KINETICS OF MEMORY CONSOLIDATION: ROLE OF AMNESIC TREATMENT PARAMETERS

By Arthur Cherkin

PSYCHOBIOLOGY RESEARCH LABORATORY, VETERANS ADMINISTRATION HOSPITAL, SEPULVEDA, CALIFORNIA. AND DIVISION OF ANESTHESIA, UNIVERSITY OF CALIFORNIA (LOS ANGELES)

Communicated by Linus Pauling, May 2, 1969

Abstract.—The consolidation theory states that with the passage of time the engram of a recent learning experience grows increasingly resistant to disruption by amnesic treatment. The time required to reach complete resistance ("consolidation time") is a controversial issue; current estimates range from 10^1 to 10^5 seconds. The present study suggests a parsimonious interpretation of the divergence, namely, that weak amnesic treatments fail to block memory consolidation but do slow its rate, so that post-treatment consolidation inflates the retention scores measured 24 hours later and leads to variably shortened "consolidation times."

This study utilized 2880 neonate chicks trained in a one-trial avoidance paradigm. Retrograde amnesia was induced by treatment with flurothyl (CF_3CH_2-OCH_2CF_3) vapor. Apparent "consolidation times" determined by conventional data analysis varied widely as a function of flurothyl concentration and exposure time, ranging from 4 minutes under weak amnesic conditions (0.85% flurothyl; 1-min exposure) to 24 hours under strong conditions (1.7% flurothyl; 8-min exposure). With 1.7% flurothyl, the consolidation half-time was found to be 9.8 hours.

The brain takes time to consolidate information input into a durable memory trace. How much time has yet to be settled; estimates based on clinical data and retrograde amnesia experiments range from seconds to days.[1, 2] Weiskrantz[2] has pointed out that "it is extremely difficult to discover any consistent factor which would account for the differences in maximum interval over which retrograde amnesia extends" and has suggested that the explanation is to be found in impairment of memory retrieval rather than of consolidation. I propose the alternative interpretation that the responsible factor is a methodological factor, namely, the use of incomplete amnesic treatments that decrease the rate of consolidation but do not stop it, so that retention measured 24 hours later is inflated by post-treatment consolidation and misinterpreted as reflecting a steep consolidation gradient.

Glickman[3] postulated that "the interval following a learning trial, during which time interference with retention can be produced, is a direct function of the degree of physiological severity of the interpolated procedure (amnesic treatment)." The severity of ECS (electroconvulsive shock) treatment is a function of current and duration. There are reports that the critical factor is duration but not current,[4] or current but not duration,[5] and that at a constant duration amnesia is current-dependent[6–10] or current-independent.[11, 12] Dose-dependence has been reported with convulsant drugs,[13] and concentration-dependence[14, 15] and

duration-dependence[11, 14] have been reported with anesthetic agents. This report describes the concentration-dependence and duration-dependence of retrograde amnesia induced in chicks by inhalation of flurothyl, a chemoconvulsant known to be amnesic in mice.[15, 16] The results confirm that interference can be produced 24 hours after a learning experience[15, 17] and suggest a quantitative interpretation of variable consolidation gradients within the framework of consolidation theory.

Materials and Methods.—Neonate chicks are advantageous for quantitative memory studies; they peck a suitable target but learn in one trial to avoid that target if it is coated with an aversive liquid when first pecked.[18, 19] We used 2880 two-day-old White Leghorn cockerels (Kimber K155), individually housed in cartons 8.5 cm diameter by 16.5 cm deep. The target was a 3 × 5-mm microminiature lamp (5 v rating, operated at 2.6 v) cemented to the tip of a 3.5 × 200-mm plastic tube and coated with liquid methyl anthranilate[20] ($NH_2C_6H_4COOCH_3$, Givaudan). Flurothyl ($CF_3CH_2OCH_2CF_3$; b.p. 63.9°C) was obtained from Ohio Medical Products.

Environmental conditions: The carton temperature was 32.5° to 34.5°C, the relative humidity was 40 to 46%, the illumination was 23 footcandles, and the masking white noise level was 76 db. Chicks were acclimated to the carton for 2 hr before the training trial. Each chick remained in its carton throughout the experiment and was not fed, watered, or touched.

Experimental and control groups: Three parameters were varied: (1) interval between training trial and flurothyl treatment, termed the *training-treatment interval* (4 to 2880 min),[21] (2) *concentration of flurothyl vapor* (0.43 to 3.0% v/v),[22] and (3) duration of exposure to the vapor, termed the *exposure time* (1 to 16 min).[21] For each experiment on a batch of 240 chicks, the training-treatment interval and the flurothyl concentration were constant, while the exposure time was varied. Five experimental groups ($N = 40$) from a single shipment had exposure times of 1, 2, 4, 8, or 16 min; a sixth group ($N = 40$) received no flurothyl treatment and served as a nontreated control.

Training trial: The training was 1-trial avoidance conditioning. A transparent plastic cover with a 3-cm circular aperture was centered over the chick carton. The microminiature lamp was dipped into methyl anthranilate, passed through the aperture, and hand-held approximately 1 cm in front of the beak. A timer was started when the chick oriented to the lamp, typically within 0.5 sec. Ten sec later the lamp was withdrawn. The latency of the first peck was recorded to the nearest 0.1 sec; the range of median latencies in 70 groups was 0.8 to 1.9 sec. Six per cent of chicks failed to peck in 10 sec; they were replaced to maintain 40 trained chicks per group.

Amnesic treatment: Liquid flurothyl was dispensed into each carton and a tight lid was applied. The volume of 21, 42, 84, or 150 μl was calculated to produce a vapor containing 0.43, 0.85, 1.7, or 3.0% v/v flurothyl, respectively.[22] Full tonic convulsions with opisthotonos occurred in all chicks treated with 42 to 150 μl of flurothyl. After 1, 2, 4, 8, or 16 min (all plus 1 min for uptake and distribution[21]) the flurothyl vapor was replaced by room air.

Test trial: Conditions for the memory retention test, applied 20 to 24 hr after flurothyl treatment, were identical with training conditions except that the lamp was dry. All testing was "blind"; each carton was coded with a random number, the cartons were mixed, and the code was not broken until the results were recorded.

The criterion of amnesia was a peck at the lamp within 10 sec. The "experimental score" is the percentage of a group of flurothyl-treated chicks that met this criterion. The "control score" is the corresponding percentage of a parallel group of nontreated control chicks. The control score varied from day to day (Table 1). An "induced peck score" was defined to represent amnesia assignable to the flurothyl treatment, as follows: induced peck score = 100 (experimental score-control score/100-control score).

Results.—Learned avoidance response: The anthranilate-induced inhibition of

pecking was established within the 10-second training trial; the median number of pecks at the anthranilate-coated lamp was two, compared with seven at a water-coated lamp.[23] The learned avoidance persisted at least nine days.[23]

A dissimilar target (a 3-mm stainless steel ball fixed to a 1-mm wire) elicited prompt pecking in chicks trained to avoid the anthranilate lamp, proving that peck performance was unimpaired by methyl anthranilate and that the learned avoidance was not a generalized avoidance response.[23] The possibility that flurothyl interfered with peck performance rather than with memory was ruled out in separate experiments; for example, of 89 anthranilate-trained, flurothyl-treated chicks that avoided the lamp, 88 pecked the ball.[23]

Retrograde amnesia: It is conventional to consider that retrograde amnesia is exhibited when the raw experimental response differs significantly from the raw control response in the predicted direction.[4, 5, 11, 15, 16, 19, 24] When this conventional analysis is applied to the chick data, flurothyl appears to produce amnesia when administered four minutes to 24 hours after training. The longest intervals at which significant differences were observed (χ^2 test; $p < 0.05$) depended upon the flurothyl treatment parameters. For a one-minute exposure to either 0.85 per cent or 1.7 per cent flurothyl, the interval was four minutes. For exposures of 2, 4, 8 or 16 minutes to 0.85 per cent flurothyl, it was 64 minutes; for the same exposures to 1.7 per cent flurothyl, the interval was 1440 minutes. The divergence from 4 to 1440 minutes occurred solely as a result of manipulating the amnesic-treatment parameters rather than reflecting the progress of memory consolidation. Thus, the conventional analysis does not seem to be justified.

Dependence of amnesia upon exposure time and concentration of flurothyl: The induced peck scores at exposure times of 4, 8, and 16 minutes differed only slightly (Fig. 1); they were pooled for determining the concentration dependence

TABLE 1. *Induced peck scores for flurothyl exposure times of 4, 8, and 16 minutes (pooled).*

Flurothyl concentration (% v/v)	Training-treatment interval (min)	Proportion Pecking at Test Trial* Experimental N	Experimental (%)	Control N	Control (%)	p†	Induced peck score‡
0.43	4	120	38.4	40	27.5	0.3	14.9
	64	120	23.3	40	22.5	>0.9	1.0
0.85	4	120	92.5	40	20.0	0.001	90.6
	64	120	58.3	40	15.0	0.001	50.9
	256	120	58.3	40	37.5	0.03	33.3
	1440	118	39.8	40	22.5	0.08	22.3
1.7	4	114	97.4	40	30.0	0.001	96.2
	64	119	86.5	40	22.5	0.001	82.6
	256	115	69.5	40	35.0	0.001	53.2
	1440	113	56.6	40	27.5	0.002	40.2
	2880	110	24.5	38	26.3	>0.9	−2.4
3.0	256	96§	59.4	40	20.0	0.001	49.3§

* Experimental groups were treated with flurothyl vapor for 4, 8, or 16 minutes; control groups were not treated.

† Significance level of the difference between experimental and control groups (χ^2 test with Yates' correction).

‡ Equals 100 (experimental %-control %) ÷ (100-control %). The pooled score is the mean of the scores at 4, 8, and 16 min, weighted for number of chicks (*N*).

§ Only 28 chicks were available for the 3.0%, 16-min group; of these, 9 did not survive this severe flurothyl treatment. The unweighted mean is 52.3%.

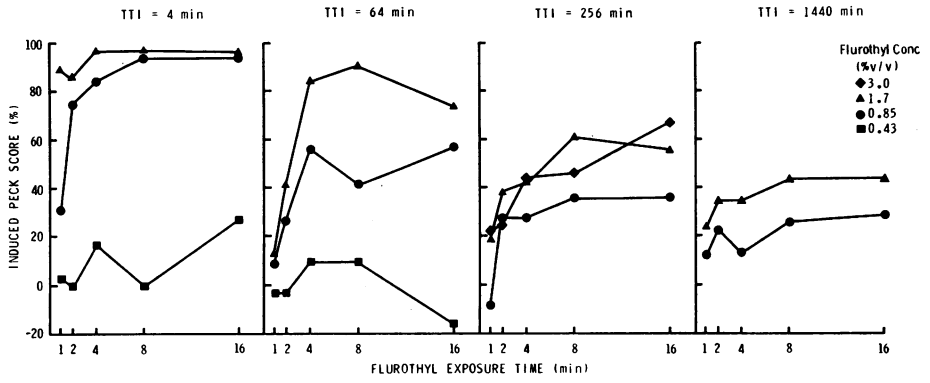

FIG. 1.—Dependence of induced peck score upon flurothyl exposure time[21] and concentration.[22] TTI = training-treatment interval. Each point represents 33 to 40 experimental chicks, except that for 3.0% flurothyl, 16-min exposure (N = 28) which is uncertain because of excessive mortality (32%).

(Table 1; Fig. 2). The sensitivity of the concentration-response relationship was found to be a function of the consolidation interval (Fig. 1). The 64-minute interval was optimal for discriminating between 0.43, 0.85, and 1.7 per cent flurothyl; 0.85 per cent flurothyl produced significantly less amnesia than 1.7 per cent flurothyl (χ^2 = 22.2; $p < 0.001$) and was therefore incompletely amnesic. At the 256-minute interval, the amnesia produced by 3.0 per cent flurothyl did not significantly exceed that produced by 1.7 per cent flurothyl.

Discussion.—The chick results confirm numerous experiments with rats and mice that demonstrate increased amnesia with increased intensity of various amnesic treatments.[4-8, 10, 13-15] It remains to develop a parsimonious interpretation of this reliable effect, as follows.

Retrograde amnesia experiments have (1) a training trial to convey new information to an animal, (2) an amnesic treatment to block consolidation of that information input, and (3) a test trial to estimate the engram present *at the moment just following the amnesic treatment.* The test, however, is delayed for 24

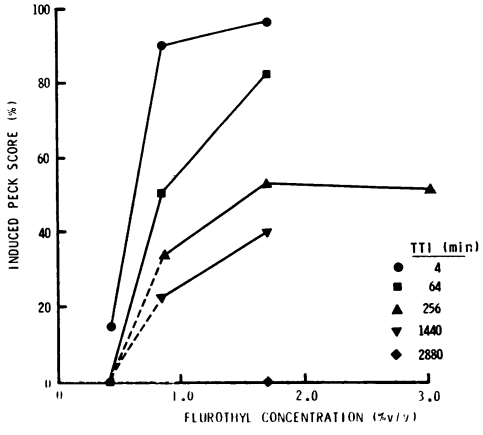

FIG. 2.—Dependence of induced peck score upon flurothyl concentration.[22] Each point represents pooled data for flurothyl exposure times of 4, 8, and 16 min (N = 110 to 120 except for 3.0% flurothyl, where N = 96). The broken lines are extrapolations.

hours to avoid the confounding effects of residual short-term memory, pro-active performance deficits, and circadian variations. An amnesic treatment must meet three criteria: no destruction of consolidated engram, complete block of ongoing consolidation (so that no additional engram is formed before the test trial), and no effects upon performance or memory retrieval at the test trial.

In this experiment, the evidence for the first criterion is that the effect of flurothyl decreased as the training-treatment interval increased, whereas engram destruction would cause the same amount of amnesia after every interval.

Evidence for complete block of consolidation has not been provided in any retrograde amnesia experiment. The conventional assumption that tonic convulsion is an acceptable criterion[4-6, 15, 24] is clouded by observations of a poor correlation between seizure pattern and retrograde amnesia in rodents[5-7, 10, 25, 26] and chicks,[9] and by our results; chicks could experience little amnesia despite full tonic convulsion (Fig. 1, TTI = 64 min, 1-min exposure to 0.85% and 1.7% flurothyl). The amnesic effect of flurothyl appears to approach a maximum in this experiment at a concentration of 1.7 per cent to 3.0 per cent flurothyl with an exposure time of 8 to 16 minutes (Fig. 1, TTI = 256 min).

Evidence for the absence of performance or retrieval effects is provided by separate experiments,[23] in which (1) the marked performance deficit observed after one hour largely disappeared after 24 hours and (2) the amnesic effect persisted for nine days, the maximum tested.

Interpretation of concentration-dependence: The interpretation of the smaller retrograde amnesia found with 0.85 per cent flurothyl compared to 1.7 per cent flurothyl (Fig. 3) is that the lower concentration slowed but did not block memory consolidation, so that additional engram formed during the 20 to 24 hours before the test trial.

To quantitate the concentration-dependence it is convenient to shift attention from amnesia to memory retention, as indicated by avoidance of the lamp during the test trial. The induced avoidance score is equal to 100-induced peck score. Memory retention is a function of the interval between the training trial and the flurothyl treatment. The probit of the avoidance score, y, appears to be linearly related to the logarithm of this interval, t, over a considerable range.[27] Assuming complete block by the amnesic treatment the empirical linear relation is $y = \alpha + \beta \log t$, where α is the intercept at $\log t = 0$ and β is the slope constant. I have suggested[27] that α may be an empirical measure of "learning strength" and that β is an empirical measure of the rate of consolidation. The linear relationship does not imply a unitary process because the transfer function between the engram and the measured behavioral response is unknown.

To account for post-treatment consolidation we require a third term, $\Delta y = (1\text{-}k)\ \beta \log t'$, where k is a factor (0 to 1) that describes the effectiveness of an incomplete amnesic treatment and t' is the time between that treatment and the test trial. Two arbitrary assumptions are required: (1) no consolidation occurs during the acute phase of the convulsion and (2) post-treatment consolidation commences at the end of the acute phase. From the experimental data for 1.7 per cent flurothyl (Fig. 3), with $k = 1.00$ and t in seconds, I have calculated α as 1.27 probits and β as 0.82 probits per log second. The value of k for 0.85 per

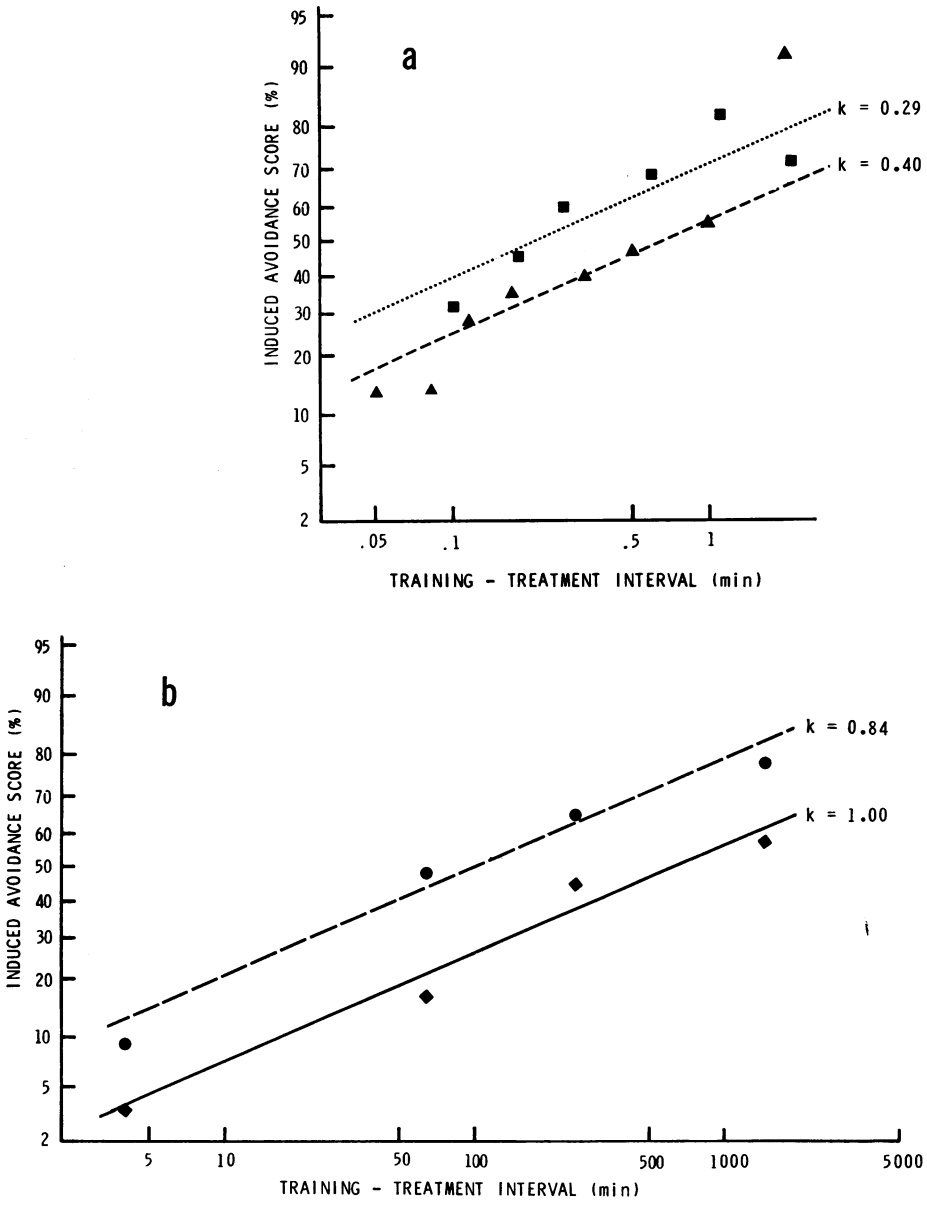

FIG. 3.—"Consolidation" gradients with various amnesic treatments in chicks. The ordinate is on the probit scale, the abscissa on the logarithmic scale. The values of k represent the relative amnesic effectiveness compared with that of 1.7% flurothyl. The regression lines represent $y = 1.27 + 0.82 \log t + 0.82 (1 - k) \log t'$, as explained in the text. The points represent experimental data. ◆, 1.7% flurothyl (a value of 102.3% at 2880 min is not shown); ●, 0.85% flurothyl; ▲, from ECS data of Magnus, Kanner and Hochman;[28] ■, from ECS data of Lee-Teng and Sherman[19] (5 plateau values after 2.1 min, of 67 to 83%, are not shown).

cent flurothyl is 0.84, calculated as $y = 1.27 + 0.82 \log t + 0.82 (1\text{-}k) \log t'$. The linear regressions are plotted in Figure 3, compared with regressions using ECS data in chicks.[19, 28]

It is clear that the less effective the amnesic treatment, the shorter the observed "consolidation time." The consolidation half-time (CT_{50})[27] calculated for $k = 1.00$ by setting $y = 5.00 = 1.27 + 0.82 \log CT_{50}$, equals 35,400 seconds or 9.8 hours. The apparent CT_{50} calculated for $k = 0.84$ (obtained with 0.85% flurothyl) equals 1.6 hours. Variability of amnesic effectiveness occurs with dissimilar agents as well as with quantitative differences in other treatment conditions, e.g., in the ECS current *at the brain sites critical to consolidation*[5, 8, 10, 26] or in the partial pressure of inspired ether vapor.[29]

The interpretation that divergent consolidation gradients arise from variable amnesic treatments is parsimonious and plausible, but it must be qualified because of the uncertainty of interpreting retrograde amnesia experiments. Deutsch[30] has made a critical analysis of this uncertainty. Weiskrantz,[2] for example, has suggested a quite different interpretation, namely, that consolidation is vulnerable to disruption for less than 30 seconds after information input[11, 19, 24, 28, 31] and that the "amnesia" observed after longer intervals reflects impaired retrieval. The critical prediction that such "amnesia" must disappear with time[2] has been supported[32] and denied[33, 34] in recent reports. The amnesia induced by flurothyl in chicks persisted for nine days, the maximum studied.[23] Conceivably, amnesic treatments impair both retrieval and consolidation.[34] More detailed studies of retention as a function of the time after amnesic treatment should permit a clearer delineation of the role of consolidation and retrieval effects in retrograde amnesia phenomena.

I thank Professor Linus Pauling for his valued interest in this work and for critical revision of earlier drafts. Miss Mayme Bailey and Mrs. Mary Garman carried out the experiments and Mr. Daniel Cherkin assisted with the calculations.

[1] Booth, D. A., *Psychol. Bull.*, **68**, 149 (1967); Rosenzweig, M. R., and A. L. Leiman, *Ann. Rev. Psychol.*, **19**, 55 (1968).

[2] Weiskrantz, L., in *Amnesia*, ed. C. W. M. Whitty and O. L. Zangwill (New York: Appleton-Century-Crofts, 1966), pp. 1–35.

[3] Glickman, S. E., *Psychol. Bull.*, **58**, 218 (1961).

[4] Alpern, H. P., and J. L. McGaugh, *J. Comp. Physiol. Psychol.*, **65**, 265 (1968).

[5] Miller, A. J., *J. Comp. Physiol. Psychol.*, **66**, 40 (1968).

[6] Weissman, A., *J. Comp. Physiol. Psychol.*, **56**, 806 (1963). See also *ibid.*, **57**, 248 (1964) and Weissman, A., *Arch. Intern. Pharmacodyn.*, **154**, 122 (1965).

[7] Jarvik, M. E., and R. Kopp, *J. Comp. Physiol. Psychol.*, **64**, 431 (1967); Dorfman, L. J., and M. E. Jarvik, *Neuropsychologia*, **6**, 373 (1968).

[8] Pagano, R. R., D. F. Bush, G. Martin, and E. B. Hunt, *Physiol. Behav.*, **4**, 1 (1969).

[9] Lee-Teng, E., *J. Comp. Physiol. Psychol.*, **67**, 135 (1969).

[10] Ray, O. S., and R. J. Barrett, *J. Comp. Physiol. Psychol.*, **67**, 110 (1969).

[11] Paolino, R. M., D. Quartermain, and N. E. Miller, *J. Comp. Physiol. Psychol.*, **62**, 270 (1966).

[12] Lee-Teng, E., *Proceedings of the 75th Annual Convention of the American Psychological Association* (1967), p. 87.

[13] Weissman, A., *Intern. Rev. Neurobiol.*, **10**, 167 (1967).

[14] Quinton, E. E., *Psychonomic Sci.*, **5**, 417 (1966).

[15] Alpern, H. P., and D. P. Kimble, *J. Comp. Physiol. Psychol.*, **63**, 168 (1967).

[16] Bohdaneckỳ, Z., R. Kopp, and M. E. Jarvik, *Psychopharmacologia*, **12**, 91 (1968).

[17] Nieschulz, O., *Arzneimittel-Forsch.*, **17**, 1151 (1967).

[18] Cherkin, A., and E. Lee-Teng, *Federation Proc.*, **24**, 328 (1965).

[19] Lee-Teng, E., and S. M. Sherman, these PROCEEDINGS, **56**, 926 (1966).

[20] Dr. Morley R. Kare kindly suggested this repellant.

[21] The operational interval between training and addition of liquid flurothyl was increased by 1 min to allow for uptake and distribution of flurothyl, as estimated from the latency of opisthotonos. The operational exposure time was decreased by 1 min for the same reason.

[22] The stated concentrations are nominal; actual concentrations were reduced by diffusion through the carton and uptake in chick tissues. Monitoring by vapor phase chromatography and infrared spectrophotometry indicated a loss of approximately 2.5%/min. The relative partial pressures at the site of action remain the same as the nominal concentrations, standing in the ratios 3.0:1.7:0.85:0.43. The mean induction times (±S.D.) for tonic extension were: 59.2 ± 12.0 sec in 0.85% flurothyl, 39.6 ± 8.0 sec in 1.7% flurothyl, and 24.6 ± 5.1 sec in 3.0% flurothyl. The CD_{50} (concentration convulsing 50% of chicks within 10 min, and 95% confidence limits) was 0.40% (0.33 to 0.49) in cartons and 0.49% (0.44 to 0.54) in glass; the lethal concentration (LD_{50}) was 10.3% (8.6 to 12.5) in cartons and 10.1% (9.2 to 11.2) in glass.

[23] Cherkin, A., manuscript in preparation.

[24] Quartermain, D., R. M. Paolino, and N. E. Miller, *Science*, **149**, 1116 (1965).

[25] McGaugh, J. L., and H. P. Alpern, *Science*, **152**, 665 (1966).

[26] Dorfman, L. J., and M. E. Jarvik, *Physiol. Behav.*, **3**, 815 (1968); Kesner, R. P., and R. W. Doty, *Exptl. Neurol.*, **21**, 58 (1968).

[27] Cherkin, A., these PROCEEDINGS, **55**, 88 (1966); *Psychonomic Sci.*, **4**, 169 (1966).

[28] Magnus, J. G., M. Kanner, and H. Hochman, unpublished results.

[29] Cherkin, A., *Psychonomic Sci.*, **13**, 255 (1968).

[30] Deutsch, J. A., *Ann. Rev. Psychol.*, **20**, 85 (1969).

[31] Chorover, S. L., and P. H. Schiller, *J. Comp. Physiol. Psychol.*, **59**, 73 (1965).

[32] Zinkin, S., and A. J. Miller, *Science*, **155**, 102 (1967); Kohlenberg, R., and T. Trabasso, *J. Comp. Physiol. Psychol.*, **65**, 270 (1968); Lewis, D. J., J. R. Misanin, and R. R. Miller, *Nature*, **220**, 704 (1968); Nielson, H. C., *Exptl. Neurology*, **20**, 3 (1968).

[33] Chevalier, J. A., *J. Comp. Physiol. Psychol.*, **59**, 125 (1965); Luttges, M. W., and J. L. McGaugh, *Science*, **156**, 408 (1967); Herz, M. J., and H. V. S. Peeke, *Science*, **156**, 1396 (1967); Herz, M. J., and H. V. S. Peeke, *Physiol. Behav.*, **3**, 517 (1968); Geller, A., and M. E. Jarvik, *Psychonomic Sci.*, **10**, 15 (1968).

[34] Riddell, W. I., *J. Comp. Physiol. Psychol.*, **67**, 140 (1969).