70 percent of the Hi-Dis group and 60 percent of the Hi-Mod group improve, while for the Lo-Dis and Hi-Hi groups there is improvement by only 40 percent and 33 percent, respectively. This effect is revealed in statistically significant fashion on a number of different analyses of the learning performance. Thus we have been able to bring the learning performance of Hi-Dis subjects under a similar degree of control with verbal-cognitive manipulations, as has been possible with the Hi-Mod group by use of variations in shock intensity.

Next we turn to physiological data to determine whether changes noted above in cognitions and learning extend to the noncognitive component of pain. Figure 2 presents mean galvanic skin resistance (GSR) data for each of the four main groups, subtracting a subject's GSR to each of the first three shocks in list 2 from each of the first three shocks in list 3. These results clearly parallel those obtained at each of the other two levels of analysis. The control group given high shock throughout shows an increase in physiological responsiveness to shocks, while, as expected, lowering the shock for the Hi-Mod group produces a decrement in GSR. What is dramatic, however, is the fact that the Lo-Dis group again mirrors the Hi-Hi control, while the Hi-Dis group behaves physiologically as if shocks (of constant intensity) did not hurt as much after commitment as they had before ($F = 3.05$, $p < .05$; Hi-Dis versus Lo-Dis: $t = 2.00$, $p < 05$; control groups: $t = 2.24$, $p < .05$). These differences remain significant even after covarying changes in basal skin resistance.

Evidence has been presented which appears to validate the position derived from the theory of cognitive dissonance that voluntary commitment for minimal justification to a behavior which is discrepant with a motivational state can effectively limit the impact of that motive upon behavior. Recent studies in our laboratory and elsewhere (*6*) lead us to believe that this motivational control is demonstrable across a wide range of primary and socially acquired drives.

PHILIP G. ZIMBARDO
ARTHUR R. COHEN
MATISYOHU WEISENBERG
LEONARD DWORKIN, IRA FIRESTONE
Department of Psychology, University College, New York University, New York 10453

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6. This research was financed by an NSF grant (GS-226) to New York University (P. Zimbardo, principal investigator) and represents part of a larger project initiated by A. R. Cohen before his recent death. A fuller report of this study is in preparation and will appear as a monograph published by Scott, Foresman and Company, Chicago.

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Phenylketonuria in Rats: Reversibility of Behavorial Deficit

Abstract. *Phenylketonuria was induced in hooded rats by the conventional procedure of feeding excessive quantities of* L-*phenylalanine after weaning. Although this procedure reliably induced large, dose-dependent deficits in performance on a water maze, the behavioral deficits were completely eliminated after cessation of phenylalanine loading. These results cast doubt on the assumption that this animal preparation adequately simulates the irreversible intellectual impairments found in the child with late-detected phenylketonuria.*

Most laboratory investigations of phenylketonuria (PKU) have used an experimental animal preparation which has been more or less tacitly assumed to be an analog of human PKU in most important respects (*1*). One common procedure for producing this PKU preparation has been to administer excessive quantities of phenylalanine, of related compounds, or of both to weanling or older rats to simulate certain biochemical signs of PKU—mainly, elevated concentrations of phenylalanine in plasma and excessive excretion of phenylketones in urine. Although there is somewhat general agreement that this preparation provides an adequate model of PKU for biochemical studies, there is considerable disagreement about whether it also simulates the principal behavioral effect of PKU, that is, the irreversible and usually profound mental retardation seen in the child who has a late stage of untreated PKU (*1, 2*).

Recently several researchers have investigated the permanence of behavioral effects of phenylalanine loading begun during fetal or neonatal life (*3*). The net result of these experiments is equivocal. Our experiment was designed to determine whether a permanent behavioral deficit could be demonstrated in the weanling rat—the preparation most likely to be used by other investigators because of the obvious technical convenience of phenylalanine administration in the diet. Although it must be acknowledged that this preparation is less likely to sustain a permanent PKU-related behavioral deficit because of its relatively advanced stage of development, we considered the experiment important because of a singular omission in the growing body of literature on this problem: No one has adequately established in the same or related experiments both (i) that there are detrimental behavioral effects of PKU in animals (to establish the sensitivity of the behavioral test) and (ii) that these behavioral effects are eliminated after cessation of phenylalanine administration.

Our previous research has shown that rats fed phenylalanine-enriched diet (P-diet) from weaning perform less well than controls in a multiple-unit, water–T-maze learning task (*4*). A more recent and extensive experiment with pair-fed, littermate controls (*5*), concerned with the direct and interactive effects of dose of phenylalanine (3-, 5-, and 7-percent P-diets) and duration of administration (10, 25, 40, 55, and 70 postweaning days), revealed that P-diets induce deficits in maze performance which are directly related to dosage, regardless of sex or duration of administration (see *5*).

Although these experiments demonstrated large, reliable, and dose-dependent behavioral effects of chronic dietary administration of excessive phenylalanine, they did not answer the question of whether the behavioral deficits resulted merely from acute reactions to excessive phenylalanine or its related metabolites—an effect which should subside when feeding of P-diet is stopped. Accordingly, the present experiment was designed primarily to test the permanence of the behavioral deficit induced by P-diet administration begun at weaning.

A second goal of this experiment was to determine if P-diet administration during any one, or any combination of the first three 10-day intervals after weaning (20 to 29, 30 to 39, and

40 to 49 days of age) is sufficient to produce as large a behavioral deficit as has been shown to result from P-diet feeding for 30 continuous postweaning days. In other words: Is there a "critical interval" of development in the first 30 postweaning days for inducing detrimental behavioral effects by P-diets?

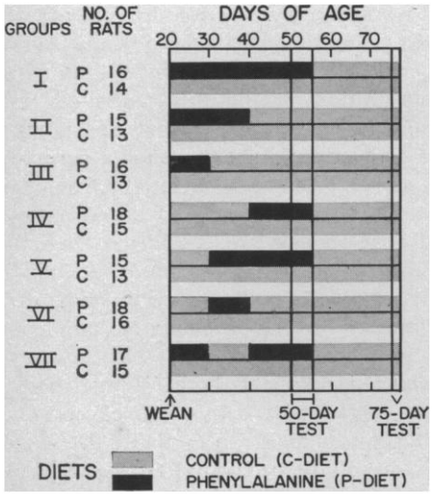

Fig. 1. Diet schedule, number of rats per group, and behavioral testing schedule for the seven P- and C-groups. P-groups were fed P-diet during those postweaning intervals indicated by solid bars, and C-diet as indicated by stippled bars. C-groups were fed C-diet throughout, individual rats being pair-fed to their respective P-group siblings.

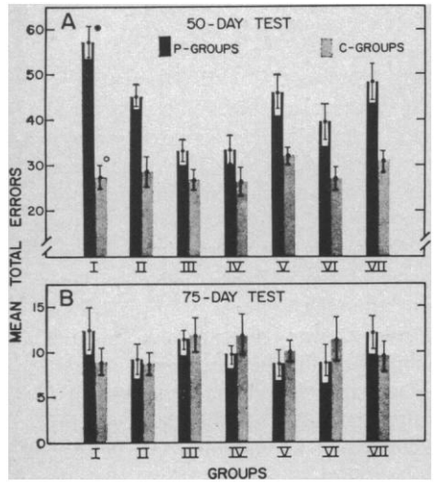

Fig. 2. (*A*) Mean (± one standard error of the mean) total errors made by the seven P-groups (solid bars) and associated pair-fed, littermate C-groups (stippled bars) during days 2 through 6 of the 50-day water-maze test. Solid circle beside the bar for groups I-P and open circle beside the bar for group I-C represent the mean total errors of groups similarly treated (see text) in a previous experiment (*5*). (*B*) Mean (± standard error) total errors made during the 2 days of the 75-day test by the same groups as in Fig. 2*A*.

The 214 Long-Evans strain, hooded rats used were bred and raised in a closed colony and obtained from litters of no more than eight or no less than six pups. When weaned at 20 days of age, each pup of a litter was ranked on the basis of body weight, and one member of each successive pair of ranked pups was randomly designated the pair-fed, littermate control (C-group) for the other pup, which was randomly assigned to one of the seven P-diet conditions. Assignment to one of the seven groups was random without replacement in successive litters so that no more than one pair of rats from a given litter was assigned to a given group. Each weaned pup was housed in an individual cage, weighed every 10 days, and fed the diet indicated in Fig. 1 for the designated intervals. Each P-group rat was fed a 5-percent P-diet (*6*), and the quantity consumed was recorded daily (*7*). Each C-group rat was fed the same quantity of C-diet (*6*) that its paired P-group sibling consumed on the previous day.

Figure 1 shows that P-groups differed by the combinations of the three 10-day intervals during which P-diet was fed. Group I-P received P-diet during all intervals; it corresponded to a previously studied group (*5*) fed P-diet from weaning and tested at 45 days of age. Each of the four groups fed P-diet in the last 10-day interval (groups I, IV, V, and VII) was maintained on P-diet during the 6 days of the 50-day test, but beginning at 56 days of age, even these groups were fed C-diet until the end of the experiment. Thus all P-groups were fed C-diet at least 20 days before the 75-day test.

Rats were tested in a water maze at 50 and 75 days of age. The 50-day test followed the standard 6-day procedure (*5*). Briefly, on the first test day each rat was run for five trials through a channel 120 cm long and filled with water at 20°C. Speed of swimming this straight channel was used as an index of ability to swim. On test days 2, 3, and 4 the rats received five trials per day through a six-unit, water-filled T-maze. On test days 5 and 6, five trials per day through the reverse path of the maze were given. Both swimming speed and errors (entries into a blind alley) were recorded, but the total number of errors made during test days 2 through 6 constituted the basic test datum (see *5* for justification).

The 75-day test consisted of 2 days in which five trials per day were again administered. A new problem was presented, however, by running the rats through the same six-unit T-maze in its mirror image. The total number of errors made in these 2 days was used as the basic datum of the 75-day test.

The results of the 50-day test are presented in Fig. 2*A* (*8*). There were no significant differences between the seven C-groups (*9*). Also, the performances of groups I-P and I-C in this experiment were comparable to those of similar groups (symbolized in Fig. 2*A* by the solid and open circles) in our previous experiment (*5*). These data indicate that the performance of control rats in this maze test is relatively invariant, and that there is a large and replicable decrement induced by 5-percent P-diet for 25 to 30 postweaning days.

Analyses (*9*) of the data on total errors (Fig. 2*A*) revealed that each P-group made significantly more errors than did controls. Nevertheless, each of the 20- or 30-day P-groups made significantly more errors than did any of the 10-day P-group (I = II = V = VII > III = IV = VI > C-groups). These results clearly support rejection of a "critical interval" hypothesis and suggest instead that the magnitude of the induced behavioral effects is directly related to total quantity of P-diet consumed during the 30 postweaning days, regardless of when the P-diet is fed. Moreover, the fact that P-groups fed C-diet during testing made as many errors as did comparable P-groups fed P-diet during testing (III or VI versus IV; II versus V or VII) indicates that an acute toxic reaction to excessive phenylalanine cannot by itself account for the behavioral deficit on the 50-day test.

The most important finding of the present experiment (Fig. 2*B*) was that all P-groups performed normally on the 75-day test (*9*, *10*). Thus it may be concluded that the deficits in maze performance in the 50-day test were not the result of permanent behavioral impairment. Rather, the deficit was completely eliminated when C-diet had been fed for 20 days or more.

Thus, at least under the present combination of behavioral testing procedure (*11*), species, and method of PKU-induction, an irreversible behavioral deficit was not obtained. Although the liver phenylalanine hydroxylase activity of the laboratory rat is at least ten times that of the monkey or human (*13*), and may therefore preclude serious and permanent damage to the

central nervous system, it seems more reasonable to assume tentatively, on the basis of existing data, that the method or timing of PKU induction is a more important factor.

It is indeed likely that administration of excessive phenylalanine during fetal or neonatal life, or during both, would have a greater and perhaps a permanent behavioral effect. The results of such experiments are contradictory, however. Woolley and van der Hoeven (*3*) have reported that mice administered DL-phenylalanine and L-tyrosine from birth until 7 or 8 weeks of age showed "subnormal learning ability as measured in the maze test" after being fed normal diet for at least 3 days. On the other hand, three other groups of investigators (*3*) have failed to detect an impairment in complex behavior of rats administered phenylalanine under an impressive variety of prenatal and neonatal treatments. Therefore, although the balance of evidence seems to point to the absence of permanent behavioral effects of fetal or neonatal treatment, the question is by no means settled. Nevertheless, the most important implication of the results of the present experiment is that the weanling or older rat is not the appropriate experimental animal preparation for further research on this problem.

V. J. POLIDORA
Department of Psychology, University of Wisconsin, Madison
ROBERT F. CUNNINGHAM
Central Wisconsin Colony and Training School, Madison
HARRY A. WAISMAN
Department of Pediatrics, University of Wisconsin Medical School

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6. Five percent P-diet, prepared by adding 5 g of NRC (National Academy of Sciences–National Research Council) grade L-phenylalanine to every 95 g of ground Rockland rat diet (C-diet), was selected as an optimal concentration on the basis of our previous findings (see *5*).
7. Mean phenylalanine dosage (grams per 100 g of body weight per day) actually administered during the three 10-day intervals (in the order 20-29, 30-39, 40-49, respectively) for the seven P-groups were: I—9.1, 8.5, 6.6; II—8.3, 7.8; III—8.3; IV—6.5; V—7.4, 6.2; VI—7.1; VII—8.5, 6.2.
8. Swimming speeds on the first test day were comparable for all groups. Swimming speeds through the maze on test days 2 to 6 showed a pattern of differences between groups identical to that indicated by the error data reported (compare *5*).
9. Analyses of variance of unweighted means were performed on the data on total errors in the 50-day test (Fig. 2*A*) and the 75-day test (Fig. 2*B*). For the 50-day test, the effects of diets (P- versus C-groups), groups (I through VII), and their interactions were statistically significant (diets $F = 94.13$, $df = 1/200$, $p < .001$; groups $F = 6.17$, $df = 6/200$, $p < .001$; diets by groups interaction $F = 2.97$, $df = 6/200$, $p < .01$). Multiple *t*-tests subsequent to the analysis of variance revealed that each P-group made significantly more errors than did the corresponding C-group ($p < .001$ in each case). Duncan's new multiple-range tests established that: the seven C-groups did not differ significantly from one another ($p > .05$); the three, 10-day P-groups did not differ ($p > .05$), nor did the three, 20-day P-groups differ from each other or from the 30-day group ($p > .05$); each of the 20- or 30-day groups made significantly ($p < .01$) more errors than did any of the 10-day groups. For the 75-day test, no main effect or interaction was significant. Statistical tests were taken from B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962).
10. Approximately the same number of errors *per day* were made by C-groups in the 50- and 75-day tests; the apparent lack of comparability (compare Fig. 2*A* and 2*B*) stems from the fact that the data of the 50-day test were based on 5 days and the 75-day test, on 2 days.
11. Selection of any single "valid" behavioral testing procedure for animals must, of course, be questioned (*1*, *12*) and cross-validating results must be sought. Our justification for using the described water-maze test in this series of experiments is threefold: (i) Maze-learning in rats has been extensively studied and shown to be a sensitive measure of a wide range of experimental treatments upon behavior. (ii) A test based upon the rats' obvious and persistent motivation to escape from cold water as quickly and efficiently as possible is a naturally occurring tendency which avoids most of the confounding problems associated with behavior motivated by electric shock, or by deprivation of food or water (which affects phenylalanine intake). (iii) The present and previous experiments have demonstrated that this test is a reliable index of relatively complex behavior in normal rats, and it also detects replicable and dose-dependent behavioral deficits associated with PKU. Thus we have to a certain extent avoided a judgment of test validity; we have sought instead to base the firmness of our conclusions upon the degree to which related experiments, each using the same behavioral test, produce internally consistent and replicable results.
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Anticholinesterase-Induced Amnesia and Its Temporal Aspects

Abstract. *Injection of the anticholinesterase drug diisopropyl fluorophosphate into the hippocampi of rats, 30 minutes after escape learning, produces partial amnesia with full recovery 5 days after injection. No such amnesia is produced if the injection takes place 3 days after learning. However, with injections 5 days after learning there is again an effect, and at 14 days amnesia is complete though no normal forgetting occurs within this period.*

Experimentally induced amnesia has been widely used as a tool in the investigation of the physiological basis of memory (*1*). Our study suggests that amnesia can be produced by interference with at least two processes which differ in their temporal characteristics and that such amnesia may be temporary.

Amnesia was induced in rats by the injection of the anticholinesterase drug diisopropyl fluorophosphate (DFP) into the hippocampi after learning. Flexner, Flexner, and Stellar (*2*) have recently produced amnesia by the intracerebral injection of puromycin, a protein synthesis inhibitor which produces an effect on cholinesterase activity (*3*). Cholinesterase may play an important role in learning (*4*).

Male rats (Sprague-Dawley, Holtzman strain, 350 g) were trained in a Y-maze to run into the illuminated arm to escape from shock (0.75 ma) applied to the grid floor. The position of the safe arm was varied randomly from trial to trial. A series of ten consecutive correct choices was the criterion of learning both during initial learning and retraining. The rats were divided into nine groups, six experimental and three control. The groups were run in small sections randomly interspersed with others, so that possible variations in procedure and drug sample could be counterbalanced. The differences between the experimental and control groups were large, and the variance within these groups was small, an indication of consistency of the effect that was independent of the time when a section of a group was tested. There were no significant differences in initial learning scores between the groups.

The first three experimental groups were used to determine if DFP pro-