Genetic Aspects of Learning and Memory in Mice

The study of differences among strains and individual subjects is a most promising topic in psychobiology.

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One of the most controversial issues in biology since the time of Locke and Leibniz has been that of the role exerted by heredity and environment—what is inborn and what is acquired. Two factions arose in attempting to explain this delicate balance. One minimized the role of genetics as a determinant of behavior, and the other (mainly represented by ethologists and geneticists) emphasized the importance of heredity (*1*).

As a matter of fact, the genetic determinants of behavior have been confused with, or identified with, fixed action patterns currently classified as instinctive. Heredity, however, also affects many forms of behavior less organized or less systematic than nest building, sexual behavior, or some forms of highly organized aggressiveness.

Each individual or species is provided with genetic baggage responsible for the so-called spontaneous motor activity, for the search for new stimuli (which are the grounds of the exploratory behavior and curiosity), and, more generally, for all types of motivational and emotional behavior. Besides, any type of adaptive behavior, like the aptitude to respond to a conditioned stimulus, to learn, and to remember, is genetically determined. Heredity plays an important role in different forms of adaptive behavior, since individual reactions to environmental influences and the ability of man to benefit from cultural heritages depend on hereditary factors.

Due to the difficulties of controlling the genetic and environmental determinants and the role played by cultural factors in human societies, it seems necessary to start with experimentation on animals in order to confront the problem of "nature and nurture" in men. The use of other animal species involves a less-complex approach. The problem of nature and nurture constitutes the target of interest in today's psychobiology, a new branch of biology that affords a tentative bridge between neurophysiology and comparative psychology, and ethology. Psychobiology attempts to study whether, and to what extent, learning behavior is genetically determined.

Behavioral Genetics

One of the first extensive experiments in the field of psychogenetics was Tryon's selective breeding program (*2*). In mating rats which displayed characteristic traits, Tryon selected those among their offspring with the highest or lowest performance in a standardized maze and bred their offspring. Bright maze learners produced bright progeny, whereas dull maze learners produced dull progeny. Tryon's experiments, based on carefully controlled breeding and environmental factors, established the inherited nature of this adaptive behavioral trait. In light of these experiments it seemed advisable to use animals bred selectively, from genetically homogeneous strains to assess the effects of heredity on behavioral aptitudes (*3*).

In agreement with Hall (*4*), the main objectives of psychogenetics are (i) to discover whether a given behavior pattern is transmitted from generation to generation, (ii) to determine the number and nature of the genetic factors (*5*) involved in the trait, and (iii) to locate the genes on the chromosomes. We discuss here the first of the above problems.

Scott, Frederickson, and Thompson (*6*) demonstrated that inbred strains of mice differ not only with respect to certain morphological traits but also with regard to behavioral traits. As far as adaptive behavior is concerned, the acquired behavior of inbred mice has been studied by the use of a reward as motivation; in a few investigations, avoidance conditioning has been used (*7*).

Individual and Strain Performances in Avoidance Behavior

Our experiments with different conditioning tests for screening the action of psychotropic agents demonstrated that the large individual variability of a heterogeneous population was an obstacle to the assessment of the factors affecting the patterns of learning and retention, characteristic of a given species. By the use of different strains of inbred mice, we developed an avoidance technique similar to one which we had used for the rat. In this paper we describe results obtained by studying the avoidance behavior of different strains of mice in a shuttle box.

Double-Compartment Grill-Box Technique

The apparatus used for the study (*8*) of escape and avoidance responses is a two-compartment cage adapted from Warner's model (*9*). Each apparatus consists of a rectangular plexiglass box divided into two equal compartments which are connected by a small opening (Fig. 1). The floor is a tilting platform of stainless steel rods. On each trial the conditioned stimulus (a lamp which lights the compartment where the animal is) is preceded by a constant interval (5 seconds), and the unconditioned stimulus is represented by a continuous electric shock administered through the grid floor. An unconditioned escape response is recorded when the mouse shuttles into the adjacent compartment after the onset of the shock. A conditioned avoidance response is recorded when the mouse avoids the shock by running into the other compartment within 5 seconds after the onset of the light. A series of

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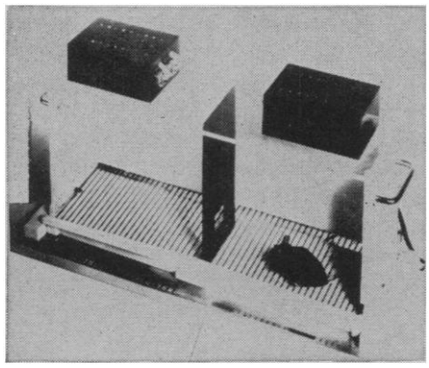


Fig. 1. Shuttle box used for the study of avoidance learning in mice.

eight automated shuttle boxes driven by the same programming device was used.

An advantage of using the shuttle box in experiments on avoidance lies in the type and the constancy of the motivation adopted. Staying alert to avoid and escape from predators is one of the basic requirements for survival for a wild species (*10*). A technique based on an escape reaction gives reliable and homogeneous results in that it appeals to a type of inborn behavior.

Other reasons support the study of escape and avoidance behavior. (i) In contrast to what happens in operative behavior, and typically in a lever-pressing apparatus, it is possible to study the initial learning phases in a shuttle box, because escape and avoidance reactions are closely related. (ii) The anticipatory nature of the acquired responses makes this type of learning the prototype of an intentional "forward looking" behavior different from other types of "contiguity" learning (*11*). The complexity of this task is also reflected in the large number of trials required; this is in contrast to the "single-trial" tasks used to measure other types of learning. This complex task allows analysis of the different phases of the learning processes.

The use of an aversive stimulus in the Warner cage has been widely criticized. The use of electric shock gives the advantage of a constant and adjustable motivation but might also be responsible for emotional components which can be variously interpreted.

Harlow (*12*) claimed that "the double compartment grill box is without doubt the most efficient torture chamber which is still legal." Actually, the intensity of the electric shock generally used for testing small laboratory rodents is only sufficient to keep the animal alert. A real state of anxiety is evident only during the first trials, and the experimenter can select conditions which minimize this phenomenon. Fear and anxiety, which may be an emotional reaction of freezing, are largely dependent on species and strain. One of the reasons for using mice is that in most of the strains this emotional component can almost be ignored.

In an amusing experiment we showed that when a "bridge" was placed between the mouse pen and the shuttle box where the animal had been trained, the mouse spontaneously returned to the shuttle box, presumably because of curiosity, in spite of the risk of receiving an electric shock.

Behavioral Variability of a Common Noninbred Strain

In establishing a learning curve, the experimenter has the same difficulties as a man who is training a dog or taming a beast. These troubles have often been matters of perplexity or jokes. For instance, Scott (*13*) says that "there is an old joke among biologists known as the Harvard law of animal behavior: when a stimulation is repeatedly applied under conditions in which environmental factors are precisely controlled the animal will react exactly as it pleases."

When Warner (*9*) described his technique for testing avoidance behavior he was confident that it would become an "ideal and universal yardstick for the measurement of the learning capacity." However, the results did not always support his hopes. Also, with animals of different species trained in a shuttle box, individual differences between subjects of the same species and even of the same colony represent a bias and sometimes make interpretation of the results hazardous.

Even when the techniques used are not considered, individuals may appear refractory to any training because the behavior may differ as widely between two animals of the same colony as among fish, birds, rats, or even monkeys. In this respect, our findings agree with numerous reports on conditioning, maze learning, or operative behavior.

A specific example (*14*) shows the extent of the individual differences in groups of Swiss Webster mice from one colony and trained in a shuttle box (Fig.

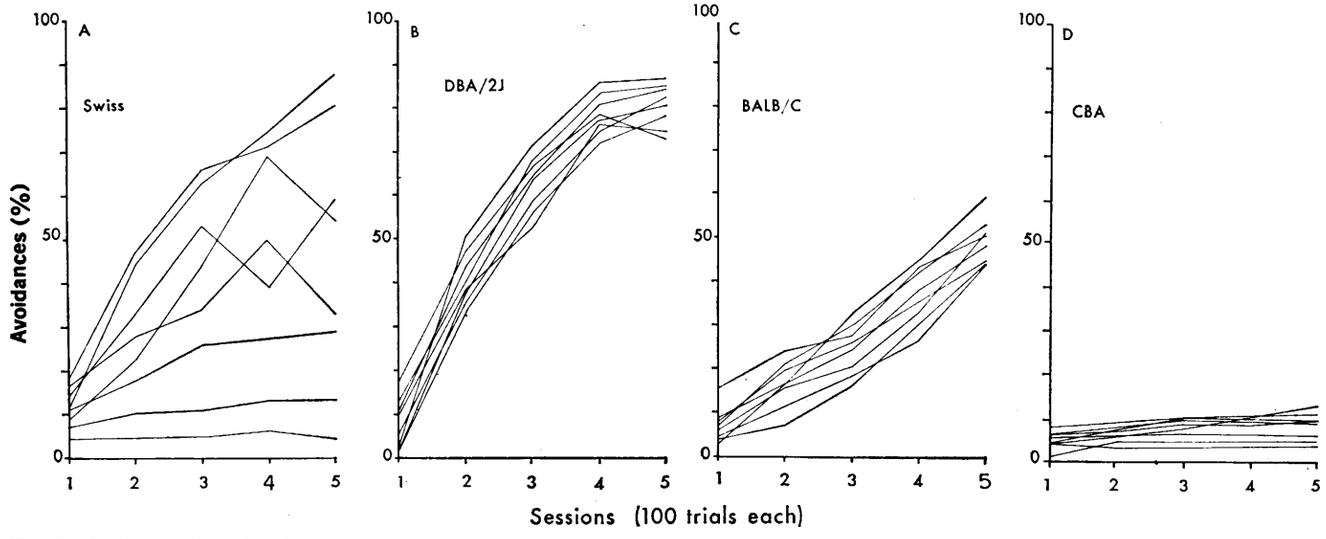


Fig. 2. Avoidance learning in (A) a heterogeneous population of Swiss mice; (B) DBA/2J mice; (C) BALB/c mice; (D) CBA mice. Each curve represents the individual performance of a mouse during five avoidance sessions of 100 trials each.

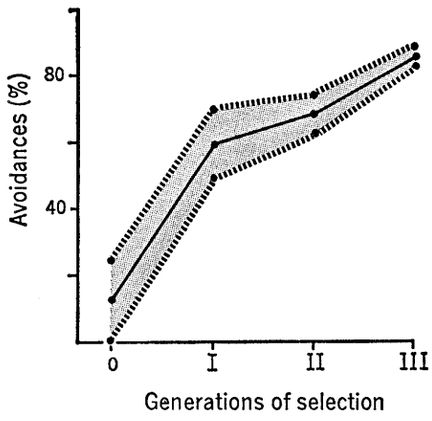


Fig. 3. Selective breeding for avoidance learning in the mouse (Swiss Webster). Each point represents the performance reached at the end of five 100-trial sessions (solid line). Dotted lines, fiducial limits of the mean.

2). A comparison between the individual response curves shows that different levels of performance are attained by each subject. Some animals reach a high rate of response during the first session, whereas other mice seem to be unable to associate the conditioned stimulus with the escape response (*15*).

The level of performance of a given animal shows sharp fluctuations from session to session. This last finding, that is, a sharp decrement in performance between consecutive sessions, can be interpreted in different ways. From an ethological standpoint, if the behavior of a laboratory animal is compared to that of the species, we might ascribe this type of inhibitory behavior to the reaction of immobility usual in the presence of danger, a type of instinctive behavior well known among the naturalists. From a psychological viewpoint, freezing behavior has generally been interpreted as a type of emotional behavior associated with experimental neuroses. In our experiments we used strains of mice in which this type of emotional behavior is absent or at a minimum.

The extreme variability in a heterogeneous population of Swiss Webster mice led us to initiate a selective breeding experiment to obtain a line of mice with high performance levels in the shuttle box. Previous experiments had shown that within the span of a few generations it was possible to breed two lines of rats characterized by patterns of high or low avoidance (*16*).

From Fig. 3 we note that a large group of Swiss mice (32 subjects) attains a rather low performance at the end of five 100-trial sessions. If the high avoiders are mated together performance improves within a generation from the original 15 percent to 60 percent. Selective breeding, continued until the third generation, produced a population of mice with a performance level of 85 percent.

Selective breeding not only produced a line of high avoiders, but was also accompanied by progressively reduced individual variability. The fiducial limits of the mean decreased from the original level of 23 percent to 4 percent in the third generation.

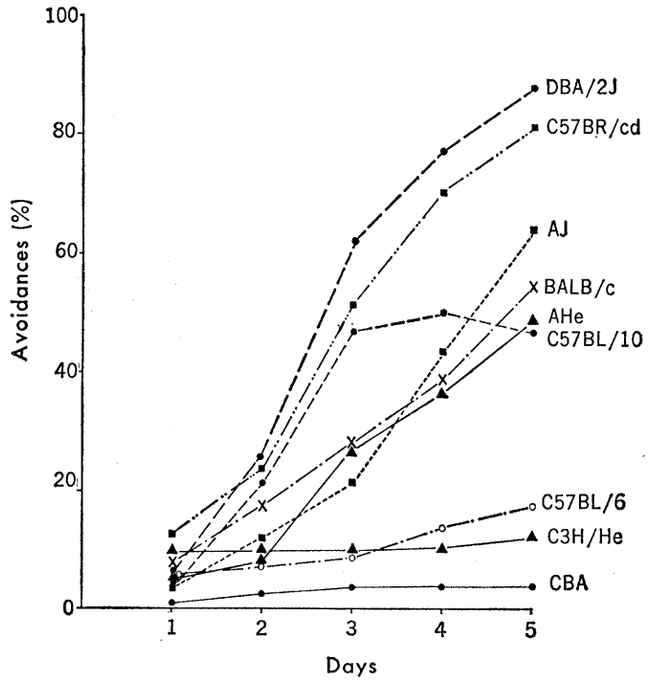
Strain Differences in Avoidance Acquisition

Similar to what we witnessed in experiments based on the selection of a given trait, a comparison between the performances of different strains of mice shows that avoidance learning is genetically determined. The advantage of using mice comes from the many strains now available for research.

Figure 4 gives the avoidance performance of nine strains assessed by training different groups of naive animals. A comparison between the shuttle-box performance attained during a cycle of five daily sessions under rigid control of the experimental conditions shows the following.

1) Three strains (CBA, C3H/He, and C57BL/6) were quite poor in acquisition of avoidance learning. The mean of the responses during the last session was lower than 20 percent (*17*).

2) In four strains (A/He, A/J, BALB/c, and C57BL/10) the mean percent of avoidances had reached, at the



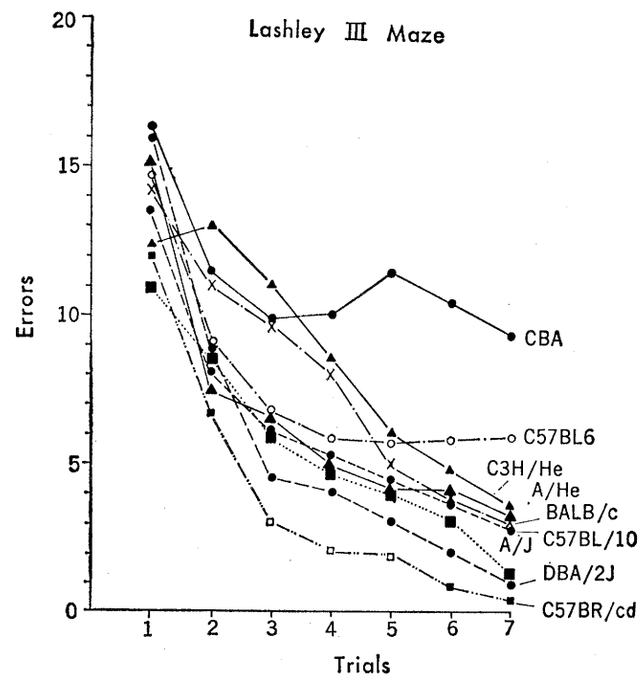


Fig. 4 (left). Avoidance conditioning of nine strains of inbred mice during five consecutive daily sessions of 100 trials. Fig. 5 (right). Maze learning (Lashley III maze) in nine strains of inbred mice. Each point represents the mean errors of 16 mice given one daily trial for ten consecutive days.

end of the experiment, values ranging between 40 and 70 percent.

3) Finally, two strains (C57BR/cd and DBA/2J) attained a very high level and during the last session reached an 80-percent level of performance.

The results obtained with genetically related strains show that the three strains from the group C57, although they belong to the same "strain family," attain strikingly different levels of performance. The CBA and C3H/He strains are both derived from a crossing of DBA mice; the DBA strain reached a high percentage of avoidances whereas the CBA and C3H/He remained at a very low level (*18*).

Our results for C3H/He and DBA/2J mice are in agreement with those reported by Royce and Covington, by Royce, and by Meier and Foshee for solving ability in a water maze (*19*). There is, however, a discrepancy between the performance of the strains tested in this research and that reported by Schlesinger and Wimer, which can be explained by the fact that different techniques were used (*19*).

The importance of the genetic factors is evident from the differences among strains and from the homogeneity of performance in animals of the same strain. The homogeneous behavior of inbred mice contrasts with the heterogeneous performance of randomly bred mice. The fiducial limits of the mean are in fact very low (< 10 percent) in the strains DBA/2J, C57BR/cd, and BALB/c, and lower than 20 percent in the remaining six strains (Fig. 2).

The wide variability of adaptive behavior of animals in a homogeneous population which, for almost a century, have been selectively bred for their activity or absence of aggressivity, is astonishing. It is to be expected that in a wild species the variability would be even greater (*20*).

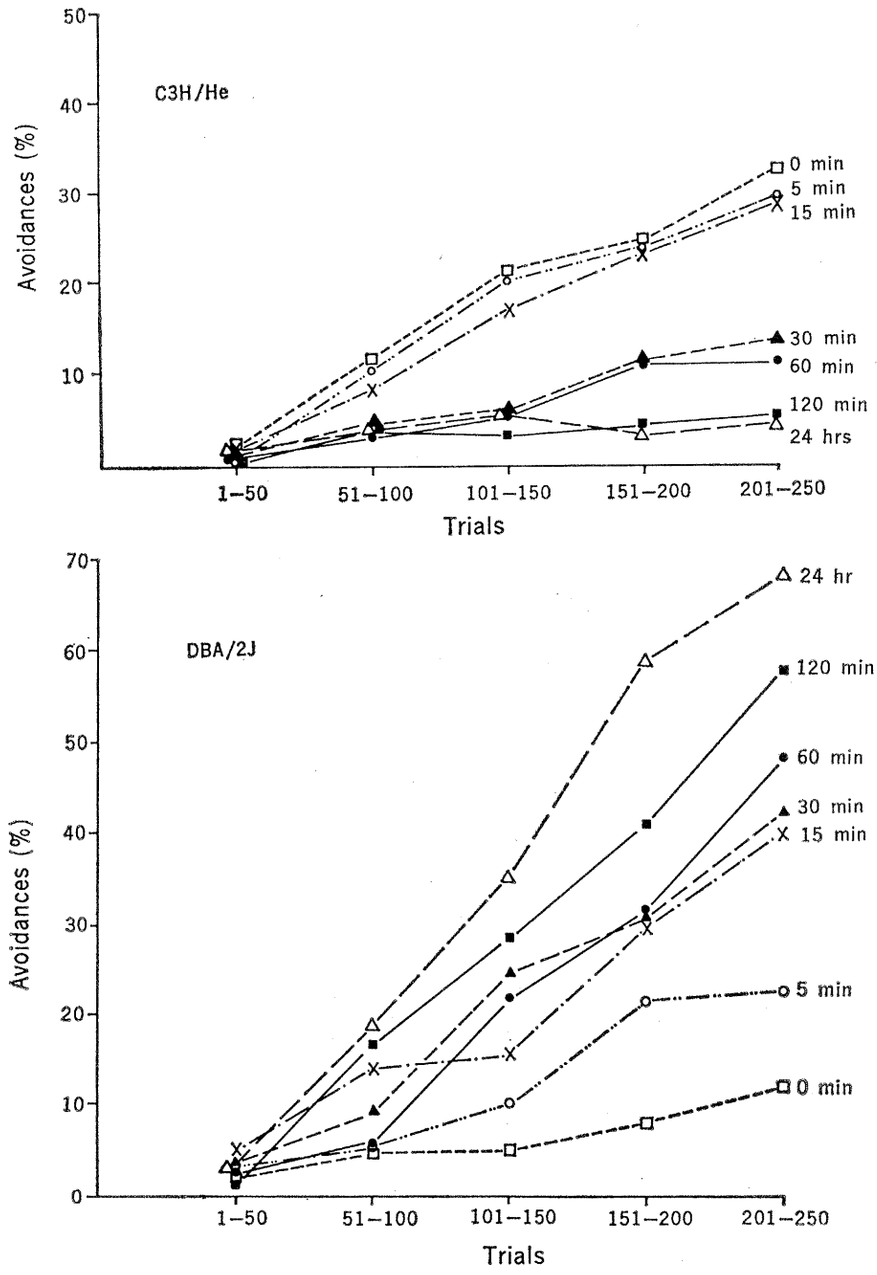


Fig. 6. Comparison of the performances of the strains C3H/He and DBA/2J when various time intervals between five sessions of 50 trials are used. The numbers at the end of the curves represent the length of the interval.

Relations between Avoidance and Maze-Solving Behaviors

Different strains of inbred mice attain different levels of performance when trained in a shuttle box. On the basis of these results and of previous findings showing sharp differences among inbred strains with respect to ability as judged by performance in a maze (*6*), we trained nine strains of mice in a small Lashley III maze. Figure 5 shows the performance of the different groups of animals given one trial a day for ten consecutive days. In agreement with previous results (*19*), ability to get through a maze also appears to be genetically determined, in that some strains (DBA/2J and BALB/c) of mice quickly attain good performance, whereas other strains (C57BL/6 and C3H/He) are poorer learners. The agreement between our findings and those of the authors quoted above (*19*) is particularly interesting in that those strains of good maze learners also attained a better performance when trained in the shuttle box. However, though these findings appear suggestive, it seems premature to extend the meaning of this particular problem-solving or learning ability.

Strain Differences in Learning Patterns

Can behavioral data be used to enable the synthesis of a concept of the process of retention? Having observed the striking differences in the performance of learning tasks among strains of inbred mice, we analyzed the nature of these different types of behavior by studying the effect of variable schedules of conditioning. The rather surprising conclu-

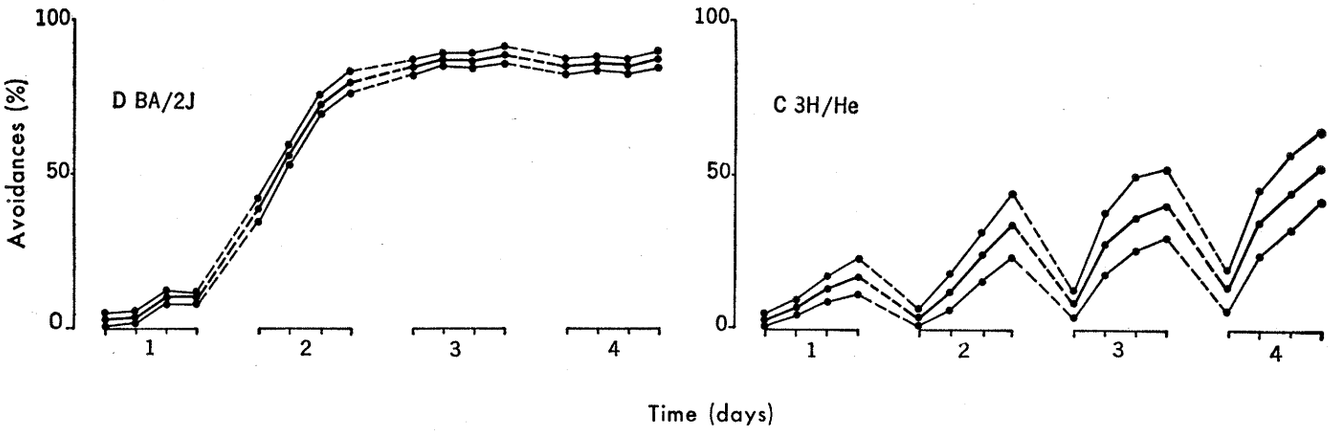


Fig. 7. Progression of the performance within each session and in the interval between consecutive sessions in C3H/He and DBA/2J mice. Solid lines, mean percentage of avoidances in groups of 32 mice during four daily sessions of 200 trials each. Dotted lines, fiducial limits of the means.

sion was that the differences between the various strains were not only quantitative but also qualitative, and that at least two different mechanisms of retention seem to be involved in learning processes of the various strains.

Dependence of Learning Differences on Massed or Distributed Practice

We demonstrated that different strains of mice are characterized by different levels of performance. Further analysis revealed that these differences are not only quantitative but also qualitative as evidenced by the shape of the curve representing acquisition. An example of this fact comes from comparing the response of strain DBA/2J to that of strain C3H/He (*21*).

Let us start by asking whether our mice prefer a long uninterrupted training session or a series of short ones, and, in case they choose the second solution, how long the break should be to take advantage from the practice. Actually this is one of the most controversial and debated problems in social psychology and experimental pedagogy.

The experiments were conducted under comparable, rigid conditions. The interval between each trial consisted of 30 seconds; a total of 250 trials was massed in a single uninterrupted session (interval 0 minute) or spaced in five sessions (each session consisting of 50 trials) the interval between each session being 5, 15, 30, 60, 120 minutes, or 24 hours. The performances achieved by the two strains under these conditions are completely opposite (Fig. 6). Strain C3H/He attains good performance only when the trials are massed in a single (125-minute) session, whereas performance is very poor when the interval between each session is 2 or 24 hours. By contrast, strain DBA/2J performs better under distributed practice when the interval between each session is increased.

Another way to space or mass the trials is by increasing the length of the interval between trials. By this means we can compare performances of two groups of mice subjected to a single 200-trial session in which the trials were spaced by 30 or 120 seconds. In this case (by giving in the same period of time a different number of trials), C3H/He mice perform better when the interval between trials is shorter (30 seconds), whereas DBA/2J mice attain a higher level of response when the trials are more widely spaced (interval between intertrials, 120 seconds).

Differences between strains can be analyzed by studying the variations of performance during each session and during the interval between consecutive sessions (*21*). Figure 7 gives the results of an experiment in which mice of two strains (C3H/He and DBA/2J) were given four sessions of 200 trials each with intersession intervals of 24 hours. The results show different patterns of behavior. If the variations of performance during the interval between successive sessions are considered (that is, between the level observed at the end of a given session and that observed at the beginning of the following session) the results show: (i) a decrement of performance in the strain C3H/He; and (ii) a marked improvement of performance between the last trials of session 1 and the first trials of session 2 in DBA/2J mice.

The type of learning pattern evident in DBA/2J mice is the one most frequently observed in the other strains studied. An "intermediate" pattern was evident in BALB/c mice. The first results of experiments conducted on strains of rats and guinea pigs show that avoidance learning in these two species often takes place in a "DBA/2J-type" (*21*).

Performance after Rest between Long Avoidance Sessions

A third experiment was conducted in which recovery of the performance at the end of a prolonged session was compared in the two strains, in the context of research on the effects of reactive inhibition (fatigue) and rest on avoidance performance of mice subjected to prolonged avoidance sessions (*22*). In a first experiment groups of trained mice were given a 2000-trial session (16 hours and 30 minutes). A decrement of the performance was evident in both C3H/He and DBA/2J mice after the first 3 to 4 hours (400 to 500 trials). This decrement progressively became more pronounced; at the end of the session the rate of avoidance responses was very low. For both strains a rest period of at least 16 hours was needed before they could again perform at their initial level. Figure 8 shows the difference between C3H/He and DBA/2J naive mice when both strains were subjected to the same type of session.

A progressive increment in the rate of responding (higher in C3H/He mice) is evident during the first part of the session. As far as trained mice are concerned a progressive decrement in performance, evident 4 to 5 hours after

the beginning of the session, became more evident until the end of the session.

A second difference in the behavior of the two strains (besides that observed in conditioning rates) was in their performance after different rest intervals after the end of the 2000-trial session. After 16 hours, the performance of the C3H/He mice returned to levels similar to, or lower than, the highest (peak) level reached during the uninterrupted session. It was rather surprising to observe that after a similar rest period DBA/2J mice attained high performance (about 70 percent), a highly significant improvement as compared to the highest performance attained during the previous session.

In the most general terms the results show that after the rest period, the responding was a function of the retention mechanisms characteristic of the strain. Under these experimental conditions, as in the previous experiment, consolidation mechanisms were active in DBA/2J mice and defective in strain C3H/He.

Strain Differences in Inhibitory One-Trial Task for Avoidance Learning

The results obtained for the two strains of mice show that there are two time-dependent processes in memory storage. However, we were concerned about the possible dependency of this phenomenon on the technique used. In other words, did the differences observed in DBA/2J and C3H/He mice with respect to patterns of learning and consolidation result only because a particular active avoidance task was presented?

In order to investigate this problem, groups of ten mice belonging to the two strains received a single "training" trial on a task involving inhibitory avoidance (*23*) and a "retention" trial given at time intervals from 2 minutes to 24 hours after the first trial (*24*). On each trial a mouse was placed on a small metal platform leading to a dark chamber. As the mouse stepped through the hole into the box he received a foot shock of about 3 milliamperes. The amount of time the mouse spent on the platform before he stepped into the box was recorded. Figure 9 shows that when the "retention" trial was given 0 or 2 minutes after the training trial, the median latencies to step through the hole were not different in the two strains. However, when longer intervals of time are considered, differences are evident between the two groups of mice. An increment in median latencies is evident in C3H/He mice when the "retention" trial is given 10 or 30 minutes after the "training" trial. After this time a decrement in latencies is evident, and the performance at 24 hours is lower than that at 10 or 30 minutes. This indicates a decrement of memory in time. In DBA/2J mice a poor retention of the "training" experience is evident when short intervals of time separate the "training" from the "retention" trial (2, 10, or 30 minutes). For longer intervals the latencies increase—indicating an improvement of retention. These findings therefore seem to be consistent with the recent interpretation given by McGaugh and by Jarvik on the multiple mechanisms involved in passive avoidance learning (*25*).

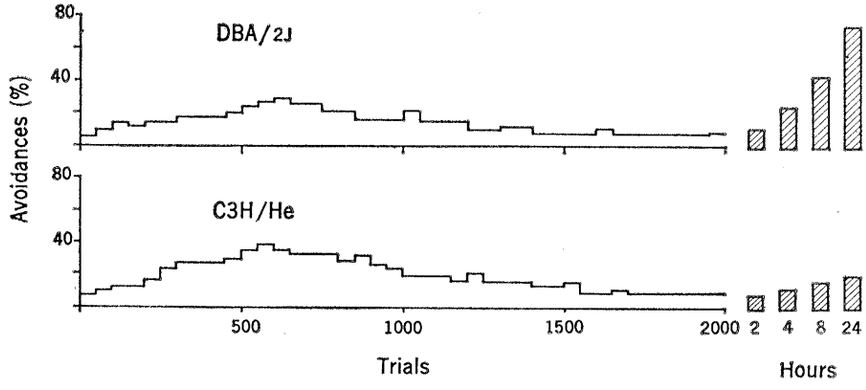


Fig. 8. Performance of two different strains of mice during an uninterrupted (16.5-hour) avoidance session consisting of 2000 trials. Columns at the right represent the performance of four different groups of mice given an additional 100-trial session after different intervals of rest from the end of the previous session.

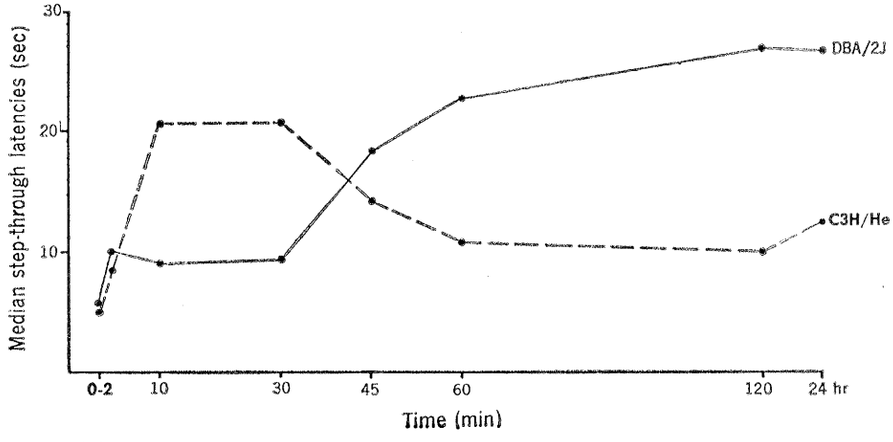


Fig. 9. One-trial passive avoidance learning in two different strains of mice.

Interpretation Based on Two-Stage Concept of Memory Storage

Our results show that not only the speed of conditioning but also the nature of the mechanisms involved depend on strain, and are genetically determined. An interpretation of the studies on reminiscence is often rather complex because of the number of variables which frequently are not controlled. Two types of explanations have been put forward to describe the usual superiority of distributed practice and to explain the reminiscence phenomenon in motor learning.

The first explanation probably does not apply to the type of avoidance learning used in our experiment. It postulates that during the rest pauses there is a recovery from the effects of practice that are detrimental to performance. Key words in these various hypotheses have been "fatigue," "work decrement," "disinhibition," "maturation of anxiety." Hull, in his classic theory, viewed learning as the acquisition of a "reaction potential" at the same time as "inhibitory" elements are building up. He felt that, with distributed practice, inhibitory processes would by and large disappear. Thus, he concluded, more improvement should result when practice periods were spaced (*26*).

A second and more probable interpretation postulates the role of an active consolidation process in reminiscence. The hypothesis of two-phase or multiple-stage retention is to be considered. During an early phase, the labile trace of the process of sensorial or ideational registration process corresponds to the so-called short-term memory. It is followed by the formation of a stable latent trace, potentially capable of subsequent evocation, which corresponds to the so-called long-term phase of memory. The hypothesis has been advanced that dynamic processes such as reverberation consolidation or perseveration, integration, and organization could act during the phase of storage. During this process of storage the labile trace may go through an autonomous decay or may be modified by new experiences such as a learning or interference (*27*).

A neurophysiological model of a dual mechanism of memory storage based on neurophysiological circuits (during the first phase) and on a molecular coding (during a second phase) has been suggested by Hebb, Gerard, and Young (*28*). Biochemical and pharmacological studies provide support for molecular coding (*29*). The experimental data shown above concerning the discrepancies of the learning curves of two strains of inbred mice strongly support the hypothesis of such a dual mechanism. In agreement with this concept, the strain C3H/He should be characterized by a good short-term memory and by autonomous decay, or by a rather ineffective consolidation mechanism. However, the processes of consolidation are a determinant in avoidance learning of DBA/2J mice. This interpretation at the same time explains the differences of performance that depend on massed or distributed practice (such as the patterns of improved or impaired performance evident during the intersession interval), the latent learning in long sessions, and the strain differences in the passive avoidance task.

Effects of Electroconvulsive Shock, Age, and Drugs on Consolidation

The interpretation already proposed concerning the nature of the differences in the learning patterns of C3H/He and DBA/2J mice is further supported by experiments dealing with the action of electroconvulsive shock, aging, and administration of convulsant agents after the trial. Electroconvulsive shock, a therapy used in psychiatric treatment

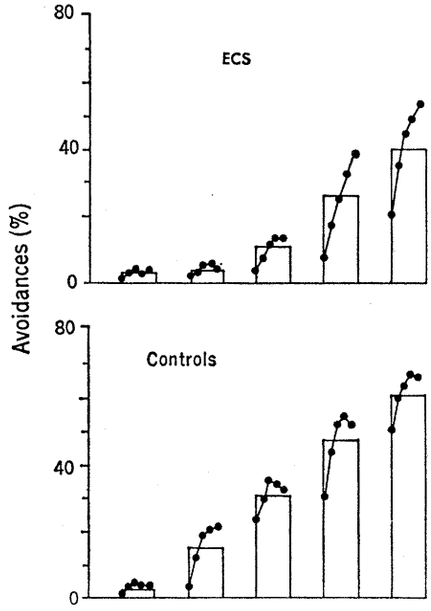


Fig. 10. Effects of posttrial electroconvulsive shock (ECS) on consolidation of shuttle-box learning. Shock (15 ma, 100 msec) was administered to DBA/2J mice immediately after the end of each 50-trial session. In additional experiments shock treatment was ineffective if administered 15 minutes after the end of each session. The curves within each session represent the mean avoidances (percent) during five blocks of ten trials each.

(*30*), reportedly produces transient impairments of memory. Retrograde amnesia affects from 50 to 75 percent of the patients treated with shock, although amnesia may not be complete.

Clinical implications aside, the use of electroconvulsive shock opened a new area of research in the psychobiological sciences. The first findings are from Duncan (*31*), who reported that administration of shock after a trial impaired consolidation in rats. This effect was no longer evident if time elapsed between the end of the training session and the application of shock. Analogous experiments were undertaken by the use of other species of animals and other training procedures. Although most of these experiments dealt with "one-trial" learning procedures, we adopted longer training schedules consisting of sessions of 50 trials with intervals of 24 hours between each session (*32*). The results showed that retrograde amnesia occurred if shock treatment was applied immediately after the end of each training session (Fig. 10). The disruptive effects were only evident if shock was administered within 2 minutes after the end of the session. The time course of the consolidation interval shows a linear regression after log-probit transformation in agreement with results of one-trial learning experiments plotted by Cherkin (*33*).

The analysis of the learning curves within each session enables a distinction to be made between the effects of shock on learning (short-term memory) and on the consolidation processes which occur in the interval between two consecutive sessions (long-term memory). In fact, although the performance of control mice improves between the end of one session and the beginning of the following session, a decrement is evident during the same period in animals treated with shock. With the exception of the initial level of performance, the shape of the learning curves shows no sharp differences between controls and animals treated with shock.

We now examine a second group of results relating to the performance of different groups of animals belonging to the same strain (DBA/2J) but tested at different ages (*34*).

As shown by Fig. 11, four groups of mice (21, 60, 180, and 360 days old) were tested in a task involving avoidance learning during five consecutive days. In agreement with data collected on rats by Verzar-McDougall and others and on mice by Meier (*35*), our adult mice showed the best performance when compared to younger (21 days) or older (180 to 360 days) animals. However the most striking result was not the difference in the overall performance but the variations of responding (enhancement or decrement) evident between two consecutive sessions. Briefly, during the interval between successive sessions, active processes of consolidation took place in the adult, and decay occurred in young or old mice. These findings further support the interpretations of Kirby and of Doty and Doty (*36*).

The role played by mechanisms of consolidation in avoidance learning of mice is also evident from the effects of administration of drugs after trials. Injections of strychnine and picrotoxine facilitate learning, as shown by the experiments of McGaugh and his associates (*37*). By contrast, experiments by Pearlman, Sharpless, and Jarvik (*38*) gave evidence that anesthesia after the trial results in an impairment of learning.

Our results (*39*) show that the effects of treatment with drugs on the rate of acquisition of avoidance behavior is particularly evident when performance at the beginning of the second session

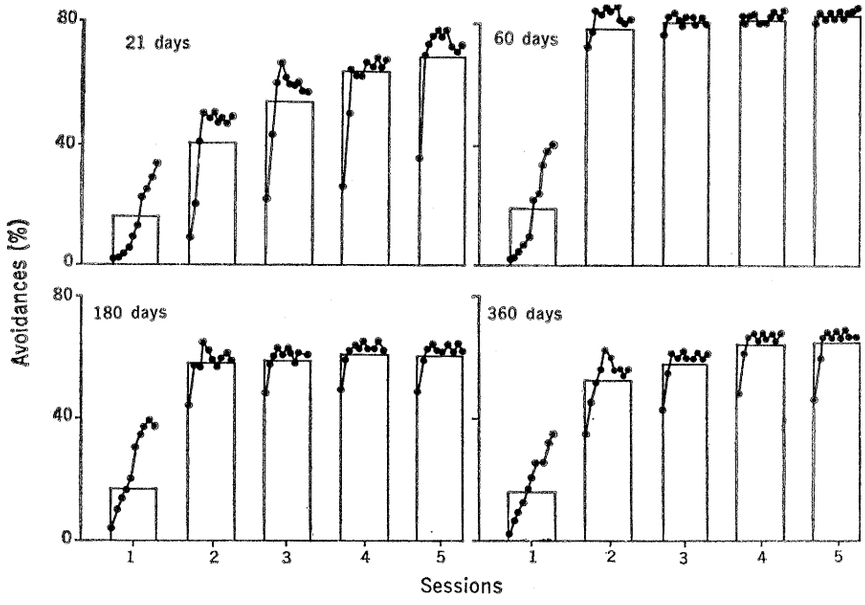


Fig. 11. Differences in the learning and retention curves in mice of different ages during sessions of 400 trials. The curves represent the patterns of performance within each session; each point represents the mean of 40 trials.

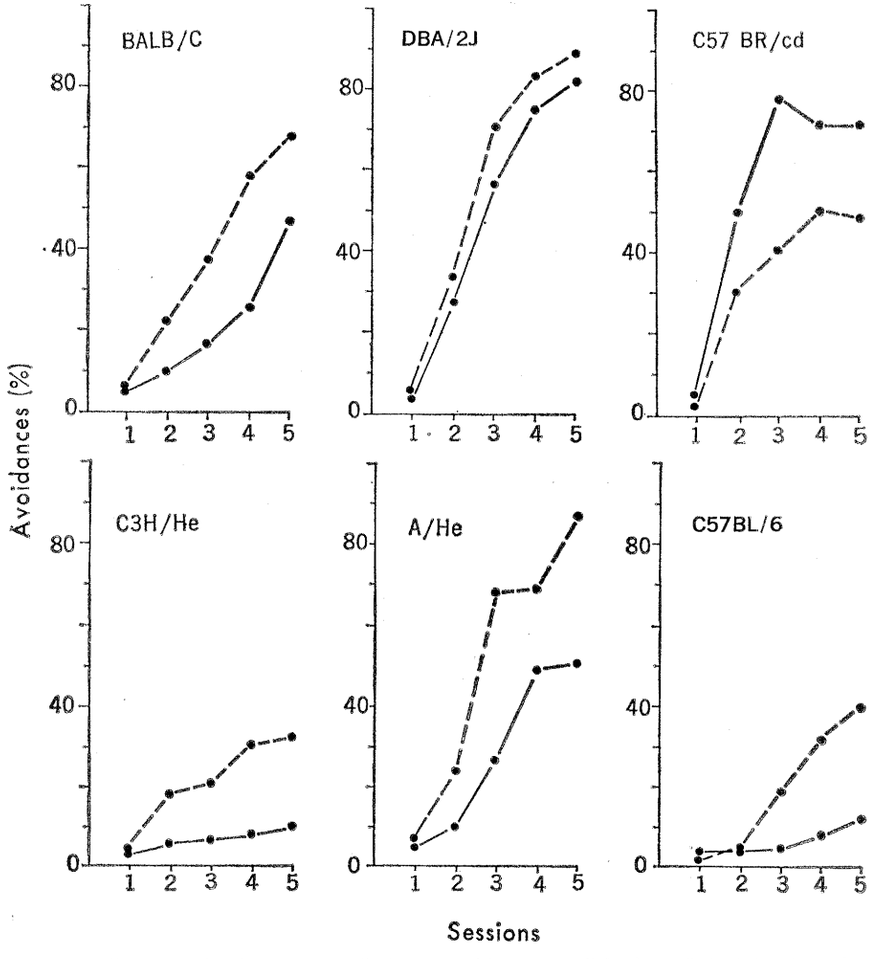


Fig. 12. Effects of nicotine (0.5 mg/kg) on avoidance conditioning of six inbred strains of mice. Dotted lines, nicotine-injected groups; solid lines, control groups.

is compared to performance at the end of the previous session. In the group of animals injected after a trial with strychnine or picrotoxine, the percentage of avoidances during the first trials of session 2 are much higher than the level during the last trials of session 1. The enhancement, due to the effect of convulsants on consolidation mechanisms, is higher than that in control mice. Similarly, an impairment of the storage was observed when nitrous oxide was administered immediately after a short training session.

The results of two series of experiments, under identical conditions and in which stimulants and depressants were used on the central nervous system, are consistent with a view of memory storage as a process involving several traces. Furthermore, these results are in agreement with the findings that learning patterns are affected by aging and that retrograde amnesia occurs when shock is administered after a trial.

Pharmacogenetics of Centrally Acting Drugs

There is a large body of results on the different reactivity of different species or strains of animals to the action of psychotropic agents. Various strains of mice have different pharmacological responses to a wide variey of drugs such as *d*-amphetamine, iproniazid, or chlorpromazine (*40*).

On the basis of the electrophysiological and pharmacological evidence of a central cholinergic transmission and on the hypothesis that there is a cholinergic system regulating arousal (*41*), the effects of nicotine and arecoline on learning and retention were tested. During investigations on drugs which facilitate elementary forms of learning, we observed, in 1963, that treatment with nicotine improved avoidance learning of naive rats. We used inbred mice (*42*) to extend these results and to study the pharmacological implications of the genetic differences in avoidance learning.

The effect of the same dose of nicotine (0.5 milligram per kilogram) was compared in nine inbred strains subjected to the same avoidance schedule in a shuttle box. The results show that under similar conditions, the various strains attain quite different performance, and that there are important differences between the effects of nicotine in the various strains. Nicotine had

a facilitating effect on six out of nine inbred strains: the incidence of avoidance increased to 35 percent above the level of the control (C3H/He) mice. The same dose had a smaller effect in DBA/2J mice (15 percent), and an impairing effect on the performance of two other strains (Fig. 12).

In contrast to nicotine (a typical cholinergic agent), arecoline did not facilitate avoidance learning. On the contrary, a decrement of performance was evident after treatment with various dosages of this drug. This impairment was strain dependent, as shown by a comparison between the effects of arecoline on DBA/2J and BALB/c mice (Fig. 13).

A correlation between the pharmacological effects of these cholinergic agents and the significance of the short- and long-term memory balance in the strains of the mice tested is still hypothetical. However, the facilitating effect of nicotine is generally higher in the strains characterized by low performance levels.

Effects of Environment on Learning and Memory

How can we determine whether a given type of behavior depends on heredity or environment? The interdependence of these factors shed light on studies in which we attempted to find out to what extent either of these elements could be responsible for any behavioral variation.

The role of genetic patterns in the determination of simple behavioral traits has been determined by studies on selective breeding and the comparative behavior of inbred strains of laboratory rodents. However, relatively little is known about the limits of the effects of the environment on learning aptitudes and memory. Here are a few examples of the advances of the research in this field.

1) Concerning sexual behavior, the effects of environmental factors on gonadotropic hormones and the nervous system have been widely studied. Light, diet, and olfactory stimuli are some of the most important nonsocial patterns modifying the sexual behavior of the male and the estrus cycle and the spontaneous activity of the female (*43*). The implications of these and other social factors on the dynamics of population of confined house mice have been extensively studied (*44*).

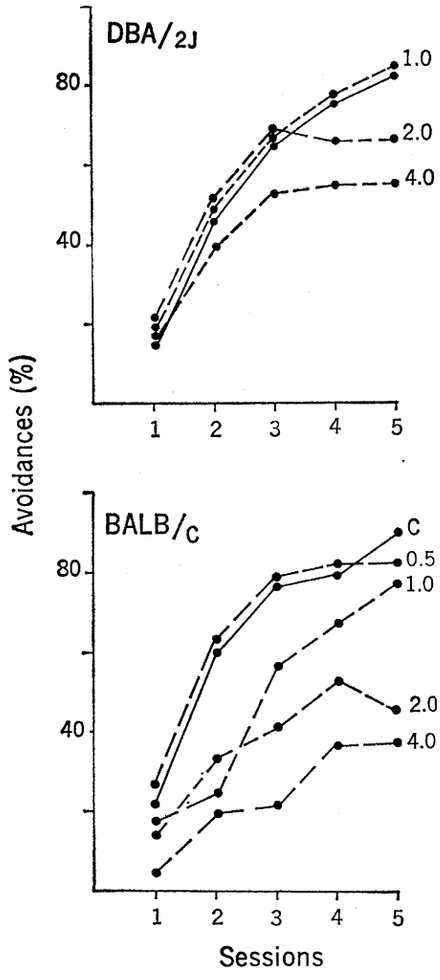


Fig. 13. Effects of arecoline on avoidance learning of BALB/c and DBA/2J mice. Each point represents the mean of eight mice during five consecutive daily sessions of 100 trials. *C*= controls. The numbers indicate the dose of the drug.

2) Many experiments have been conducted by Rosenzweig and his associates (*45*) on the effects of an enriched or impoverished environment in the rat. The most striking results deal with modifications of the weight and morphology of the brain and with changes in cerebral chemistry. Some evidence has been furnished that an enriched environment leads to an improvement of ability to get through a maze.

3) Imprinting is, according to the ethologists, a species-specific type of learning that occurs (mostly in birds) within a limited period of time, early in the life of the organism, and is relatively unmodifiable thereafter. Although this concept does not seem immediately applicable to the behavior of small rodents, many experiments have dealt with the effects of early experience. As demonstrated by Sluckin (*46*), a distinction may be drawn between the lasting effects of early learning in a form of training and the effects of other kinds of early experience. The value of a very early training is that it may be, according to Hebb's theory "half-transferable" from early to later learning.

The fact that the lack of any training often precludes the acquisition of skills and habits, or may adversely affect the learning ability, was shown by studies dealing with the nature of free environmental experience and with the effects of early visual and motor experience as determinants of the rat's ability to solve problems in a maze (*47*). Various experimental factors, such as the exposure to high and low temperatures, bright and dim light, noise, electric shock, and drugs, have been manipulated to discover their long-term effects (*48*). Levine (*49*), for example, reported that stimulation such as momentary handling could facilitate the rat's ability with regard to conditioning. Denenberg (*50*) reported that the shocking of infant mice furthered subsequent classical conditioning.

Of particular interest are the studies on the effects of the preweaning environment on behavioral patterns; it was demonstrated that behavior of mice reared by alien foster mothers was affected by fostering, or that it resembled behavior of the alien strain (*51*). The results of these experiments support findings (*52*) that emotionality of the offspring is affected by maternal environment.

By the use of a shuttle box in experiments on avoidance, we demonstrated (*53*) that the usual patterns of avoidance behavior were not affected by cross-fostering two inbred strains of mice (DBA/2J and C3H/He), whereas some emotional components of behavior were strongly affected. In particular, we found, by measuring avoidance and "freezing" behavior with the same technique, that modifications of preweaning environment affect the emotional behavior, whereas variations of learning patterns are due to the concomitant appearance of freezing behavior.

Figure 14 shows that DBA/2J mice, which in control conditions reach a high performance and do not present freezing behavior, when reared by alien foster mothers appear to be more emotive. On the other side, the emotivity is enhanced by fostering C3H/He mice by alien mothers in which this type of emotional behavior is absent. In general terms, the results show that a modifi-

cation of the environment, as that produced by cross-fostering, affects the emotional but not the adaptive behavior (*54*).

Conclusions

Among laboratory mammals the mouse is used in most genetic studies and is bred in many inbred strains which are currently available. The findings reported here suggest that there are at least two reasons for using inbred strains in psychobiology: (i) the extreme behavioral homogeneity of the individuals belonging to the same strain, and (ii) the characteristic differences in behavioral traits of each strain. These inbred strains provide the psychobiologist with unlimited groups of individuals presenting a homogeneous adaptive behavior. This availability is particularly important in view of previous difficulties and problems surrounding the establishment of learning and retention curves in laboratory animals.

Particularly in the field of learning, the average individual is, as Tryon stated (*2*), "a man made fiction." The individuals in an inbred strain form a kind of family—a multitude of one-egg twins. Psychobiologists studying the comparative psychology of individuals take advantage of this anomalous and almost unnatural situation.

It is also important to point out the "artificial" nature of the inbred strains and the profound differences among inbred strains and the general concept or "race."

Of his classic experiments on the dancing mouse Yerkes (*55*) wrote: "All dancing (mice) are alike in certain important respects but to the trained observer of animal behavior, their individual peculiarities are quite as evident and even more interesting than their points of resemblance."

We have seen how the use of different strains of inbred mice is a useful tool in the study of the mechanisms of retention. The findings obtained by analyzing the learning curves of two strains of mice support the two-stage concept of memory storage. In agreement with this theory, a type of short-term memory is peculiar to the C3H/He strain, whereas DBA/2J mice have long-term memory storage.

It is probable that the individual differences observed in the "adaptive" behavior of a heterogeneous population are not only quantitative, and that they derive from the different mechanisms of memory that characterize each subject. A similar line of reasoning might explain the different effects exerted by some psychotropic agents in different strains.

The findings reported until now in the literature and in the present paper suggest that there can be no clear-cut determination of the role of the environmental factor modifying learning

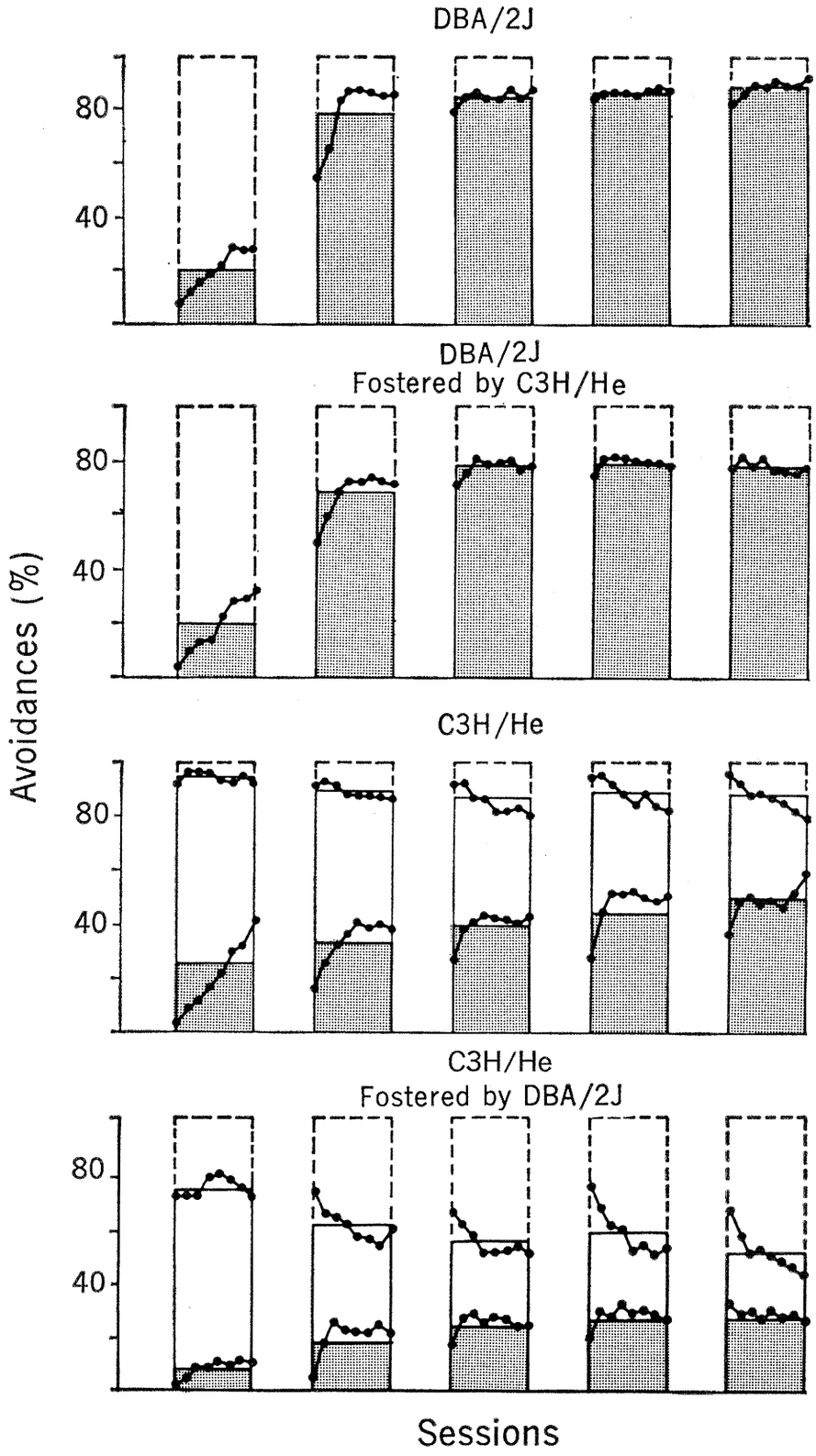


Fig. 14. Mean percentage of avoidance (dark columns) and freezing responses (white columns) of control and cross-fostered DBA/2J and C3H/He mice during five sessions of 400 trials each. The curves within each session represent the responses in eight blocks of 50 trials each.

and retention aptitudes. In a general way our findings show that adaptive behavior, less than emotional behavior, is subject to the influences of environment (*56*).

As an answer to the question which Hull raised concerning the role exerted by the genetic factors in the determination of the individual variability in adaptive behavior, the findings reported here illuminate the important role exerted by heredity in the determination of learning and retention mechanisms (*53*).

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57. The experiments described here were carried out in the Department of Pharmacology of the University of Sassari and the University of California School of Medicine, Los Angeles. Supported by grants from the Italian Consiglio Nazionale delle Ricerche, from the American Medical Association, ERF Committee for Research on Tobacco and Health, and from a USPHS general research support grant to the University of California, Los Angeles. Many citations were furnished by the Brain Information Service, Biomedical Library, University of California at Los Angeles.