measure of the possible synergistic action of CS's and US's on base-rate responding. As in experiment 1, a measure of base-rate responding was obtained during the adaptation session.

There was a significant acquisition of CR's over days in the paired CS-US condition [$F(4,96) = 22.9$; $P < .001$] which reached terminal levels (mean ± SEM) of 45 ± 7 percent ($N = 27$) by the last 2-day block of training. There was no evidence of CR acquisition in the unpaired CS,US condition, in that responding to the tone averaged 1.7 percent throughout training. Moreover, base-rate responding was not elevated, since it (1 percent) was essentially equivalent to that observed during adaptation (2 percent) when no stimuli were presented. Therefore, as with peripheral US's (*8*), the acquisition of conditioned NMR's produced by the pairing of a tone CS with a brain stimulation US was due to associative factors and not to such nonassociative factors as sensitization, pseudoconditioning, or an alteration in base-rate responding.

Examination of the data for the three groups of animals in the paired CS-US condition revealed no significant differences in acquisition or terminal CR performance as a function of US intensity. Consistent with this finding, there was also no significant difference in the mean UR amplitudes of the three control groups. The locus of electrical stimulation was, however, critical in determining terminal CR performance. The five animals with electrode tips located within the abducens nucleus (Fig. 1A) acquired CR's and reached the highest level of asymptotic performance, 74 ± 9 percent (Fig. 2). Stimulation at sites outside of the abducens nucleus, though eliciting UR's, resulted in more variable acquisition and overall lower terminal levels of performance. Specifically, ten animals with electrode tips located in the pontine reticular formation (Fig. 1A) demonstrated asymptotic performance of only 55 ± 13 percent (Fig. 2) with three animals responding near base rate, 3.6 ± 2.2 percent. The lowest level of asymptotic performance, 16 ± 8 percent (Fig. 2) occurred in seven animals with electrodes located in the medial longitudinal fasciculus (Fig. 1B), with five animals responding near base rate, 3.8 ± 1.2 percent. Four additional animals had electrodes located outside these regions (Fig. 1B) and their overall CR performance in the last block of training was 36 ± 20 percent. The differences in terminal CR production for the paired CS-US groups (Fig. 2) were significant [$F(2,19) = 5.27$; $P < .05$]. In addition, relating terminal CR performance to linear distance from the abducens nucleus for the rabbits with electrode tips depicted in Fig. 1A indicates that the closer the electrode tip was to the abducens nucleus, the higher the overall CR performance. For the rabbits with electrode tips located in the reticular formation (Fig. 1A), increased distance from the abducens nucleus, in general, appears to have decreased the efficacy of the US. Taken together, these results indicate that optimum conditioning is obtained by direct stimulation of the abducens motor neurons as the US. Moreover, the observed conditioning was associative, since stimulation of the abducens nucleus, or other brainstem structures, in the unpaired CS,US condition (Fig. 1C) failed to provide any evidence of CR acquisition (Fig. 2).

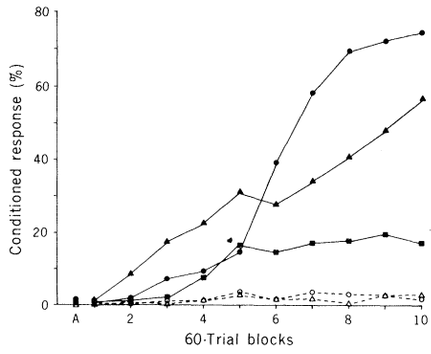

Fig. 2. Mean percent conditioned responses as a function of blocks of 60 trials. For the paired CS-US condition, separate acquisition functions are presented for animals with electrodes in: the abducens nucleus (●, $N = 5$), pontine reticular formation (▲, $N = 10$), and medial longitudinal fasciculus (■, $N = 7$). For the unpaired CS,US condition, data are given for animals with electrodes in the abducens nucleus (○, $N = 4$) and in other structures (△, $N = 8$).

The results of these experiments provide, to our knowledge, the first unambiguous evidence that electrical stimulation of a motor nucleus can effectively serve as the US in classical conditioning. Moreover, these results indicate that the rabbit NMR procedure appears to be ideally suited for determining the neural pathways involved in learning.

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10. R. F. Thompson has informed us that similar results have been obtained in his laboratory as well as in that of J. W. Moore.
11. Supported by NSF grants BNS 76084561, NIMH grant MH16841, and NIMH postdoctoral award MH07470 (F.W.M.). We thank K. H. Knight, S. E. McMaster, and J. L. Jensen for their valuable and dedicated assistance.
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Gamma Rays: Further Evidence for Lack of a Threshold Dose for Lethality to Human Cells

Abstract. *In experiments designed to measure human cell survival with ± 2 percent accuracy it was found that low doses (21 to 87 rad) of γ-rays inactivated the colony-forming ability of cultured human cells with a probability of 0.00226 ± 0.00012 per rad. There appears to be no threshold for the lethality of radiation to human cells in vitro.*

Three important effects of ionizing radiations on human cells are mutagenesis, carcinogenesis, and cell reproductive death. These effects are thought by some to occur through a common molecular mechanism (*1*). Whether or not this is the case, it would be useful to know with certainty whether any of these effects has a dose threshold. The alternative to the existence of a dose threshold is the "linear hypothesis," according to which there is no dose too small to produce an effect, so that dose response curves should be linear at very low doses. We tested the validity of the linear hypothesis for radiation lethality to human cells at low doses and obtained data that strongly support it.

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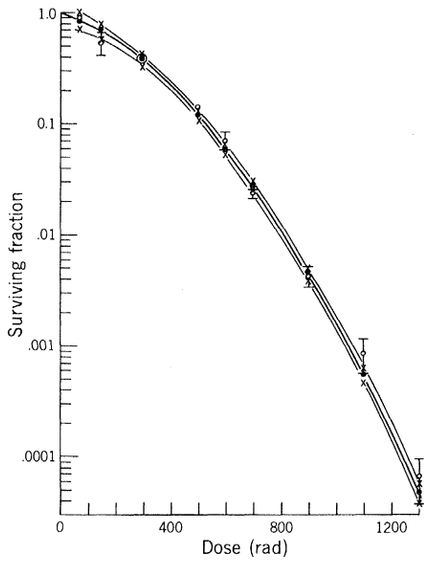

Fig. 1. Dose-response data for the inactivation of cultured human kidney (T-1) cells (*7*) by ^{60}Co γ-rays. Single suspended cells were allowed to attach to 6-cm Falcon plastic culture dishes for 4 hours before they were irradiated with graded doses of ^{60}Co γ-radiation from a Gammacell 220 (Atomic Energy of Canada) with a dose rate of 138 rad/min. The colonies formed after 12 to 14 days were stained with 0.1 percent aqueous methylene blue. About 500 colonies were counted to determine each survival point. Equation 2, represented by the middle line and dots, was fitted to the data, represented by circles and error bars, by a weighted nonlinear least-squares method (*6*). The upper and lower curves and x's represent 95 percent confidence limits of the fitted curve.

One of the most accurate ways to assess human cell death is to measure the ability of irradiated cells to multiply into colonies in cell cultures. The results of such experiments are recorded as survival curves, in which the logarithm of the fraction of cells capable of forming colonies (relative to unirradiated controls) is plotted against radiation dose, as in Fig. 1. If the threshold hypothesis is valid such a curve will have zero slope at zero dose; if the linear hypothesis is valid it will have a negative initial slope. Several attempts have been made to measure this initial negative slope (*2*, *3*). One approach is to use survival equations that have an initial negative slope and determine the statistical accuracy with which they fit experimental survival data. The following two equations provide examples:

$$S = e^{-CD}[1 - (1 - e^{-BD})^m] \quad (1)$$

$$S = e^{-CD - AD^2/2} \quad (2)$$

in which S is the fraction of cells able to form colonies after dose D, and A, B, C, and m are coefficients, usually derived from least-squares curve-fitting by computer. Equation 1 is the "single-hit times multitarget" (*4*) and Eq. 2 is the "linear-quadratic" (*5*) relationship. In both equations, the coefficient C is the slope of the logarithm of survival at zero dose. This coefficient must be significantly different from zero to support the linear hypothesis.

When the data of Fig. 1, for example, were fitted to these equations by a gradient-search least-squares fitting method developed for this purpose (*6*), the value of C was found to be 0.00328 ± 0.00033 and 0.00233 ± 0.00020 rad^{-1} in Eqs. 1 and 2, respectively (the errors are 95 percent confidence intervals). Similar values for the survival curve slope at zero dose have been found consistently in our experiments with these human kidney (T-1) cells (*7*) over the past 15 years (*4*, *6*).

The fitting of survival data with multiparameter functions may not provide sufficient evidence to validate the linear hypothesis for cell inactivation. We therefore exposed large numbers of cells to low doses of γ-rays to obtain a direct measurement of the initial slope of the survival curve. Survival data obtained with asynchronous cell populations are given in Fig. 2, which shows that survival at the lowest doses was measured with an accuracy of ± 2 percent. Clearly, doses as low as 21 rad lead to measurable cell inactivation. Furthermore, the survival curve in this dose range is consistent with a straight line with a slope 0.00226 ± 0.00012 rad^{-1}. This value strongly supports with improved error limits the nonzero initial slope (0.00222 ± 0.00049 rad^{-1}) reported in the pioneering x-ray studies of Barendsen (*2*).

A characteristic of linear, or "single-hit," cell inactivation is its lack of dependence on damage that must be accumulated to be lethal (sublethal damage). The repair of sublethal damage, expressed as an increase in survival with dose fractionation (*8*), would argue in favor of the threshold hypothesis. Therefore, to further demonstrate that 21 rad is not a subthreshold dose, cells were exposed to four separate doses each of 21 rad delivered 3 hours apart. This 3-hour interval provides enough time for complete repair of sublethal damage (*8*) in this cell line (*9*). A series of subthreshold doses 3 hours apart would presumably lead to insignificant cell inactivation; however, this series of doses led to a surviving fraction of 0.81 ± 0.04. This is not significantly different (at the $P < .01$ level) from the survival of 0.71 ± 0.10 measured when the entire dose was delivered in a single fraction, and is essentially equal to the value of 0.83 predicted by the linear hypothesis with C = 0.00226 rad^{-1}. Note that after exposure to 84 rad, the cell survival is 0.80 in Fig. 1 and 0.85 ± 0.06 in Fig. 2. The absence of detectable repair of sublethal damage after exposure to these low doses is further evidence against the existence of a dose threshold for the lethality of radiation to human cells.

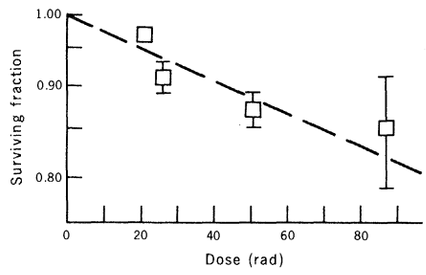

Fig. 2. Dose-response data for the inactivation of cultured human kidney (T-1) cells by low doses of ^{60}Co γ-rays. Single suspended cells were allowed to attach to 10-cm Falcon plastic culture dishes for 4 hours before they were irradiated as described in Fig. 1. Colonies were counted at a magnification of ×3. Eight asynchronous cell populations were used so that survivals at each of the three lowest dose points were determined by counting at least 10^4 colonies. Fewer colonies were counted at the highest dose. The plotted survival values are weighted mean survivals from eight experiments. The line through the points was fitted by the weighted least-squares method and has a slope of 2.26 ± 0.12 × 10^{-3} rad^{-1}. Its intercept is 1.000 ± 0.016 based on the standard error of the mean control colony counts. A fitted line not forced through the origin has an intercept of 1.018 ± 0.022. These intercepts do not differ at the $P < .01$ level (two-tailed Student's t-test).

We conclude that the linear hypothesis is a valid description of human cell inactivation by low doses of γ-rays. Each rad is capable of inactivating one cell in approximately 450. This confirmation of the linear hypothesis for cell inactivation should stimulate a more intense experimental investigation of the applicability of the linear hypothesis to human mutagenesis and carcinogenesis (*10*).

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Movement Disorders of Aged Rats: Reversal by Dopamine Receptor Stimulation

Abstract. *When placed in a tank of water, aged rats (24 to 27 months old) showed marked impairments in swimming. Compared with young adult rats (3 to 4 months old), the older animals moved their limbs less vigorously and were less successful in keeping their heads above water. The young, but not old, rats maintained a position nearly horizontal to the water surface and planed across it. These movement dysfunctions of aged rats resemble those seen in young adult animals that have sustained injury to brain dopamine-containing neurons. The swimming impairments of the aged rats were reversed by the dopamine receptor stimulant apomorphine and by the biosynthetic precursor of dopamine,* L-*dopa. Thus, age-related alterations in brain dopaminergic systems may be responsible for some of the movement disturbances associated with senescence.*

While movement disturbances of aged humans have been well documented, those of aged animals have not been systematically examined. As reviewed by Wellford (*1*), elderly persons need more time to carry out movements, pace themselves poorly in tasks requiring continuous performance, and fail to adjust their movements to compensate for their errors. To determine the motor capacities of aged rats, we have examined their capacity to swim. Swimming, which requires the coordinated movement of the limbs and trunk, is a common measure of the motor abilities of rodents. Its ontogeny (*2*) and neurological basis (*3*, *4*) have been investigated. We now report that 2-year-old rats have deficient ability to sustain vigorous and effective swimming. However, when given apomorphine or L-dopa they swim as well as young adult animals, which suggests that a deterioration of brain dopamine neurotransmission may contribute to some of the movement disorders of advanced age.

Male rats (Fischer 344 strain, retired breeders, Charles River) delivered by cesarean section were housed in metal cages. The swimming of rats 24 to 27 months old was compared with that of 3- to 4-month-old rats. Each rat was placed in a cylindrical tank (46 cm in diameter by 46 cm deep) of water (22° to 24°C) for 15 minutes. The rats could not keep their nostrils above water while supporting themselves on the bottom of the tank, nor could they jump out. At 1-minute intervals, the observer rated swimming along two dimensions: (i) the vigor with which the animal moved its limbs, and (ii) the success that each rat had in maintaining its head above the water surface (*5*).

Young adult rats swam with vigorous limb movements and held their heads above water. They typically kept their body axes nearly horizontal to the surface, moving all four limbs so as to plane across it (Fig. 1A). The vigor and success with which the young rats swam declined gradually during the 15-minute test (Fig. 2A). In contrast, the aged animals swam vigorously for a few minutes but were significantly impaired by minute 6 (Fig. 2A). Moreover, the aged rats were unable to sustain a horizontal position in the water. They lapsed into a more vertical posture (Fig. 1C) that necessitated their struggling to keep their heads above water. After several minutes they repeatedly sank and intermittently fought their way back to the surface. For the 15-minute test, the two age groups differed significantly on both measures of swim performance [F (1,17) = 27.8, for vigor, 96.4 for success, $P < .001$] (*6*). The interaction between group and time was significant for both measures, indicating a more rapid deterioration of swim performance in aged than in young adult rats [F (14,238) = 6.80, $P < .001$, for vigor; F (14,238) = 2.35, $P < .005$, for success].

The poor performance of these aged rats resembles the impaired swimming of

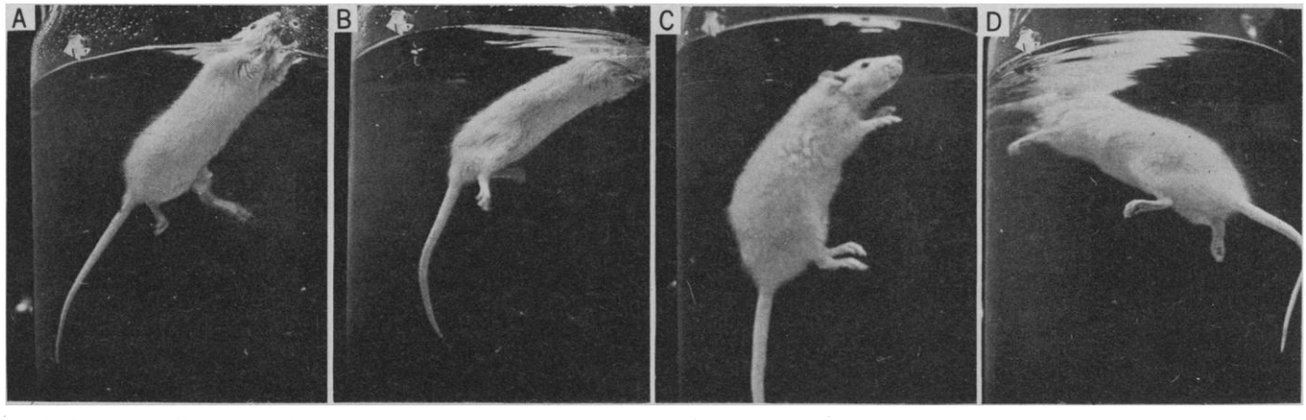

Fig. 1. Swimming of young adult (A and B) and aged (C and D) rats given apomorphine (0.50 mg/kg) or its vehicle. Note the vertical position assumed by the aged rat given vehicle (C). After apomorphine treatment, the aged animal adopted a more horizontal position (D), similar to that of young adult rats given apomorphine (B) or its vehicle (A).

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