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Genetic Effects on PCP-Induced Stimulation in Recombinant Inbred Strains of Mice

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FREED, W. J., S. CRUMP AND D. V. JESTE. *Genetic effects on PCP-induced stimulation in recombinant inbred strains of mice.* PHARMACOL BIOCHEM BEHAV 21(1) 159–162, 1984.—The stimulation of motor activity by phencyclidine was found to differ significantly in BALB/c and C57B1/6By inbred strains of mice. Phencyclidine-induced stimulation was compared for these strains, their reciprocal F1 hybrids, and their recombinant inbred offspring. There were significant differences in responsivity among the strains, suggesting a genetic influence on the PCP response; however, the strains did not segregate into two distinct groupings, suggesting that this genetic influence was not carried by a single gene. In addition, there was no relationship between the responsivity of these strains of mice to PCP and their previously-reported responses to amphetamine or scopolamine, which suggests that PCP-induced stimulation is not a simple cholinergic or amphetamine-like response.

PCP-induced stimulation Phencyclidine Inbred mouse strains

THE use of the recently-developed recombinant inbred strains of mice affords a new method of analyzing genetic influences on pharmacological responses. This model, developed by Bailey in 1971 [1,2], begins with two different but highly inbred strains, C57B1/6By and BALB/c in the most commonly used system. The two strains are crossed (male × female and female × male) and the offspring F1 hybrids (CB6F1 and B6CF1) are again mated. A large number of offspring, or recombinant, strains, can be produced and subsequently inbred to genetic homogeneity by brother-sister mating. In this way, the original genetic material of the parent or progenitor strains (BALB/c and C57B1/6By) is mixed and later resegregated into a number of recombinant inbred strains, each with homogenous yet unique genetic properties. Thus several unique strains are produced having various combinations of the genetic material from only two progenitors.

In the area of psychopharmacology, this model has previously been used to investigate the behavioral responses to amphetamine [12,14], scopolamine [14], and phenylethylamine (Jeste, Stoff, Rawlings and Wyatt, submitted). Phencyclidine (PCP) is a stimulant and psychotomimetic drug with complex pharmacological properties. In some respects, the behavioral effects of PCP resemble those of amphetamine [7, 9, 11, 13], and can be blocked by neuroleptics including haloperidol and pimozide [3, 10, 11, 13, 16, 17]. Certain behavioral effects of PCP, such as locomotor stimulation in mice, are, however, relatively insensitive to blockade by the neuroleptics pimozide and haloperidol, but are readily blocked by phenothiazines and methiothepin [4,5]. PCP-induced stimulation in rats also has a serotonergic component [5,9]. Although PCP has cholinergic properties [3, 8, 15, 18] the central effects of PCP do not seem to be cholinergic [6]. Thus, although PCP has amphetamine-like properties, it is also possible that the stimulatory effects of PCP involve a variety of neurochemical effects in addition to dopaminergic stimulation.

The purpose of the present study was to determine whether the genetic determinants of the stimulant response to PCP in mice are associated with the genetic determinants of the previously-reported responses of these same strains to amphetamine, scopolamine, or the endogenous amphetamine-like compound phenylethylamine. The same recombinant inbred strains of mice, derived from BALB/c and C57B1/6By, that have previously been employed in psychopharmacological studies were used.

METHOD

Animals

Animals consisted of parent strain mice (BALB/c and C57B1/6By), the F1 hybrid strains (CB6F1 and B6CF1), and six recombinant inbred strains (CXBD, CXBE, CXBG, CXBH, CXBI, and CXBJ) obtained from Jackson Labs., Bar Harbor, ME. Animals were housed in groups of 4 to 8 with free access to food and water. Mice were tested when they weighed at least 20 grams. Due to variations in growth and availability of the strains, the number of mice of each strain that were tested varied considerably.

Drugs and Injections

PCP (phenylcyclohexyl piperidine HCl), obtained from Philips-Roxane Inc., St. Joseph, MO, was dissolved in normal saline on the day of use and injected IP in a volume of 10 ml/kg. The dose used, 5 mg/kg, has been found to produce

approximately a two-fold stimulation of motor activity, which is about 67% of the maximal response that can be elicited, in random-bred Swiss-Webster mice [4].

Procedure

Animals were tested exactly as previously described [4]. Each mouse was placed in one of eight similar Motron-Produktor Co. activity meter units, using both the horizontal photocell banks (photocells under the cage floor illuminated from above by deep red lights) and vertical photocell banks (photocells 2.0 cm above the floor illuminated by red lamps across the cage). The horizontal photocell banks measure locomotion, while the vertical photocell banks measure a combination of locomotion and rearing activity. Each animal was tested for 30 min. The activity meters were turned off for 5 min during which drug was injected, and the animals were then tested for an additional 30 min. Animals were tested eight at a time, and animals were non-systematically assigned to testing groups so that animals from several strains were always tested at the same time.

Data Analysis

The data were analyzed statistically by a multivariate analysis of variance using the Statistical Analysis System General Linear Models Procedure (SAS Institute, Inc., Box 8000, Cary, NC). The following six variables were used: vertical and horizontal activity counts for the first and second 30 min, and the ratio of activity counts during the second 30 min to those during the first 30 min. Individual group comparisons were made by Duncan's multiple range test.

RESULTS

There were large differences among the strains in their response to PCP. Of the progenitor strains, the BALB/c mice showed a much greater response than did the C57Bl/6By mice (Fig. 1). The F1 hybrids and the recombinant inbred strains showed a wide range of responses, but there was no indication that the recombinant inbred strains could be segregated into two discrete groupings on the basis of the degree of response to PCP (Table 1).

The statistical analysis revealed significant differences among the strains in the following measures: (1) Horizontal activity counts after administration of PCP, $F(9,211)=8.88$, $p<0.0001$. (2) Horizontal activity count ratio, $F(9,211)=3.63$, $p<0.0003$. (3) Vertical activity counts before administration of PCP, $F(9,211)=5.51$, $p<0.0001$. (4) Vertical activity counts after administration of PCP, $F(9,211)=7.82$, $p<0.0001$. (5) Vertical activity count ratio, $F(9,211)=3.31$, $p<0.0009$. Post-hoc comparisons for the individual variables by Duncan's multiple range test revealed a number of differences between the various strains and several overlapping groupings of the strains. Some of these data are shown in Fig. 2 and Table 1. Again, it did not seem that the strains could be grouped into a small number of discrete categories.

From the previously-published data on responsivity of the strains to amphetamine, and scopolamine [12,14], and to phenylethylamine (Jeste *et al.*, submitted) a ratio of activity counts after drug administration, divided by activity counts before or without administration of drug was determined. There was no significant correlation between response to PCP and any of these measures for all ten strains (Pearson's $Rho=0.33$, $p<0.2$). For the recombinant inbred strains only, there was also no significant correlation between PCP re-

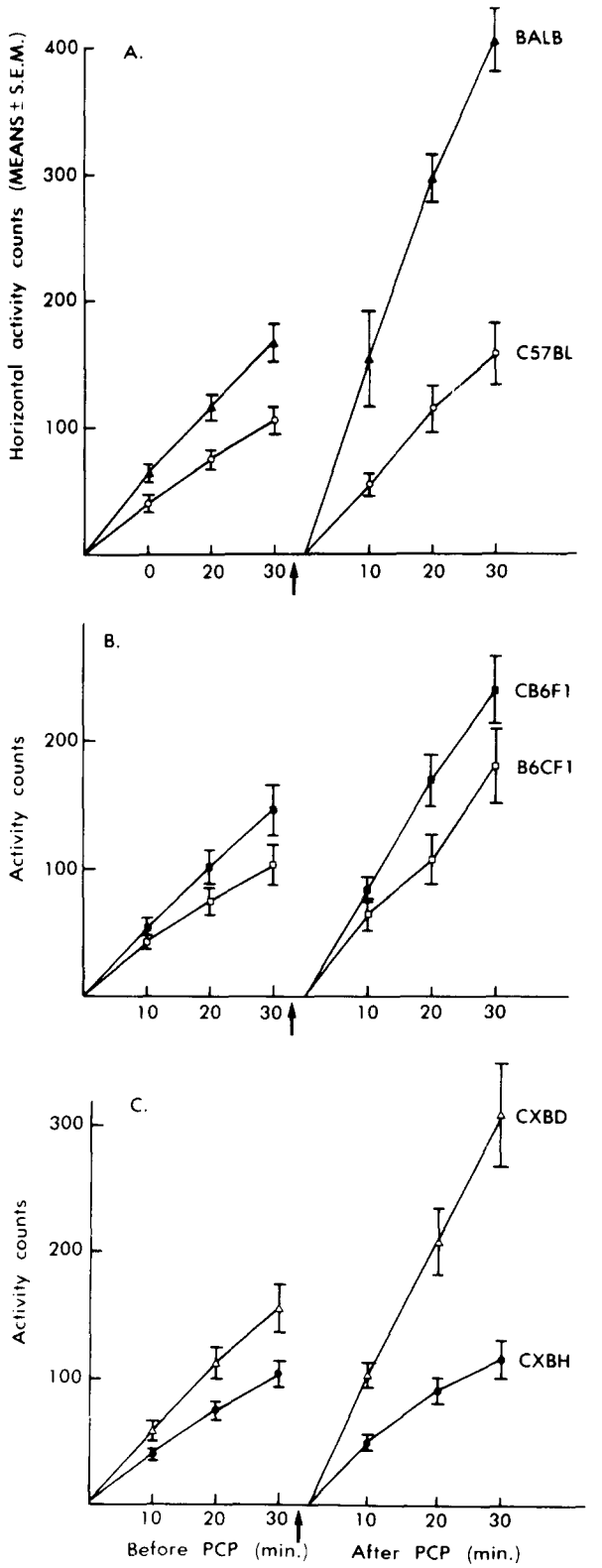

FIG. 1. PCP-induced stimulation in the two parent strains (A) their reciprocal F1 hybrids (B), and two examples of recombinant inbred strains (C). Activity counts obtained from the horizontal photocell banks are shown as a function of time for the 30 min before PCP administration and for the 30 min following administration of PCP. The arrow shows the point at which PCP was administered. Vertical bars indicate standard errors of the mean.

TABLE 1

HORIZONTAL ACTIVITY BEFORE AND AFTER PCP ADMINISTRATION FOR THE TEN MOUSE STRAINS

Strain	N	Activity Before PCP (A)	Activity After PCP (B)	Ratio (B/A)
BALB/c	11	159 ± 15 (a)	406 ± 25 (a)	2.79 ± 0.30 (a)
C57B1/6By	37	106 ± 11 (a)	158 ± 25 (d)	1.63 ± 0.20 (b,c)
CB6F1	29	147 ± 19 (a)	241 ± 26 (b,c)	1.78 ± 0.15 (a,b,c)
B6CF1	11	103 ± 16 (a)	181 ± 30 (d,c)	2.50 ± 0.73 (a,b)
CXBD	14	157 ± 18 (a)	309 ± 40 (b)	2.07 ± 0.23 (a,b,c)
CXBE	28	112 ± 12 (a)	181 ± 21 (c,d)	1.85 ± 0.26 (a,b,c)
CXBG	28	131 ± 27 (a)	250 ± 27 (b,c)	2.70 ± 0.36 (a)
CXBH	37	103 ± 9 (a)	117 ± 13 (d)	1.24 ± 0.13 (c)
CXBI	7	87 ± 21 (a)	138 ± 17 (d)	2.02 ± 0.41 (a,b,c)
CXBJ	20	108 ± 13 (a)	150 ± 17 (d)	1.52 ± 0.20 (b,c)
F*		1.58	8.88	3.63
p (overall)		0.123	0.001	0.001

*Multivariate analysis of variance: df=9,211.

In each column, values with the same letter in parentheses are not significantly different at the 0.05 level (Duncan's multiple range test).

sponse and any responses to other drugs ($p<0.2$), except that there was a slight tendency for PCP response to be correlated with response to amphetamine reported by Oliverio and his colleagues [14] (Rho$=-0.64$, $p=0.17$).

DISCUSSION

There were significant differences among the recombinant inbred strains in their responsivity to PCP. Thus, among these strains of mice, their genetic differences apparently influence their responsivity to PCP. There was, however, no indication that the strains could be categorized into two groups on the basis of their responsivity to PCP.

A continuous distribution of responsivity among the recombinant inbred strains is generally considered to be indicative of polygenic transmission of the characteristic. On the other hand, if the response is controlled by a single gene, the recombinant inbred strains should have only one of two categories of response, because they can have only one of two forms of the controlling gene: either that which originated in the BALB/c mice or that which originated in the C57B1/6By mice. Therefore, although there appeared to be a genetic influence on PCP responsitivity among the recombinant inbred strains studied here, this genetic influence was not carried by a single gene.

Moisset [12] reported that these strains of mice segregated into two discrete groupings on the basis of the decrease in grooming behavior induced by amphetamine. Jeste *et al.* (submitted) found a similar segregation of these recombinant inbred strains into two groups on the basis of their responsivity to phenylethylamine. Oliverio and his colleagues [14] studied changes in behavioral activity in these strains of mice in response to the administration of amphetamine and scopolamine. These strains could be segregated into two groupings on the basis of their responses to scopolamine, suggesting that this response was controlled by a single gene. Responses to amphetamine, on the other hand, did not fall into two discrete categories, suggesting a polygeneic control

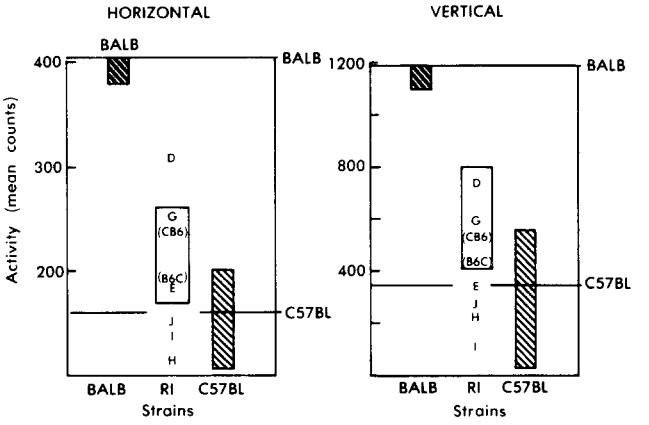

FIG. 2. Responses to PCP for the various strains of mice. The graphs show the activity following PCP administration for vertical (left) and horizontal (right) activity counts. The vertical bar on the left encompasses, horizontally, all the strains not differing significantly from the BALB/c mice. The vertical bar on the right, conversely, is drawn to encompass those strains not differing significantly from the C57B1/6By mice. The strains encompassed by the box in the center did not differ significantly from each other. The designation of each strain is shown at the vertical position which indicates the activity on the left axis.

of this response. In this respect, responses to amphetamine were most similar to the responses to PCP observed in the present investigation, in that both appeared to be under a polygenic influence.

On the other hand, we did not find any significant correlations between responsivity to PCP and any of the behavioral measures reported by Oliverio and his colleagues [14] or Moisset [12]. The only significant correlation that was observed was between the response to amphetamine reported by Moisset [12] and the response to phenylethylamine

reported by Jeste *et al.* (submitted) for the six recombinant inbred strains (Rho=0.97, $p<0.003$) or for all ten strains (Rho=0.82, $p<0.004$). It is particularly interesting to compare the response to PCP to those reported for amphetamine, as PCP-induced stimulation has been suggested to be akin to that induced by amphetamine [6,11]. The test employed here was similar to that employed by Oliverio *et al.* [14]. Yet, of the parent strains, the C57Bl/6By mice were more stimulated by amphetamine while the BALB/c mice were more stimulated by PCP. Both the F1 strains had an intermediate response to PCP as well as to amphetamine. Of the recombinant inbred strains, CXBH, CXBI, and CXBJ were least responsive to PCP. For amphetamine, CXBJ was the least active, CXBI was intermediate, and CXBH had the greatest activity of all the strains after amphetamine administration.

Thus, in conclusion, the stimulatory response to PCP measured here was under a polygenic influence, and was not under the control of a single major gene. In addition, there were substantial differences between the responsivity of these strains of mice to PCP and their previously-reported responsivity to amphetamine. Although it is tempting to suggest that the differences among the strains were due to differential brain responsivity to the drugs, it is also possible that pharmacokinetic differences among the various mouse strains were responsible. These data are, however, consistent with the hypothesis that the behavioral effects of PCP do not consist simply of amphetamine-like stimulation.

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