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Levodopa, Fertility, and Longevity

Abstract. *High concentrations of the dopaminergic drug levodopa* (L-dopa, L-3,4-*dihydroxyphenylalanine) administered to mice in their diet affected fertility to a moderate degree and prolonged the mean life-span by a maximum of 50 percent.*

Because of its effects on Parkinsonism and other motor disturbances, the dopaminergic drug levodopa is being consumed by many persons (*1*) most, but not all, of whom are beyond the childbearing age (*2*). Preliminary observations suggested that large concentrations of levodopa in the diets of male mice enhance fertility and longevity (*3*). Our study continues these observations. It extends these observations to both sexes, in which the maximal tolerated concentrations of dietary levodopa were determined and were given either during breeding or over the entire life-span of these mice.

Approximately 1100 male and 1500 female Swiss Albino mice were reared on Purina chow, which remained as the diet of all control animals. All experimental mice were shifted at 4 to 5 weeks of age to diets containing increasing amounts of L-3,4-dihydroxyphenylalanine (L-dopa, levodopa) given in 5-mg increments ranging from 1 to 100 mg per gram of Purina chow (hereafter referred to as "mg/g"). The maximum concentration that caused no deaths for 22 dyas was 40 mg/g for males and, notably, 80 mg/g for females.

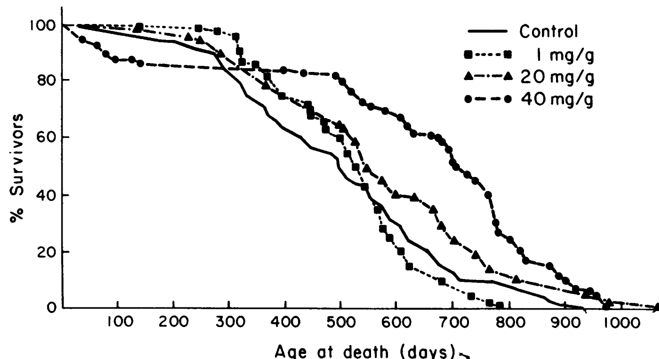
During mating experiments, the maximum levodopa concentration in the diet was 40 mg/g to protect the males. Concentrations of 0, 10, 20, and 40 mg/g were each continuously fed to groups of mating partners as follows: females only, males only, and both. Matings were started in week 10 of life by keeping two females in a cage with one male for 4 days. All young were examined during day 1 of life. Their numbers per litter and their weights were recorded at birth and checked weekly for 3 weeks. This procedure was followed for five matings at 8-week intervals, the total number of females being 1152 and the total number of offspring 6669.

Levodopa did not affect significantly the number of pregnancies (50 to 68 percent of females), the numbers of young (6 to 8 per litter), or the weights of the young (1.5 to 1.8 g per mouse) except when 40 mg/g was fed to the females, in which case significant decreases emerged in each of these items ($P < .01$). The effects were less when levodopa was fed only to the males and were not additive when it was fed to both partners. The young appeared normal on inspection, and cannibalism was not noted. These results demonstrate the feasibility of rearing sequential generations of mice on levodopa. Experiments of this type have been used to enhance nutritional effects in the past (*4*) and might prove useful in testing for effects on longevity.

In the beginning of our longevity experiments, we assigned 4- to 5-week-old males to groups of 100 in such a way that the body weights were not significantly different from group to group. This was done in view of the increased longevity induced by undernutrition (*5–7*). The animals on levodopa, however, in time developed differences in weight, shown in Table 1 as percentages of the weights of the corresponding controls. An impressive diminution in weight emerged during the first month on 40 mg/g. The other differences, even when statistically significant, were small by comparison to those induced during experiments on undernutrition (*5, 6*). The grams of food consumed per mouse per day were estimated on 15 occasions after the first month on levodopa. The controls consumed 4.8 ± 0.7 g/day, and the food consumption by the other groups showed no significant differences.

Survival curves for the four groups of males receiving 0, 1, 20, and 40 mg/g are shown in Fig. 1. The distributions of the survival times were found to be normal for the 1 mg/g, 20 mg/g, and control groups, while the 40 mg/g group appeared to be a mixture of two normal distributions with 13 percent having a life-span of ≤ 130 days and 86 percent having a much longer life-span.

A summary of the data is given in Table 2 both for the entire group and for the censored group, using only those that lived more than 130 days. The median life, the mean life, the standard deviation (S.D.), and the range for the treatment increased with dosage, although the



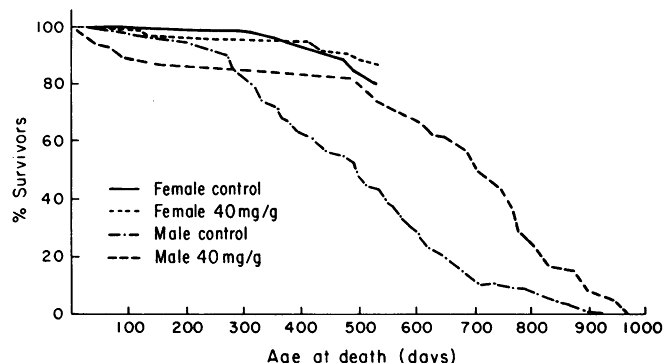


Fig. 1 (left). Survival curves comparing control male Swiss Albino mice with similar mice consuming levodopa in their diets. Fig. 2 (right). Survival curves of control male and control female Swiss Albino mice and of those consuming levodopa (40 mg per gram of chow). See text.

Table 1. Body weights as percentages (means ± S.D.) of controls at L-dopa doses per gram of chow.

Time (months)	Males at: 1 mg/g	20 mg/g	40 mg/g	Females at 40 mg/g
1	94.6 ± 11.2	97.8 ± 7.2	66.2 ± 7.6	88.9 ± 9.4
4	100.6 ± 12.0*	95.7 ± 6.3	87.4 ± 5.7	83.6 ± 9.4
11	104.6 ± 10.8	96.2 ± 9.8	88.3 ± 6.5	83.0 ± 8.1
16	103.9 ± 15.2	92.7 ± 8.9	87.4 ± 7.1	88.9 ± 9.2
19	102.9 ± 12.3	89.5 ± 10.2	86.9 ± 8.0	88.7 ± 10.3
21	100.0 ± 13.6*	91.4 ± 16.4	88.4 ± 9.0	
27	(All dead)	84.1 ± 9.7	74.7 ± 5.4	
	Last ten animals per group			
	100.3 ± 13.3*	91.7 ± 8.0*	73.7 ± 21.2	

*Denotes significant difference from the controls.

Table 2. Data pertaining to the longevity experiments on male Swiss Albino mice. Longevity was measured in days; S.E.M., standard error of mean.

Group	Median	Mean	S.D.	S.E.M.	~ 99 percentile	Range
			Not censored			
40 mg/g	710	634.10	264.48	26.45	1050.4	933
20 mg/g	545	568.63	205.84	20.58	1048.3	920
1 mg/g	528	513.91	124.62	12.46	804.3	539
Control	495	494.66	191.02	19.10	939.7	855
	Censored at 130 days (1 mg/g and 20 mg/g groups not affected)*					
40 mg/g	758	725.98	142.91	15.41		841
Control	503	516.60	171.19	17.47		730

*The censored data were obtained by subtracting all deaths occurring prior to 130 days of life (see text).

standard deviation and range of the 1 mg/g group were smaller than those of the control.

An analysis of variance on four groups was carried out and the F-test for the difference among group means was significant ($P < .001$). For more detailed pairwise comparison of group means, Scheffé's tests for simultaneous comparison were used. For the 40 mg/g group compared to the control, P was $< .001$; for the 20 mg/g group compared to the control, the significance was $.01 < P < .025$; but for the 1 mg/g group compared to the control and the 40 mg/g group compared to the 20 mg/g group the tests did not show significant differences. The 95 percent confidence interval for the difference between the means of the 40 mg/g group and that of the control was (46.59, 232.29), and between the 20 mg/g group and the control it was (5.49, 142.45).

Bartlett's test for equality of all variances gave $P < .001$, indicating a difference in the variances. In the analysis of variance above, since all sample sizes are equal, the lack of normality in the 40 mg/g group and the inequality of variances will not seriously affect the results.

Assuming normality of distribution of survival time for the control, the 1 mg/g group, the 20 mg/g group, and the censored 40 mg/g group, estimates for their 99 percentile would be approximately normal; the difference between the three treatment groups and the control was indicated by P values of .002, .046, and .014, respectively.

For the female mice, 100 animals each in the control and 40 mg/g groups were observed up to 527 days, at which time the experiment was terminated. Of these mice, 73 of the controls and 86 of the 40 mg/g group survived up to that time. The survival curves are given in Fig. 2. When these curves were plotted on normal-probability paper, they appeared parallel to the corresponding curves of the males but with increases in mean survival time. For these censored observations a Wilcoxon-Gehan test for difference in distributions was significant, with $P < .006$.

Additional observations were made in the course of these experiments. During the second and third months the males receiving 40 mg/g lost their hair, and the dense new hair, which grew within another month, gave them a youthful appearance. This sequence was also shown by the females consuming 80 mg/g. Corneal opacities developed as the mice aged, in controls of both sexes and to a more pronounced degree in the test animals; this suggests a propensity of the strain for opacities which is accentuated by levodopa. Motor activity, measured periodically in an Animex activity meter (*8*), did not differ significantly from group to group.

The highest concentrations of levodopa used here are much lower than the 13 percent levodopa content of the velvet bean (*9*), on which farm animals have been reared (*10*). The finding that the highest doses of levodopa given by us affected the mean life-span more than the total life-span suggested a lesser incidence of intercurrent diseases in the test animals than in the controls (*6, 7*), perhaps because of some immunological response similar to that found in animals whose life was prolonged by dietary restriction (*11*).

The sum of the above is compatible with the reports by Markham *et al.* (*12*) and by Sweet and McDowell (*13*) that in Parkinson's disease (a nonlethal affliction terminated by intercurrent disease), levodopa prolonged the lives of patients in comparison with those studied by Hoehn and Yahr (*14*) prior to the advent of levodopa. Our own hitherto unpublished calculations on 11 deaths concord with the above. In view also of the findings on aging by Finch (*15*), we are in favor of studying effects of this and related dopaminergic drugs on the process of aging.

George C. Cotzias*
Samuel T. Miller
Lily C. Tang
Paul S. Papavasiliou
Departments of Neurology, New York Hospital–Cornell Medical Center and *Memorial Sloan-Kettering Cancer Center, New York 10021*, and *Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973*
Ying Yao Wang
Biostatistics Laboratory, Memorial Sloan-Kettering Cancer Center, New York 10021

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* Address all reprint requests to G.C.C., Room 1918, Memorial Sloan-Kettering Cancer Center.

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Classical Conditioning with Auditory Discrimination of the Eye Blink in Decerebrate Cats

Abstract. *Cats were subjected to complete lower brainstem transection, and were then tested for learning ability according to a classical conditioning paradigm. An auditory stimulus was systematically paired with a brief shock to the eyelid. Within a few weeks after the operation, the decerebrate cats could learn the conditioned response with a tone frequency discrimination and then a discrimination reversal. Our results support the notion that the brainstem reticular formation can support a conditioned response which is behaviorally similar to that obtained in the intact animal.*

The problems encountered in investigating the neurophysiology of learning are due not only to the complexity of the nervous system but also to the difficulty in defining "learning" broadly enough for the definition to be behaviorally meaningful yet sufficiently constrained so that it can be analyzed (*1*). Our current approach to this problem is to delimit the "minimal" mammalian brain capable of sustaining a conditioned motor response with formal properties similar to those in intact animals. By reducing the volume and circuitry of the neural tissue which may participate in a conditioned response, the task of determining the processes involved in learned associations should become simpler.

The classically conditioned eye-blink response was selected as the standard learning task because it has been widely studied and there are considerable normative data available on the behavioral (electromyogram, EMG) response elicited (*2*). Moreover, neurophysiological analysis of this response should be simpler than that of other somatic conditioned responses, for example, leg flexion, which are complicated by postural reflexes and feedback mechanisms. In the intact cat, a conditioned eye-blink response can be established by systematically pairing a neutral acoustic conditioned stimulus (CS), which does not produce a response (*3*), with an unconditioned shock stimulus (US), which produces an unconditioned blink response (UR). After repeated pairings, the CS begins to evoke a conditioned blink response (CR).

The conditioned eye-blink response has several properties which provide important definitional guidelines for determining the validity of conditioning in our experimental preparations. From CS onset the conditioned eye-blink response has a variable latency with an absolute minimum of 100 to 125 msec (*2*), in contrast to shorter, fixed latency, acoustic reflex responses (*4*). The CR also reflects the temporal relationship of the CS and US, so that the maximum amplitude of the CR tends to occur toward the end of the CS-US interval (*2*). In a discrimination paradigm, the blink response can be conditioned selectively to a particular stimulus dimension, for example, tone frequency, by pairing one frequency (the CS+) with the US and not reinforcing the second frequency (the CS−). Finally, when the CS and US are presented randomly without temporal association, the CS does not elicit the blink response. We, as well as others (*2*), have utilized these features to define the conditioned eye-blink response that we have chosen to investigate.

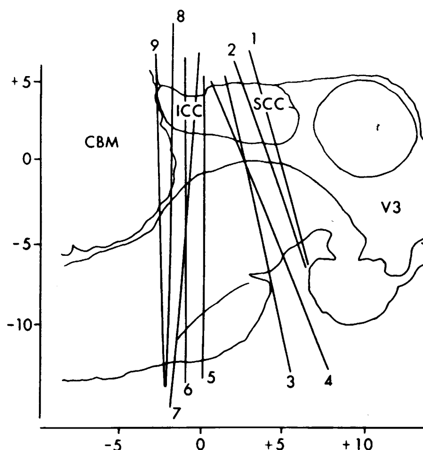

Fig. 1. Mid-saggital brainstem plane showing levels of transection of cats included in this study. The numbers correspond to those in Table 1.

In a previous study, the conditioned eye-blink response was shown to develop essentially normally in the absence of both cerebral hemispheres (*5*). Because components of the remaining diencephalon in these preparations showed retrograde changes, we hypothesized that the lower brainstem alone might be sufficient to support this simple form of learning. Here we report data from a subsequent study of eye-blink conditioning in the decerebrate cat.

The brains of adult cats were transected at the mesencephalic or rostropontine levels, under sterile conditions (*6*). The left occipital lobe was elevated and the underlying brainstem was exposed; subsequent aspiration with a fine probe, which could be visually guided except at the extreme upper right aspect of the brainstem, usually resulted in a complete transection. The general extent of the lesion could be observed directly during surgery, and was in all cases (except one) verified postmortem by means of both gross and histological examinations of the brains. The extent of the decerebration was determined by projecting Nissl-stained sections through the area of the lesion onto relevant brain atlas sections. Typically, all ascending and descending fiber tracts (lemnisci and peduncles), as well as the central tegmentum, were entirely sectioned so that the brainstem was completely separated from the forebrain (Table 1). Figure 1 shows the planes of section in the decerebrate cats enumerated in Table 1. Because we found no systematic relation between the plane of section and the behavioral results, we considered all the animals as a single group.

The general postoperative care of these decerebrate animals (*6*) was particularly focused upon proper temperature control, because all the animals were poikilothermic. By means of a thermister implanted in the cranium or in the rectum, an infrared heater or a cooling fan was automatically controlled so as to maintain body temperature at 37° ± 0.5°C. In spite of these precautions, accidental overheating caused the deaths of several of our animals; no ill effects were ever encountered by cooling, even when body temperature dropped as low as 32°C. In contrast to hemispherectomized animals (*5*), these animals were never able to feed themselves and were maintained on a liquefied diet of canned cat food, water, and vitamins given by intubation two to three times daily. Urine density and volume were normal with this diet and weight remained relatively constant up to 3 or 4 months at which time a general weight loss often began which was diffi-