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6. Age of menarche was recorded to the tenth of a year in the CRC and BGS Studies and in months in the HSPH Study; the latter were converted to years and tenths of a year. Height and weight data were given for half yearly (CRC and BGS) and yearly (HSPH, after age 11) intervals. Height and weight at menarche were found by interpolation. Interpolation was within a half year for 71 percent of the girls, within a year for the remainder. Three dates of menarche were missing. Fourteen girls were discarded (12, HSPH; 2, CRC) because of growth data intervals greater than a year at the menarcheal year. The means of age of menarche and height and weight at age 18 of the discarded girls did not differ from those remaining in the study. Three girls (2, BGS; 1, HSPH) with weights at menarche greater than 70 kg, which a study of the distribution of menarcheal weights showed to be clearly abnormal, were omitted.
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Interanimal "Memory" Transfer: Results from Brain and Liver Homogenates

Abstract. *Sixty mice received either shock or no shock in a shuttle box, or nonspecific stress in another apparatus. Brain and liver homogenates from these animals were then injected into 120 naive recipients, who were all tested in the shuttle box. Subjects receiving brain or liver from shocked or stressed donors had significantly higher latencies than control counterparts. These results are interpreted in terms of stress, rather than a memory transfer hypothesis.*

Babich *et al.* (*1*) found that RNA extracts taken from the brains of trained donors and injected into naive recipients facilitated performance of the latter when compared to animals receiving homogenates from untrained donors. This was interpreted as a demonstration of memory transfer. In the many attempts to replicate these results, there have been reports of both successes (*2*) and failures (*3*). This raises a question about the validity of the original interpretation. The discrepancy between successful and unsuccessful replications may involve the type of task used in each; "successes" have frequently employed passive avoidance tasks, and "failures" generally employed positive reinforcement situations. In the avoidance task, an increase in latency of recipients when compared with those of donors is taken as evidence of interanimal memory transfer. But such changes in latency could be the result of performance variables completely unrelated to learning, such as increased emotionality, fatigue, or stressful side effects of the particular experimental procedure. Thus, while these variables may have a pronounced effect on latencies, they may have only a marginal effect on either trials to criterion or error scores, the most common dependent measures in positive reinforcement tasks. These uncontrolled variables could account for the ambiguous results in the literature and raise the issue of whether the results of Babich *et al.*, and others, were due to (i) a specific memory transfer, possibly involving mediation by RNA (*4*) or large protein molecules (*5*), or (ii) the transfer of some factor affecting performance (*6*), such as stress. The present experiments were designed to separate these possibilities by contrasting the hypothesis that memory can be transferred from a trained to an untrained animal, with the hypothesis that "apparent" interanimal transfer in an aversive situation is mediated by nonspecific stress substances.

The subjects were 180 male albino mice, of the CD1 strain from the Charles River Laboratories, weighing 25 to 30 g. The subjects were divided into three groups, each comprised of 20 donors and 40 recipients. Each donor was randomly assigned to two recipients, one for brain homogenate and one for liver homogenate. The liver injection served to provide high concentrations of nonspecific RNA, and also to control for volume of foreign matter injected into the intraperitoneal cavity.

The behavioral apparatus was a rectangular box, 42.5 by 12 by 11 cm, one half of which was painted white and the other black, with the two sections separated by a center partition. The floor consisted of a grid of 0.2 by 12 cm brass rods placed 0.3 cm apart. The black side had a removable opaque cover, and the white side was illuminated with a high-intensity lamp producing 3300 lu/m^2 at the top of the box. The dependent variable for the donors was the number of seconds the subjects took to enter the black section from the white section through an opening in the center partition. Each donor was given only one trial in the apparatus, after which the maze was first cleaned with "Windex" spray containing ammonia and then distilled water to remove any odor-producing steroids which might affect subsequent animals. On the basis of previous research, all prospective donors taking longer than 30 seconds to enter the black section were discarded as atypical (this amounted to five animals across all three groups).

The shock-group donors received 5 seconds of scrambled shock (1.2 ma a-c) after entry into the black section of the box. The procedure for donors in the no-shock group was identical to that of shocked donors except that the former were not shocked while detained in the black section.

The stressed, control donors were placed in a ventilated glass jar, 5.5 cm in diameter and 7 cm tall, and rolled back and forth five times across a distance of approximately 15 cm.

Immediately following the procedures described above, each donor was decapitated, and the brain and liver quickly removed, weighed, and individually homogenized with an equal weight of distilled water. The entire liver was used in that homogenate, whereas the brain preparation did not include the olfactory bulbs and the cranial nerves. Next, a single injection of either brain or liver substance was given intraperitoneally in the upper left abdominal area of the appropriate recipient, who was first lightly anesthetized with ether to reduce pain. The volumes of the two

homogenates from each donor were equated on the basis of volume obtained for the brain substance, and ranged between 1 and 1.5 ml per injection. The entire procedure, from initial test of donor to intraperitoneal injection of recipient, was completed within 5 minutes.

On the basis of preliminary work, we observed that injected recipients walked with difficulty and seemed lethargic for periods of up to 2 hours. After 3 hours, we noted that in all cases normal home cage activity resumed. Therefore, each recipient was tested 6 hours after injection to permit unequivocal recuperation from any temporary disabling effects of the injections. Recipient testing was identical to that described for the donors given experience in the straight alley, except that no shock was administered upon entry into the black section. All testing was done by an experimenter who was naive as to which recipients had been given brain homogenate and which had been given liver.

Statistical analyses revealed a treatment effect significant at $P < .05$ ($F = 3.29$, d.f. $= 2/54$) (*7*). We found longer latencies in subjects receiving homogenates from the shocked and stressed donors than in subjects receiving homogenate from the nonshocked donors. In addition, we noticed consistently longer latencies in the recipients of homogenates from donors in the nonspecific stress group than in recipients of homogenates from shocked donors. The mean latencies of brain and liver recipients are summarized in Table 1.

The data from the groups that received brain homogenate from shocked and unshocked donors, respectively, could be interpreted as supporting the hypothesis that a specific memory is transferrable from trained donors to naive recipients. As expected, subjects receiving brain homogenates from donors who were shocked in the testing apparatus generally remained outside the black section longer than subjects receiving brain homogenates from nonshocked donors. However, when data from recipients of homogenates from the nonspecific stress group are considered, our results suggest that the increased latencies in recipients of homogenates from trained subjects cannot be adequately explained by the memory transfer hypothesis. This hypothesis, as proposed by Rosenblatt (*8*) or Unger (*9*), would not be able to account for the long latencies observed in the subjects whose donors were given no experience with relevant learning cues, that is, donors in the nonspecific stress group. However, this occurrence is understandable as a result of transfer of an as yet unidentified stress substance affecting general activity.

As behavioral testing proceeded, we noticed that some of the recipients were slower than their donors, while others had latencies almost identical to those observed in the donor mice. Because of this rather extensive variance, we decided to determine whether "fast" or "slow" subjects, so designated by inspection of the data, were differentially affected by the treatments. For the purpose of this analysis, subjects whose latencies were above the median of their respective group were called "slow," and those below, "fast" (*10*).

Of the mice whose latencies were above the median, those receiving brain homogenate from the shocked donors had significantly longer latencies than recipients of brain homogenate from unshocked mice ($P < .01$). In addition, the mice receiving homogenates from stressed donors had longer latencies than those receiving from no-shock donors ($P = .025$ for brain and $P < .05$ for liver homogenates). Other comparisons among groups were not significant.

In the below-median latency groups, the recipients from stressed mice took significantly longer to enter the dark side of the box than recipients from shocked mice, given either brain ($P < .01$) or liver ($P < .05$) homogenates. The other comparisons were not significant.

A chi-square analysis was also used to determine the percentage of subjects having response latencies longer than 20 seconds (Table 2). We chose this baseline figure because it represents approximately twice the mean of all donor latencies (11.65 seconds) in our experiment. The results paralleled the latency data; recipients of brain homogenate from the no-shock group were significantly faster than recipients from shocked donors ($\chi^2 = 9.6$, $P < .01$, d.f. $= 1$) and recipients from stressed donors ($\chi^2 = 7.9$, $P < .01$, d.f. $= 1$). No significant differences were found for the recipients of brain from shocked and stressed donors, respectively, or in any liver comparisons.

The individual differences in recipient latencies, and the stressful nature of the experimental situation, suggested that there might be an interaction between emotionality and memory transfer in the cases of similar scores for the donor and recipient. This interference could result from injecting substance from highly emotional donors into nonemotional recipients, and from nonemotional donors into emotional recipients. Because of the possibility of this interaction, it seemed necessary to perform a second experiment before reaching any definite conclusions about the accuracy of our general stress hypothesis. Consequently, mice for the next experiment were pretested on two independent measures of emotionality with the intention of creating groups consisting of three emotionally similar animals, one donor and two recipients. It was expected that this matching would decrease the overall variance.

The subjects were 180 male albino mice, of the CD1 strain from the Charles River Laboratories, weighing 25 to 30 g. These were assigned to the three groups described in the first experiment.

Two independent tasks were used to match triad members. The apparatus consisted of a straight alley and an open field maze. The first was painted a medium gray; it measured 103 by 11.5 by 16 cm, and had a free-flowing water spout at one end. The open field maze measured 46 by 46 by 15.5 cm and was demarcated into 36 squares.

For the 2nd through 5th days following arrival in the laboratory, the mice were placed on a 23 hour 45 minute water-deprivation schedule, and received water only in the straight alley. On the 6th day, each mouse was allowed to run to water ten times. A mean of the latencies for these ten trials was recorded for each animal.

Approximately 3 hours after testing in the straight alley, each mouse was permitted 3 minutes of exploration in the open field maze. A count was made of the squares traversed during that time.

The mean latencies and the open field scores constituted the measures according to which the members of each triad were selected. Within each triad, the difference between the shortest and longest mean latencies was limited to 1.5 seconds (group mean latency = 6.26 seconds, S.D. = 2.84), and the difference between the lowest and highest open field scores was lim-

ited to 25 squares (group mean = 255.93, S.D. =53.33).

The training, injection, and test and data-analysis procedures were identical to those described in the first experiment. Our analysis of the data revealed a significant difference between subjects receiving brain injections and liver injections ($P < .05$, $F = 4.30$, d.f. = 1/57). Thus, for the brain recipients, the longest latencies were found in recipients from the nonspecific stress donors, the shortest, in recipients from the no-shock donors, and those in recipients from the shocked donors were intermediary. For the liver recipients, the latencies in recipients from no-shock donors were also the shortest, and the latencies in recipients from stressed and shocked donors, respectively, were virtually the same. Within each treatment group, the liver recipients had significantly longer latencies than their respective brain-recipient counterparts. The mean latencies are summarized in the lower half of Table 1.

As in the first experiment, the data were analyzed with the Mann-Whitney U-test, for above-median and below-median latency scores.

Of the brain recipients with above-median scores, recipients from shocked and stressed donors, respectively, had significantly longer latencies than recipients from the no-shock donors ($P < .01$ and $< .025$, respectively). Similar findings were obtained for the liver recipients; that is, recipients from shocked and stressed donors, respectively, had longer latencies than recipients from the no-shock donors ($P < .05$ and $< .025$, respectively). The two remaining comparisons were not significant.

Of the recipients with below-median latencies, there were also four significant differences observed. For brain recipients from shocked and stressed donors, respectively, the latencies were longer than those of recipients from unshocked donors ($P < .025$ and $< .01$, respectively). For the subjects receiving the liver homogenate, recipients from shocked donors had longer latencies than both recipients from the no-shock donors ($P < .025$) and recipients from the stressed donors ($P < .01$). The other differences were not significant.

The results of the chi-square analysis for frequency of subjects with response latencies greater than 20 seconds revealed that recipients from shocked donors took significantly longer than recipients from no-shock donors, in the case of both brain recipients ($\chi^2 = 26.3$, $P < .001$, d.f. = 1) and liver recipients ($\chi^2 = 7.5$, $P < .01$, d.f. = 1). Recipients from stressed donors also took significantly longer than recipients from no-shock donors, in the case of both brain recipients ($\chi^2 = 16.5$, $P < .001$, d.f. = 1) and liver recipients ($\chi^2 = 7.5$, $P < .01$, d.f. = 1). No significant differences were found between recipients from shocked or stressed donors for both brain and liver injections.

Table 1. Mean latency of response (seconds) for recipients in experiments 1 and 2.

Recipients of	Source of injection		
	Shocked donors	Non-shocked donors	Stressed donors
	Experiment 1		
Brain	16.05	9.2	18.62
Liver	12.95	13.59	14.82
	Experiment 2		
Brain	18.7	11.35	26.45
Liver	31.0	20.1	30.55

In addition, Pearson correlation coefficients were obtained between the paired donor pretest scores and the recipient testing latencies for recipients from shocked, no-shock, and stressed donors, respectively; the purpose of this was to determine if the two pretests could predict performance in the test apparatus, as had been intended. These correlations were very low, ranging from $-.26$ to $+.25$, and were not significant.

The results of these two experiments indicate that support for the hypothesis of specific memory transfer may require further study with additional controls for stress-related substances. Although we obtained increased latencies in subjects receiving homogenates from the shocked donors of our experiment, we observed even greater increases in the recipients from the nonspecific stress donors; furthermore, increased latencies were observed for all recipient groups, regardless of whether the homogenate injected was from brain or liver. At this point, one could ask if two independent agents are active in producing the observed effect, a specific memory substance in the brain, and some hormonal stress element in the liver. This possibility is negated by our finding that substantial increases in latency occurred with injections of brain homogenate taken from subjects given the nonspecific stress treatment. These recipients, whose donors were stressed in a part of the laboratory far removed from the testing apparatus, had the longest latencies of all experimental animals receiving brain homogenates.

Table 2. Percentage of subjects in each group with latencies greater than 20 seconds. (This baseline represents approximately double the mean of all donor latencies, from both experiments.)

Recipients of	Source of injection		
	Shocked donors	Non-shocked donors	Stressed donors
	Experiment 1		
Brain	25	15	33
Liver	20	20	25
	Experiment 2		
Brain	45	10	35
Liver	50	30	50

If the specific memory hypothesis is questionable, what alternative explanations would provide better understanding of this phenomenon? On the basis of our experimental findings, we would tentatively suggest that a general stress factor may be responsible for the observed increases in latency. Furthermore, it seems necessary to take into consideration individual differences in emotional reactivity. Such individual differences, produced by environmental and genetic variables, have been shown by several investigators (*11*) to be important factors in predicting an animal's responses to different experimental treatments. Although we attempted to predict emotionality in the second experiment by using various pretests thought to assess arousal, we were unable to obtain significant correlations between the scores of the donors on the pretests (the open field maze and the straight alley) and the appropriate recipient latencies in the test apparatus. However, by matching donors with recipients, our results became less variable even though our measures of emotionality did not correlate perfectly. Future studies of interanimal transfer should seriously consider the prominent role of emotionality and arousal in determining recipient behavior. Therefore, the finding of pretests capable of designating a well-defined range of emotional types is of primary importance at the present time.

Since our studies were done with either whole brain or liver homogenates, no statement can be made regarding the effect of injections of pure RNA extract in interanimal transfer studies (*2*, *3*). We have shown, however, that

an effect similar to that obtained with RNA can be demonstrated with stress-affected whole brain or liver substance. Thus, if controls are not made for such factors as stress, it seems inappropriate to conclude that the RNA specific memory hypothesis is adequate, or even accurate.

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13. Requests for reprints may be addressed to Donald G. Stein.

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Olfactory Stimuli and the "Pseudo-Extinction" Effect

Wasserman and Jensen (*1*) demonstrated that continuously rewarded rats showed a decrease in starting speed on a runway recently traversed by other rats undergoing experimental extinction. They showed a less clear-cut effect on mean running speed. Their conclusion was that their "results indicate that the odor trace of a rat undergoing experimental extinction can significantly disrupt the performance of a subsequently run animal that was continuously reinforced."

Another observation that they made was that all rats undergoing experimental extinction urinated while none of the other experimental rats did. Thus one might conclude that the observed effect was produced by (i) an odor emitted by extinction rats as hypothesized by Wasserman and Jensen, (ii) an odor emitted by the urine of such rats, or (iii) an odor emitted by the urine of any rat. In the absence of further information, I would prefer the last of these hypotheses, which requires the postulation of no psychological mechanism, but merely a simple physical interference by the odor of urine with the ability of the experimental rat to catch the scent of the reward pellet. A delay in picking up the scent would affect starting speed more than running speed; hence this mechanism would also explain the differences observed between figures 1 and 2 of Wasserman and Jensen. They do not explain these differences, but speculate that "the repeated testing procedures had differential [sic] effects on running than on starting times."

To test which hypothesis is correct, another control experiment could be conducted in which rats are continuously rewarded on a runway that has been treated with the urine of rats not undergoing experimental extinction. As Wasserman and Jensen state at the end of their paper, "Control for odor effects would seem desirable if interpretation of experimental outcomes is to be unambiguous."

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Some of the comments by Deutsch appear to be answered by a careful reading of our paper (*1*). We attributed the "pseudo-extinction" effect to discriminable odors emitted by rats undergoing experimental extinction. We felt, and still feel, that this conclusion is consistent with our data. Contrary to Deutsch's contention, we did not speculate as to the exact nature of the olfactory stimuli involved. We explicitly stated that our experiment did not identify precisely what these olfactory stimuli were, ". . . particularly whether these stimuli are isolable from those of the excretory products deposited by the ET (extinction trace) animals." The question of the exact origin and chemical composition of the odors involved is interesting and important in its own right, but it was peripheral to our problem (the explanation of pseudo-extinction), our hypotheses, and our conclusions.

Deutsch's other comments appear to rest on an unusual and possibly naive hypothesis regarding the cues that control the behavior of rats on the runway. He suggests that urine deposited by ET animals caused a "delay in picking up the scent" of reward pellets in the runway in animals subsequently placed on the runway (odor recipients). This hypothesis presumes that the performance of the rat on the runway was controlled by olfactory cues from reward pellets in the goal box rather than by habit and expectancy which have been conditioned to handling and apparatus cues. This hypothesis is, however, inconsistent with the behavior of ET rats. If the hypothesis were correct and if the animals running in the alley were "picking up a scent" of reward pellets, then on the first extinction trial ET animals would show decreased starting and running speeds since food and food odor were not present. No such effect was observed in ET animals when they were first placed on extinction (*2*).

While Deutsch's hypothesis may appear simpler than our hypothesis of differential sensitivities of starting and running speeds to experimental manipulations, his hypothesis is refuted by our data. Even though Deutsch's hypothesis has been found to be implausible, it was testable and scientifically meaningful. Such cannot be said for his distinction between "simple physical" and "psychological" mechanisms.

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