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Genetic Differences in Locomotor Activation in Mice

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CRABBE, J. C. *Genetic differences in locomotor activation in mice.* PHARMACOL BIOCHEM BEHAV 25(1) 289–292, 1986.—Two highly inbred strains of mice were found to differ in habituation of activity repeatedly assessed in a toggle-box exploration task. The recombinant inbred (RI) strains derived from their cross resembled either one or the other parent strain, suggesting that a single gene exerts a marked influence on this behavior. The influence of an acute ethanol injection on activity in an open field was found to differ among 19 inbred strains. In 6 strains significant decreases in activity from the previous day's scores were seen; in two strains activity increased; and in 11 strains, no significant change was seen. Genetic specificity must, therefore, be considered in the interpretation of pharmacologic substrates for activity in mice. Lines of mice selectively bred for high and low open-field activity are suggested to offer an ideal subject population for neuropharmacologic studies.

Inbred mouse strains Pharmacogenetics Behavior genetics Open-field activity
Toggle-box exploratory activity Habituation Ethanol H and L selected lines

EXPERIMENTS with the goal of assessing the neuropharmacological substrates of activity in rodents typically employ randomly outbred stocks of rats or mice. Most such animal stocks are inbred at some unknown proportion of gene loci, possibly in excess of 50 percent, while they retain polymorphism at other genes. Variability in a group of untreated outbred animals on some measure of motor activity is generally assumed to represent experimental or measurement error. When specific pharmacological manipulations are performed in an attempt to understand which neural systems are important for a given behavior, the responses measured represent an uncontrolled mixture of genetic and non-genetic influences. Several genetic strategies may be employed to enhance the resolution of such pharmacological analyses. These share in common a recognition that genetic information need not be noise in the experimental system; rather, genetic differences may offer powerful tools for mapping behavioral responses to their appropriate neuropharmacological substrates [10].

First, there may be large genetically-determined differences in a given measure of motor activity. For example, we tested mice for exploratory activity in a toggle-box apparatus. Crossings from chamber to chamber in a darkened box were recorded each 2 min for 10 min. Thirty days later, a retest was performed. We tested inbred strains C57BL/6By and BALB/cBy, their reciprocal F_1 hybrids, and 7 recombinant inbred (RI) strains developed from the F_2 cross between the inbreds. Strains differed significantly in habituation on this task (See Fig. 1). One hybrid and 4 RI strains resembled the C57 parent strain in showing little habituation, while the other F_1 and 3 RI strains resembled the BALB parent strain, showing more marked habituation. Besides demonstrating that there are marked genetically-determined differences in activity on this measures, this suggests that a single gene clearly influences habituation on this response [3].

Perhaps the most commonly used measure of motor activation in rodents is the open field, the development and uses of which have been the subject of an excellent review [12]. We tested male mice from 19 inbred strains for their activity in an open field under dim illumination. Each mouse was injected with saline and tested 30 min later for a 3 min period. Number of beam interruptions was automatically recorded. Strains differed significantly in activity, $F(18,169)=9.7$, $p<0.001$, by analysis of variance. Mean number of crossings ranged from 81 for the least active strain (CBA/J) to 211.9 for the most active (C57BR/cdJ). One day later, each mouse was retested 30 min after injection of ethanol (2.0 g/kg IP, 20% v/v). The effects on activity, shown as a difference from Day 1 scores in Fig. 2, differed significantly among strains, $F(18,169)=7.0$, $p<0.001$. Six strains had significantly reduced activity, while two (C58/J and BALB/cAnN) had significantly increased activity. Eleven strains did not respond significantly to ethanol. Strain differences in blood ethanol concentration measured at the time of testing, while significant, did not correlate meaningfully with the activity scores. The strain differences probably represent qualitative differences in neural sensitivity to ethanol [1].

Similar analysis of the response to ethanol in the open field in two inbred strains of mice, C57BL/6N and DBA/2N, revealed that DBA/2N mice always respond to ethanol with

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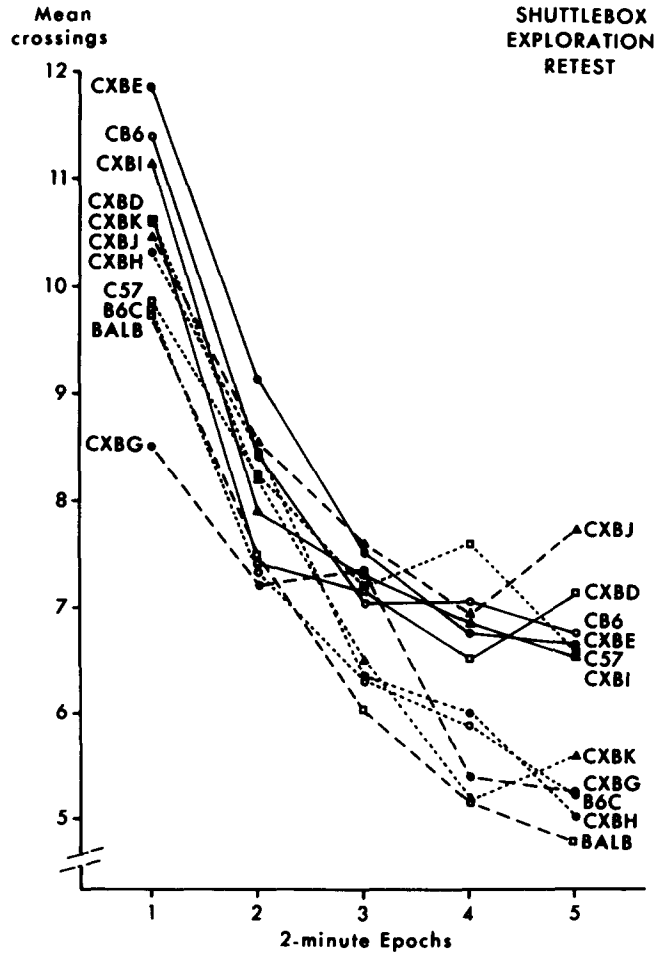

FIG. 1. Habituation of toggle box exploration on successive 2 min epochs during a retest session 30 days after the initial trial. Data from 436 mice of inbred strains C57BL/6By (C57), BALB/cBy (BALB), the two reciprocal F_1 hybrids B6C and CB6, and the seven recombinant inbred strains CXBD, E, G, H, I, J, and K are shown. Pattern during epochs 3–5 suggest single-gene influence on the trait. From [3], with permission.

increases in activity, regardless of dose or time after injection. On the other hand, C57BL/6N mice respond with a transient increase in activity which is followed by a long-lasting depression [2].

Other studies of the genetic influences on motor activation after ethanol injection in mice have also reported that qualitative as well as quantitative differences in response characterize different genotypes [6]. Whether or not the reduction and elevations in activity are negatively genetically correlated, as one might suppose on a common sense basis if similar mechanisms are involved at the neurochemical level, is unresolved [1,6]. Tolerance develops to the depression, but not to the increase in activity [2,11]. Detailed examinations of activity increases after ethanol in genetically heterogeneous mice support the finding that tolerance to the stimulant properties of ethanol does not develop readily, if at all [9].

If the case has been adequately defended that genetic differences can influence measures of motor activity in mice, a legitimate question is whether this has any practical utility for the analysis of neuropharmacological substrates. One group has examined this issue in three inbred strains of mice, an improvement over the usual comparison of two strains. Their analysis of the biphasic response to ethanol in open-field activity in BALB/c, DBA/2 and C57BL/6 mice reported stimulation by an acute 1.35 g/kg dose of ethanol in BALB/c and DBA/2 strains when activity was cumulated over a 30 min test [11]. The C57BL/6 strain did not respond, probably because their stimulant response is transient [2]. BALB/c mice showed increased formation of striatal DOPA, an effect not seen in the other two strains until higher ethanol doses were given. All three strains showed significantly reduced DOPAC levels, an effect which appeared to be smallest in the C57BL/6 strain [8]. While these experiments do not unequivocally link enhanced dopamine synthesis and inhibited release with behavioral stimulation, they demonstrate the necessity for awareness of genotypic determinants of motor activation that may not be monolithic.

Finally, one of the most powerful behavior genetic techniques is artificial selection. Through systematically intermating of extreme-scoring individuals, lines of mice have been bred to be high (H) or low (L) in activity during two 3

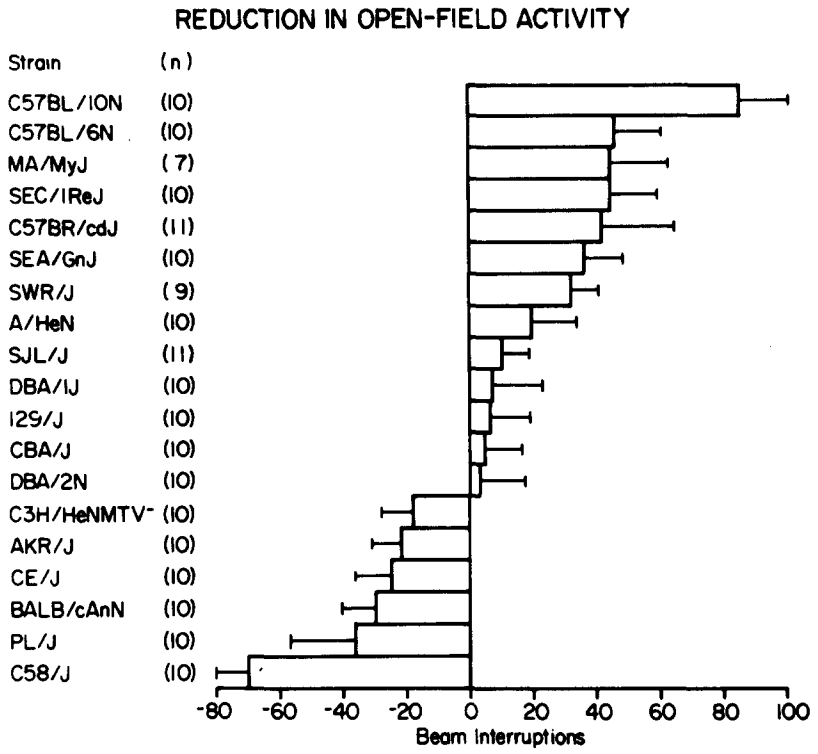

FIG. 2. Reduction from previous day's score (after saline injection) in open-field activity during a 3 min test 30 min after injection with 2.0 g/kg ethanol. Mean±SE for the numbers shown in parentheses of adult male mice of 19 highly inbred strains.

min tests in an open field on consecutive days. After 30 generations, the average activity scores of the H and L mice were approximately 40-fold different, with no overlap between the distribution of scores in the two lines [4]. These lines differ in activity in a number of other behavioral tasks in addition to open-field activity [5]. Only one attempt has been made to determine the pharmacological bases for this enormous difference. This group failed to detect large differences between H and L mice in various pharmacologic parameters of GABA activity [7]. However, they studied whole brain homogenates, so regional differences in GABA cannot really be ruled out. The H and L mouse lines provide an ideal test system in which to investigate neurochemical substrates of motor activation. There are replicate H and L lines, as well as two nonselected control (C) lines. The existence of replicates allows the investigator to demand that any relevant pharmacological change must appear in both pairs of genetically independent H and L lines, thus increasing the likelihood that differences detected between the lines are true genetic correlates of open-field activity.

In summary, studies with genetically-defined populations of mice demonstrate a clear influence of genotype on behavioral measures of motor activation, and the alterations in activity induced by drugs. While such genetic differences may add a level of complexity, they also offer opportunity for the investigator. The H and L genetically selected lines of mice are ideally suited for studies of the neurochemical substrates of activity.

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