEPRENYL ($n = 22$), RESPECTIVELY, BELONGING TO THE 'SLUGGISH' GROUP BEFORE TREATMENT

Rats were tested once a week until death. For methodological details see: [28].

No. of animals	*Number of tests in which the animal displayed mounting (M), intromission (I) and ejaculation (E)*					
	Saline-treated			*(−)Deprenyl treated*		
	M	*I*	*E*	*M*	*I*	*E*
1	21	3	0	101	97	60
2	20	12	0	98	83	50
3	18	12	0	98	75	43
4	20	11	0	95	70	32
5	21	12	0	60	49	13
6	23	18	0	77	57	14
7	16	10	0	65	48	15
8	23	0	0	72	47	9
9	29	16	0	72	59	10
10	16	10	0	95	72	23
11	21	14	0	69	48	20
12	26	11	0	69	46	19
13	21	13	0	72	52	20
14	20	9	0	57	41	6
15	21	9	0	97	68	42
16	21	4	0	57	47	11
17	27	18	0	71	52	12
18	14	7	0	74	53	10
19	28	12	0	75	50	11
20	22	13	0	74	51	22
21	22	9	0	75	50	22
22	22	8	0	66	42	17

copulator category. In the (−)deprenyl-treated group 13 rats completed or lived longer than the TLS^r and 7 rats lived more than 4 years.

The 'sluggish' rats, i.e. the most active ones at the start of the experiment created the longest living group. As can be seen in Fig. 3, the saline-treated rats in this group died later than their saline-treated peers either in the 'non-copulator' and the 'mounting' group. The (−)deprenyl-treated males in this category too, were much longer living than their peers in the two other groups. Only two out of the 22 (−)deprenyl-treated rats belonging to this category did not reach the TLS^r, only four members of this group died before completing their 4th year, 16 rats lived longer than 4 years and the last animal died during the 226th week of its life.

The average lifespan in the saline- and (−)deprenyl-treated animals, grouped according to their sexual performance before treatment, is shown in Table XV.

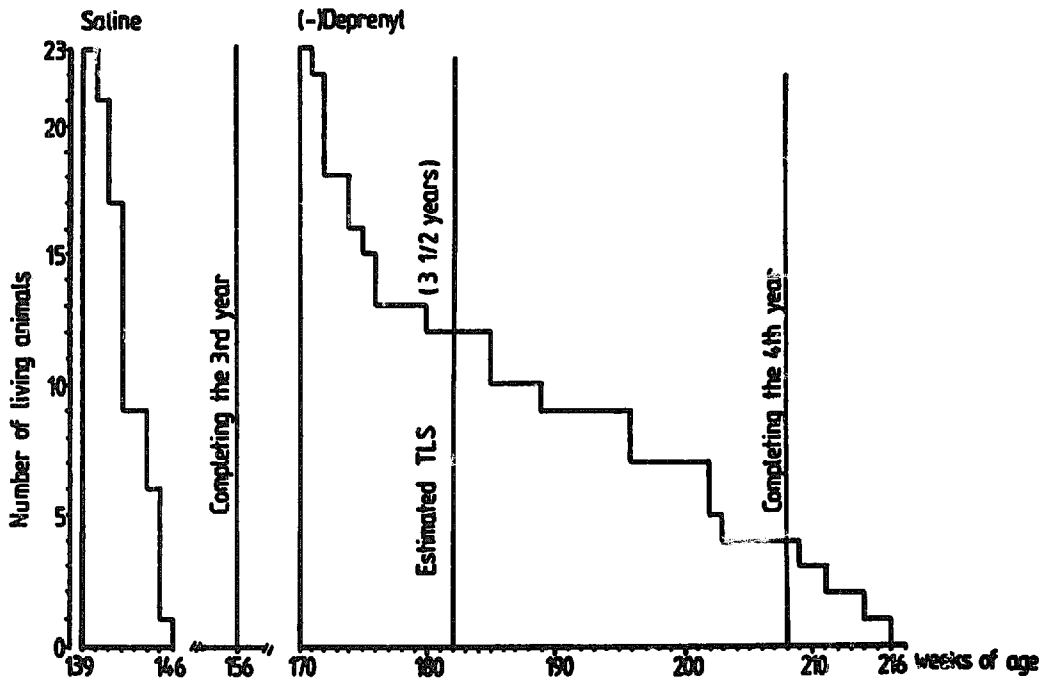

Fig. 1. The death rate of rats treated with saline ($n = 23$) and (−)deprenyl ($n = 23$), respectively, which proved to belong to the 'non-copulator' group before treatment. TLS = technical lifespan.

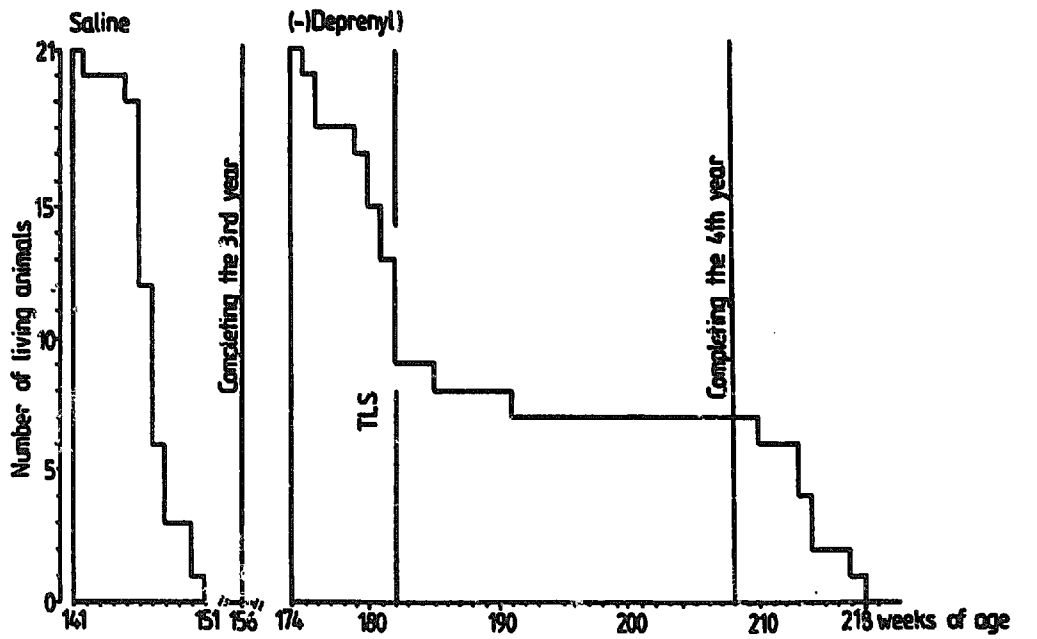

Fig. 2. The death rate of rats treated with saline ($n = 21$) and (−)deprenyl ($n = 21$), respectively, which proved to belong to the 'mounting' group before treatment. TLS = technical lifespan.

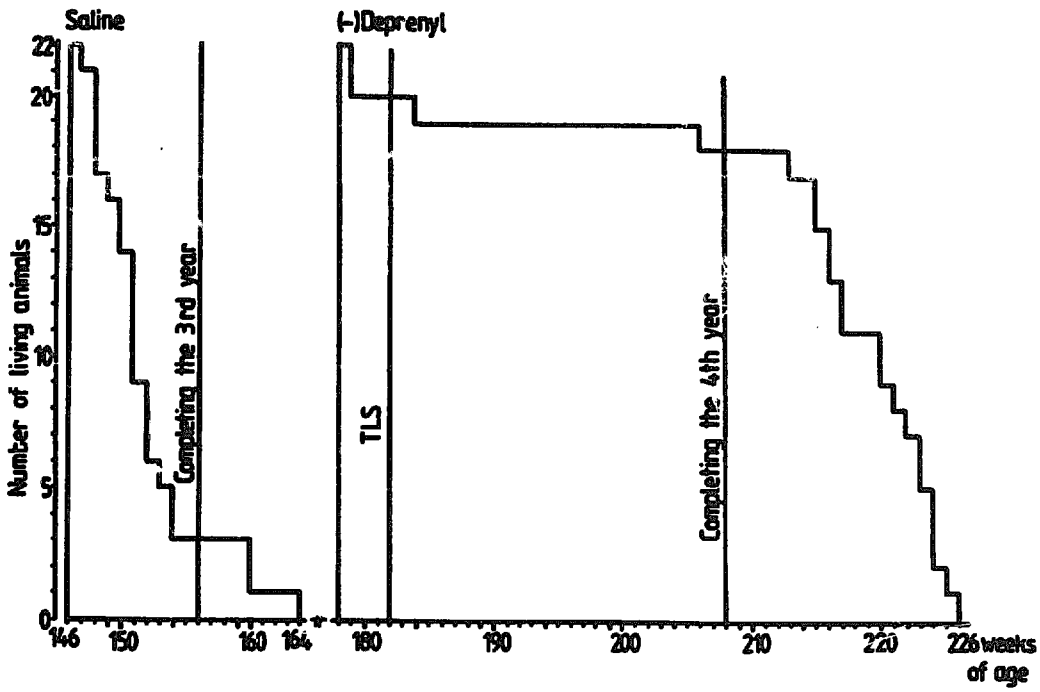

Fig. 3. The death rate of rats treated with saline ($n = 22$) and (−)deprenyl ($n = 22$), respectively, which proved to belong to the 'sluggish' group before treatment. TLS = technical lifespan.

TABLE XV

LIFE SPAN OF RATS TREATED WITH SALINE ($n = 66$) AND (−)DEPRENYL ($n = 66$), RESPECTIVELY

Classification of the groups according to sexual performance before treatment	*No. of animals*	*Average lifespan (weeks)*	
		Saline-treated	*(−)Deprenyl-treated*
Non-copulators	23	142.74 ± 0.38 A	187.90 ± 3.27 D
Mounting rats	21	146.95 ± 0.42 B	191.95 ± 3.59 E
Sluggish rats	22	152.00 ± 0.92 C	214.05 ± 3.07 F
Total	66	147.05 ± 0.56 G	197.98 ± 2.36 H

Significances according to the Student's t-test for two means:

A:B $P < 0.001$	C:F $P < 0.001$
A:C $P < 0.001$	D:E $P > 0.05$
B:C $P < 0.001$	D:F $P < 0.001$
A:D $P < 0.001$	E:F $P < 0.001$
B:E $P < 0.001$	G:H $P < 0.001$

The data reveal that, on the one hand, in all the three subgroups of the (−)deprenyl-treated rats the average lifespan was longer than the TLS^r, on the other hand, the sexually more active animals lived significantly longer in both the saline- and (−)deprenyl-treated groups of rats.

Thus, we succeeded to transform a population of rats, by changing the status of striatal dopamine via the continuous administration of (−)deprenyl, to have an average lifespan beyond the TLS^r. This is, according to our knowledge, the first instance that a well-aimed medication increased life-span of members of a species beyond the limit taken as life-span maximum. Even if the rat served in the history of experimental pharmacology as a reliable and useful model for the elaboration of drug therapy in humans, also the important differences between the two species are obvious. The proof of the pudding is in its eating. To protect the nigrostriatal dopaminergic neurons in human from its natural aging by taking 2–3 tablets of (−)deprenyl weekly, from the beginning of the 5th decade of life, is now open for clinical scrutiny. As a consequence of this safe and reasonable medication there is a hopeful possiblity to extend the average human lifespan, improve the quality of life in senescence and lower the incidence of age-related CNS illnesses, like Parkinson's disease, Alzheimer's disease, senile dementia and involutional depression.

A NOTE ON THE WEISMANNIAN EVOLUTIONARY THEORY OF AGING

Weismann, who wrote in 1885 his immortal paper "The continuity of the germ-plasma as the foundation of a theory of heredity", also established the first evolutionary theory of aging in his 1886 study "On the significance of sexual reproduction in the theory of natural selection". He assumed, in harmony with Darwin's concept, that aging and death serve the selection of individuals beyond their sexually active period (for review see [52]). The most interesting reflections in modern literature on the Weismannian theory of aging are critical remarks by leading scientists [53,54] who are philosophical in nature. My study recalls this old, almost forgotten, view from practical aspect.

I really found that the sexually more active male rats live longer than their less active peers and the striatal dopaminergic machinery, which probably plays the main role in maintaining the sexual drive in males, is the most rapidly aging system in the brain. Thus, the striatal dopaminergic system seems to represent a life-terminating machination, built in by nature, and the possibility, that a few similar non-genome-determined brain mechanisms may also exist, deserves serious attention.

That aging and death are somehow genetically determined is a popular and widely held view. As a matter of fact, the simplest explanation for the existence of a characteristic lifespan maximum in all species of mammals, is the concept

that it is written into the genetic message. The finding that cultured mammalian cells can make a limited number of divisions only [55] substantially supports this view.

The survival of male rats beyond the lifespan maximum just by maintaining one part of the catecholaminergic system in the brain on a higher activity level is, however, in variance with a hypothesis that in the rats the maximum age of death, i.e. the 182-week limit, is programmed. This is of great practical importance.

In the example shown in this paper, the maximum age of death of rats was shifted from 182 weeks to 226 weeks by a specific drug-manipulation of a non-genome-determined mechanism. The obtainable upper limit of the lifespan maximum via similar, still unexplored, brain mechanisms is unpredictable at present. A further extension of lifespan maximum is theoretically imaginable in the far future by influencing genome-determined mechanisms. Thus, it is a long experimental way to find out the final TLS^r, which, due to the second law of thermodynamics, cannot be surpassed anymore by any kind of intervention. Experiences with the rat might hopefully serve the benefit of the human race.

My suggestion is that the basic difference between the individuals is in their ability to generate that kind of specific activation of the brain (for review see [10]), which determines the efficiency of their drive-motivated behavior. The intensity of the drives determines the time spent with purposeful activity and, as a result, the chances to reach the goals giving satisfaction [10]. This ability seems to have a decisive influence on the aging of the brain and of lifespan. According to this working hypothesis, the more intensive are the drives, the slower is the mental decay and the longer is the span of life. The nigrostriatal dopaminergic machinery seems to be one of the probably very few brain mechanisms which control the drives, i.e. determine the efficency of drive-motivated behavior.

The data presented in this paper that (−)deprenyl-induced repair of the striatal dopaminergic neurons in 2-year-old rats restores the lost activity level, suggest that the natural decay of the striatal dopaminergic system is partially responsible for the well known lower efficiency of the senescent animals in behavioral tests compared to their younger peers. This change – as we learned from the experiments – has a determinant influence also on the lifespan of the animal.

As the behavioral studies clearly indicate that the catecholaminergic system, as a whole, plays a decisive role in the activation of the CNS, it seems to be reasonable to develop new compounds which activate on the long run in a (−)deprenyl-like safe manner and with utmost selectivity, on the one hand, the limbic dopaminergic system, on the other hand, the noradrenergic mechanisms in the basal ganglia. A sophisticated and safe complex medication which keeps the whole catecholaminergic brain system on a high activity level and protects it from aging, may offer new chances for fighting against the age-dependent mental decay and may also substantially decrease the incidence of age-related mental diseases.

To follow the path opened up by (−)deprenyl-induced longevity of male rats is compelling, as to improve quality of life in senescence is beyond all question the highest priority project in a rapidly aging society.

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