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Genetic Influences on Locomotor Activating Effects of Ethanol and Sodium Pentobarbital

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DUDEK, B. C., T. TRITTO AND K. A. UNDERWOOD. *Genetic influences on locomotor activating effects of ethanol and sodium pentobarbital.* PHARMACOL BIOCHEM BEHAV **48**(3) 593–600, 1994.—The paradoxical capability of sedative-hypnotics to produce behavioral disinhibition varies among genotypes. In DBA/2 mice ethanol (ETOH) produced strong locomotor stimulation with the peak of the biphasic curve at 1.5 g/kg IP. C57BL/6 mice showed no activation, and F_1s were intermediate. These characterizations held for a variety of behavioral indices derived from 15 min tests, such as distance, speed, and rest time, at doses in the 0–2.0 g/kg range. Analogous studies with sodium pentobarbital (0–40 mg/kg) yielded a similar pattern of strain differences in locomotor stimulation. In contrast, loss of righting reflex durations (60 mg/kg PENTO, IP) were similar in the two strains, indicating dissociation of activating and sedative effects. In complementary studies, long- and short-sleep mice, which were bred for differences in soporific effects of ETOH, showed similar activation profiles at ETOH doses up to 1.5 g/kg and PENTO doses up to 30 mg/kg. These studies provide support for an hypothesis of common genetic control of the activation effect for ETOH and PENTO.

Genetics Ethanol Barbiturate Pentobarbital Locomotor activity

MANY sedative-hypnotic drugs of abuse have a paradoxical action producing behavioral arousal at low doses or for short time periods after administration (32). These psychomotor stimulant properties may show sensitization rather than tolerance with repeated administration (19,28), and have been argued to be related to abuse potential (40). Such activating effects may play a central role in liability to at least one subtype of alcoholism (4). Because these theories on drug abuse etiology (particularly alcoholism) focus on genetic antecedents, the role of hereditary factors in psychomotor stimulant sensitivity requires investigation. Genetic variation in these psychomotor stimulant effects of ethanol (ETOH) and pentobarbital has been demonstrated with laboratory mice (8–12, 27–30). Such studies are useful in facilitating an understanding of mechanisms of drug action, as well as serving as models for aspects of human drug abuse liability [cf. (20)].

Whether the observed activation following administration of various sedative-hypnotics is due to common mechanisms, pathways, or modulatory actions, perhaps genetically based, is a question that remains unanswered. Genetic methodologies offer a unique capability of addressing this question of commonality. If genetically based correlations between activating effects of different drugs can be demonstrated, then a commonality hypothesis would be viable. In an initial effort to assess similarities of genetic influences on response to ETOH and pentobarbital, the present paper reports dose-response curves for ETOH and sodium pentobarbital effects on a variety of behavioral indices related to psychomotor stimulation. We worked with two breeding systems. In one, we examined the C57BL/6 inbred strain which shows little or no activation to ETOH and the DBA/2 strain which shows marked locomotor stimulation to ETOH in photocell apparatuses (5,9,12, 24,33). In the second, we evaluated the selectively bred long- and short-sleep mice which were bred for differential sedative effects of ETOH (21). In both systems, the primary question was whether the genetic influences on psychomotor phenotypes were similar for ETOH and pentobarbital.

EXPERIMENT 1

Numerous reports demonstrate that the C57BL/6 (B6) mouse strain has an aberrant locomotor activity dose-response curve to ETOH (5,9,12,24). The B6 strain may show slight activation shortly after injection or under certain lighting conditions (5,24) but, in many studies, the activational limb of the biphasic curve is absent [e.g., (9,12)]. Genetic

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influences on barbiturate sensitivity are also known (39), and although some reports indicate that the DBA/2 (D2) strain might be more sensitive than B6 to the soporific effects and chronic toxicity of sodium pentobarbital (26,37), we are not aware of reports comparing their psychomotor responses at low doses. The lack of an activating effect of pentobarbital in B6 mice would strongly suggest common genetic foundations for the paradoxical stimulation produced by low doses of ETOH and pentobarbital. This experiment reports on the characterization of dose-response curves for ETOH and pentobarbital in these two strains using a computerized animal activity apparatus, which expands on the range of indices available from the simple photocell counts reported in older literature. These indices include distance traveled, speed, number of movements, movement time, and rest time. Such a battery permits a fuller characterization of the activating phenomenology than is available with simple photocell apparatuses.

Method

Animals. Male and female mice of the C57BL/6Abg and DBA/2Abg inbred strains, and their reciprocal F_1 hybrids were obtained from our breeding colony. Mice ranged in age from 55 to 130 days. Within each study, mice were randomly assigned to drug treatment groups and mean ages for genotype/sex/dose groupings differed by a maximum of only 10 days. Mean age was 88 days in the ETOH study and 95 days in the pentobarbital study. Lighting cycle in the mouse colonies was 16 h light to 8 h dark, and temperature was approximately 22°C. Food (Agway RMH-3000) and water were available ad lib.

Procedure. Mice were tested in Omnitech automated activity monitors (Model CCDIGI(16)TAO). This is a photocell apparatus where mice were placed into a 40 cm^2 acrylic chamber with a 30 cm ceiling. The activity monitors were enclosed in a larger sound-attenuating chamber, and all testing was done in complete darkness. A ventilating fan provided a masking background noise. The monitors produced counts of total horizontal and vertical photobeam breaks in various locations in the chamber. The PC-compatible software derived the following indices: a) distance traveled (cm), b) number of movements, c) movement and rest time, as well as a variety of additional measurements that we do not report here for the sake of brevity. We derived two additional indices that facilitated the characterization of behavioral activation. These were an average speed index (distance/movement time; cm/s), and an average movement length index (distance/number of movements; cm/movement). Of these indices, we focused on horizontal counts (HORZ), vertical counts (VERT), distance (DIST), rest time (REST), speed (SPEED), and movement length (LENGTH). The VERT index is an indirect assessment of rearing activity.

Testing protocol followed that of earlier work [e.g., (12)], where normally fed/watered mice were removed from their home cage, weighed, injected, and immediately (< 10 s) placed into the monitors for 15 min. Mice were placed in the middle of the activity chambers by grasping the tail with forceps. Behaviors were recorded in three successive 5-min blocks of time. Mice were tested in squads of four and were randomly assigned to one of four test chambers. In most cases, no individual squad contained more than one mouse of the same genotype/sex/dose grouping. Activity chambers were cleaned with a dilute isopropyl alcohol solution after each mouse.

The first study generated alcohol dose-response curves for the two strains and their F_1s. ETOH doses (0, 1.0, 1.5, and 2.0 g/kg) were prepared in 0.9% NaCl and administered in a 20 ml/kg volume IP. The second study assessed pentobarbital dose-response curves in drug-naive mice of the same genotypes and sodium pentobarbital doses (0, 10, 20, 30, and 40 mg/kg) were prepared in 0.9% NaCl and administered IP in a 10 ml/kg volume.

Additional mice were evaluated for sedative effects of pentobarbital by the use of the loss of righting reflex test. Male B6 and D2 mice were treated with 60 mg/kg of sodium pentobarbital (IP). Duration of narcosis was assessed as length of time from loss of the reflex to three rights within 30 s.

Data analysis. Data for all indices were examined with analyses of variance. Because several of the activity apparatus measures were derived from common variables, a multivariate analysis of variance was inappropriate. The focal indices were total horizontal counts (HORZ) and distance (DIST), and all six indices outlined above were analyzed with individual univariate ANOVAs. Although a main effect of sex was present in some instances (females more active), sex never interacted with the dose and genotype variables and, thus, all analyses presented below ignore the sex effect for the sake of simplicity of presentation. This lack of sex differences is in contrast to a recent a report (25) that used a different type of apparatus and testing procedure.

As expected from earlier work [e.g., (12)] reciprocal F_1 groups were not statistically different in either study, and were not distinguished in analyses reported here. The ANOVAs were accomplished with the conservative approach of the general linear models regression methodology because sample sizes were unequal. Planned orthogonal contrasts for the genotype factor involved a) direct comparison of the two parent strains, and b) comparison of the F_1 groups with the average of the two parent strains as an assessment of dominance. Interactions of these contrasts with dose and trend components of dose provided the major statistical tests of strain differences and dominance. Additional post hoc tests of pairwise group comparisons were accomplished with the Tukey HSD test with $\alpha = 0.05$.

Results and Discussion

ETOH produced dose-response curves in the B6 and D2 inbred strains that resemble earlier reports for simple photobeam counts (Fig. 1). We report only total 15 min data because the general pattern of genotype differences did not vary across the three 5-min blocks of time. The HORZ index is most similar to the traditional photobeam counts, and D2 mice showed the expected strong activation at all doses. B6 mice showed sedation in HORZ at most doses and the F_1 curve was intermediate to the two parent strains for this measure. The DIST, REST, and SPEED indices provided considerable insight into the reasons that total horizontal counts in this study, and in older literature, show the differing pattern between the two strains. D2 mice, when ETOH-treated, traveled greater distances, rested less, and showed increments in mean running speed throughout this dose range. B6 mice showed an increment in mean DIST and HORZ at only the lowest dose (although nonsignificant, Tukey HSD test). No dose of ETOH produced a significant decrement in REST or a significant increment in SPEED of B6 mice. Genotype by dose interactions were significant for all four measures ($p < 0.001$) and simple main effects of dose were significant in all three genotypes for all four of these indices ($p < 0.002$). However, specific planned comparisons were more informative. The quali-

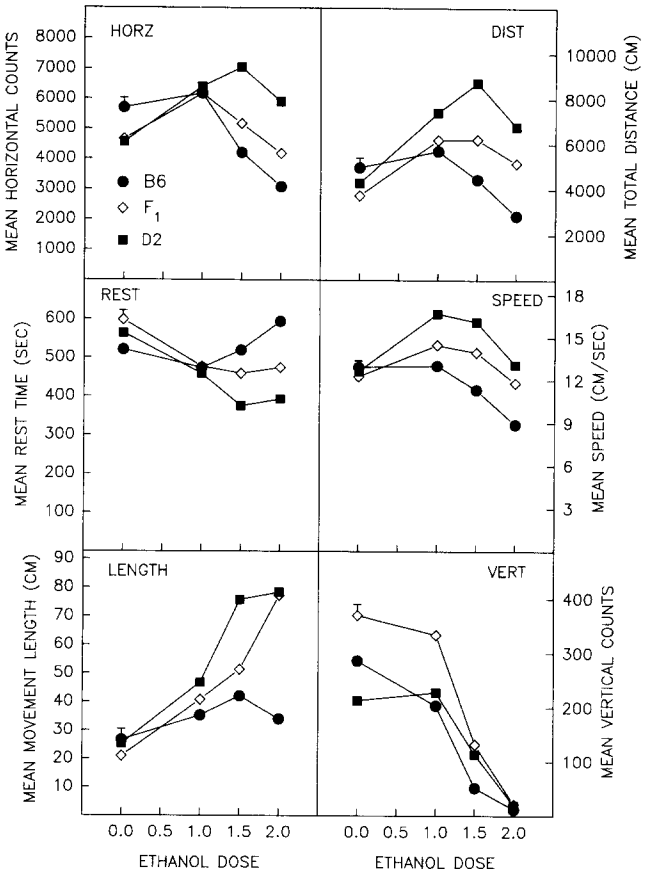

FIG. 1. Dose-response curves for 15-min ETOH effects on six behavioral indices in C57BL/6Abg (B6) and DBA/2Abg (D2) inbred strains and their F_1 hybrids. HORZ is total horizontal photocell counts, DIST is distance traveled, REST is time spent without locomotion, SPEED is movement rate, LENGTH is average distance traveled per movement, and VERT is total vertical photocell beam counts. Sample sizes ranged from 8 to 27 for each group (smaller *ns* in F_1 groups). Data points reflect data for both males and females, because sex differences were not present. Vertical bars represent generalized standard errors of the mean derived from analysis of variance error terms.

tative difference in ETOH response of the two strains was substantiated by significant interaction contrasts (D2 vs. B6 × dose interaction) in ANOVAs for each of the HORZ, DIST, REST, and SPEED indices (all $p < 0.001$).

The LENGTH index revealed more of a quantitative difference between the two strains (interaction contrast significant, $p < 0.001$). Although the D2 mice showed more marked increases in LENGTH following ETOH, B6 mice also showed increments. This pattern for the B6 strain provides an interesting contrast with the four indices described above, in that it reflects an ETOH-induced disinhibition not revealed by the other measures. Thus, one type of disinhibitory effect of ETOH was present in B6 mice. The vertical activity index revealed similar ETOH effects in the two strains, with a hint that 1.0 g/kg ETOH in D2 mice might increase VERT (Tukey test). Although ETOH strongly inhibits vertical activity across the whole dose range, clear evaluation of the genetic influences is difficult because of such large baseline (control groups) differences in vertical activity.

A general pattern of intermediate inheritance of F_1s, reflecting no dominance, was seen in HORZ, DIST, REST, and SPEED. The contrast of the F_1 values with the midparent point did not interact with the dose variable for any of these indices. This absence of significance might have obscured some tendency for dominance in the D2 direction at 1.0 g/kg, and further study of additional doses in this range is needed. For the LENGTH measure, a clear departure from the midparent point was significant (interaction contrast with dose, $p < 0.001$), but this was largely due to the resemblance of the F_1 to the D2 strain at the highest dose. Assessment of dominance in the VERT index is complicated by the large baseline differences and the major suppressive effect of ETOH in all genotypes. However, if one examines only the 1.0 g/kg dose, the appearance of a B6/D2 difference and an intermediate ETOH response of the F_1s suggests a pattern similar to the HORZ, DIST, REST, and SPEED measures. However, the post hoc nature of this description reiterates the above point that further study of the 0–1.0 g/kg dose range is indicated.

Of the six indices, DIST appears to be the single best character for assessment of the B6/D2 difference in ETOH activation. HORZ, which is total horizontal photobeam breaks, lacks specificity for general utility. VERT and LENGTH are less clear discriminators of the biphasic dose-response curve. DIST has a heuristically useful scale, reflects the absence of dominance, and has statistical properties that are compelling. The partial eta squared for interaction contrast of dose with the parent strain comparison is a crude heritability estimate, and was large for DIST (0.28). This interaction contrast accounted for a smaller proportion of variation in REST (0.21) and SPEED (0.23).

The pentobarbital study revealed both similarities and differences in the pattern of genotype influences to the pattern seen with ETOH for the 15-min test (Fig. 2). The three genotypes showed characteristically different dose-response curves for all six indices (interactions all significant; $p < 0.003$). D2 mice were clearly activated by 10 and 20 mg/kg, and B6 mice showed slight activation at 10 mg/kg. This characterization holds for the HORZ, DIST, REST, and SPEED indices. The contrast of B6 vs. D2 interacted with dose for each of these four variables ($p < 0.008$). The activation shown by D2 mice to 10 and 20 mg/kg was significant (Tukey tests) for these measures. In contrast, the tendency toward activation shown by B6 means for HORZ, DIST, and SPEED at 10 mg/kg was not significant for any of these three measures, but the decrement for REST at 10 and 20 mg/kg was significant (Tukey tests). As was the case for ETOH, both strains increased LENGTH following pentobarbital administration, but this effect was larger in D2 mice ($p < 0.001$). The VERT index appeared to reveal a strain difference in the dose-response curves that mirrored the ETOH difference, but the strain by dose interaction contrast was not significant. Like ETOH, pentobarbital produced a small (Tukey test; NS) increment in D2 levels of VERT at 10 mg/kg, but the general influence of pentobarbital on VERT was a strong attenuation in all genotypes (main effect of dose significant, $p < 0.001$).

In contrast to the mode of inheritance seen in the ETOH study, a pattern of intermediate inheritance of the F_1s was less clear in the pentobarbital study. In the activational limb of the curves (0–20 mg/kg), the slope of change in HORZ, DIST, REST, and SPEED was close to the average of the two parent strains. For ANOVAs done on just the 0, 10, and 20 mg/kg doses, the interaction contrast of F_1 vs. the midparent point by dose was not significant for any of these four indices. However, at higher doses, resemblance to the B6 strain some-

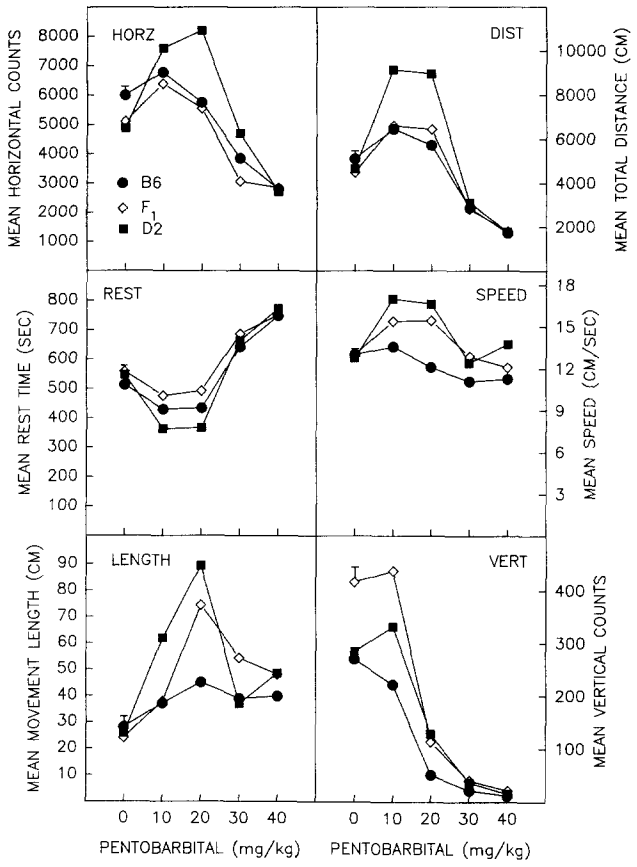

FIG. 2. Dose-response curves for 15-min pentobarbital effects on six behavioral indices in C57BL/6Abg (B6) and DBA/2Abg (D2) inbred strains and their F_1 hybrid. Each panel represents a different behavioral measure as described in the text and in Fig. 1. Sample sizes ranged from 19 to 22 for each group. Data points reflect data for both males and females, because sex differences were not present. Vertical bars represent generalized standard errors of the mean derived from analysis of variance error terms.

times appeared. This yielded a significant departure of the F_1 curve for the whole dose range from the midparent curve for HORZ and REST, as well as for LENGTH and VERT, where the F_1s resembled the D2 strain (all $p < 0.03$).

This picture of no dominance in the activational limb of the pentobarbital dose-response curve is somewhat misleading. Unlike for ETOH, pentobarbital effects and their genotype-dependence changed considerably across the three 5-min blocks of time. A representative indicator of this change across time is the DIST measure (Fig. 3). Two features of this change are clearly different than the simple consistency of genotype differences shown in response to ETOH. The first is the resemblance of the F_1s to the D2 strain in the first block of time, and the switch to resemblance to the B6 strain in the second and third blocks of time. This characterization is validated by the significance of a three-way interaction contrast involving the comparison of the F_1 values to the midparent values. This comparison showed a three-way interaction with dose and time, indicating that the change in dominance was significant ($p < 0.001$). The second distinction with the ETOH pattern is that although D2 mice show strong activation in the activational limb of the curve, marked sedation is visible in the second and third blocks at the 30 and 40 mg/kg doses. Therefore, the large degree of activation is not due to a genotype-based resistance to sedative properties. This dissociation of genetic influences on activating and sedative components of the pentobarbital dose-response curve has also been reported in extensive genetic analyses for ETOH-induced activation and sedation (12).

A further assessment of sedative effects of pentobarbital is available from the loss of righting reflex test. Following 60 mg/kg IP D2 and B6 mice lost the righting reflex for similar durations (D2 = 105.99 ± 9.27 min and B6 = 106.52, 9.92 min; t-test not significant, $n = 6$ per group). Although some previous literature (37) had reported slightly longer durations for pentobarbital sleep time in the D2 than in the B6 strain, neither those data nor the present data give reason to conclude that greater locomotor activation in D2 mice is due to resistance in the sedative limb of the biphasic dose-response curve.

EXPERIMENT 2

The exact shape of biphasic dose-response curves for locomotion is determined by a mix of genotype-specific sensitivities in both the activational and sedative limbs of the curve. The data from Experiment 1 indicate that B6 do not show less

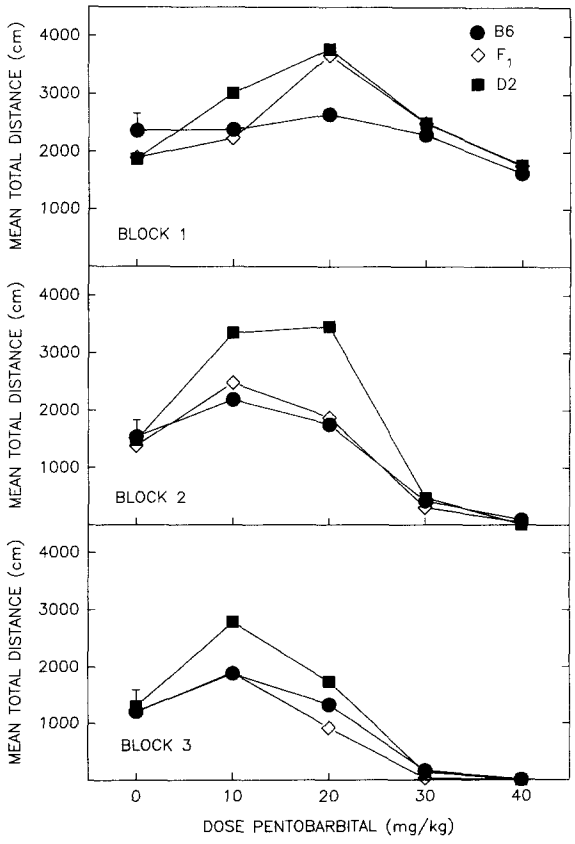

FIG. 3. Pentobarbital effects on distance traveled across three successive 5-min blocks of time. Data are from the same B6, D2, and F_1 mice whose total 15 min data are shown in Fig. 2. Vertical bars represent a generalized standard error of the mean derived from the analysis of variance error term.

activation than D2 because of a greater sensitivity to the sedative influences of pentobarbital. Diminished activation produced by sedative-hypnotics could, in principle, result from genetically based resistance to activational effects, or increased sensitivity to sedative effects. Therefore, study of ETOH and pentobarbital in additional genetic systems is required to further address these possibilities.

In this context, studies with the selectively bred long- and short-sleep mice (LS and SS) have been useful. These mice were bred for differing soporific response to ETOH (21), as indexed by loss of the righting reflex. Their biphasic ETOH dose-response curves for locomotor activity have been heavily studied with traditional photocell and open-field apparatuses (3,7-9,11-13,30,35,36). A general conclusion from these studies is that the sedative limb of the ETOH dose-response curve is shifted to the left for LS mice and to the right for SS mice. This results in strong activation over a wide range of doses for SS mice (30). In addition, the lines are comparable in the slope of the activational limb of the curve up to 1.5 g/kg, and F_1 hybrids show curves intermediate to the parent lines. The LS and SS lines are generally thought to show similar sensitivity to the soporific effect of pentobarbital or slightly greater sensitivity in SS mice [(11,16,17,18); cf. (23)]. However, one report suggests that SS mice may also show a greater degree of locomotor activation to lower sodium pentobarbital doses (8). SS mice may also show more EEG activation than LS mice following pentobarbital administration (34).

The present experiment examined ETOH and pentobarbital dose-response curves in two studies using the computerized activity monitors described above. The purposes of this experiment were a) to characterize the dimensions of the activation effect with the same multiple indices used in Experiment 1, and b) to assess whether the genetic effects on ETOH and pentobarbital activation might be dissociable as earlier work (8) suggested.

Method

Animals. Male LS and SS mice of the Albany Colony were produced in our laboratory and maintained as previously described (13). Mice ranged in age from 55 to 102 days, and were randomly assigned to drug conditions in both the ETOH and pentobarbital studies. Differences in mean ages among the line/dosage groups were no larger than 4 days in each of the two studies. Mean ages were 74 days in the ETOH study and 62 days in the pentobarbital study.

Procedure. The testing procedures of Experiment 2 were the same as for Experiment 1, except that the pentobarbital study employed only 0, 10, 20, and 30 mg/kg doses. The data analytic approach was the same as for Experiment 1, as was the derivation of the primary indices: HORZ, DIST, REST, SPEED, LENGTH, and VERT. An additional study examined effects of 0 and 20 mg/kg doses of pentobarbital in mice tested in the circular (60 cm) LVE photocell activity apparatus used for most of our previous work with these lines. Mice were placed in the LVE activity monitor immediately following injection for a 15-min test, as was the case for the studies using the Omnitech activity monitors.

Results and Discussion

The differing shapes of the 15-min ETOH dose-response curves for HORZ in LS and SS mice (Fig. 4) are very similar to many reports on their ETOH response in other photocell apparatuses. The patterns of LS/SS differences described here were consistent across the three time periods and, thus, only the 15-min data are presented. The current study offers the additional perspective of the other five indices. The genotype by dose interaction was significant for all six indices ($p < 0.001$). In all but VERT, both genotypes showed ETOH effects that were similar in the two lines up to 1.0-1.5 g/kg. But the sedative limb of the biphasic curve was clear in LS mice at 2.0 g/kg while SS mice were most activated at 2.0 g/kg. Thus, across all ETOH doses reported here, SS mice ran faster and farther, and rested less than saline-treated controls. The LS mice had a clearly biphasic curve that returned to baseline at 2.0 g/kg for HORZ, DIST, and REST, and nearly returned to baseline levels for SPEED and LENGTH. A linear trend component was significant in SS mice for these five indices ($p < 0.001$; accounting for at least 90% of the variance attributable to the dose effect in each index). In contrast, the quadratic component was significant in LS mice for each of these five indices ($p < 0.001$). VERT was strongly attenuated in LS mice as was the case for B6 in Experiment 1. In contrast, 1.0 g/kg increased mean VERT in SS mice (nonsignificantly) and then clearly decreased it at higher doses, paralleling the observation of VERT in D2 mice reported above. The main effect of dose was significant for VERT ($p < 0.001$), but the line by dose interaction was not.

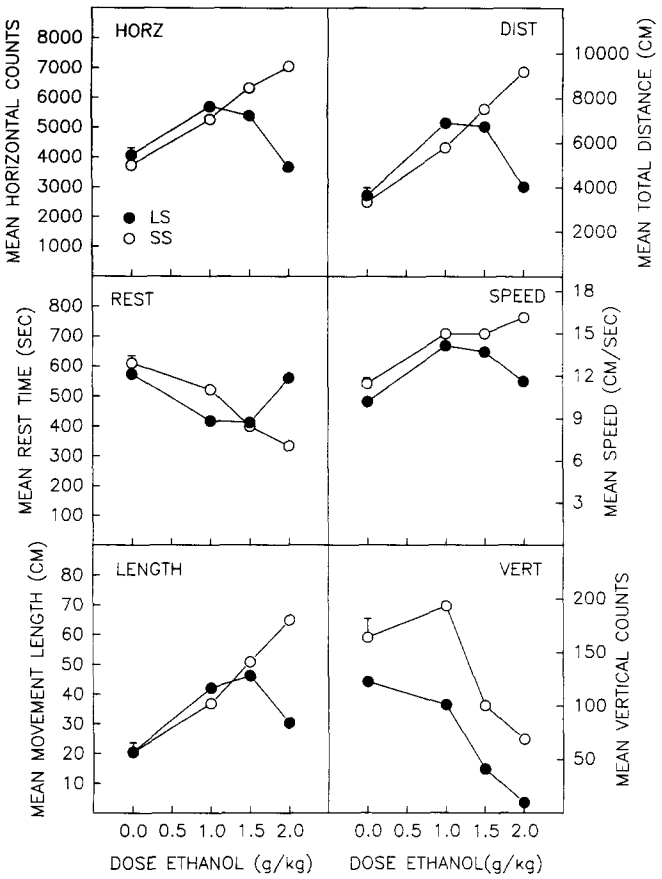

FIG. 4. Dose-response curves for 15-min ETOH effects on six behavioral indices in male long-sleep (LS) and short-sleep (SS) selectively bred lines. Each panel represents a different behavioral measure as described in the text and in Fig. 1. Sample sizes were 18 for LS and 23 for SS groups. Vertical bars represent generalized standard errors of the mean derived from analysis of variance error terms.

The HORZ and DIST dose-response curves for sodium pentobarbital in the LS and SS mice show clear biphasic effects for the 15-min data (Fig. 5), with strong activation at 10 and 20 mg/kg, and no line difference. This locomotor activation was accompanied by decreased resting time (REST) and increased SPEED. Both of these indices also showed evidence of a sedative limb in the biphasic curve at the 30 mg/kg dose. LENGTH showed consistent increments across the dose range in both lines, and VERT showed a pattern similar to the D2 data from Experiment 1 where no decrement was apparent at 10 mg/kg, but strong inhibition of VERT at 20 and 30 mg/kg. This characterization is supported by the absence of line by dose interactions for each of the six indices. A main effect of dose was significant for each index ($p < 0.001$). Linear trend components of the dose factor were significant for each index ($p < 0.001$), as was the quadratic component for HORZ, DIST, REST, SPEED, and VERT ($p < 0.05$).

Because some of the genotypic patterns in the Experiment 1 pentobarbital study varied across the three blocks of time in the 15-min test, we also examined this possibility in the DIST index in the LS/SS data set. The two lines showed a similar change in DIST across time, but the evidence of a sedative limb of the biphasic curve at 30 mg/kg was stronger in blocks 2 and 3 (Fig. 6). The two-way interaction of dose and time was significant in a repeated measures ANOVA ($p < 0.001$).

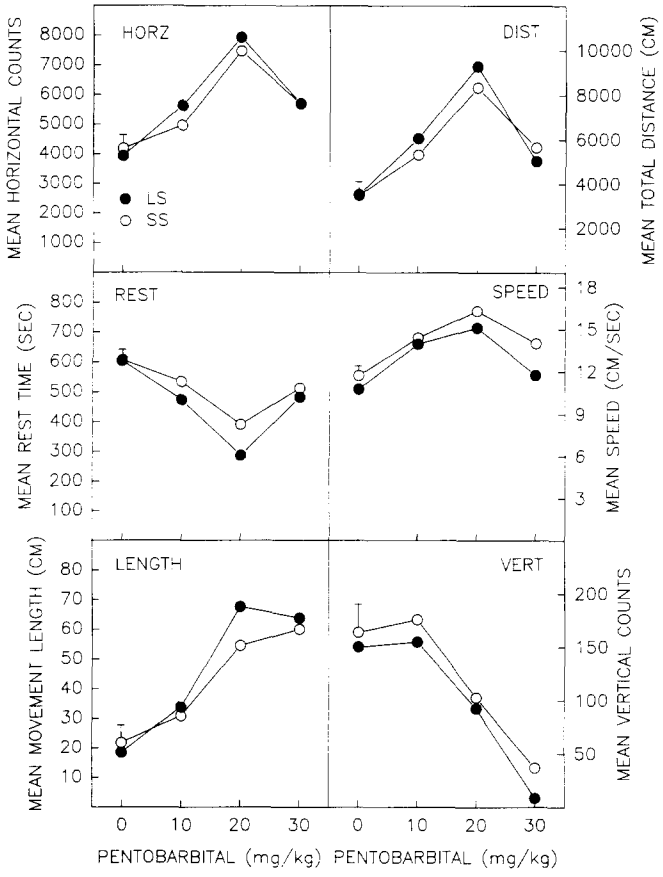

FIG. 5. Dose-response curves for 15-min pentobarbital effects on six behavioral indices in male long-sleep (LS) and short-sleep (SS) selectively bred lines. Each panel represents a different behavioral measure as described in the text and in Fig. 1. Sample size was 10 in each group. Vertical bars represent generalized standard errors of the mean derived from analysis of variance error terms.

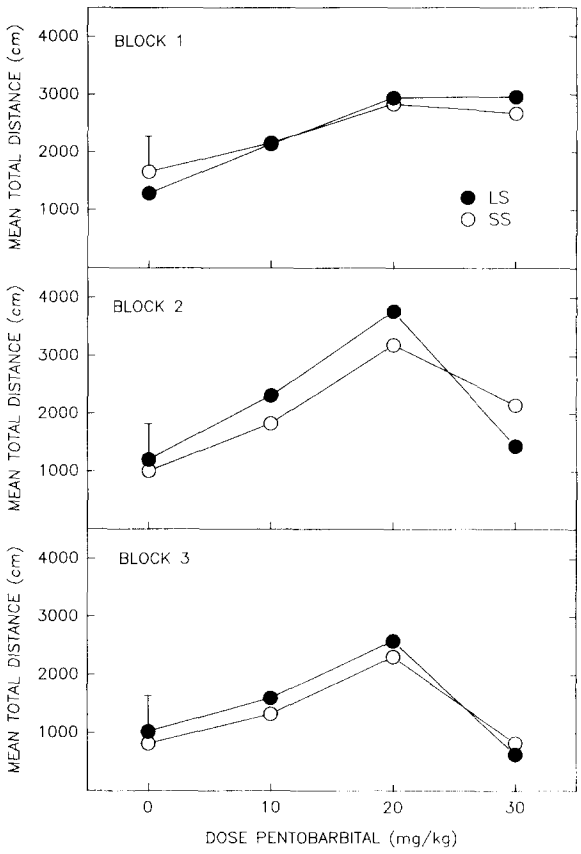

FIG. 6. Pentobarbital effects on distance traveled across three successive 5-min blocks of time. Data are from the same LS and SS mice whose total 15 min data are shown in Fig. 5. Vertical bars represent a generalized standard error of the mean derived from the analysis of variance error term.

This lack of an LS/SS difference in pentobarbital's activating effect was surprising, given earlier work from our laboratory that indicated that SS mice showed more locomotor activation to this drug in a comparable dose range to that tested here (8). Sanders (35) had previously published data where SS mice appeared to show greater locomotor activation to pentobarbital than LS mice, but a line by dose interaction was not reported. It is possible that apparatus differences account for the discrepancy of the current finding of genotypic similarity to the finding of greater SS activation in the earlier work (8). That study used a circular photocell apparatus, where mice were tested inside a small square chamber that had a grid floor for assessment of coordination. We tested an additional set of LS and SS mice in the same circular apparatus, with an acrylic floor (60 cm) rather than the grid. These data (Table 1) also indicate a similarity of the two lines. Pentobarbital effectively increased photocell counts (dose, $p < 0.001$), but to a similar degree in the two lines (dose by line interaction not significant). Thus, the earlier report of greater activation in SS mice apparently resulted from the introduction of additional sedative sensitivity in LS mice with the use of the grid floor, or possibly the use of a nonsimultaneously tested con-

TABLE 1

MEAN (±SEM) PHOTOCELL COUNTS FOLLOWING SODIUM PENTOBARBITAL

	Dose of Sodium Pentobarbital (mg/kg)	
Line	0	20
LS	676 ± 133 (3)	1329 ± 27 (4)
SS	817 ± 120 (4)	1225 ± 111 (4)

Sample sizes in parentheses.

trol group for the most discrepant doses reported in our earlier study (8).

GENERAL DISCUSSION

The most striking finding reported here is that B6 mice do not show the typical biphasic curve for either ETOH or pentobarbital. The small hint of activation in B6 at the lowest doses for both drugs is only a fraction of the size of the activation seen in D2 mice or either of the LS or SS lines. This outcome is consistent with the possibility that some or all of the same genes that control the B6/D2 difference in ETOH activation are relevant for their difference in pentobarbital activation as well. While we are aware of the limitations of strong conclusions about genetic correlations from the use of only a pair of inbred strains [e.g.,(6)], the qualitative difference of the B6 curve from other genotypes is provocative [cf. (12)]. Because the number of genes responsible for the ETOH activation difference may be small (10), the possibility exists for identification of genes common to both ETOH and pentobarbital activation [cf. (2,15,22,31)].

Sodium pentobarbital produced clear locomotor activation in four of the five genotypes tested here (all but B6). This activation was seen within 5 min of injection, and extended up to a dose of 30 mg/kg. At a dose this high, activation began to wane by the third 5-min block of time in both the data from the D2 and B6 strains in Experiment 1 and from the LS and SS lines in Experiment 2. This time dependence contrasts with the activating effect of ETOH on locomotor activity which shows strong activating effects throughout the 15-min time period at doses up to 1.5 g/kg (and higher in SS mice). Such a difference may be attributable to a stronger role for factors involving absorption and distribution of pentobarbital than ETOH. ETOH is very rapidly absorbed to a peak blood level within less than 5 min following an IP injection (14). In contrast, peak brain and blood levels of pentobarbital may be reached more slowly than ETOH because of its higher lipid solubility, and the rate constant for absorption may vary among genotypes [e.g. (16,18,37)]. Whether absorption profiles can account for the difference in the direction of dominance seen with F_1 mice in Experiment 1 can only be answered by further study. The general question of whether the basic strain difference in pentobarbital effects can be traced to genetically based differences in pharmacokinetics can be partially addressed by available literature (37). D2 mice were shown to have higher blood pentobarbital levels than B6 mice over a time period from 30–180 min following injection, but not at a 10-min time period (37). This pattern would explain (in part) why D2 mice, in that paper, showed longer loss of righting reflex durations following pentobarbital administration. They appeared to absorb pentobarbital to a higher peak, and eliminate it more slowly than B6 mice. However, in our study, loss of righting reflex durations did not differ. It is possible that D2 have a relative CNS resistance to sedative effects of pentobarbital, but that absorption/elimination differences counteracted this to produce equivalent loss of righting reflex durations to B6. This hypothesis would lead to predictions different than the outcomes reported here. D2 mice should either show more sedative sensitivity to pentobarbital, and a left shift of the sedative limb of locomotor activity dose-response curve (relative to B6), or the two might have been predicted to have similar dose-response curves if the absorption/elimination variables were not crucial. However, the finding that B6 lacks activation is not addressable by any such patterns of absorption/elimination of pentobarbital.

The strong similarity of the LS/SS dose-response curves for pentobarbital and ETOH doses less than 2.0 g/kg further illustrates the apparent specificity of the LS/SS selection for genes relevant to ETOH's sedative potency. Because these are genes with implications for CNS function (38), it is interesting that even though genes for degree of pentobarbital activation can be postulated (on the basis of Experiment 1 strain differences), these genes are apparently not different in the LS and SS lines. Such a conclusion is of interest in contrast with the reports of differences in GABA receptor functioning in the LS and SS lines (1).

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