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A Classical Genetic Analysis of Two Apomorphine-Induced Behaviors in the Mouse

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CABIB, S. AND S. PUGLISI-ALLEGRA. *A classical genetic analysis of two apomorphine-induced behaviors in the mouse.* PHARMACOL BIOCHEM BEHAV **30**(1) 143–147, 1988.—Apomorphine (3 mg/kg) produced in C57BL/6 (C57) mice a clear-cut increase in locomotor activity and climbing behavior in comparison with saline, while in DBA/2 (DBA) mice it produced a clear-cut decrease in locomotion and a small reduction in climbing behavior. Genetic analysis involving F1 and F2 hybrids and the backcross populations (F1×C57; F1×DBA) indicated that apomorphine-induced locomotion and climbing are inherited through different modes of inheritance. With regard to climbing behavior the mean analysis of apomorphine parameters showed that the additive-dominance model fitted adequately, while this single model did not fit the locomotor activity data for which the best fitting model involved epistatic parameter. Moreover, a zero correlation between the two behaviors in the F2 generation resulted, indicating that no relationship exists between these apomorphine-induced behaviors under our experimental conditions. These results suggest that the horizontal locomotion and climbing are distinct behaviors controlled, at least in part, by different genetic factors related to different dopaminergic mechanisms.

Locomotor activity Climbing Apomorphine Dopamine Inbred mice Genotype Inheritance

A body of evidence exists indicating that dopaminergic agents produce behavioral effects through multiple populations of dopamine (DA) receptors located in different brain structures [5,10]. Most of the behavioral evidence collected in this regard comes from the study of apomorphine-induced stereotyped behavior in the rat. Apomorphine induces in this species a repetitive occurrence of classes of stereotypic behaviors that are dose-dependent. Moreover, intracerebral injections of DA agonists showed that different brain regions of the rat forebrain are involved in the various classes of behavior.

Pharmacological studies of dopaminergic-controlled behavior in the mouse have mainly utilized two tests: measurement of horizontal locomotor activity [1, 3, 7, 9, 15–18] and climbing behavior [2–4, 6, 9, 17]. Both tests allow easy behavioral scoring and are reliable in detecting the effects of pharmacological manipulations of brain DA systems. However, the powerful locomotor stimulant amphetamine is only able to induce a weak effect on climbing, and active doses of non-dopaminergic drugs that affect horizontal activity are totally incapable of modifing this behavior [9,14]. On the other hand, climbing behavior was shown to be enhanced by DA agonists that stimulate locomotor activity and is depressed by low doses of apomorphine that are known to decrease locomotion [9,14]. Thus, not all researchers agree that climbing and horizontal locomotion are two different classes of behavior [6,12].

A number of studies [3, 11, 16–18] have shown major strain differences in the effects of apomorphine on locomotor activity and climbing behavior in the mouse. In particular, apomorphine increases climbing behavior in mice of the C57BL/6, AKR/J and BALB/c strains while it has no effect on climbing behavior on DBA/2 mice at doses ranging from 0.1 to 4 mg/kg [3, 11, 17]. On the other hand, it was observed that apomorphine induced a dose-dependent reduction of locomotor activity in mice of the DBA/2 and BALB/c strains, while it enhances locomotion in a biphasic way in C57BL/6 mice [3, 16, 18]. Contrasting results have been reported concerning the effects of apomorphine on locomotor activity in C57BL/6 mice [17] possibly as a result of the different experimental methods used in that study. Taken together, results indicated that the genotype plays some role in the dopaminergic modulation of these behaviors and that horizontal locomotion and climbing are distinct behaviors possibly mediated by different dopaminergic mechanisms which themselves are dependent on the genetic makeup of an individual or a strain.

The purpose of the present study is to assess whether there are identical or different genetic determinants responsible for responsiveness to apomorphine in climbing behavior and locomotor activity. C57BL/6 and DBA/2 mice, which differ significantly in their response to apomophine-induced climbing behavior and locomotion, were used together with classical genetic crosses to produce reciprocal F1 hybrids, reciprocal backcross progeny and F2 progeny.

METHOD

Animals

Male DBA/2 (DBA and C57BL/6 (C57) (Charles River Labs., Calco, Como, Italy), their F1 and F2 hybrids and their backcrosses, aged 11–12 weeks and weighing 23–25 g at the

moment of testing were used throughout this study. B6D2F1 and D2B6F1 hybrid strains were obtained from reciprocal crosses of the two progenitor strains. Male F2 progeny were obtained from selfcrosses of F1 hybrid females to F1 hybid males. Backcross progeny were obtained by crossing F1 hybrid females to parental males from each strain. Litters were culled to a maximum of eight pups and weaning day was day 21 postpartum. Mice were housed in groups of six per cage and maintained with food (standard pellets, Italiana Mangimi) and water ad lib in a 12/12 light-dark cycle (lights were on from 07.00 to 19.00 hr) under constant temperature (21±2°C) and were always tested during the second half of the light period (between 14.00 and 16.00 hr). Mice were tested only once.

Behavioral Testing

Locomotor activity was measured by an automated apparatus consisting of eight toggle-floor boxes [3], each divided into two 20×10 cm compartments. For each mouse, the number of crossings from one compartment to the other was recorded by means of a microswitch connected to the tilting floor of the box. Climbing behavior was scored by a trained observer, as previously described [2,3]. The observer did not know which treatment had been given to the tested animals. Animals were put into individual cylindrical cages (12 cm diameter, 14 cm high, with walls of vertical metal bars, 2 mm diameter, 1 cm apart surmounted by a smooth surface). Their behavior was scored as follows: 4 paws on the floor (0); forefeet holding the bars (1); 4 paws holding the bars (2). Scores were evaluated every 2 min starting 5 min after the injection during a 60-min test session.

Both tests were carried out in soundproof cubicles where a 30-W lamp was the only source of illumination. The temperature of the cubicles was constant. Testing sessions started 5 min after treatment and lasted 60 min.

Drug Administration

Apomorphine dosages and time courses used in this research were determined on the basis of previous experiments [3]. Mice were injected either with apomorphine hydrochloride (Sigma) (0.25, 0.5, 1, 3 mg/kg) dissolved in saline (0.9% NaCl) immediately before use, or with saline alone. All injections were made subcutaneously in a volume of 10 ml/kg.

Eight to twelve mice of the DBA, C57 and B6D2F1 strains were used in order to assess the behavioral effects of each apomorphine dose tested. Naive mice from parental strains, F1 (B6D2 and D2B6) and F2 hybrids and backcross progeny (n=51 to 77) were subsequently injected with the dose of apomorphine that produced the maximal difference in behavioral effects (locomotion and climbing) between the parental strains. Such a dose was 3 mg/kg.

As far as F2 progeny is concerned a further 60 naive male mice were assigned at random to two groups of 30 subjects, one of which was tested first for locomotor activity and the other one for climbing behavior. Ten days later the first group was tested for climbing and the second one for horizontal activity. Mice were injected with 3 mg/kg of apomorphine before testing. Pearson's r coefficient on locomotor activity and climbing behavior data was calculated.

Statistics

For each behavior, data were statistically analysed by a two-factor analysis of variance (ANOVA), the factor being strain (three levels: DBA, C57, B6D2) and treatment (5 levels: saline and apomorphine 0.25, 0.5, 1, 3, mg/kg). Further analysis for individual between-group comparisons was carried out with post hoc tests (Duncan multiple range test).

TABLE 1

GENETIC MODELS FOR MEAN ANALYSIS

Generation	Genetic Models: Additive-Dominance	Genetic Models: Full Model With Epistasis
P1 (DBA)	m +[d]	m +[d]+[i]
B1 (F1×DBA)	m + 1/2[d]+ 1/2[h]	m + 1/2[d]+ 1/2[h]+ 1/4[i]+ 1/4[j]+ 1/4[l]
F1	m +[h]	m +[h]+[l]
F2	m + 1/2[h]	m + 1/2[h]+ 1/4[l]
B2 (F1×C57)	m − 1/2[d]+ 1/2[h]	m − 1/2[d]+ 1/2[h]+ 1/4[i] − 1/4[j]+ 1/4[l]
P2 (C57)	m −[d]	m −[d]+[i]

Genetic models used to estimate of genetic parameters: m, midparent value; [d], summation of additive genetic effects (the extent to which the effects of alleles sum up according to gene dosage); [h], summation of dominance deviations (the deviations of actual genotypic value from the additive genotypic value); [i], epistatic interactions between homozygous pairs of alleles (epistasis is the interaction of alleles at different loci); [j], epistatic interaction between homozygous and heterozygous pairs of alleles; [l], epistatic interactions between heterozygous pairs of alleles.

Genetic Analysis

A classical F2 and backcross design was utilized in order to examine the genetic factor influencing apomorphine-induced climbing behavior and locomotor activity. Data from the parental strains (P1 and P2) and their derived F1, F2 and backcross generations were used. Various genetic parameter such as additive genetic variance (VA = 2VF2 − (VB1 + VB2), dominance variance (VD = VF2 − VA − VE) and environmental variance (VE = VP1 + VP2 + VF1/3) from the generation variances [8] were estimated.

Environmental factors, genetic factors including additive dominance and epistatic factors, and gene-environment interactions all contribute to the variation within a population of a character of interest. Heritability provides an indication of the relative importance of genetic factors, since it has been defined as the proportion of the total variation in an observed population that is due to genetic factors. There are two types of heritability that can be calculated utilizing the previously mentioned variance estimates. Broad-sense heritability (h^2_B = VA + VD/VF2) is the proportion of variation due to all sources of genetic variation, regardless of whether the genes operate in an additive or nonadditive manner. Narrow-sense heritability (h^2 = VA/VF2) is the proportion of the total variation due solely to additive genetic variance.

The adequacy of single-gene models can also be tested with this design [13]. The results expected (E) from a single gene system if there are two alleles that have dominant and recessive effects can be compared with the observed (O)

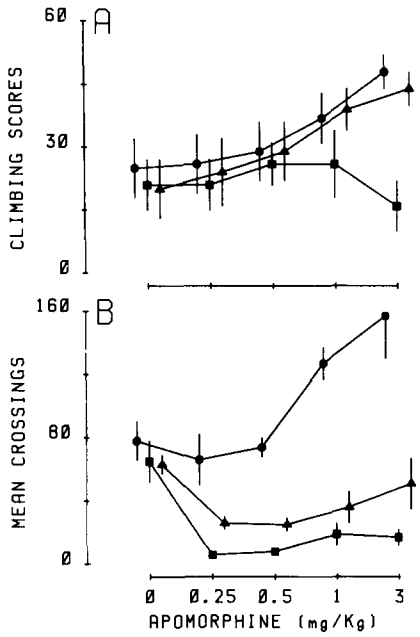

FIG. 1. Effects of apomorphine on climbing behavior (A) and locomotor activity (B) of DBA, C57 and B6D2F1 mice. Results are expressed in terms of climbing scores (mean±S.E.) and number of crossing (mean±S.E.). ■=DBA; ●=C57; ▲=B6D2F1. For statistical analysis see the text.

proportions of responses at a given dose. Moreover, the goodness-of-fit of the model can be tested by calculating a χ^2 statistic, $\chi^2 = (O - E)/E$.

This design allows to the hypothesis concerning the underlying mechanisms of apomorphine action on climbing behavior and locomotor activity to be tested. If the brain mechanisms affecting apomorphine-induced climbing are the same as those affecting locomotor activity they should be characterized by the same mode of inheritance.

RESULTS

With regard to climbing behavior ANOVA showed a significant strain main effect, $F(2,165)=4.33$, $p<0.02$, and a significant drug-treatment main effect, $F(4,165=2.75$, $p<0.05$. Between-group comparisons involving the overall means of the three strains (DBA, C57, B6D2) showed a significant difference between DBA mice and C57 ($p<0.01$) and B6D2 ($p<0.01$) mice respectively, while no significant difference between C57 and B6D2 was evident. As can be seen in Fig. 1 the dose-response curve of B6D2 hybrids parallels that of C57, thus suggesting that apomorphine-induced climbing is inherited through a dominant mode of inheritance.

With regard to locomotor activity, ANOVA showed a significant strain main effect, $F(2,105)=45.98$, $p<0.001$; a significant drug-treatment main effect, $F(4,105)=6.48$, $p<0.001$; and a strain × drug-treatment interaction, $F(8,105)=5.32$, $p<0.001$. Within each strain, individual between-group comparisons showed significant differences between saline- and apomorphine-injected mice. Apomorphine at all doses used significantly decreased locomotor activity in DBA mice, while in C57 mice it significantly enhanced locomotion at the dose of 1 and 3 mg/kg (Fig. 1). These results confirm our previous experiments [3]. Between-group comparisons showed that the dose-response

TABLE 2

EFFECTS OF APOMORPHINE (3 mg/kg) ON CLIMBING BEHAVIOR (CLIMBING SCORES, MEAN ± S.E.) AND LOCOMOTOR ACTIVITY (CROSSINGS, MEAN ± S.E.) IN DBA AND C57 MICE, AND THEIR F1, F2, F1×DBA AND F1×C57 HYBRIDS

Generation	Climbing Behavior	Locomotor Activity
P1 (DBA)	15.4 ± 3.4 (53)	10.8 ± 2.7 (52)
B1 (F1×DBA)	25.2 ± 4.0 (58)	25.4 ± 9.3 (56)
F1	42.0 ± 3.0 (63)	44.5 ± 8.4 (61)
F2	28.2 ± 2.7 (74)	50.2 ± 10.4 (77)
B2 (F1×C57)	39.3 ± 2.9 (57)	42.7 ± 11.9 (61)
P2 (C57)	48.0 ± 2.9 (51)	161.8 ± 17.2 (60)

In brackets the number of animals.

TABLE 3

GENETIC ANALYSIS OF APOMORPHINE-INDUCED CLIMBING BEHAVIOR

Generation	Observed (O) Mean	Expected (E) Mean	(O−E)
P1 (DBA)	15.42	12.26	3.16
B1 (F1×DBA)	25.20	24.80	0.40
F1	42.03	37.33	4.70
F2	28.28	33.47	−5.19
B2 (F1×C57)	39.33	42.14	−2.81
P2 (C57)	48.03	46.96	1.07

Genetic Parameter Estimates

m = 29.61 ± 1.86
[d]=−17.35 ± 1.24
[h]= 7.72 ± 2.19
$\chi^2(3) = 2.40$, $0.50<p<0.40$

The additive-dominance model was applied to apomorphine (3 mg/kg)-induced climbing behavior. The non-significant χ^2 estimate indicates a good fit of the data to the model. Genetic parameter estimated are: m, midparent value ± S.E.; [d], summation of additive genetic effects ±S.E.; [h], summation of dominance deviations ±S.E.
In brackets degree of freedom.

curve of B6D2 hybrids was intermediate between those of DBA and C57 progenitors, while no significant difference between saline-injected mice was evident in our experimental conditions.

To investigate further the relative contribution of genetic factors in the effects of apomorphine on climbing behavior and locomotor activity we decided to study the effects of the dopamine receptor agonist on these two behaviors in DBA, C57 and their F1, F2 and backcross generations. The dose of 3 mg/kg, which produced the largest differences between parental strains in both climbing behavior and locomotor activity, was used for this experiment. Since F1 hybrids resulting from crossing C57 females with DBA males (B6D2) were not significantly different from the reciprocal crosses DBA×C57 (D2B6) in climbing behavior and locomotor activity the data of both F1 hybrids were pooled in the F1 population.

The results concerning climbing behavior are shown in Table 2 and Table 3 together with the parameter estimates

TABLE 4

GENETIC ANALYSIS OF APOMORPHINE-INDUCED LOCOMOTOR ACTIVITY

Generation		Additive-Dominance		Epistatic	
	(O)	(E)	(O−E)	(E)	(O−E)
P1 (DBA)	10.83	10.28	0.55	10.26	0.57
B1 (F1×DBA)	25.40	26.98	−1.58	29.56	−4.16
F1	44.52	43.67	0.85	48.84	−4.32
F2	50.29	51.49	−1.12	54.44	−4.15
B2 (F1×C57)	42.73	76.00	−33.27	37.69	5.04
P2 (C57)	161.85	108.34	53.51	151.63	10.42

Genetic Parameter Estimates

Additive-dominance	Epistatic
m = 59.31 ± 5.81	m = 60.03 ± 5.81
[d] = −49.03 ± 5.6	[d] = −49.77 ± 5.60
[h] = −15.54 ± 5.63	[h] = −11.19 ± 5.60
	[j] = −166.53 ± 2.65
$\chi^2(3) = 41.14$	$\chi^2(2) = 2.68$
$p<0.001$	$p>0.20$

An additive-dominance model and a model including epistatic interactions were applied. Several combinations of genetic parameters including the epistatic ones were tested and the model presented is that model which best represents the data. The nonsignificant χ^2 for epistatic model indicates a good fit of the data to that model. Genetic parameters presented in the table: m, [d], [h]: see Table 3; [j], interactions between homozygous and heterozygous pairs of alleles ±S.E. (O)=observed mean; (E)=expected mean. In brackets degree of freedom.

for a weighted least-squares regression analysis. The nonsignificant χ^2 estimate for climbing scores suggests that the additive dominance model accounts for the data adequately. In an alternate genetic approach, the generation variances were estimated and used to calculate the heritability for apomorphine-induced climbing behavior as described in the method section. A heritability estimate of $h_B^2=0.42$ was obtained which indicates an important genetic influence on this apomorphine-induced behavior.

Locomotor activity data are shown in Table 2 and in Table 4 where the parameter estimates concerning weighted least-squares regression analyses are also reported.

The single additive-dominance model did not fit the data. Therefore, more complicated models to account for epistatic interactions were fitted to the data. The best-fitting model was found to involve an additional epistatic parameter, which accounts for interactions between loci with homozygous alleles at one locus and heterozygous alleles at the other. More complicated models did not improve the best-fitting.

A heritability estimate of $h_B^2=0.52$ was obtained which suggests a substantial genetic influence on this apomorphine-induced behavior.

Lastly, when the correlation between locomotor activity and climbing behavior induced by 3 mg/kg of apomorphine in F2 generation was considered, a Pearson's r coefficient of 4.89×10^{-3} was obtained which indicates a zero correlation between these two behaviors.

DISCUSSION

These results show that apomorphine induces a dose-dependent reduction of locomotor activity in mice of the DBA strain, while it enhances dose-dependent locomotion in C57 mice. On the other hand, apomorphine increases climbing behavior in C57 mice while it is ineffective in modifying climbing behavior in DBA mice. These results seemed to indicate that horizontal locomotion and climbing were different behaviors possibly controlled by different dopaminergic mechanisms depending on the genetic makeup and that C57 mice were characterized by a non specific activation stimulated by the drug acting through a single neural mechanism. When B6D2F1 hybrids were considered, the dose-response curve of apomorphine concerning climbing behavior parallels that of the C57 parental strain, while it is intermediate between the parental strains with regard to locomotor activity.

On the basis of these results the hypothesis arises that the two apomorphine-induced behaviors are controlled by different genetic factors underlying different neural mechanisms.

In order to assess such a hypothesis a classical genetic analysis of the two apomorphine-induced behaviors was carried out on DBA C57, their F1 and F2 hybrids and F1 × DBA and F1 × C57 backcrosses, using the test dose of 3 mg/kg of the drug.

With regard to climbing behavior an important genetic influence was envisaged. Moreover, the means analysis of apomorphine parameters showed adequate fit of the additive-dominance model, a result that may support the single-gene model since more complicated models involving epistatic interactions between loci need not to be evoked. However, since the F2 generation deviated consistently from the expected value a more complicated pattern of inheritance cannot be ruled out. This discrepancy between observed and expected values for F2 generation may be a result of not testing enough animals.

As far as locomotor activity is concerned, the simple additive-dominance model did not fit the data, while the best-fitting model was found to involve the j epistatic parameter. The j parameter accounts for interactions between loci with homozygous alleles at one locus and heterozygous alleles at the other. These results indicate different modes of inheritance of the apomorphine-induced climbing behavior and locomotor activity.

Furthermore, when phenotypic correlation between the two behaviors in the F2 generation was considered, a zero correlation resulted indicating that no relationship exists between these apomorphine-induced behaviors in our experimental conditions. It therefore appears that genetic recombination can lead to the separation of the inherited determinants of susceptibility to apomorphine-induced climbing behavior from those determining susceptibility to apomorphine-induced locomotor activity. These complex genetic determinants controlling the effects of apomorphine on climbing and locomotion can then assort to produce new phenotypes which are characterized by different behavioral responses to apomorphine in comparison with the parental strains.

In conclusion, these results suggest that climbing behavior and locomotor activity in the mouse are distinct behaviors controlled, at least in part, by different genetic factors related to different dopaminergic mechanisms. Further experiments involving recombinant-inbred strains will possibly give more information about single- vs. multiple-factor models of inheritance of these two apomorphine-induced behaviors in the mouse.

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