Developmental effects of the A^Y substitution on mouse activity level¹

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Short-term activity level was tested with inbred mice of four genotypes in an open-field apparatus. These data supplement earlier results for long-term exploratory activity using the same genetic stocks. The results indicate that the effects of A^Y in suppressing locomotor activity are not restricted to long-term experimentation nor to a specific apparatus in mature (125 day old) Ss. Different results are found at each of the three age levels studied, however. The interpretation utilizes indirect evidence from earlier strain-difference studies and is open to experimental challenge. It is also noted that components of sexual and agonistic behavior patterns may provide stable phenotypes for behavior-genetic investigation.

This paper reports a partial replication and extension of an earlier experiment (Hawkins, 1965). In that study the stocks of mice used here were tested in a large (1 x 4 x 4 ft.) multiple-unit maze over a long (165-min.) test period. It was found that A^Y , which has numerous pleiotropic structural effects, also affects the amount of exploratory activity exhibited by 125 day old C57BL/6J mice. Of interest here was whether or not similar differences in performance would be found with a different activity measure and with a shorter testing period. Also of interest was the course of behavioral development over time.

Subjects

The Ss were C57BL/6J mice maintained with forced heterozygosis at the agouti locus. One-half of the animals were black (genotype aa); the rest were yellow (genotype $A^Y a$). The Jackson Laboratory stock to which they can be traced were N = 22, F = 11. Independent groups of 10 animals, of each sex, from each color variety, were run at three age levels: 35 (+ or - 3), 80 (+ or - 11), and 125 (+ or - 9) days. In this study, all groups included siblings produced by matings of A^Y bearing parents of both sexes.

Apparatus

A Lehigh Valley Electronics, automatically recording, open-field activity apparatus was used. This device consists of a metal drum, painted flat-black, which is 16 in. high and 18 in. in diameter. Six infra-red beams cross each other at the base. Three parallel beams 4 in. apart lie along one plane; three equivalent beams are at right-angles to them. A plywood insert was used as the running surface. Thus, a 16 unit grid was formed across an homogenous field. Each bank of parallel beams has its own counter. The data counts used were the sum of both counter totals for each S.

Procedure

Ss were weaned at 30 days and housed by experimental groups (5 to a cage) in wire mesh cages 8 x 10 x 12 in. Custodial and laboratory conditions were as described previously (Hawkins, 1965). In testing, each animal was released in the open-field and given one 15-min. trial. Ss were run under normal room illumination during the light portion of the usual 12 hr. dark/light cycle.

Results

An analysis of variance gave as significant sources, locus, sex, age, locus by age, and sex by age effects. Locus by sex and locus by sex by age variance was non-significant at the .05 confidence level. Figure 1 graphs the performance of each genotype.

The t tests (df = 38, 2 tail) within each sex and within each color variety between ages gave the following results: (1) 35 vs 80 day comparisons; all tests ($t = 1.86$ or less) nonsignificant. (2) 80 vs 125 day comparisons; males ($t = 2.95$, $p < .01$), aa ($t = 4.92$, $p < .001$), females and $A^Y a$ ($t = 2.02$ or less) non-significant. (3) 35 vs 125 day comparisons; males ($t = 5.06$, $p < .001$), aa ($t = 5.10$, $p < .001$), females and $A^Y a$ ($t = 1.46$ or less) non-significant.

The t tests (df = 18, 2 tail) between sexes and between color varieties within age levels gave these results: (1) 35 day old groups, male vs female ($t = 3.78$, $p < .01$).

Fig. 1. Performance of each genotype at each age level.

- (2) 125 day old groups, *aa* vs *A^ya* ($t=6.38, p<.001$).
 (3) All other comparisons non-significant ($t=1.12$ or less).

Discussion

This study indicates that the yellow lethal gene *A^y* produces the same sort of behavioral differences on a short term open-field activity test, that were previously found with long-term exploratory activity. That is, C57BL/6J-*A^ya* mice exhibit much less activity than do their siblings homozygous for *a*. To a certain extent then, this study confirms and extends the results found earlier. However, it also restricts some of the conclusions which may be drawn. The replication of results applies only to the 125 day old animals.

As in the previous study, locus by sex interaction is nonsignificant. The significant interactions that do appear are primarily attributable to changes in performance by *aa* and by male Ss at successive age levels. Significant sex differences are found at 35 days while agouti locus differences are absent. At 80 days there are no significant differences attributable to either sex or locus. Finally, at 125 days, locus differences are found but not those attributable to sex.

Two hypotheses can be advanced with regard to such data. First, it can be postulated that the *aa* genetic constitution predisposes animals of this strain to high locomotor activity when fully mature. Alternatively, the action of *A^y* through the period of maturation may suppress the activity level which such animals would otherwise have attained.

An argument based on reevaluation of previous strain difference studies (by post hoc consideration of the agouti locus constitution) could be used in support of the first position. For example, in Thompson's classic study of exploratory activity (1953), there is a preponderance of strains homozygous for *a* high in activity as opposed to those further down the list. Also, the first major change in his tabled values of *p* differences comes with a strain homozygous for *A^W* (*white bellied agouti*). His least active strain, however, is homozygous for *a* and most investigators would probably contend that multiple differences in the total gene complement between strains is most likely the basis for such results.

With regard to the hypothesis that *A^y* acts as a suppressor, there are several indirect sources of support. First, Thiessen (1965) in his review of spontaneous activity notes: "... relative strain differences tend to be maintained, with the order of activity from high to low, C57, DBA, and C3H." In those experiments which Thiessen reviewed that involved strictly locomotor activity, there are no reversals between C57 and DBA Ss. Since both of these strains are homozygous for *a*, it is apparent that agouti locus constitution is not a necessary variable in activity

difference experiments. Next, when stovepipe emergence activity is considered, four experiments (Lindzey et al, 1960, 1963) have shown a complete reversal with DBA Ss showing shorter latency than C57 Ss. It thus appears that each strain represents a stable behavioral phenotype but that strain reversals may be found with different kinds of activity measures.

This conclusion is supported when one considers measures of the components of sex behavior. McGill & Blight (1963) report on 14 measures of sex behavior using C57BL/6J and DBA/2J Ss. There are 10 measures in which C57 vs DBA comparisons are significant. Eight of these can be viewed as activity measures. In all eight, the C57 Ss are the more active strain. One of the components not considered here as an activity measure is "percentage of bites." While none of the C57 males bit the females at ejaculation, twenty percent of the DBA males did. This, too, might prove to be a stable phenotype. In ecologically oriented free-breeding tests, Calhoun (1956) found that DBA Ss were less successful in reproduction than were C57 Ss due, in part, to more frequent and more intense fighting among themselves—extending even to male attacks on females.

In summary, locomotor activity and stovepipe emergence appear to be stable behavioral phenotypes when comparisons are made between strains. The data of this experiment suggest that *A^y* acts as a suppressor of locomotor activity in C57BL/6J mice at maturity. There is some evidence that certain components of sexual and agonistic patterns might also provide stable phenotypes when considered as activity measures. The effects of *A^y* in such contexts are unknown.

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Note

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