Table 1
Sequences of Correct Responses for All Subjects During 39 Trials*

Degrees of Divergence	4	5	6	7	8	9	10	11
Representative	12345	12354	12435	12534	13425	15342	14253	31524
Sequences	54321	54312	45312	35412	23154	41325	52413	25143
Absolute Number Choices	0	2	5	21	35	57	53	60
Weighting Factor	1/2	1/4	1/14	1/32	1/18	1/28	1/14	1/8
Degree of Preference	0	.5	.4	.7	1.9	2.0	3.8	7.5

One S did not complete the first trial

successive trials. The probability of behaving in this manner as a result of random choices is less than three in a million.

RESULTS
Learning Rate

All six animals attained the criterion of learning. The best learner required 7 trials, the poorest 32; the mean number of trials was 14.7. These findings contrast with corresponding data for the undifferentiated five-path problem, in which the best learner required 35 trials, the poorest, 138 trials, and the mean number of trials was 93.4 (Lachman, 1965).

Choice Sequence: Stereotypy and Variability

Statistical treatment suggests a definite avoidance of adjacent paths and a preference by Ss for selecting pathways that diverge most from each other on successive runs; that is, there was avoidance of the sequences having minimum divergence (4 units) and strongest preference for the successions of pathways having maximum divergence (11 units), with definite preference trends in the minimum-to-maximum divergence direction for the sequences of intermediate divergence values (Table 1).

DISCUSSION

Results indicate that rats in a free-choice, five-path elimination problem with stimulus-differentiated pathways solve the problem dramatically more readily than rats confronted with the identical problem without such differential-path cues. The distributions of scores for comparable groups confronted with differentiated and undifferentiated five-path problems do not overlap. These results suggest that stimulus differentiation of the pathways markedly facilitates learning. The mean number of trials (19.4 trials) for attaining the criterion of learning on the *three-path* problem with *uniform runways* (Lachman, 1966) was greater than the mean number of trials for attaining the same criterion of learning on the *five-path* problem with *stimulus-differentiated runways* (14.7 trials).

Three aspects of these results are worthy of note: (1) Ss did not develop stereotyped sequences of response, i.e., they did not adopt any precise order or pattern of path elimination, either prior to or after attainment of the learning criterion.

(2) There was a trend toward employing different response sequences to solve the problem on consecutive days, while maintaining high accuracy of performance. (3) Successive pathways selected in any given pattern (or day's trial) tended to diverge maximally, i.e., the Ss tended to select on a given run a pathway which was maximally different in terms of spatial orientation from the pathway chosen on the immediately previous run.

These results are in harmony with results of previously reported research on undifferentiated multiple-path problems involving three, four, and five paths

(Lachman & Brown, 1957; Lachman, 1965, 1966).

The results are also consistent with and support the idea that there is a need for stimulus variability. Apparently, there is something inherently attractive about a *change* of stimulus. Perhaps the organism, given the opportunity, displays an innate preference for a change in stimulus and tends to search for it; such an innate preference is accommodated by this kind of learning situation.

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Chemical changes in the rat brain following escape training

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Sprague-Dawley albino rats were given maze exploration and escape training in a straight-runway maze. Ss in Control Group 1 were given only maze exploration, and those in Control Group 2 had no maze experiences. Half the Ss from each of the groups were sacrificed immediately, and the remainder were sacrificed 2 h after completing training. The brains were analyzed for protein, RNA, and total nitrogen. A significant increase in brain protein was found for the 2-h sacrifice escape condition. Moreover, estimates of NPN (nonprotein nitrogen) were significantly lower in this experimental condition.

In previously reported donor-recipient transfer experiments with rats (Kleban, Altschuler, Lawton, Parris, & Lorde, 1968;

Altschuler, Kleban, Gold, & Lawton, 1968), we found an increase in the amount of brain protein following shock-escape and shock-avoidance training conditions. This increase was found in the donor brain extract. The present experiment is an attempt to determine the effect of sacrifice time interval (immediate vs delay) on brain protein synthesis following training. Brain RNA and total nitrogen were also analyzed.

METHOD

The experimental apparatus was a straight-runway, shock-escape maze. The start box and stem, 26 in. long, was painted flat black and had a grid floor. The goal box, 16 in. long, was painted flat white and had a wooden floor. [Refer to Kleban et al (1968) for a more complete description of the apparatus.]

The Ss were 72 female Sprague-Dawley albino rats between 60 and 75 days of age. The animals were assigned randomly to six cells in a 3 by 2 design situation. There were three behavioral conditions: (1) shock-escape training, (2) exploration experience (Control Group 1), and (3) no

Table 1
Statistical Indices for Control and Escape Conditions for Protein and Nonprotein Nitrogen

Item	N	M mg/gm wet weight	σ M
I. Protein			
Combined control condition	48	21.52	0.286
Immediate sacrifice escape condition	12	22.17	0.457
Two hour sacrifice escape condition	12	24.74	0.461
II. Nonprotein Nitrogen			
Combined control condition	48	1.720	0.080
Immediate sacrifice escape condition	12	1.804	0.106
Two hour sacrifice escape condition	12	1.077	0.195

maze experience (Control Group 2). Half of the animals were sacrificed immediately and the remainder were sacrificed 2 h following completion of training.

The experimental Ss were given 3 min to freely explore the maze, followed by 10 1.0-mA shock-escape trials, with 60- to 90-sec intervals between trials. Shock came on instantly with the raising of the start-box guillotine door, and the animals could escape shock only by running into the goal box. Half of these Ss were sacrificed immediately after training and the other half after a 2-h interval. Two control conditions were used: (1) Ss that received 3 min of free exploration, and (2) Ss that had no experience with the maze. These Ss were given the same carrying cage experience as their experimental counterparts, and half of them were sacrificed immediately with the other half sacrificed after 2 h.

Following the training described above, the animals were killed by decapitation with a Harvard guillotine. The brains were removed and placed into ice-chilled beakers containing approximately 10 ml of ice-cold saline. The brains were cleaned of meninges, blotted with powder paper to remove surface blood, weighed, labeled, and placed into liquid nitrogen.

The frozen brains were homogenized with ice-cold saline in an ice-packed "Vitris 23" homogenizer, set at a "high" setting for 45-60 sec. Each brain was analyzed separately. All procedures were carried out at 4 deg C. The resulting homogenate was placed in a prechilled glass beaker, and mixed with a stirring bar at approximately 100 rpm for 24 h. This was followed by centrifugation in a refrigerated Sorval RC-2 centrifuge at 30,000 x g for 15 min. All analyses were carried out on the supernatant obtained by this procedure. RNA was extracted by the method of Schmidt & Thanhauser (1945), and quantitatively determined according to the colorimetric procedure of Mejbaum (1939). Protein was analyzed using the procedure of Lowry (1951) and the total nitrogen was assayed by micro-Kjeldahl method (1924). Thymus RNA and Versatol were used as standards for RNA and protein, respectively. All samples were run in duplicate.

RESULTS

There were no significant statistical differences found for the RNA and total nitrogen among the experimental conditions. The 3 by 2 analysis of variance for brain protein had significant effects. An F of 8.99 (df = 2/66, p < .01) was found for the escape as compared to the two control conditions. The reason for the significant main effect was the extent of the increase in protein synthesis in the 2-h sacrifice escape condition (Table 1). The protein level in this group was much greater than in all the other groups. The other conditions had equivalent levels of protein, clearly indicating that a combination of escape training and a 2-h sacrifice interval allows the occurrence of increased protein synthesis.

NPN was estimated from the nitrogen and protein measures [nonprotein nitrogen (NPN) = total nitrogen − 0.16 (protein concentration)]. A 3 by 2 analysis of variance indicated a significant difference among the escape and control conditions (F = 4.99, df = 2/66, p < .01). Once again, this significant difference was attributable to the significantly lower NPN in the 2-h sacrifice escape group (Table 1).

DISCUSSION

The results indicate that the levels of brain protein were quite responsive to this aversive experience, which confirms our previous finding (Kleban et al, 1968). In the latter, recipient Ss injected intraperitoneally with donor supernatant showed a marked initial tendency to avoid the grid. We cannot conclude, however, that the elevated protein levels in the supernatant were responsible for the donor-recipient transfer since the injected supernatant contained other substances besides protein. One cannot discount the possibility, however, that the elevated protein levels may have played a major role in the donor-recipient transfer. Such is the theoretical position of Rosenblatt, Farrow, & Rhine (1966) and Ungar & Irwin (1967).

The donor-recipient paradigm is primarily used for the measurement of transferred memory from donor to recipient Ss. Flexner & Flexner (1968), Barondes & Cohen (1968), and Agranoff, Davis, Casola, & Lim

(1967) have been using specific protein inhibitors, such as puromycin, acetoxycycloheximide, and cycloheximide, to establish the importance of protein in recall. For example, Barondes & Cohen (1968) were able to grossly block protein synthesis with intracisternal injections of cycloheximide without reducing the S's ability to learn the aversive training task. The inhibition of protein appeared related to the blockage of "long-term" memory. The present experiment demonstrates a quantitative rise in protein synthesis which has been postulated to be necessary for the transfer of memory from donor to recipient Ss.

The role of other nitrogenous compounds in this type of aversive training procedure is also worthwhile to consider. Since there was a rise in protein with concomitant fall in nonprotein nitrogen in the supernatant, those results might reflect the utilization of amino acids for polypeptide synthesis. If we make the assumption that the change in NPN reflects the amino acid pool, certain inferences may be drawn. There is a postsacrifice period at which time protein synthesis is maximal and at which point the amino acid pool may be sufficiently depleted to limit further protein synthesis. It should also follow that new learning tasks introduced on depleted amino acid pools should result in memory deficits similar to those found in the protein inhibition studies. In this case, the protein inhibition would result from the depleted amino acid pool. Further investigations will obviously be necessary to provide experimental confirmation of these speculations.

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