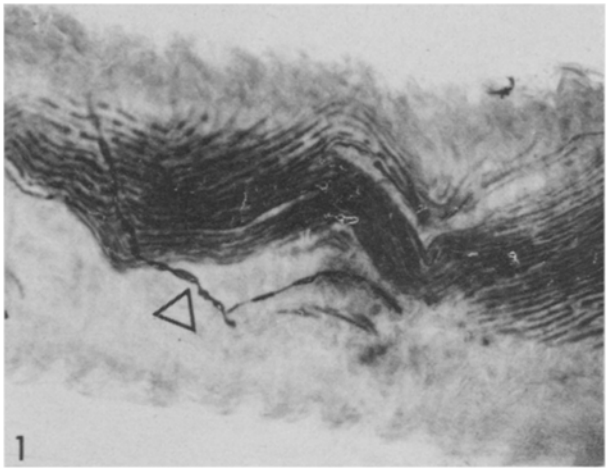

Fig. 1. Axonal beading in the sciatic nerve (Holmes silver). Low magnification.

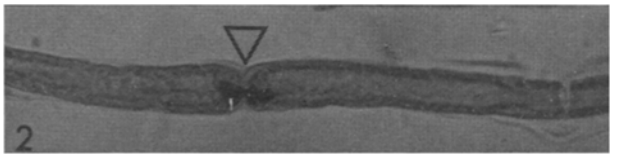

Fig. 2. Perinodal myelin breakdown (arrow). Marchi. Glycerin dissection.

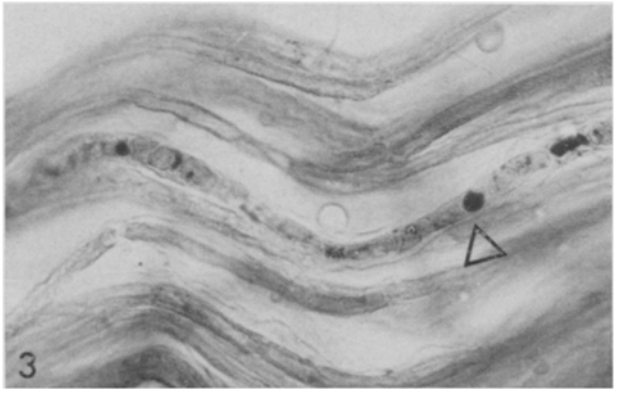

Fig. 3. Paranodal myelin breakdown (arrow). Marchi. Glycerin dissection.

silver, Nauta-Gyggax, luxol-fast-blue, PAS, oil-red-O. The Marchi preparations were dissected in glycerin. The tissues were examined with a polarizing microscope.

The histological examination of the sciatic nerves showed that in all the rats, except the controls, there was axonal hypertrophy, beading and degeneration (Holmes silver, Nauta-Gyggax), associated with widespread perinodal and segmental breakdown of myelin (Marchi, ORo, Polarizing scope) (Figures 1–3). This picture is interpreted as representing early changes of a neuropathy, which is primarily axonal with secondary myelin breakdown. The cause of the neuropathy is MnBK inhalation and the possible role, if any, of MEK in the second group of rats is now being investigated.

Résumé. Dix-sept rats sont exposés pendant 6 semaines 5 jours par semaine, 8 h par jour, à une atmosphère contenant soit du méthyl-*n*-butyl kétone seul soit un mélange de MnBK et de méthyl-éthyl kétone. Tous les rats présentèrent une faiblesse musculaire généralisée après l'inhalation qui dura de quelques h à 24 h avec récupération motrice totale. En dépit de cette apparence normale, l'examen histologique révéla une hypertrophie, un ballonement en grains de chapelet, et une dégénérescence des axones, associée à une démyélination secondaire, habituellement située dans la région des nodes de Ranvier. La toxicité du MnBK est prouvée, celle du MEK est encore à l'étude.

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Molecular Coding of Maze Learning; Demonstration by Bioassay

Since 1965 an increasing number of publications have reported detection of behavior-inducing substances in the brain of trained animals by means of behavioral bioassays[1–5] and two of the active substances have been isolated and identified[6,7]. Although evidence has been produced for the specificity of the method[8–11], it still remains controversial. The experiments reported in this paper bring further support to the validity and specificity of the behavioral bioassay.

Experiment I. The first series of 3 experiments, done at Baylor College of Medicine, used a maze (Figure 1, bottom) consisting of a white plastic outer shell into which a balsa-wood system of partitions could be inserted. Swiss albino mice (male, 25 g), water deprived 48 h prior to the first training session, were trained to run the maze (up to a maximum of 5 min) until they reached the water cup and drank for a few sec. With 1 daily training session, the

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Table I. Experiment II

	N	Mean running time	S.D.	t vs. control supernatant [a]	P
Control supernatant	15	559.8	± 189.5	—	—
Straight alley control supernatant	15	568.6	± 209.0	0.11	NS
Experimental supernatant	16	280.6	± 91.6	4.90	< 0.005
Dialyzate of RNA	20	323.8	± 140.0	3.96	< 0.005
Non-diffusible RNA	19	612.4	± 159.7	0.30	NS

[a] Computed by DUNNET's *t*-test[14].

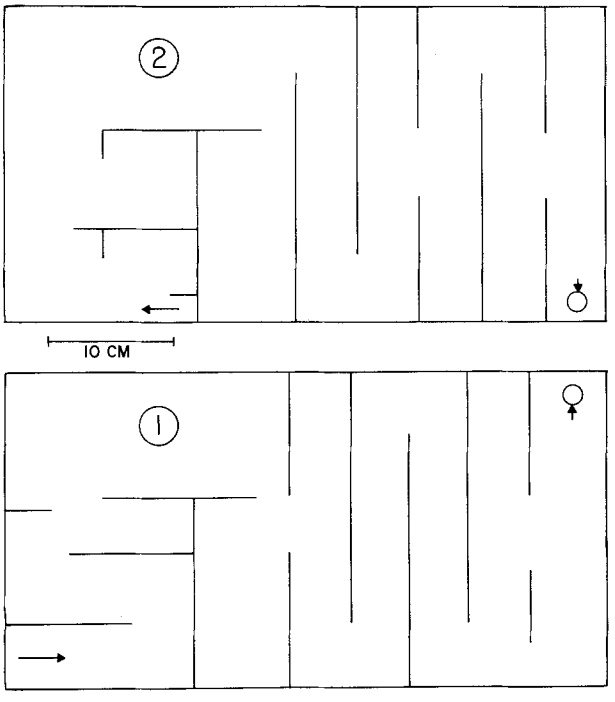

Fig. 1. Diagram of the mazes used in the experiments. → Starting point; →○ endpoint with water cup.

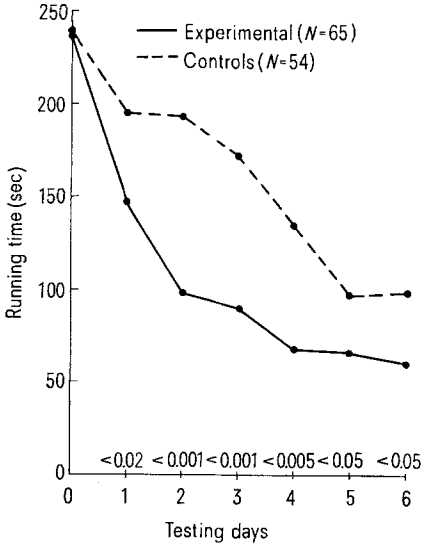

Fig. 2. Pooled results of first series of experiments (combined experiments) Abscissa: days of testing; ordinate: mean running time in sec. —, mice injected with brain from maze-trained mice; – – –, mice injected with brain from untrained water-deprived mice.

criterion of 30 sec or less running time on 3 consecutive days was reached in 9 to 14 days. 4 h after the last trial, the brains of the mice were frozen on dry ice and homogenized in 2 ml of distilled water per g of brain. After centrifugation (30 min at 25,000 rpm at 5 °C) the supernatant was used for injections. Extracts of control brains taken from resting water-deprived mice were prepared by the same procedure.

The recipient mice were pretested once and were distributed into an experimental and a control group having approximately equal mean scores. After 24 h water deprivation, they were injected i.p. with the equivalent of 1 g of donor brain in a volume of 0.25 ml. The first testing done after a further 24 h period of deprivation was followed by daily tests all done under blind conditions. The results were expressed in terms of the time required by each mouse to reach the drinking cup (running time). Figure 2 summarizes the results of the 3 tests in this series. In each one, the experimental animals ran the maze significantly faster than the controls. The results were pooled because there were no significant differences between the running times of the 3 control groups (*t*-test or the Duncan's Range Test for multiple comparisons[12]).

Experiment II. The second series of experiments, carried out at Washington University Medical School, verified the results previously obtained and supplied some preliminary information on the chemical properties of the active material. An additional control group (Group 2, Table I) was injected with extracts taken from donors trained to run for water in a straight alley, equivalent in length to that of the correctly run maze.

The results are summarized in Table I. The first 3 groups were injected with extracts of brain prepared as described above. Groups 4 and 5 received the dialyzable and non-diffusible fractions of a crude RNA preparation of trained brain to verify the assumption that the active material was a comparatively small molecule that can be separated from a complex formed with RNA by dialysis at low pH[13]. The results of this experiment were assessed by Dunnet's test for multiple comparisons with the same control group[14]. They showed no significant differences between the controls and the recipients of straight alley trained donors. The differences, however, were highly significant between the controls and the recipients of maze trained brain preparations, whether it was the supernatant of the homogenate or the dialyzate of the RNA extract; the non-diffusible fraction of the latter proved inactive.

Subsequent experiments have shown that the active material is inactivated by incubation with trypsin or chymotrypsin and is, consequently, probably a peptide.

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Table II. Analysis of variance of the results of Experiment III

Source of variation	SS	df	MS	F	P
Recipient maze	48.0	1	48.0	0.05	NS
Donor maze	5,232.67	2	2,616.33	3.11	NS
Interaction	13,056.975	2	6,528.48	7.76	< 0.01
Error	35,292.14	42	840.29	—	—

Its elution characteristics from Sephadex G-25 suggests 16 to 20 amino acid residues.

Experiment III. The 3rd experiment, done again at Baylor College of Medicine, was aimed at defining more closely the specificity of the information contained in the brain material, whether it was merely motivational (indication of reward at the end of the maze) or included clues for the shortest way to run the maze.

To decide among these alternatives, groups were trained either in maze 1 or maze 2 (Figure 1). Brain extracts from these donors and from untrained control mice were injected into the following recipient groups: (1 → 1); (2 → 2); (1 → 2); (2 → 1); (C → 1); (C → 2). The first number indicates the maze in which the donors were trained (C is control), the second the maze in which the recipients were tested. Each experimental group consisted of 10 mice.

Figure 3 summarizes the results of this experiment and shows that mice tested in the same maze in which their respective donors were trained (1 → 1 and 2 → 2) learned faster than those that were trained in a different maze (1 → 2 and 2 → 1). The latter did not perform better than the controls.

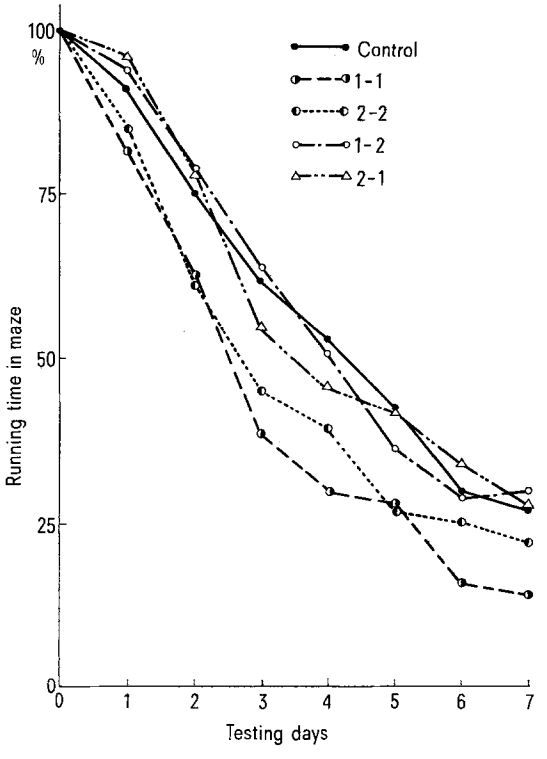

Fig. 3. Effect of brain extracts from maze-trained mice on performance of mice tested in the same or a different maze. Abscissa: days of testing; ordinate: mean running time in percent of the initial, preinjection running time. For identification of groups see text.

Overall analysis of variance of the total running time between day 1 and day 6 revealed no significant main effect of the maze in which the recipients were tested, or of the maze in which the donors were trained[15]. There was, however, a highly significant ($F_2 = 7.76$, $p < 0.01$, Table II) interaction effect between these two variables. The results of this experiment were further investigated by the comparison of individual groups using a Duncan's Range Test for multiple comparison between means[12]. It showed that 'like' groups (1 → 1) and (2 → 2) were significantly different from their control groups (C → 1) and (C → 2) at the < 0.01 and < 0.05 levels respectively. Also group 1 → 1 when compared with 2 → 1 and group 2 → 2 when compared with 1 → 2 were significantly different at $p < 0.01$. However, neither groups 2 → 1 nor 1 → 2 were significantly different when compared with the controls. Complete analysis of covariance, taking prestest scores into account, revealed an even more significant difference ($p < 0.001$) between 'like' and 'unlike' recipients.

These results suggest that the active material contained in the extracts of brain taken from maze-trained mice may encode some surprizingly specific information. Considering the possible number of peptide chains formed by 16 amino acids ($20^{16} = 6.5 \times 10^{20}$), their capacity to record all the necessary information is by no means impossible, even if one takes into account the constraints imposed by the organization of neural pathways[16–20]. Attempts will be made to define the limit of specificity by even more specific modifications of a maze[21].

Résumé. Des injections d'extraits de cerveau de souris ayant appris à trouver passage dans un certain labyrinthe ont notablement facilité aux souris qui les ont reçues l'apprentissage de la circulation dans ce même labyrinthe mais non pas dans un labyrinthe de configuration différente. Les substances responsables de ces effets ont les propriétés chimiques de peptides.

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[21] Supported by HEW grant No. NIE 2-0062 and USPH grant No. NH-7081. The authors thank Drs. L. G. SHARPE and V. J. PEREZ of the Laboratory of Neuropsychology of the Washington University Medical School for the use of space and equipment, and for their help and advice.