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Differential Convulsive Susceptibility of High-Activity and Low-Activity Selected Mice in Response to GABA Antagonists

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McINTYRE, T. D. AND H. P. ALPERN. *Differential convulsive susceptibility of high-activity and low-activity selected mice in response to GABA antagonists.* PHARMACOL BIOCHEM BEHAV **26**(1) 71–75, 1987.—Lines of mice selectively-bred for High and Low-Activity in an open-field maze were tested for seizure susceptibility to three analeptics: flurothyl, pentylenetetrazol and bicuculline. The major finding was that two replicate High-Activity lines were more susceptible to myoclonic convulsions but less susceptible to clonic convulsions than their respective replicate Low-Activity lines. The major exception to this finding was that the High and Low-Activity lines did not differ for bicuculline-induced clonus although females tended to conform to the general pattern. These results are interesting because they demonstrate that diametrically opposite susceptibility to myoclonus and clonus is not an isolated phenomenon. Similar seizure susceptibility patterns and activity differences have also been reported for the Long-Sleep and Short-Sleep selectively-bred mouse lines. Further, since the progenitor population of the High-Activity and Low-Activity lines were developed from strains that were also part of the progenitor population of the Long-Sleep and Short-Sleep lines, it is hypothesized that some of the same alleles underwent selection in both selective-breeding programs.

Selected lines Activity Pharmacogenetics GABA convulsants

TWO separate bidirectional selective-breeding programs have established lines of mice that display similar differences in open-field maze activity [8, 9, 17, 18], even though one program employed duration of ethanol-induced hypnosis as the selection criterion [17,18]. The Long-Sleep (LS) and Short-Sleep (SS) lines that were selected for different hypnotic reactions to a sedative dose of ethanol are interesting because recent evidence suggests that they also display similar reactions to a wide variety of CNS hypnotic-depressants, and thus, they may have been selected for an attribute other than specific sensitivity to alcohol. The hypothesis that the hypnotic reactions displayed by these lines are alcohol-specific originated with a study that examined how these lines responded to the soporific effects of ethanol, methanol, n-butanol, pentobarbital, paraldehyde, chloral hydrate and trichloroethanol [11], and showed that only the aliphatic alcohols separated the two lines. In a reanalysis of those data, however, it was shown that every CNS depressant employed actually differentiated the two lines [3]. Further, several other studies show that the LS line is more sensitive to the soporific effects of alcohols [11,14], barbiturates [3, 4, 21], benzodiazepines [21], general anesthetics [20], and other miscellaneous agents such as L-phenylisopropyl adenosine [10]. The conclusion, therefore, that these lines display a unique sensitivity to aliphatic alcohols is no longer tenable. Another avenue of investigation supporting this interpretation shows that these mouse lines can also be differentiated by their reaction to a number of convulsive agents. Specifically, the analeptics flurothyl, methyl-β-carboline and pentylenetetrazol all induce myoclonus more rapidly in SS mice than in LS mice, but clonus more rapidly in SS mice. Bicuculline, however, induces both myoclonus and clonus more rapidly in the SS line in comparison to the LS line. Additionally, caffeine, picrotoxin and strychnine induce only clonus, and always more rapidly in SS mice than LS mice [22]. As argued in more detail elsewhere [20], the cumulative evidence supports the conclusion that these lines were probably selected for differences in some general aspect of brain excitability. For what follows it is important to note that the LS and SS lines were derived from a heterogeneous strain (HS) that was created by systematically intercrossing mice from eight inbred strains (A, AKR, BALB/c, C3H/2, C57/BL, DBA, Is/Bi and RIII) and subsequently maintained by random mating [19]. Animals from this stock population were

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TABLE 1

MEAN LATENCIES (SEC) ±S.E. FOR FLUROTHYL (5.0 μl) INDUCED MYOCLONUS AND CLONUS FOR HIGH AND LOW-ACTIVITY REPLICATE LINES

	H1	L1	H2	L2	C1	C2
			Myoclonus			
Male	25.1 ±2.1*	35.1 ±3.9	21.1 ±1.8†	26.1 ±1.8	36.1 ±7.2	32.6 ±4.4
Female	25.2 ±2.1	27.5 ±2.1	25.7 ±4.6*	38.7 ±3.2	29.0 ±2.9	35.7 ±3.6
			Clonus			
Male	200.1 ±17.6*	40.4 ±11.6	248.8 ±50.7*	11.1 ±2.9	263.4 ±68.1	196.5 ±53.8
Female	343.8 ±43.0*	25.1 ±19.0	320.1 ±56.1*	37.0 ±12.0	278.8 ±54.4	123.0 ±29.8

*$p<0.05$ and †p approaches 0.05 for one-tailed protected t-test comparisons.

TABLE 2

MEAN LATENCIES (SEC) ±S.E. FOR PENTYLENETETRAZOL (70.0 mg/kg) INDUCED MYOCLONUS AND CLONUS FOR HIGH AND LOW-ACTIVITY REPLICATE LINES

	H1	L1	H2	L2	C1	C2
			Myoclonus			
Male	58.6 ±5.2†	67.1 ±5.4	50.6 ±1.2†	59.5 ±5.4	92.3 ±3.8	64.9 ±2.5
Female	54.1 ±3.5†	64.1 ±5.5	45.0 ±1.7*	60.0 ±4.4	74.5 ±1.9	57.5 ±1.4
			Clonus			
Male	223.4 ±59.9*	33.9 ±8.6	83.5 ±8.2†	33.6 ±8.8	99.6 ±22.1	56.3 ±15.7
Female	276.6 ±59.9*	19.5 ±5.4	99.1 ±9.3*	22.3 ±6.2	118.4 ±14.4	45.0 ±15.0

*$p<0.05$ and †p approaches 0.05 for one-tailed protected t-test comparisons.

then examined with respect to loss of their righting response subsequent to an intraperitoneal injection of ethanol. Animals that displayed a long hypnotic duration were mated together as were animals that displayed a minimal hypnotic reaction. After 16 generations of mating using these criteria there was no overlap in the sleep time distributions of the mouse lines.

In the other bidirectional selection study, replicate selected lines and replicate control lines were derived using open-field maze activity as the selection criterion [6–9]. Briefly, an F3 generation was created by mating mice from two inbred strains (C57/BL and BALB/c) known to be quite distinct in their open-field maze behavior. This F3 generation served as the foundation population for subsequent selection and the least active and most active from each of 10 randomly selected F3 litters were designated progenitors of selected lines Low 1 (L1) and High 1 (H1). In a similar fashion the Low 2 (L2) and High 2 (H2) lines were derived. Further, a male and female from each of 10 additional F3 litters were randomly selected and mated to produce one Control Line (C1), while representatives from each of 10 other F3 litters served as progenitors of the second Control Line (C2). All lines are currently in the fifty-fourth generation, with active selection pressure having been relaxed subsequent to the thirtieth generation. Although these lines have been in existence for a greater period of time than the LS and SS lines, little data are available to show mechanistically why the High and Low-Activity lines differ in the open-field maze. One hypothesis is that the High and Low-Activity lines also differ in some general aspect of brain excitability, and thus, they may share some of the same alleles as the LS and SS lines. This is not unreasonable, since both selection programs used certain mouse strains in common (C57/BL and BALB/c), and further, since SS mice are highly active in the open-field maze in comparison to LS mice. To test

TABLE 3

MEAN LATENCIES (SEC) ±S.E. FOR BICUCULLINE (6.0 mg/kg) INDUCED MYOCLONUS AND CLONUS FOR HIGH AND LOW-ACTIVITY REPLICATE LINES

	H1	L1	H2	L2	C1	C2
			Myoclonus			
Male	71.2 ±14.6*	105.6 ±17.1	80.0 ±7.9*	104.9 ±14.6	84.5 ±8.9	70.5 ±14.3
Female	62.5 ±2.5†	84.5 ±5.4	47.7 ±4.5*	84.5 ±7.8	59.6 ±5.6	77.8 ±11.2
			Clonus			
Male	128.9 ±55.7	146.8 ±51.2	169.3 ±48.9	115.9 ±42.0	174.4 ±62.4	132.4 ±52.1
Female	90.1 ±17.9	53.3 ±15.3	90.8 ±13.7	50.1 ±15.8	31.9 ±7.2	161.1 ±63.2

*$p<0.05$ and †p approaches 0.05 for one-tailed protected t-test comparisons.

whether the High and Low-Activity lines also differ with respect to brain excitability, the onset of myoclonus and clonus in these lines was evaluated after administration of the analeptics flurothyl, pentylenetetrazol and bicuculline.

METHOD

This experiment used a total of 144 male and 144 female H1, H2, L1, L2, C1 and C2 mice 150–200 days old, that were offspring of mating pairs (generations 49–50) from the Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309. Littermates were housed together but randomly distributed across experimental groups. Animals were maintained on a 12 hr light/dark cycle, with food and water continuously available. It should be noted that at least 30 days prior to the experiment all animals served as controls in another study; they received a single intraperitoneal injection of saline and were placed in an open-field apparatus for a very brief period of time.

Eight males and eight females from each of the 6 lines (H1, H2, L1, L2, C1 and C2) were administered a single dose of one of three convulsants: flurothyl, pentylenetetrazol or bicuculline. Five microliters of the inhalant flurothyl (bis [2,2,2-trifluoroethyl] ether) were introduced into a 431.6 ml testing chamber via a Hamilton microsyringe [1]. Latency to myoclonus (first jerk of the head and neck musculature) and latency to clonus (generalized convulsion with loss of body posture) were recorded. If an animal did not exhibit clonus within 8 minutes, it was given that time for a score. For bicuculline, animals were intraperitoneally injected with 6.0 mg/kg of (+) bicuculline dissolved in 0.9% saline (pH 2.5), while for pentylenetetrazol, animals were intraperitoneally injected with 70.0 mg/kg dissolved in physiological saline. After receiving one of the latter two agents an animal was placed in the same chamber as was used for flurothyl; all other aspects of the procedure remained the same. Testing took place between 0830 and 1130 to minimize the influence of circadian rhythms [4,28]. The doses of the convulsants employed were those that had been used previously with the LS and SS lines; and as a result of pilot dose-response studies with these drugs, care was taken to select doses that would yield convulsions in the High and Low-Activity lines, but not supramaximal ones which would obscure any group differences.

RESULTS

Although the design of the experiment is somewhat complex in that several factors were examined, the results, with some exceptions, are remarkably simple and consistent. When compared with the Low-Activity lines the onset of myoclonus seemed to be much shorter for the High-Activity line, but the opposite outcome was found for the onset of clonus (see Tables 1, 2 and 3). One key departure from this generalization is that males given bicuculline do not appear to show a line difference for clonic susceptibility.

For each drug, myoclonus and clonus data for the High-Activity and Low-Activity lines were analyzed separately with three-way analyses of variance (Line × Sex × Replicate); where significant, individual group comparisons were made with protected t-tests (see Tables 1, 2 and 3). The statistical analyses confirm that Line differences were the most ubiquitous and robust effects. In certain instances, however, Sex and Replicate effects were apparent. The specific effects found are: for flurothyl myoclonus, Line, $F(1,56)=13.68$, $p<0.001$, Sex × Replicate, $F(1,56)=9.16$, $p<0.01$; for flurothyl clonus, Line, $F(1,56)=116.89$, $p<0.001$, Sex, $F(1,56)=6.38$, $p<0.05$, Sex × Line, $F(1,56)=5.26$, $p<0.05$; for pentylenetetrazol myoclonus, Line, $F(1,56)=11.73$, $p<0.001$, Replicate, $F(1,56)=5.45$, $p<0.05$; for pentylenetetrazol clonus, Line, $F(1,56)=43.79$, $p<0.001$, Replicate, $F(1,56)=13.23$, $p<0.001$, Line × Replicate, $F(1,56)=13.65$, $p<0.001$; for bicuculline myoclonus, Line, $F(1,56)=15.42$, $p<0.001$, Sex, $F(1,56)=7.54$, $p<0.01$; for bicuculline clonus, Sex, $F(1,56)=5.78$, $p<0.05$. When the line effect is further dissected by comparing individual groups (see Tables 1, 2 and 3), for myoclonus, in every case the High-Activity lines exhibited a lower mean latency than their corresponding Low-Activity counterparts. Nevertheless, for females given flurothyl, one set of replicates (H1 and L1) were not different, and in certain other instances, most notably for animals given pentylenetetrazol, three group com-

parisons very closely approached statistical significance at the 5% level. Further, for clonus, bicuculline failed to distinguish the High and Low-Activity lines, although females given bicuculline tended to respond similarly to females given flurothyl or pentylenetetrazol. The large variability in clonus values for animals that had been given bicuculline undoubtedly contributed to masking any effect that might have been present. It should be mentioned that the Control Lines' data were not included in the data analyses, but their means and standard errors are included in Tables 1, 2 and 3. It is readily seen that there is no consistent trend in their pattern of response to the three drugs: in some cases their values fall between the High-Activity and Low-Activity lines, but in other instances they do not.

DISCUSSION

The results of this experiment clearly demonstrate that the High and Low-Activity selected lines differ in their responsiveness to three analeptics. Of particular significance is the finding that for myoclonus, flurothyl, pentylenetetrazol and bicuculline had, or tended to have, a proconvulsant effect on the High-Activity lines in comparison to the Low-Activity lines, but for clonus, flurothyl and pentylenetetrazol were preconvulsant for the Low-Activity lines. These diametrically opposite effects are important because flurothyl and pentylenetetrazol induce a similar pattern when administered to the LS and SS selected lines, with the SS line behaving like the High-Activity lines [2, 13, 26]. Since C57/BL and BALB/c inbred mouse strains were progenitors of the High and Low-Activity lines and were also used along with other inbred strains to form the progenitor Heterogeneous Stock for the LS/SS selection study, and since these latter lines also display distinct differences with respect to behavioral activity [5], it is not unreasonable to conjecture that some of the same alleles underwent selection in both selective breeding programs.

Two additional points concerning the results deserve comment. First, the fact that, with one exception (flurothyl-induced myoclonus in female mice), all High-Activity replicate lines exhibited shorter myoclonic latencies than their corresponding Low-Activity lines, underscores the reliability of the line differences found in this experiment. The one exception just noted is either a chance occurrence or due to genetic differences between the replicate lines for female mice. The latter is supported by the fact that for myoclonus the magnitude of the line difference for females is greater in the second replicate pair in response to all three analeptics. Second, Sex and Sex × Line effects attenuated the magnitude of the Line effect. For pentylenetetrazol, Sex was an inconsequential factor, but for flurothyl males tended to be more sensitive than females and for bicuculine the reverse was true. In fact, if one examines the clonus data for bicuculline (see Table 3), male scores are much more variable than those of females, which tends to obfuscate the proclivity of Low-Activity females to be more sensitive than High-Activity females. Perhaps another dose of bicuculline would have more clearly differentiated the High and Low-Activity lines for clonus, although the dose of bicuculline selected was based on the findings of a pilot study showing that this dose was representative of other midrange doses, and distinguished convulsive susceptibilities in the selected lines. In fact, a case can be made that two doses of bicuculline should have been used, since the dose used took significantly longer to induce myoclonus in the High and Low-Activity lines but took significantly less time to induce clonus in the High-Activity lines when compared to the latencies for the other two analeptics.

Since at least two of the convulsants employed here are GABAergic antagonists (bicuculline and pentylenetetrazol), and since several reports have indicated a relationship between behavioral activity and GABAergic transmission [12, 15, 25], differences in general CNS excitability for the selected lines may be related to GABAergic activity. Interestingly, unpublished data from this laboratory demonstrate that the Low-Activity lines are significantly more sensitive than the High-Activity lines to the soporific effects of the benzodiazepine chlordiazepoxide, which has been shown to exert its soporific, anxiolytic and anticonvulsant properties via an interaction with the benzodiazepine-GABA receptor-chloride ionophore [23, 24, 26, 27].

In summary, these findings show that in mice the reversal of convulsive susceptibility to certain analeptics originally found for the LS and SS lines (i.e., compared to SS, LS display a longer latency to myoclonus but a shorter latency to clonus) is not a unique phenomenon. Further, these findings support the conjecture that two bidirectional selective breeding programs, which used different selection criteria, may have selected many of the same alleles.

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