ples showed d-type specificities (ratio 4.7), and 11 samples showed y-type specificities (ratio 0.17).

Of the total 85 serums, four samples showed interesting variations from the simple ad and ay subsets (Table 1, group 5). Sample No. 28 showed clearly an ay subtype by immunoprecipitation with antiserum to y, and, in addition, a faint precipitin line was observed with antiserum to d. The radioimmunoassay showed a variation in the count rate ratio with antibodies to d and to y: 0.48 compared to a mean value of the ay group of 0.16. As will be obvious from the experiments on specificity, this increased ratio was due to an elevated count rate with the labeled antibody to d—844, in replicate analyses, compared to a mean value of 382 for the group. Thus, a logical interpretation was that this serum sample contained not only predominantly a and y specificities, but also contained d specificity to a limited extent. This result suggested that this serum contained a large number of virus particles that were carrying ay determinants and a smaller number carrying the d determinant. It is also possible that a laboratory contaminant was introduced, but we feel this was unlikely. An alternate possibility is that the serum contained an antigen other than y that interacted with the antiserum to y. We are attempting to trace the source of the serum to verify the result with another sample from the donor.

In the low-titered samples containing the d specificities, there were three samples that did not fit the normal pattern. Serums No. 49512 and 50275 showed abnormally low count rates with iodinated antibody to a—148 and 281 which are in the range of normal serums. Thus these two samples showed a relative "absence" of the group-specific determinant a. One possible explanation for the low reactivity is that the antigens could be complexed with antibody to a in the original serum. In the same group, the radioimmunoassay specificities for sample 49673 indicated the presence of a, d, and y specificities, similar to serum No. 28. Unlike sample 28, the specificities could not be confirmed by immunoprecipitation. These four samples require additional study to ascertain whether they represent variants of the hepatitis B antigen.

In this group of 85 serums from commercial blood donors, 50 were of the d type and 35 were of the y type, as shown by radioimmunoassay. Of the 43 samples that could be typed by immunoprecipitation, 29 were d and 14 were y. In the radioimmunoassay tests the specific activities of the labeled antibodies to a, d, and y were roughly similar (20 μc/μg). In each subset, the count rate observed with antibody to a and the type-specific antibody were grossly similar. For example, in group 1, 2040 count/min were found with antibody to a and 2446 with antibody to d. In group 2, 3094 and 2341 count/min were found with antibody to a and antibody to y, respectively. This suggested that the relative availability of the group-specific determinant a and of the type-specific determinant d or y was quantitatively similar.

The results confirmed that a majority of the samples found positive by our radioimmunoassays procedure were truly hepatitis B virus–associated antigen, since they followed the identical subtyping specificities expected for this antigen. The studies also indicated that there were no substantial differences between low-titer serums and high-titer serums, regarding the antigenic subsets. The relationships of the recently characterized w and r determinants were not dealt with in this study. The guinea pig antiserums were produced with adw and ayw antigens. Therefore, in the cross absorptions to prepare type-specific antiserums, the w antibodies, if present, were probably included in antibody to a, but absent from antibody to d and antibody to y. We have in progress studies utilizing antiserum produced with adr antigens; these studies should give information relating to the contribution of the w and r determinants to the immunologic specificities.

C. M. Ling, H. Irace
R. Decker, L. R. Overby
Department of Virology,
Abbott Laboratories,
North Chicago, Illinois 60064

References and Notes

1. C. M. Ling and L. R. Overby, *J. Immunol.* **109**, 834 (1972).
2. L. R. Overby, J. P. Miller, I. D. Smith, R. H. Decker, C. M. Ling, *Vox Sang.*, in press.
3. C. Levine and B. S. Blumberg, *Nature* **221**, 195 (1969).
4. G. L. LeBouvier, *J. Infect. Dis.* **123**, 671 (1971).
5. ———, *Amer. J. Dis. Child.* **123**, 420 (1972).
6. C. Y. Kim and J. G. Tilles, *J. Infect. Dis.* **123**, 618 (1971).
7. W. H. Bancroft, F. K. Mundon, P. K. Russell, *J. Immunol.* **109**, 842 (1972).
8. P. V. Holland, H. J. Alter, R. H. Purcell, personal communication; G. L. LeBouvier, W. H. Bancroft, P. V. Holland, personal communication.
9. A. L. Ginsberg, W. H Bancroft, M. E. Conrad, *J. Lab. Clin. Med.* **80**, 291 (1972).
10. We thank Dr. Martin Goldfield and Dr. Alfred Prince for providing broad spectrum human antiserum, for confirmatory immunoprecipitating typing, and for suggestions in immunologic typing by radioimmunoassay. We thank Bruce MacDermott, Tie Steele, and Carlos Cabal for technical assistance.

17 November 1972

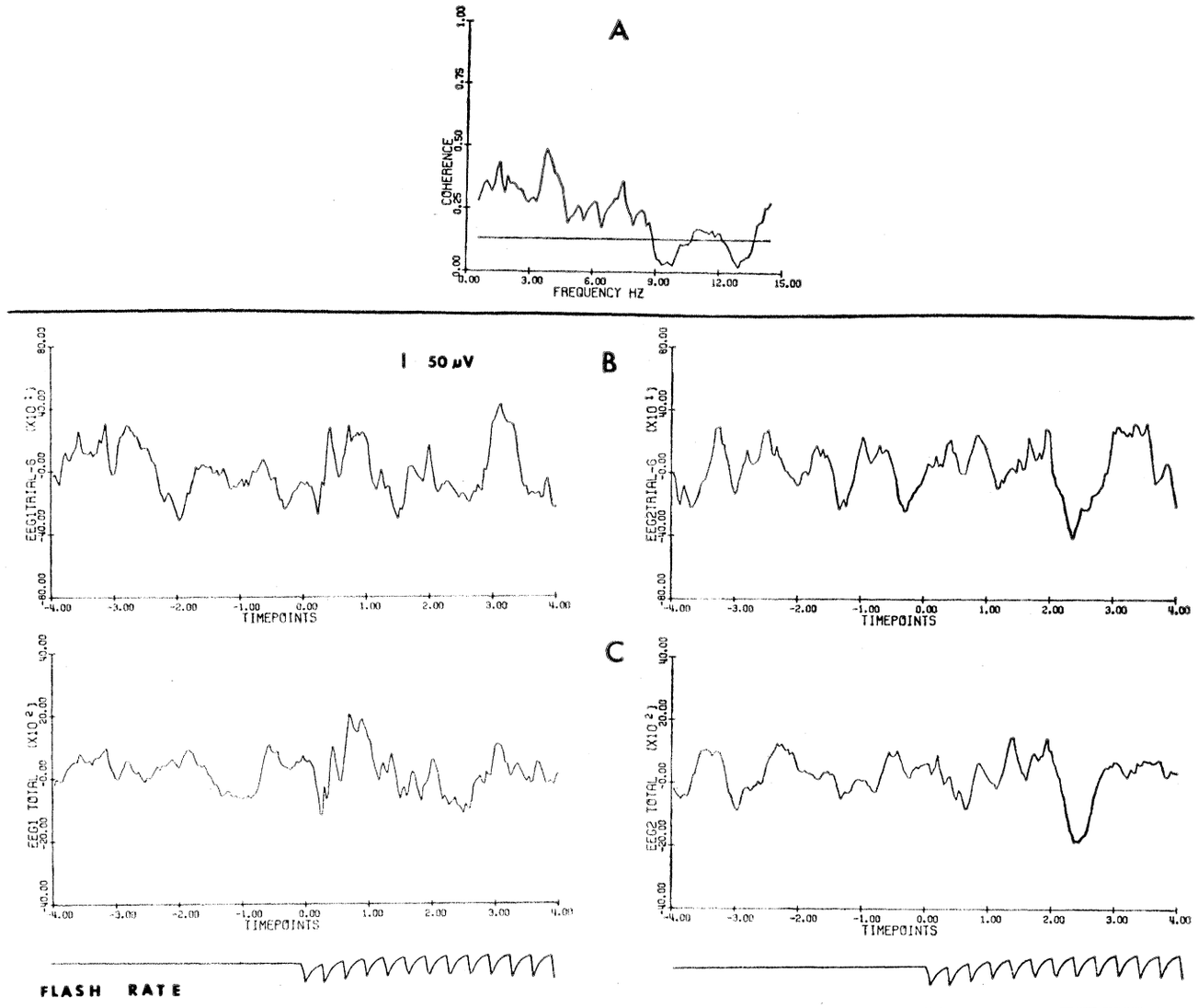
Unilateral Cortical Activity in Newborn Humans: An Early Index of Cerebral Dominance?

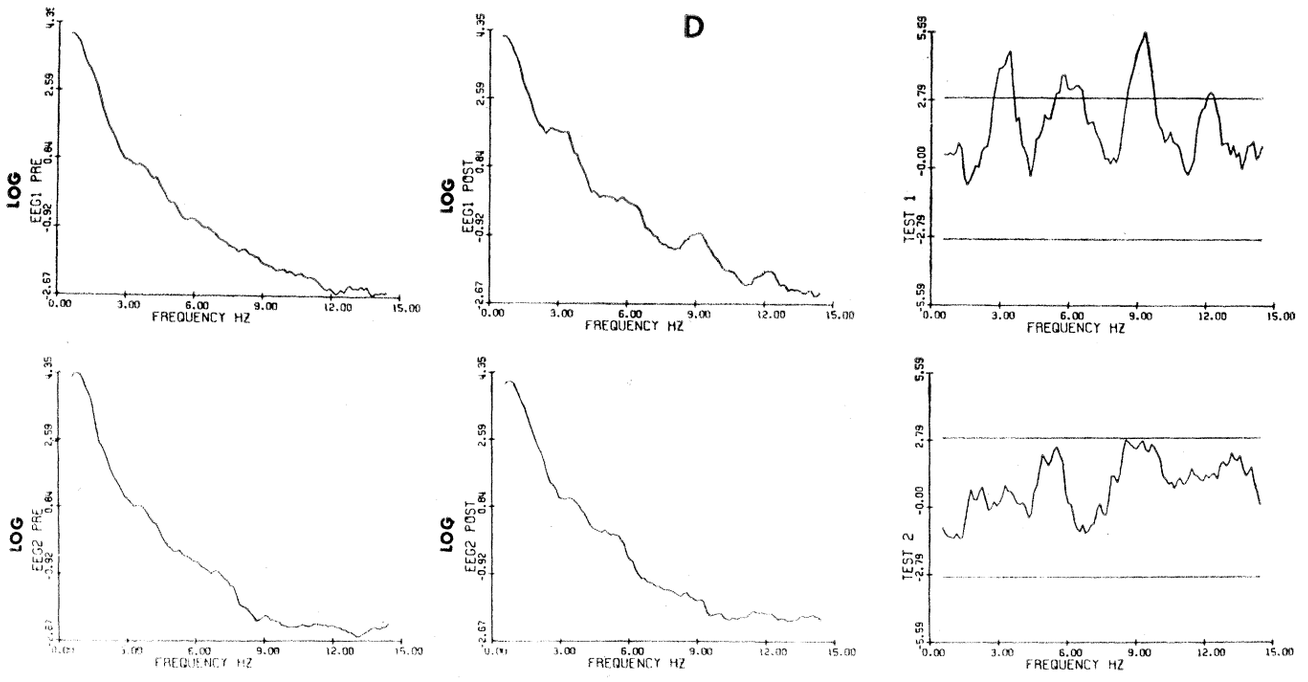
Abstract. *Spectral analyses show unilateral photic driving in newborn human infants to bilateral repetitive visual stimulation. Results are interpreted as evidence of dominance in the right hemisphere for rhythmic visual stimuli and lack of interhemispheric integration.*

Analyses (*1*) of electrical activity in brains of human newborns provide new evidence on cerebral hemispheric development. Neonatal electroencephalograms (EEG's) from homologous areas of both hemispheres show that few babies have bilateral EEG responses of driving to bilateral photic stimulation. In some newborns only a unilateral response is present and in others no response can be detected. Since a bilateral EEG response normally occurs in adults (*2*), our results with infants may be attributed to a lack of maturation. In light of this, a developmental sequence is postulated, beginning first with no driving, then right unilateral followed by bilateral photic driving. We interpret our results as an early manifestation of cerebral dominance in the ontogeny of interhemispheric integration.

The EEG's (*3*) of two samples (groups 1 and 2) of clinically normal, full-term newborn babies were recorded from right and left occipital areas referenced to the right ear, EEG 1 (O_2-A_2) and EEG 2 (O_1-A_2). Electrooculograms (EOG's) on two additional channels detected eye movement. Infants were stimulated by repetitive light flashes of 3 hertz (three flashes per second) for 4 seconds with a Grass PS-2 photostimulator, intensity setting 8, at a distance of 45.7 cm from the eyes, directly in the line of vision (*4*). After screening for artifacts (*5*), the data obtained from 97 babies (*6*) were analyzed.

The EEG's for each baby were ex-


A
COHERENCE
FREQUENCY HZ
1 50 μV
B
C
EEG1TRIAL-G
EEG2TRIAL-G
EEG1 TOTAL
EEG2 TOTAL
TIMEPOINTS
FLASH RATE


D
LOG
EEG1 PRE
EEG1 POST
EEG2 PRE
EEG2 POST
TEST 1
TEST 2
FREQUENCY HZ

amined to determine (i) whether the prestimulus activity in both hemispheres was coherent (*7*) and (ii) whether there were changes with photic stimulation. The first question is answered by estimating the coherence or degree of correlation of the waveforms between the right and left occipital brain areas. The significance level for determining whether the estimated coherence is significantly different from zero is .01. Significant interhemispheric spectral coherence at 3 hertz is present in 85 percent of the cases in both groups 1 and 2. Figure 1A is a representative example of the coherence seen in our samples.

To determine whether EEG changes, or driving, occur with photic stimulation, we developed a method (*1*) for statistically testing whether estimated spectra are the same or different (*8*). Photic driving is represented by brain waves occurring at the same frequency or harmonics of the rhythmic stimuli and by a significant increase in spectral power at the stimulating frequency or its harmonics. In this study, a more restrictive criterion of driving was adopted: a record was designated as showing driving only if the test of the EEG spectra was significant at the fundamental frequency of 3 hertz. The digitized EEG from a single trial (Fig. 1B) or the sum of all trials (Fig. 1C) illustrates the difficulty of detecting significant driving at any specific frequency from the analog tracing. The test for change between the spectra before and during stimulation, using an overall .05 significance level, precisely defines the frequencies at which significant driving occurs. Photic driving, as in *TEST 1* (Fig. 1D) is reflected in a general upward shift, indicating an increase in variance during stimulation. There is no significant driving in *TEST 2* (Fig. 1D).

Driving was present in 36 infants, or 37 percent of the total group; 16 of these showed significantly greater activity on the right side, 2 on the left side only, and 18 had driving on both sides (Table 1). Since adults respond bilaterally, these results are considered to reflect maturational factors. Based on this, the ontogenetic sequence appears to be first, no photic driving response, then unilateral, followed by bilateral photic driving. Even though our EEG derivations differ, the overall percentage of driving is within the range reported by other investigators (*9*). Although some infants had significant EEG activity at the harmonics, no pattern is evident for these responses. Neither unilateral nor bilateral driving showed any significant relation to sex or other perinatal variables (*10*).

Table 1. Driving responses of human newborns to photic stimulation (three flashes per second).

Groups	*N*	Average No. of trials	Unilateral (No.)		Bilateral (No.)	Absent (No.)
			EEG 1	EEG 2		
1	19	8.7	6 (32%)	0	1 (5%)	12 (63%)
2	78	8.9	10 (13%)	2 (2%)	17 (22%)	49 (63%)
Total	97		16 (16%)	2 (2%)	18 (19%)	61 (63%)

Of the 36 subjects with driving, 18 (or about 50 percent) had unilateral driving. More importantly, the unilateral responses were confined almost exclusively to the right hemisphere. To our knowledge, there are no other published reports with which to compare the unilateral driving. Our results should be checked against those in which other electrode placements, as well as other photic flash rates, were used (*11*).

There are no known anatomical or neurophysiological differences between the hemispheres which can account for the observed unilateral responses. The visual stimulus produces a diffusely illuminated visual field that is assumed to stimulate both retinae. Even if the visual input was unilateral, the partial crossing of the optic pathways ensures transmission to both occipital hemispheres. It cannot, however, be positively concluded that the visual field was uniformly illuminated, since eye movements of newborns are reported to be random, aimless, and independent of each other (*12*). Under the assumption that each retinal field was randomly stimulated, driving would be expected to occur equally often in both the right and left hemispheres. Our data are not in accord with this expected proportion.

In adults the driving of one hemisphere affects the opposite hemisphere (*2*). In light of this interhemispheric synergy, the unilateral driving seen in our babies is interpreted as evidence of a lack of interhemispheric integration. A tentative hypothesis is that the visual cortical matrix of the left hemisphere is slower to mature and respond. Also in adults, the hemispheres are known to be dominant and nondominant for specific functions with the right brain specialized for visual activities (*13*). In our newborns the appearance of driving activity in the right hemisphere to rhythmic visual stimuli suggests that this may be an early index of cerebral dominance. This study raises important questions about genetic components underlying right dominance, the age when bilateral driving is established, and the relevance of emerging hemispheric activity for behavioral function or dysfunction.

Fig. 1. Coherence and unilateral photic driving in a 2-day-old clinically normal, full-term female newborn, birth weight 2807 g. (A) Coherence between hemispheres for prestimulus period. The probability of the coherence exceeding the confidence line, if the true coherence is zero, is .01. Significant coherence is present for 0 to 8 hertz. (B) Example of primary tracing for *TRIAL 6*; EEG 1 (O_2-A_2), right hemisphere, and EEG 2 (O_1-A_2), left hemisphere, are digital-to-analog tracings based on 30 samples per second. Flash rate (three flashes per second) based on photo cell calibration indicates the onset of stimulation. (C) Secondary tracing or average of nine trials, *EEG TOTAL*. It is difficult to discern any photic driving in the primary or secondary tracings. (D) Spectral analyses of nine photic trials. The *EEG 1 PRE, EEG 1 POST, EEG 2 PRE,* and *EEG 2 POST* graphs are the logarithmic spectra for the 4 seconds of prestimulus (*PRE*) and 4 seconds of photic stimulation (*POST*). The photic driving on the right side, *EEG 1 POST*, is shown by the increases in power at 3 hertz and its harmonics. The tests for change between the *PRE* and *POST* logarithmic spectra are depicted in the *TEST 1* (right side) and *TEST 2* (left side) plots. The significance of the driving on the right side is confirmed by the *TEST 1* graph where the .05 confidence limits are exceeded at 3 hertz and its harmonics. No significant driving is shown in the left hemisphere, *TEST 2*.

DAVID H. CROWELL
Newborn Psychological Research Laboratory, Department of Psychology, Pacific Biomedical Research Center, University of Hawaii and *Kapiolani Hospital, Honolulu 96822*
RICHARD H. JONES
Department of Information and Computer Sciences, University of Hawaii
LINDA E. KAPUNIAI
Newborn Psychological Research Laboratory, Department of Psychology, University of Hawaii
JOANN K. NAKAGAWA
Department of Information and Computer Sciences, University of Hawaii

References and Notes

1. R. H. Jones, D. H. Crowell, J. K. Nakagawa, L. E. Kapuniai, *Computers in Biomedicine* (A Supplement to the Proceedings of the Fifth Hawaii International Conference on System Sciences) (Western Periodicals, North Hollywood, Calif., 1972), p. 18.
2. N. L. Freedman, *Science* **142**, 598 (1963).
3. The EEG and EOG signals were monitored on a Beckman type R dynograph, simultaneousl digitized with an analog-to-digital converter (Electrical Engineering Company of California, model 762) at the rate of 30 and 10 samples per second, respectively, and recorded on a Precision Instrument incremental digital recorder model 1167 F.
4. Infants were swaddled and buckled in a semiupright position in a plastic infant seat. The angle was adjusted so that the incline was approximately 45°. The stroboscope lamp was aligned so that the center was (i) perpendicular to the frontal plane of the face and (ii) directed midway between the eyes. The experimental room was dark. Photic stimulation was initiated only when the babies were quiet with eyes closed.
5. The tracing of each photic trial was inspected prior to and during stimulation to ensure a stable recording of ongoing activity: the nonstimulated peak-to-peak amplitude was scanned and used as a standard. If this criterion was grossly exceeded or if there was any departure from the test criteria for subjects or procedure, the trial was omitted.
6. Group 1 included 8 males and 11 females; group 2, 36 males and 42 females. Their ages range from 36 to 59 hours with a median of 46 hours; birth weights vary from 2466 to 4252 g with a median of 3203 g, with ar average 1 minute Apgar score of 8 [V. Apgar, *Curr. Res. Anesth. Analg.* **32**, 260 (1953)].
7. G. M. Jenkins and D. G. Watts, *Spectral Analysis and Its Applications* (Holden-Day, San Francisco, 1968), pp. 321–362.
8. Since the characteristics of the EEG signals can vary with time (nonstationarity), the estimates of the spectra are not pooled across trials. They are estimated separately for each 4-second interval with use of the smoothed periodiogram method (*1*). The statistical test is based on the difference of the logarithms of the estimated spectrum of the 4 seconds immediately preceding stimulation and the estimated spectrum of the data during stimulation. The differences of the logarithms of the spectra are then summed over trials, giving a test that is not influenced by trial to trial nonstationarity.
9. Z. Vitora and A. Hrbek, *Electroencephalogr. Clin. Neurophysiol.* **28**, 391 (1970); R. J. Ellingson, in *Advances in Child Development and Behavior*, L. P. Lipsitt and C. C. Spiker, Eds. (Academic Press, New York, 1967), pp. 53–97; G. H. Glaser and L. L. Levy, *Amer. J. Dis. Child.* **109**, 333 (1965).
10. A multivariate discriminant analysis was made with the following variables: maternal (number of pregnancies, number of fetal deaths, stimulation or induction of labor, presence or absence of maternal complications, time from membrane rupture to delivery, duration of first stage of labor, duration of second stage of labor, mother's age, amount of Demerol in 48 hours before delivery, and amount of Phenergan in 48 hours before delivery) and infant (sex, cord position, time to first cry, time to first breath, birth weight, length, Apgar score at 1 minute, and age, in hours, at time of test).
11. R. J. Ellingson and G. H. Lathrop, *Electroencephalogr. Clin. Neurophysiol.* **23**, 592 (1967).
12. G. O. Dayton, M. H. Jones, B. Steele, M. Rose, *AMA Arch. Ophthalmol.* **71**, 871 (1964); A. Peiper, *Cerebral Function in Infancy and Childhood* (Consultants Bureau, New York, 1963), p. 61. For those subjects with unilateral driving, the EOG tracings were inspected for any movement during stimulation. Of the 18 babies, 9 showed no movements and 7 had activity in less than half the trials.
13. B. Milner, *Res. Publ. Ass. Res. Nerv. Ment. Dis.* **36**, 244 (1958); H. Hecaen, in *Interhemispheric Relations and Cerebral Dominance*, V. B. Mountcastle, Ed. (Johns Hopkins Press, Baltimore, 1962), pp. 215–243; H. L. Teuber, *ibid.*, pp. 131–157; S. Weinstein, *ibid.*, pp. 159–176; J. Levy-Agresti and R. W. Sperry, *Proc. Nat. Acad. Sci. U.S.A.* **61**, 1151 (1968).

14. We are grateful to Professors Donald B. Lindsley, Guenter Rose, D. O. Walter (University of California, Los Angeles), and R. J. Ellingson (University of Nebraska) for their comments, and the University of Hawaii Computing Center. Research supported in part by National Institute of Child Health and Human Development grant HD00869 (D.H.C.), the Grant Foundation (D.H.C.), and NSF GP-31074 (R.H.J.).

22 January 1973

Multiphasic Retention Deficits at Periodic Intervals after Passive-Avoidance Learning

Abstract. *After one-trial passive-avoidance training, independent groups of rats tested promptly after training or at successive 6-hour intervals displayed a repetitive pattern of high then low retention scores. These results suggest that some physiological rhythm may interact with retention performance.*

We have evidence that the retention deficit found at intermediate intervals after avoidance conditioning, commonly called the "Kamin effect," may not represent a transitory phenomenon occurring only once after the training session. We found that rats tested for passive avoidance promptly (15 minutes) after a one-trial training procedure or at successive multiples of 12 hours after training displayed higher retention scores than did rats tested 6 hours after the training session or at successive multiples of 12 hours from this 6-hour interval.

The U-shaped retention curve of a partially learned avoidance response was first reported by Kamin (*1*) and has been replicated with a variety of procedures, including passive-avoidance tasks (*2–4*). The locus of the maximum retention deficit has been reported to vary from 1 to 8 hours after training. Such variations may have resulted from differences in procedure (such as stressing conditions and apparatus) or from an incomplete sample of training-testing intervals (TTI). Also, others have tended to omit testing for retention at intervals between 8 and 24 hours after training, between 24 and 48 hours after training, and so forth.

Several hypotheses have been offered as explanations for the Kamin effect, for example: (i) Memory consists of a multiple-stage process in which deficits at certain intervals after training may result from temporal disparities between stages (*5*). (ii) Decreases in fear or physiological processes underlying motivation for the avoidance behavior may occur during the intermediate TTI (*3, 6*). (iii) Increases in fear, inhibition, anxiety, or stress induced by the shock during training may be maximal during intermediate TTI's and thus interfere with avoidance behavior (*4, 7*). (iv) Stimulus generalization decrements or "state-dependent" effects may result from changes in the internal state of the organism between training and testing (*8, 9*). To our knowledge, in all suggested explanations for the Kamin effect it has been assumed, at least implicitly, that the retention deficit is transitory inasmuch as no impairment in retention is usually reported at 24 hours or successive multiples of 24 hours after training. The present experiment was designed to explore retention at intervals up to 24 hours and at intervals between multiples of 24 hours.

Subjects were 195 male albino rats (Sasco), weighing 314 to 376 g. All animals were housed in individual cages and given free access to food and water. They were placed on a light-dark cycle of 12 hours of light (0800 to 2000 hours) and 12 hours of dark (2000 to 0800 hours) at least 2 weeks before training began, and were maintained on this cycle throughout the experiment. Subjects were randomly assigned to one of 13 independent groups (15 animals per group). These groups received passive-avoidance training and testing and differed only in terms of TTI's. All groups were trained during the light cycle, with approximately equal numbers of subjects within each group trained in the earlier (1000 to 1345 hours) and later (1400 to 1715 hours) portions of the cycle. The 13 groups were designated by their TTI's, which were 15 minutes and 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and 72 hours, respectively.

The passive-avoidance apparatus (*10*) was trough-shaped, consisting of a smaller, clear Plexiglas chamber (start box) illuminated by neon lamp (6 watts) and a larger, darkened, black Plexiglas chamber (shock box). The larger compartment was accessible to the subject through a circular opening