mium. The shadow-cast replica is then ready for examination in the electron microscope.

By this means, we have been able to obtain excellent electron micrographs of carbon black particles showing variation in dispersion (Figs. 1 and 2). We have made pictures of blood cells at 5 kv, but much control work must still be done before proper interpretation can be made of the results. New techniques will enable us to picture bacteria and other living matter without exposure to the high vacuum of the electron microscope. We have pictured the overlapping of lead particles and structural differences that would not be resolved by the light microscope.

W. A. LADD*
W. M. HESS
M. W. LADD
Columbian Carbon Company, New York

Notes

1. W. B. Wiegand, H. A. Braendle, and M. T. Karves helped with this work. It was encouraged and financed by Columbian Carbon Company, 380 Madison Ave., New York, to whom applications for patents covering appropriate parts of it are assigned.

* Present address: Ladd Research Laboratories, Roslyn Heights, N.Y.
† Present address: Guggenheim Foundation, Institute of Dental Research, New York.

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Effects of Pentobarbital Sodium on Adaptive Behavior Patterns in the Rat

We have previously reported a relationship between the rat's behavior in our maze and cholinesterase (ChE) activity in the cerebral cortex (*1*). In each unit of our maze, the rat chooses between a lighted and a dark alley, only one of which is open. The lights and correct alleys are changed after each trial; thus following any one cue can bring only chance success. Nevertheless, the rat usually displays consistent choices ("hypotheses")—for example, consistently choosing lighted alleys or the left alleys. This hypothesis behavior represents a perceptual selectivity that is significant in the organism's normal adjustments (*1*).

The present experiment derived from the following considerations. (i) Illumination is evidently the dominant cue in our maze, since most animals start with a light-going preference. Persistence in this preference results in a light hypothesis. A spatial hypothesis requires *ignoring* the dominant visual cue. (ii) Animals with lower ChE activity display a preponderance of light hypotheses. Animals with higher ChE activity tend to abandon this preference and adopt spatial hypotheses (*1*). (iii) ChE activity is assumed to be an index of the rate of acetylcholine (ACh) metabolism. (iv) Pentobarbital sodium reduces the rate of synthesis of ACh in the cortex (*2*).

These four points can be rephrased in the following hypothesis: Animals with low rates of cortical ACh metabolism are relatively incapable of ignoring the dominant visual cue. Therefore, pentobarbital, by depressing ACh metabolism, should increase light-going behavior.

Figure 1 shows the percentage of light-going choices per trial for three groups of 80-day-old male rats. Group I (control) choices showed the initial light-going tendency but soon fell to about 50 percent and remained there. Their hypotheses during days 1–4 were distributed fairly evenly among light, dark, left, and right (Table 1). Group I was of our C strain, a cross between maze-bright and maze-dull (D strain) rats (*3*). D rats (group IV, $n = 24$) were more persistent in light-going behavior and rarely adopted other hypotheses. In comparison with C rats, D rats have lower cortical ChE activity (*1*).

Group II consisted of C rats run under pentobarbital on days 1–4. Ten milligrams per kilogram of body weight was injected intraperitoneally about 15 minutes prior to testing. This dosage did not reduce eating or increase maze running time. Figure 1 shows that their behavior was preponderately light-going as long as the barbiturate was used, most animals persistently displaying light hypotheses. When pentobarbital was not used (days 5 and 6) light-going choices fell off. Administering the drug on day 7 raised them again. The *t* test (Table 2) indicates highly significant differences between groups I and II. Peculiarities in the behavior of the drugged animals included a rapid gait and lack of exploratory and "vicarious-trial-and error" behavior. The whole picture was one of sterotypy.

To test whether the drug might have made the animals phototropic and unresponsive to the problem nature of the situation, ten animals of group II were later trained with the maze made solvable. On successive days the right, left, and dark alleys were correct. Most animals reduced their light-going behavior and followed these cues, even under the

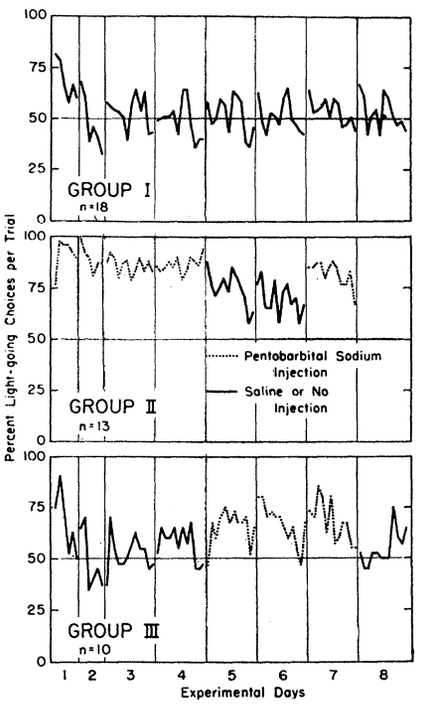

Fig. 1. Percentage of light-going choices per trial for three groups of rats.

Table 1. Frequency of various hypotheses per rat per day. An animal is credited with displaying a hypothesis if its choices during a day deviate from 50 percent, for any cue, by at least 2.5 standard deviations.

Group	Strain	Days 1–4				Days 5–6			
		Light	Dark	Left	Right	Light	Dark	Left	Right
I	C	.19	.11	.19	.15	.20	.06	.30	.08
II	C	.90	.00	.00	.05	.58	.00	.04	.04
III	C	.30	.10	.07	.10	.40	.10	.05	.10
IV	D	.36	.03	.14	.07				

Table 2. *t* Tests of differences between groups in the pentobarbital experiment (light-going scores).

Groups compared	Experimental days					
	1–2	3	4	5	6	7
I and II	7.62*	5.81*	6.15*	3.88*	3.07†	4.08*
II and III	7.61*	4.14*	3.35†	1.06	0.32	1.65
I and III	0.08	0.14	1.10	1.88	2.37‡	1.86

* *t* significant at .001 level of confidence. † *t* significant at .01 level of confidence.
‡ *t* significant at .05 level of confidence.

drug. As a further test, group III was run normally for 4 days and then under the barbiturate. Light-going behavior increased moderately under the drug but remained well below the level of group II. Group III actually displayed fewer light hypotheses while drugged than did group II when the drug was withdrawn. Clearly, pentobarbital does not create a simple phototropism.

The present data, together with our first report, seem consistent with the hypothesis that differences in adaptive behavior patterns are related to the rate of cortical ACh metabolism.

A possible additional interpretation is suggested by recent findings that afferent channels show habituation "... to stimuli which tend to be insignificant for the organism" (*4*). This habituation is due to inhibitory impulses from the brain stem reticular formation and is prevented by pentobarbital anesthesia or injury of the reticular formation. The inhibition can originate at levels above the brain stem (*5*). To initiate the inhibitory impulses may require a given rate of ACh metabolism. In our experiment, pentobarbital may have depressed the ACh metabolism enough to retard the initiation of these inhibitory impulses and thus forced the rat to "attend to" the dominant visual cue. We are here suggesting a biochemical mechanism for perceptual selectivity to complement the neurological one.

MARK R. ROSENZWEIG
DAVID KRECH
EDWARD L. BENNETT
Department of Psychology and
Radiation Laboratory,
University of California, Berkeley

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1 September 1955

Nuclei Counts on Rat Liver Homogenates

During the course of studies on the desoxyribose nucleic acid (DNA) per average nucleus in rat liver homogenates, it was observed that the number of nuclei per unit weight of liver was significantly greater than that reported by Einhorn *et al.* (*1*) and Price and Laird (*2*). These authors used the method of Price *et al.* (*3*) who had made the nuclei counts after a 15-minute homogenization in sucrose, whereas we had homogenized the tissue for a period of less than 30 seconds in 0.85-percent saline. The present study (*4*) was designed not only to determine the effect of homogenization time on nuclei counts but also to evaluate the effect of passing the tissue through a tissue press prior to homogenization.

Female rats of the Sprague-Dawley strain, given free access to Purina laboratory chow and water, were used. The animals were sacrificed by a blow on the head, and the livers were excised, weighed, and immediately cooled to 0°C. The following variations in homogenization technique were used:

1) A 10-percent homogenate in 0.85-percent saline was prepared employing homogenization times of (i) 10 seconds and (ii) 15 minutes.

2) The liver was first passed through a tissue press, then a 10-percent homogenate was prepared in 0.85-percent saline employing homogenization times of (i) 10 seconds and (ii) 15 minutes.

A modified Potter-Elvehjem tissue grinder with a motor-driven, loosely fitting, longitudinally grooved plastic pestle was used. After homogenization, a 2-ml aliquot was mixed thoroughly with an equal volume of crystal violet solution (80 mg of crystal violet in 100 ml of 6-percent acetic acid). A drop of the mixture was placed under the cover slip of a Petroff-Hausser bacteria-counting chamber, and the nuclei were enumerated. Five counts were made on each preparation, and the average of these counts was recorded. The counts on animals 1, 2, and 3 were made by one person and counts on animals 4, 5, and 6 were made by another. This was done in order to eliminate human error insofar as possible.

Table 1. Number of nuclei per gram of liver $\times 10^8$.

Rat No.	1(i) 10-second homogenate	1(ii) 15-minute homogenate	2(i) Tissue press 10-second homogenate	2(ii) Tissue press 15-minute homogenate
1	4.13	2.00	3.98	2.19
2	4.10	2.06	4.16	2.13
3	3.87	2.10	3.85	2.06
4	3.97	2.17	3.80	2.12
5	3.82	2.16	3.82	2.08
6	3.95	2.05	3.97	2.20
Mean ± S.E.*	3.97 ± 0.050	2.09 ± 0.027	3.93 ± 0.056	2.13 ± 0.023
	P†			
	1(i)–1(ii)	1(i)–2(i)	2(i)–2(ii)	1(ii)–2(ii)
	$P > .001$	$P > .7$	$P > .001$	$P > .3$

* Standard error (S.E.) computed by standard deviation (S.D.) = $\sqrt{\frac{\Sigma x^2 - (M x \Sigma x)}{n-1}}$; S.E. = $\frac{\text{S.D.}}{\sqrt{n}}$

† *P* = probability from Fischer's table "t."

Table 1 shows that mincing the liver in a tissue press has no effect on the number of nuclei obtained. However, when the tissue was homogenized for 15 minutes, the nuclei were reduced to almost half the number obtained in the 10-second homogenization. Regardless of the method of preparation, there was no significant variation in the amount of DNA per unit weight of tissue when determined by the Disch diphenylamine method (*5*): (i) 39.7 mg of DNAP per 100 g of liver with 10-second homogenization and (ii) 38.7 mg of DNAP per 100 g of liver with 15-minute homogenization. It is obvious that the DNA per nucleus in the groups that had been homogenized for 15 minutes would be approximately double that of groups that had been homogenized for 10 seconds.

These data indicated that more nuclei were destroyed during the longer period of homogenization. This was confirmed in the following manner: aliquots of the 10-second and 15-minute homogenates were taken and centrifuged at 1000 rev/min for 20 minutes, and then the DNA content of the supernatant was determined. Microscopic examination of the supernatant established that it was free of nuclei in each case. Analysis of the cell-free supernatant of the 15-minute homogenate showed an increase in DNA corresponding to the decrease in nuclei count. To evaluate the accuracy of the nuclei-counting technique, the DNA values per nucleus were determined on two samples of the same liver; one sample was used for the isolation of nuclei by the citric acid method, and the other sample was used for the determination of nuclei by the 10-second homogenization procedure described here. The values obtained for both samples agreed within 2 percent. Moreover, nuclei counts made with a