

## ANALYSIS OF THE GEOTROPIC ORIENTATION OF YOUNG RATS. IX

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### I

With lines of rats well inbred it has been shown<sup>1</sup> that the relationship between orientation angle  $\theta$  and slope of surface  $\alpha$  in the geotropic creeping of young rats is specific and quantitatively reproducible. It is also found that the capacity of individuals to exhibit variation in geotropic response (orientation) is likewise a reproducible function of the genetic constitution of these rats. The capacity to exhibit variation of response is measured by the functional connection between relative variations of  $\theta$  and the magnitudes of the performance,  $\theta$ , which in turn depend upon the intensities of the exciting force due to the acting gravitational component. It was shown that the "proportionate modifiable variation" is a constant which differs for each of several lines tested. The proportionate modifiable variation is taken as the fraction of the total relative variation of performance which is dependent upon (or modified by) the intensity of excitation, and thus upon  $\theta$  (Crozier and Pincus, 1931-32 *a*).

The evidence for the invariant character of the capacity to vary the performance  $\theta$ , as a function of the impressed intensity of excitation, is derived from several sources. The percentage of modifiable variation is independent of the size of the sample of individuals or of measurements of  $\theta$ , provided the sample is really homogeneous in this respect (Crozier and Pincus, 1931-32 *a, b*, etc.). It is also quite unaffected by experimental modifications of the curve connecting  $\theta$  and  $\alpha$ , such as are brought about by forcing the young rats to carry additional loads (Crozier and Pincus, 1931-32 *b*) or by causing them to creep more rapidly when adrenin is administered (Crozier and Pincus,

<sup>1</sup> Crozier and Pincus (1929-30; 1931-32*a*), Crozier, 1935.

1932-33 *a*). Moreover, in several pure lines which have been studied, the percentage modifiable variation of geotropic responses is the same in adult rats as in the young (Crozier and Pincus, 1931-32 *a*; 1932-33 *b*), despite the difference in appearance of the  $\theta$ - $\alpha$  graphs, and is here likewise unaffected by the action of adrenin which markedly alters the relation of  $\theta$  to  $\alpha$  (Crozier and Pincus, 1932-33 *b*).

In hybrid young rats, obtained by crossing inbred lines, the *total* variation of performance is either increased or about the same, but the dependence of the variation of observed  $\theta$  upon  $\log \sin \alpha$  is definitely less, and the percentage modifiable variation is markedly decreased (Crozier and Pincus, 1929-30; Crozier, 1935). This is due to an increase, in the  $F_1$  hybrids, of the portion of the total variation of measured performance which is not open to control as a function of the intensity of excitation. Hybridization thus brings about a kind of modification of the young rats' ability to exhibit variation of  $\theta$  which other attempts to modify the  $\theta$  vs.  $\alpha$  curve do not induce.

It has been pointed out that the evidence thus far available, from the study of other phenomena of excitation and response as well as from the present series of cases, supports the conclusion that the capacity to vary response is limited, under controlled conditions, by the intensity of induced excitation (*cf.* Crozier, 1935). This capacity must be measured as a function of the conditions of excitation, if it is to be measured at all. When this is done, the proportionate modifiable variation of geotropic performance appears to be determined by the number of appropriate excitation units open to stimulation (*cf.* Crozier, 1935), and that in each pure line these are determined by the genetic constitution of the rat. Consequently it is important to discover the nature of the heterosis-like behavior of the variability of performance in  $F_1$  hybrids; the behavior of backcross individuals, which show by comparison with  $F_1$  an expected return to the magnitudes of the variability functions characteristic of their phenotypic composition with respect to geotropic performance, suggests a purely genetic interpretation of the effect, although in certain instances (Crozier and Pincus, 1931-32 *c*) this is not sufficient as explanation. It is of interest to discover whether the decreased proportionate modifiable variation in young  $F_1$  individuals is maintained throughout their lives, or whether it is modified in the adult state. The in-

terpretation will be different in the two cases. For the heterosis-like phenomena appearing in the curve of mean  $\theta$  vs.  $\alpha$  for  $F_1$  individuals of the cross between rats of inbred lines  $A$  and  $B$  it was suggested that the effect could be understood by supposing that in the hybrid individuals relevant elementary developmental processes fail to "keep step" as in the pure line rats (Crozier and Pincus, 1931-32 *c*). In this event, the irregularities might be expected to disappear in the adult.

## II

This matter was tested by examining the geotropic orientations of a group of 5  $F_1$  adult females 7 months old from the cross  $A \text{ } \varnothing \times B \text{ } \sigma$  (*cf.* Crozier and Pincus, 1931-32 *c*). We desired to determine the variability of  $\theta$ , and its possible modification under the action of adrenin. In the adults of race  $A$  adrenin has no effect on the proportionate modifiable variation (Crozier and Pincus, 1932-33 *b*). The hybrids of  $A \times B$  have the particular advantage for this test that in all essential respects the  $B$  factors for threshold and for  $\Delta\theta/\Delta\alpha$  are all dominant over those of  $A$  (Crozier and Pincus, 1931-32 *c*). (In other cases, where the slope  $\Delta\theta/\Delta\alpha$  in different portions of the  $\theta$  vs.  $\alpha$  curve is due to factors of differing genetic origin, irregularities in the variability functions may complicate the situation. This will be discussed elsewhere.) In the cross  $B \text{ } \sigma \times A \text{ } \varnothing$  the  $\varnothing \text{ } \varnothing$  when young appeared to show less of the "heterosis effect" than the  $\varnothing \text{ } \varnothing$  from the opposite cross (Crozier and Pincus, 1931-32 *c*).

For observations on orientation these rats were prepared by being fed after creeping upward upon an inclined plane, during about 10 days. The procedure was as outlined in the account of a preceding experiment with adult  $A$  rats.<sup>2</sup> The determinations of the thresholds were made as follows: (1) the upper limit was determined by the fact that above  $\alpha = 80^\circ$  there was marked slipping and in the case of one animal, No. 1, creeping even at  $80^\circ$  was impossible; (2) the lower threshold was determined in two ways: first by the direct observation of the mode of creeping: it was noted that the injected animals at  $10^\circ$  and at  $12.5^\circ$  in general crept in the "typical" manner. Typical creeping was more obvious at  $12.5^\circ$  than at  $10^\circ$  in the injected animals, and the number of downward movements

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<sup>2</sup> We are indebted to Dr. W. H. Stavsky for painstaking assistance in these preparations.

made was much less in the injected than in the uninjected animals. The second method involved the running of the uninjected rats at angles below  $15^\circ$ ; it was then noted that the average value of apparent  $\theta$  was much higher than at  $15^\circ$ . This increase in average value of  $\theta$  is of course an artifact and is due to the fact that the animals tend to run away from the observer; the average  $\theta$  then obtained is obviously determined by the shape of the creeping plane. It is possible at the angles below the threshold to start the animals at the top of the plane and have them run down at  $\theta$ 's corresponding to those recorded. Actual measurements of downward  $\theta$ 's were not taken, however, as the rather abrupt change in  $\theta$  values from threshold to the below-threshold values was sufficiently significant. Below threshold, moreover, P.E.<sub>g</sub> rises suddenly to very high values.

These rats, although amenable to handling, were much more "nervous" than *K* strain adults. In consequence there was often quite a delay between successive orientations. Furthermore any loud noise coming from other parts of the building had a definite effect upon the animals. In certain cases it seemed that unknown events occurring during the day must have affected the animals, for on various evenings they exhibited inexplicable jumpiness. These disturbances appeared not to affect the magnitudes of  $\theta$  during geotropic progression; if anything, their influence might be expected to increase the uncontrollable variation of performance, but the results give no such indication.

### III

Determinations of mean orientation angles for adult rats of the  $F_1$  generation  $A \times B$  are summarized in Table I. The threshold slope of surface was at  $\alpha = 15^\circ$ . This agrees with that obtained for a series of adult *A* rats (Crozier and Pincus, 1932-33 *b*), although the orientation angle at threshold slope is lower. Such comparisons are not profitable, however, because the shape of the curve obtained with adults varies with the age of the individual. For this reason, comparisons can only be made among measurements secured with homogeneous material. It is noteworthy, nonetheless, that in this case the young  $A \times B$  hybrids (Crozier and Pincus, 1931-32 *c*) also show a threshold slope of  $15^\circ$ , whereas experience with other lines, including line *A*, has generally demonstrated that for the adults of any one type the threshold slope of surface is lower than with the young. The important feature of the threshold response, however, is the magnitude of the orientation angle  $\theta$ .

The general form of the relationship between  $\theta$  and  $\log \sin \alpha$  (Fig. 1), exhibiting a discontinuity in the neighborhood of  $\alpha = 45^\circ$ , is that

TABLE I  
Geotropic Orientations ( $\theta$ ) of Adult Rats,  $F_1 (A \times B)$

Slope $\alpha$	Orientation $\theta_m$
degrees	degrees
15	55.22 $\pm$ 2.32
20	56.32 $\pm$ 3.78
25	59.58 $\pm$ 1.98
35	66.94 $\pm$ 2.72
40	70.32 $\pm$ 1.10
	70.37 $\pm$ 0.83
45	70.94 $\pm$ 1.68
55	77.68 $\pm$ 1.22
70	79.97 $\pm$ 0.71
80	84.10 $\pm$ 0.56

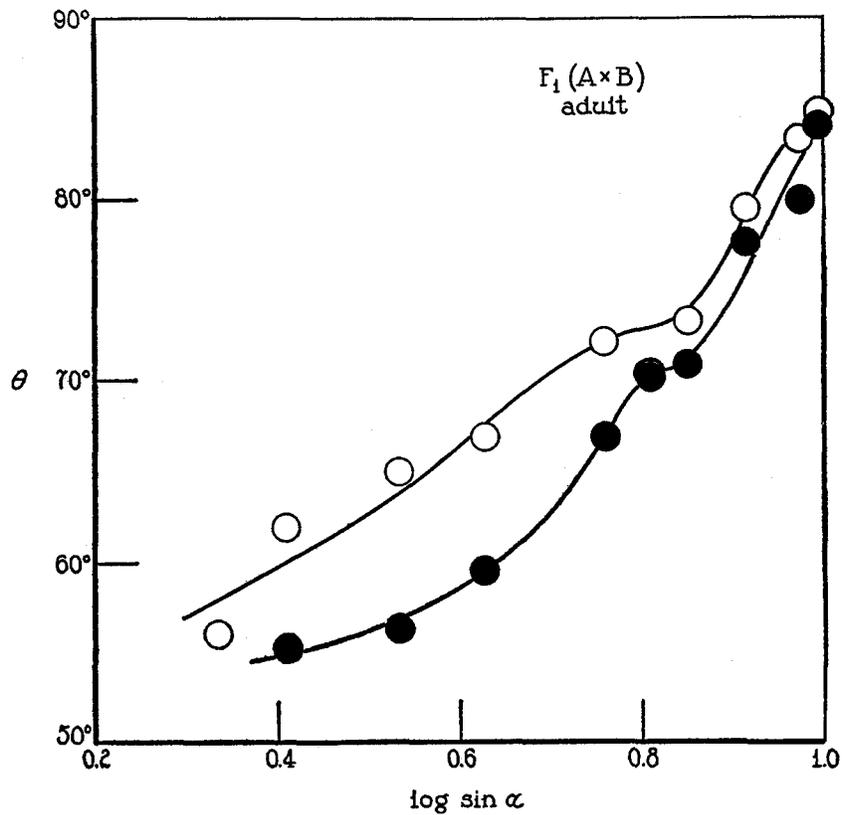


FIG. 1. Mean orientation angles for geotropic progression of adult  $F_1$  hybrid rats ( $A \times B$ ). Lower curve, normal; upper curve, after injection of adrenin 1:50,000, 1/2 ml. intraperitoneally. The points do not depart from the curve by more than their probable errors.

previously observed (Crozier and Pincus, 1932-33 *b*).<sup>3</sup> Complete smoothness and regularity is not to be expected in the relationship between  $\theta$  and  $\alpha$  obtained in such experiments, particularly when observations are made after injection of adrenin, or other experimental treatment. The method of progression alters as  $\alpha$  is increased above  $45^\circ$ , and one cannot be sure that the time-course of the action of the drug is uniform in each instance. The important consideration is that which grows out of the fact that  $\theta$  and P.E.<sub>g</sub> are intimately related.

The individuals involved in the measurements of Table I were also employed to test the action of adrenin. They received intraperitoneal injection of 0.5 ml. of 1:50,000 adrenalin hydrochloride 1/2 to 1 hour before the observations at any one slope of surface. Rats prepared in this way were more active and crept more steadily, even at very low slopes of surface.

The five individuals tested, all females, varied in weight from 184 to 206 gm.; as in all other series, there is no correlation between  $\theta$  and weight, or between variation and weight.

The effect of injection with adrenin is precisely that observed with adults of race *A*—the curve is pulled out to the left, the threshold slope of surface is definitely lowered, but at this threshold slope the minimal response observed is statistically identical with that obtained from the uninjected rats. The data are summarized in Table II.

<sup>3</sup> von Buddenbrock (1931) suggested that in the orientation process the organism is really attempting to keep its transverse axis horizontal; the idea is of course utterly at variance with the effects of added loads. This can be tested by computing, as von Buddenbrock does, the angle  $\delta$  defined by the relationship  $\sin \delta = (\sin \alpha) (\cos \theta)$ . According to von Buddenbrock this angle tends to be small and *constant*. It has been pointed out (Crozier, 1934-35) that  $\sin \delta$  computed in this way is also to be regarded, in certain cases, as the proportionate measure of a force vector normal to the path of progression and parallel to the inclined surface. It was also pointed out (Crozier, 1934-35) that, far from being constant, the angle  $\delta$  in fact must pass through a maximum. It is of some interest to test von Buddenbrock's idea by means of the data upon rats, since in this case especially the possible rôle of the bilaterally arranged statocyst mechanisms should be made clear if the inner ears do control the angle of orientation. In various series of measurements, as in Table I and Table II,  $\delta$  changes systematically as  $\alpha$  is increased; the maximum is located at  $\alpha = 45^\circ$ .

The effect of this concentration of adrenin is about the same, proportionately, as in adult *A* rats (Crozier and Pincus, 1932-33 *b*). The measurements are plotted in Fig. 1.

## IV

Since the number of observations ( $n$ ) at each point is not the same for the two series to be compared (*cf.* Tables I and II), the values of the relative variation of  $\theta$ ,  $(100 \text{ P.E.}_\theta)/\theta$ , have been multiplied by  $\sqrt{n/N}$  in each case to give units independent of  $n$  and of  $N$  (*cf.* Crozier and Pincus, 1929-30; 1931-32 *a*). The data giving the dependence

TABLE II  
*Geotropic Orientations ( $\theta$ ) of Adult Rats,  $F_1$  ( $A \times B$ ), under Action of Adrenin;  
 $N = 5, n = 45$*

Slope $\alpha$	Orientation $\theta_m$
<i>degrees</i>	<i>degrees</i>
12.5	56.00 $\pm$ 1.40
15	61.89 $\pm$ 0.66
20	64.95 $\pm$ 0.94
25	66.88 $\pm$ 0.81
35	72.13 $\pm$ 1.17
45	73.33 $\pm$ 0.59
55	79.57 $\pm$ 0.80
70	83.31 $\pm$ 0.34
80	84.81 $\pm$ 0.42

of the relative variation of  $\theta$  upon  $\theta$  are plotted in Fig. 2. For reasons already discussed (Crozier and Pincus, 1931-32 *a*, etc.), the points in Fig. 2 are expected to show a progressively wider spread toward the low- $\theta$  end, since each determination is subject to a standard deviation proportional to its own magnitude.

It is apparent that the measures of variation for the two series, normal and adrenalized, are concordant when expressed as a function of  $\theta$ . This was also found for adult *A* rats (Crozier and Pincus, 1932-33 *b*). The total observed variation (*cf.* Crozier and Pincus, 1931-32 *b*, etc.) is 117.5 units. This agrees quantitatively with the corresponding number for young rats of pure line *B*, and is much above that for young rats of race *A* (Crozier and Pincus, 1931-32 *b*), or

for adults of race *A* (Crozier and Pincus, 1931-32 *b*). It was found previously that the action of adrenin did not modify the total variation of performance in adult rats of race *A*, and this is confirmed for the adult  $A \times B$  hybrids.

It has also been shown that even in cases where the total variation of geotropic performance can be modified by experimental treatments, in young rats (Crozier and Pincus, 1932-33 *a*), the percentage of the total which is modifiable as a function of  $\theta$  remains unaltered (Crozier,

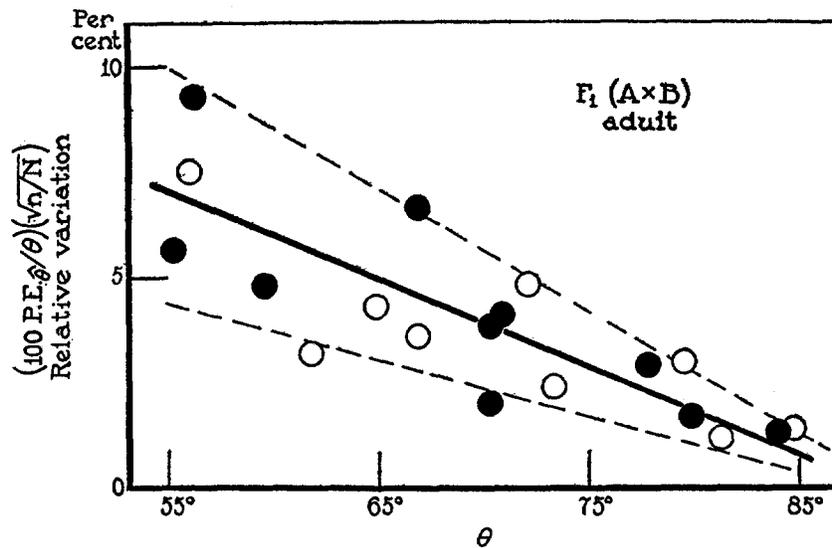


FIG. 2. The relative variation of geotropic performance, corrected for  $N$  and for  $n$ , as a function of extent of orientation, for  $F_1$  ( $A \times B$ ) rats (see Fig. 1). The variability of orientation is the same with adrenin as without.

1935). In the present case the distortion of the  $\theta$  vs.  $\alpha$  curve under adrenin does not affect the rat's capacity to exhibit variation of extent of orientation. The percentage of the total variation which is modifiable works out to be 74 per cent. This agrees precisely with the value already ascertained for young rats of race *B* (Crozier and Pincus, 1931-32 *c*).

The evidence from this experiment therefore demonstrates that the peculiar variability of geotropic performance detected in young rats obtained by crossing inbred lines tends to disappear in the course of

their development. The adult  $A \times B$  rats exhibit precisely the extent and character of ability to vary geotropic performance which are specifically associated with the  $B$  line. Since in this cross the factors derived from  $B$  are dominant, this finding is entirely consistent with the view (Crozier, 1935) that the limitation of variability of performance is determined by the genetic constitution of the rat as concerns its equipment of tension-receptive sensory units. It is also consistent with the idea that the disturbances of variability in young hybrids are due to the failure of relevant developmental processes to keep pace in the same orderly fashion as in the individuals of pure lines.

#### SUMMARY

Adult hybrid rats from the cross of races  $A \times B$  show a total capacity to vary their geotropic performance which is identical with that of their  $B$  parents. The proportionate modifiable variability of geotropic orientation also agrees quantitatively with that for the  $B$  parents. These relationships are not disturbed by the action of adrenin, which leads to a distortion of the  $\theta$  vs.  $\alpha$  curve. Young rats of the  $F_1$  generation show a greater proportion of unmodifiable variation of geotropic orientation. It is pointed out that the present findings support the conclusion that the capacity of rats to exhibit variation of geotropic orientation is limited by their genetically determined composition and that the special condition in the young hybrids may be understood as due to a kind of temporary disharmony of developmental events.

#### CITATIONS

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