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7. These assumptions reflect the serial-successive progression of processing stages inherent in the additive-factors model. Other models predict that all processes are active concurrently, although contingent upon each other's output. See, for example, J. L. McClelland, *Psychol. Rev.* **86**, 287 (1979).
8. Each matrix was composed of four rows and six columns of characters arranged as a square which subtended approximately 2.5°. One target word was presented on each trial, written horizontally, and appearing with equal probability in any of the four rows. The starting column of the target word was also randomly chosen and varied among columns 1 and 2 for RIGHT and columns 1, 2, and 3 for LEFT.
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10. See G. McCarthy, thesis, University of Illinois (1980).
11. Fifteen male students (right-handed, ages 19 to 32 years) participated. The matrix was exposed for 400 msec. The cue-to-matrix onset interval was 1000 msec with the cue's exposure duration set for 750 msec of the interval. The scalp EEG was recorded from six silver–silver chloride scalp electrodes (F_z, C_z, P_z, O_z, C_3, and C_4, according to the 10/20 system) referenced to linked mastoids. Electrodes placed above and to the side of the right eye were used to record the electrooculogram (EOG) in bipolar fashion. The EEG was amplified by a Van Gogh polygraph with a 1/2 amplitude upper cutoff of 35 Hz and with a 10-second time constant. The EOG was amplified with an upper cutoff of 15 Hz and with a 1-second time constant. Both the EEG and EOG were digitized at 5 msec per point for a period of 3.5 seconds beginning 50 msec prior to the cue stimulus and continuing until 2450 msec after the onset of the stimulus matrix. These data were stored on digital tape along with a record of the stimulus conditions and reaction time for that trial. Of the total trials, 17.8 percent were not used either because the subject failed to respond within 2000 msec, or because of eye movement artifact in the EEG.
12. The grand mean RT was 805 msec. The mean RT for "noise" trials was 266 msec longer than for "no noise" trials [$F(1, 12) = 166.6$, $P < .0001$]; the mean RT for incompatible stimulus-response mappings was 91 msec longer than for compatible mappings [$F(1, 12) = 84.5$, $P < .0001$]. Equivalent values were obtained when trials marked for eye movement artifacts were included in the analysis. "Noise" trials were associated with higher RT variance than "no noise" trials [$F(1, 12) = 32.1$, $P < .0001$]. There was a nonsignificant trend for more RT variance in the incompatible than compatible trials. Subjects performed correctly on 91.7 percent of the trials.
13. The response-aligned waveform data will be described elsewhere.
14. The large positivity seen in the "no noise" waveforms is probably a composite of two potentials: one maximum in amplitude over the centroparietal scalp sites and the other maximum in amplitude over the parieto-occipital scalp sites. The former potential we identify as P300. In the "noise" trials, these potentials are dissociated in time as the latency of the P300 component increases. On some percentage of the trials, the earlier positive component may have been used to estimate P300 latency. As this component appears relatively fixed in latency, these trials would add a fixed component to the distributions of P300 latency. It is unlikely that this affected our conclusions. The absence of any significant changes in the variances of the P300 latency distributions suggests that it is unlikely that such misreadings occurred more often in some conditions. [For more details see (*10*).]
15. The mean RT's obtained for each matrix row (from the top) were 843, 730, 783, and 883 msec [$F(3, 36) = 27.1$, $P < .0001$]. This row effect strongly interacted with stimulus discriminability [$F(3, 36) = 23.5$, $P < .0001$] because it was not present in the "no noise" trials.
16. The mean P300 peak latencies for each matrix row were 721, 665, 684, and 748 msec [$F(3, 36) = 7.9$, $P < .0004$]. As for RT, the row effect interacted with stimulus discriminability [$F(3, 36) = 11.0$, $P < .0001$] and was not present in the "no noise" trials.
17. For all experimental factors, the change in mean RT is greater than the change in mean P300 latency. This result is readily apparent in the differing slopes of P300 and RT in Fig. 2. See (*10*) for a discussion of these differences and their potential relevance to the assumptions underlying the additive-factors model.

18. This research was supported by the Office of Naval Research under contract N00014-76-C-0002, with funds provided by the Defense Advanced Research Projects Agency, and by the Air Force Office of Scientific Research, Bolling Air Force Base, Washington, D.C., under contract F49620-79-C-0233, and the Air Force Systems Command, Wright-Patterson Air Force Base, Ohio, under contract F33615-79-C-0512. We thank E. F. Heffley and C. C. Wood for helpful comments on this report.

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Cyclophosphamide-Induced Spermatogenic Effects Detected in the F_1 Generation by Behavioral Testing

Abstract. *Fischer 344 male rats were treated with cyclophosphamide (10 milligrams per kilogram of body weight) for 5 weeks and subsequently mated to females previously treated with saline or cyclophosphamide. The F_1 progeny of the cyclophosphamide-treated males exhibited behavior deficits when compared to controls. These data could indicate a chemically induced genetic effect manifested by behavioral alterations.*

Chemical mutagens induce a variety of sperm abnormalities. These may be detected at the chromosome level, for example, in Y chromosome nondisjunction (*1*), or at the cellular level, in which morphological aberrations of sperm heads (*2*) or aspermia may be observed (*3*). The genetic transmission of some of these effects has been reported (*4*), but no attempt has been made to correlate these cytological findings with any behavioral anomalies in the F_1 generation although Brady *et al.* noted a "gametotoxic" effect in rats treated with lead (*5*). We report evidence of behavioral aberrations in the F_1 generation of cyclophosphamide (CP)-treated male rats. These results suggest that the behavioral test system devised provides a sensitive and new end point to evaluate the genetic effects of mutagens in vivo. The use of this phenotypic end point in studying mutagenic agents may closely parallel the results one would expect to find in the human population after exposure to a mutagen.

A total of 30 female and 20 male adult Fischer 344 rats (*6*) were injected intraperitoneally with either 10 mg of CP per kilogram of body weight or a 0.9 percent saline solution. The animals were injected daily for 5 days, allowed to rest for the next 2 days, and then treated again for a total period of 5 weeks (*7*). Beginning 3 days after the last injection, the animals were housed in cages with a ratio of two females to one male per cage for about 2 weeks. The males were then switched to another cage according to the same breeding scheme for two additional weeks. The crosses used in the breeding scheme were as follows: saline-treated males and females (saline × saline, controls), saline-treated males with CP-treated females (saline × CP), CP-treated males with saline-treated females (CP × saline), and CP-treated males and females (CP × CP).

During breeding the animals were weighed at weekly intervals, and the dates of birth of all litters were recorded. Twelve live pups (three litters) were born from the CP × CP matings; 41 pups (nine litters) from the CP × saline group; 82 pups (ten litters) from the saline × saline group (35 of these control pups were randomly chosen for study in this report); no pups were born from the saline × CP matings (*8*). Thirteen animals (three from the CP × CP group and ten from the CP × saline) died postpartum.

One case of gross morphological abnormality occurred in the offspring from CP-exposed parents. One male pup of a CP × CP mating was born with a single eye. This condition was associated with a low body weight, compared to the littermates, which persisted to adulthood. Because of the low number of pups surviving for 1 day in some of the CP × CP and CP × saline litters, pups from the different breeding groups were fostered to females so as to provide an average nursing litter size of eight pups.

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Table 1. Open-field activity during three 1-minute trials in 14- and 21-day-old progeny of CP-treated rats. Open-field activity was measured by the number of entries that pups made on a circular field. Data are expressed as means ± standard errors.

Matings	N	Day 14 trials			Day 21 trials		
		1	2	3	1	2	3
Saline × saline	35	4.5 ± 1.0	4.5 ± 0.8	5.6 ± 1.1	16.5 ± 0.9	10.2 ± 0.9	8.2 ± 0.9
Range		0 to 13	0 to 12	0 to 13	5 to 28	1 to 23	0 to 20
CP × CP	9	9.0 ± 1.8*	12.3 ± 3.2*	10.1 ± 2.7	21.3 ± 3.4	22.6 ± 3.9*	13.0 ± 2.1†
Range		1 to 14	1 to 25	1 to 20	6 to 30	13 to 41	3 to 20
CP × saline	31	9.5 ± 1.5*	9.4 ± 1.8*	8.1 ± 1.3	18.8 ± 1.5	16.2 ± 1.3*	12.4 ± 1.4*
Range		2 to 17	0 to 20	0 to 26	5 to 28	1 to 23	0 to 20

*$P < .01$ †$P < .05$.

The body weights on days 3, 7, and 14 postpartum of the CP × CP pups and the CP × saline pups were not significantly different compared to the control animals. The weights of these pups suggest that the behavioral deficits did not result from nutritional deficiency caused by poor nursing.

Behavioral tests were performed on the F_1 pups to evaluate the effects of CP given to their male parent or to both parents. All surviving pups received each behavioral assessment through postnatal day 21. The behavioral assessments used here involve tests to evaluate motor reflex (surface righting, cliff avoidance, and negative geotaxis test), motor coordination (swimming), and locomotor activity (open-field behavior) (*9*) were conducted. Surface righting ability was tested at 3 days of age, and testing continued daily with two trials per day until criteria were reached or until day 6 (*10*). The time required for surface righting was not significantly increased in the CP × CP F_1 pups or in the CP × saline F_1 pups when compared to the saline × saline F_1 pups. However, the CP-treated F_1 pups showed developmental delay in cliff avoidance in both CP × CP and the CP × saline groups in that they were slower to retract from the edge of an elevated horizontal surface and a lower percentage reached criteria on each day tested. In the cliff avoidance test, the percentage of pups from the CP-treated animals that reached criterion at each time interval was significantly below that observed in the pups from the control group (parallel curves for CP-treated animals are seen in Fig. 1). The criterion used in this test was that the nose had to be completely behind the edge of the platform within 20 seconds; a maximum of 60 seconds was allowed, and a single trial was given per day. Testing was begun on day 4 and continued until day 10 (*11*).

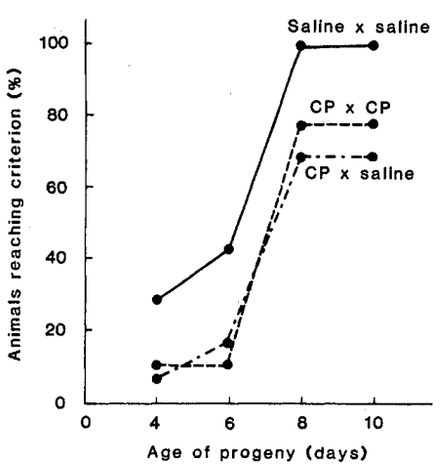

Fig. 1. Percentage of F_1 progeny reaching criterion for cliff avoidance test.

Measurements of the swimming capabilities of the F_1 pups were initiated at day 6 and repeated on days 8 and 10. The pups were placed in a tank filled with 22°C water for a maximum of 15 seconds. The movement of pups in the water was recorded and rated as follows: sinking, 0; floating, 1; swimming in circles, 2; and swimming in a straight line, 3. A large number of pups from the CP-treated groups showed developmental delay by continuing to swim in circles on all 3 days, compared to the number of pups from the saline-treated animals who swam in a straight line (Fig. 2). The angle of the pup's head in the water was also rated: below water, 0; tip of nose above water, 1; nose and top of head above water, 2; one-half of ears above surface, 3; or ears completely above the surface, 4. The pups' use of their limbs in the water was rated as: no limbs used, 0; all four limbs used, 1; and only rear limbs used, 2. In the swimming test, some developmental delay in the pups from the CP groups was measured by the large number who continued to swim with only the nose and top of head above water at day 10 compared to the number of pups from the saline × saline matings that were swimming with part of their ears above the surface of the water at this time. These observations were not accounted for by differences between litters, as determined by the Kruskal-Wallis one-way analysis of variance method. No differences in limb usage were observed in pups from any groups on any day.

The negative geotaxis test was performed on the rat pups on days 10 and 15. Pups were placed in a head-downward position on a 25° inclined plane, and the time it took them to reorient to a head-up position (180°) was measured. In this test, pups from the CP-treated groups required a similar length of time for reorientation as the control pups.

The open-field activity test was performed on days 14 and 21. The CP × CP and CP × saline pups were significantly more active on both days than the saline × saline pups [Table 1 and (*12*)]. Pups from both of these CP groups made more total entries on a 45-cm circular field, which was divided into 24 components. The increased activity of the CP pups on days 14 and 21 suggests that the deficits in cliff avoidance and swimming are not the result of a general motor disability.

Our data suggest that the behavioral effects measured during the preweaning period of the F_1 progeny of CP-treated rats were consistent in that the same animals that appeared developmentally retarded in the cliff avoidance tests were also retarded in the swimming tests. That CP induced a genetically toxic effect is supported by (i) the time interval of 14 days, which involved a period of 3 days of rest after treatment and 11 days before conception of the first litter in the treated group and (ii) the short half-life of Cy-

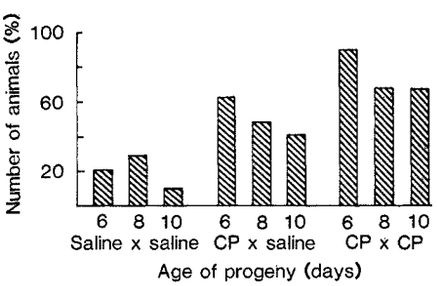

Fig. 2. Percentage of F_1 progeny exhibiting developmental deficits in the swimming test, measured by swimming in circles.

toxan in rodents and in man; half of the Cytoxan is eliminated within hours after cessation of long-term systemic treatment (*13*). These data suggest that behavioral testing of the F_1 generation might be used as an end point to detect abnormalities induced during spermatogenesis and transmitted through the sperm. This end point would then be used to evaluate genetically transmissible effects of potentially mutagenic, carcinogenic, or teratogenic compounds.

Further studies will determine if the observed differences are transient or permanent in nature. In either case, however, the procedures used in this report indicate a transmissible effect which can be used to detect genetically toxic compounds.

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5. K. Brady, Y. Herrera, H. Zenick, *Pharmacol. Biochem. Behav.* **3**, 561 (1975).
6. The male and female rats were derived from stock from the National Institute of Mental Health and were in the seventh generation.
7. Modified dominant lethal protocol as described by S. Green *et al.* [*Toxicol. Appl. Pharmacol.* **39**, 549 (1977)].
8. The number of pups in the litters of the CP ×CP crosses were 7, 4, and 1; of the CP × saline crosses, 10, 8, 7, 4, 4, 3, 2, 2, and 1; and from the saline × saline crosses, 10, 10, 10, 9, 9, 8, 8, 8, 6, and 4. These data suggest a certain degree of embryotoxicity as seen by decreased litter size in the CP-treated groups (the mean is 4.0 neonates per litter in CP × CP crosses, 4.6 in CP × saline crosses, and 8.2 in saline × saline crosses). The CP-treated females also had decreased numbers of successful matings (CP × CP, 3 out of 10 and saline × CP, 0 out of 10 compared to CP × saline, 9 out of 10 and saline × saline, 10 out of 10).
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10. Surface righting was tested daily beginning on day 3. The pups were given two daily trials with a maximum of 30 seconds per trial. The time required for the pup to right itself so that all four feet were on the surface was recorded. The criterion was that the pup had to right itself on at least one single trial in less than 2 seconds on a given day.
11. A statistical analysis of the percentage of pups in each litter reaching criterion for a given treatment was done on days 4, 6, 8, and 10. Significant effects were observed in the pups from the CP-treated groups on days 8 and 10 ($P < .05$); these effects were not due to differences between litters within treatments (Kruskal-Wallis one-way analysis of variance); S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).
12. Saline × saline progeny were compared statistically with CP × CP or CP × saline F_1 pups by using the *t*-test.
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β-Endorphin: Possible Involvement in the Antihypertensive Effect of Central α-Receptor Activation

Abstract. *Clonidine and L-α-methylnoradrenaline (but not D-α-methylnoradrenaline) increase the release of a substance with β-endorphin immunoreactivity from slices of brainstem of spontaneously hypertensive rats, but not that of normotensive rats. It was reported earlier that opiate antagonists inhibit the hypotensive action of clonidine and α-methyldopa in spontaneously hypertensive but not in normotensive rats and that β-endorphin has hypotensive effects of its own. Together, these findings indicate that release of β-endorphin by central α-receptor agonists may contribute to the antihypertensive action of these drugs.*

Opioid peptides have been implicated in the control of pain sensation, mood, and various behavioral functions (*1*). Less attention has been devoted to the possible role of these substances in the central control of cardiovascular function. Morphine and some endogenous opiates can reduce blood pressure and heart rate, effects that result partly from a reduction of sympathetic tone as opiate receptors in the medulla oblongata are activated (*2, 3*). Clonidine, an antihypertensive drug that activates α-adrenergic receptors (α-receptors) in the same brain

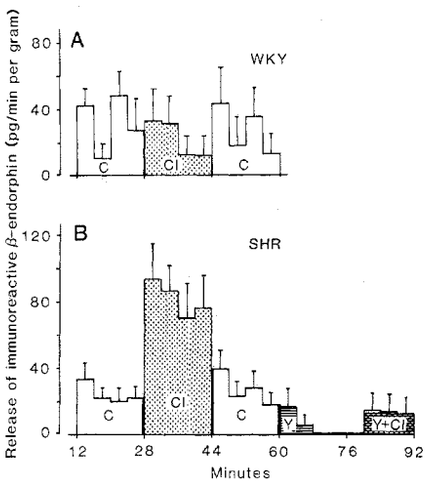

Fig. 1. The effect of clonidine on the release of β-endorphin immunoreactivity from slices of brainstem from (A) normotensive (*WKY*) and (B) hypertensive rats (*SHR*). The concentration of β-endorphin immunoreactive material in the lyophilized samples (*11*) was measured by radioimmunoassay (New England Nuclear) with ^{125}I-labeled human β-endorphin as tracer and a rabbit antibody to human β-endorphin. The antibody does not significantly cross-react with α-endorphin, α-melanocyte-stimulating hormone, leucine enkephalin or methionine enkephalin (< 0.01 percent), whereas there is 50 percent cross-reactivity with β-lipotropin. Bovine β-endorphin, which is identical to rat β-endorphin (*20*) had 14.3 ± 2.0 percent cross-reactivity at 50 percent displacement. Since cross-reactivity progressively decreased with increasing concentrations of bovine β-endorphin, it is probable that the increases we detected in human β-endorphin immunoreactivity reflect much greater increases in the release of the homologous rat β-endorphin. Although a contribution of β-lipotropin to the immunoreactivity measured is possible, this may be minimal in view of findings that in rat brain β-lipotropin represents a negligible fraction of the total β-endorphin immunoreactivity (*14*). Lyophilized samples were reconstituted in 0.2 ml of assay buffer. Standards prepared in assay buffer were supplemented with the same amount of salts present in the experimental samples. With this sample matrix, blanks bound 9.0 ± 0.9 percent and "zero" standards bound 30.8 ± 1.6 percent of the total radioactive material. The useful range of the standard curve was between 10 and 500 pg of human β-endorphin equivalent per assay tube. All incubations were done in siliconized test tubes. Separation of bound from free ligand was achieved by adsorption of free β-endorphin onto activated charcoal and centrifugation. Radioactivity in the supernatant was measured in a gamma counter. A value of zero was arbitrarily assigned to those control samples for which β-endorphin immunoreactivity was below the limit of detectability. Columns and bars represent means and standard errors from three experiments in WKY and 11 experiments in SHR. The concentration of clonidine (*Cl*) was $10^{-8}M$, that of yohimbine (*Y*) $10^{-6}M$. Open bars indicate control (*C*) values. In separate experiments (*10*), we tested the interaction of clonidine (5 μg/kg, intravenously) and naloxone (2 mg/kg, intraperitoneally) on systolic blood pressure of unanesthetized SHR and WKY. In seven WKY, clonidine reduced blood pressure from 125.2 to 113.8 mm-Hg before and from 130.0 to 117.6 mm-Hg after naloxone was given ($P > .5$). In contrast, in six SHR, the effect of clonidine (from 196.0 to 172.5 mm-Hg) was nearly abolished by naloxone (from 194.8 to 192.7 mm-Hg, $P < .001$).

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