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 Group III actually displayed fewer light hypotheses while drugged than did group II when the drug was withdrawn.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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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

bacteria-counting chamber showed no significant difference from those made with a hemacytometer.

The data presented indicate that prolonged homogenization will destroy liver cell nuclei, and that, in order to obtain accurate DNA values per average nucleus from homogenate suspensions, the homogenization time must be reduced to such an extent that the DNA in the nuclei-free supernatant fraction is negligible.

ALLAN D. BASS
A. HOPE MCARDLE
J. W. GRISHAM
Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee

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Grouping in Spatial Distributions

A number of procedures have been developed in recent years for measuring departures from randomness in the spatial distribution of points, but few analytic attempts have been made to separate the factors responsible for these deviations. Lack of randomness in a distribution of points over a given space may result (i) from the influence, on the location of the points, of regional differences in the nature of the space, and (ii) from the influence of the points on the position of one another. Evaluation of the importance of these two sources of nonrandomness in a given population has been complicated by the fact that current measures of nonrandomness are simultaneously sensitive to both of them. It is the purpose of this paper (*1*) to suggest a method whereby grouping in spatial distributions can be exhaustively described and by means of which, under certain circumstances, the afore-mentioned causes of nonrandomness can be distinguished.

It has been shown by Clark and Evans (*2*) that in a random distribution in two-dimensional space the proportion of points for which the relation of nearest neighbor is reflexive—that is, points which are the nearest neighbor of their nearest neighbor—is .6215. This expected proportion of reflexive relationships is independent of whether the density of the distribution is constant over the space occupied by it, but it is increased by any tendency for the formation of groups of two. It often happens, however, that distributional factors result in the formation of groups larger than two without appreciably affecting the number of pairs. The following generalization of the concept of reflexiveness is applicable to groups of any size.

$$dP = \frac{k}{(n-1)!}\left[\frac{\rho\pi^{k/2}}{\Gamma(k/2+1)}\right]^n r^{kn-1} e^{-\frac{\rho\pi^{k/2} r^k}{\Gamma(k/2+1)}} dr$$

Formula 1

$$k^{P}n = \int_0^\infty \frac{k}{(n-1)!}\left[\frac{\frac{k}{\pi^{\frac{2}{2}}}}{\Gamma(\frac{k}{2}+1)}\right]^n r^{kn-1} e^{-2r^k\rho}\left[\frac{\frac{k}{\pi^{\frac{2}{2}}}}{\Gamma(\frac{k}{2}+1)} - \frac{\frac{k-1}{\pi^{\frac{2}{2}}}}{\Gamma(\frac{k+1}{2})}\int_{\frac{1}{2}}^{1}(1-x^2)^{\frac{k-1}{2}}dx\right] dr$$

Formula 2

In a random distribution in k-dimensional space, consider the point X_0 and its 1st, 2nd, . . . , nth nearest neighbors, designated $X_1, X_2, \ldots, X_n$. The relation of nth nearest neighbor is reflexive for X_0 if X_n is closer to X_0 than to any other points except $X_1, X_2, \ldots, X_{n-1}$. Morisita (*3*) has obtained the probability distribution of the distance, r, to the nth nearest neighbor for a random distribution of density ρ in two-dimensional space. In k dimensions it is formula 1. Consequently, employing the reasoning of Clark and Evans (*2*), the proportion of points for which the relation of nth nearest neighbor is reflexive in a k-dimensional random distribution is formula 2, which upon integration becomes formula 3.

$$k^{P}n = \left[\frac{\frac{1}{2}\Gamma(\frac{k+1}{2})}{\Gamma(\frac{k+1}{2}) - \pi^{-\frac{1}{2}}\Gamma(\frac{k}{2}+1)\int_{\frac{1}{2}}^{1}(1-x^2)^{\frac{k-1}{2}}dx}\right]^n$$

Formula 3

For 1, 2, and 3 dimensions we have, respectively,

$$_1P_n = (^2/_3)^n$$
$$_2P_n = \left(\frac{6\pi}{8\pi + 3^3/_2}\right)^n$$
$$_3P_n = (^{16}/_{27})^n$$

Values of $_2P_n$ for $n = 1$–21 are given in Table 1.

For the purpose of this discussion, it is convenient to define a group as a collection of points in which every individual is closer to some member of the collection than to any individual outside of it. The concept of a group, as so defined, is a hierarchical one, large groups containing smaller ones within them. Groups occur in random, as well as in nonrandom, distributions. Nonrandom distributions differ from random ones in the extent to which the groups are isolated, increased isolation of groups being characteristic of aggregated distributions and decreased isolation, of distributions that tend toward uniformity. A group may be said to be completely isolated if each of its members is closer to every other member than to any individual outside of the group, from which it follows that every group of two individuals is completely isolated. The delimitation and counting of groups is not likely to facilitate distributional analysis unless the groups are highly isolated.

Although the group is ordinarily not a useful unit in distributional studies, a measure of the tendency for isolation of groups of specified size in a population is of value in describing spatial pattern. The tendency for isolation of groups of size n in a population may be called *grouping of order n,* positive, neutral, and negative grouping implying tendencies for isolation respectively greater than, equal to, and less than that expected in a random distribution. It is apparent that the observed proportion of individuals for which the relation of

Table 1. Proportions of individuals for which the relation of nth nearest neighbor is reflexive for populations in two-dimensional space.

	Size of sample		
	∞	184	179
n	Random distribution	*Lespedeza*	Prairie-dog burrows
1	.6215	.609	.642
2	.3863	.435	.246
3	.2401	.152	.134
4	.1492	.168	.084
5	.0927	.098	.028
6	.0576	.060	.011
7	.0358	.065	.006
8	.0223	.027	.006
9	.0138	.038	.000
10	.0086	.027	.000
11	.0053	.038	.000
12	.0033	.087	.000
13	.0021	.022	.000
14	.0013	.043	.000
15	.0008	.049	.000
16	.0005	.033	.000
17	.0003	.060	.000
18	.0002	.027	.000
19	.0001	.049	.000
20	.0001	.147	.000
21	.0000	.098	.000