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Effect of Low Dose Ethanol on Spontaneous Motor Activity in Alcohol-Preferring and -Nonpreferring Lines of Rats[1]

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WALLER, M B , J M MURPHY, W J McBRIDE, L LUMENG AND T -K LI *Effect of low dose ethanol on spontaneous motor activity in alcohol-preferring and -nonpreferring lines of rats* PHARMACOL BIOCHEM BEHAV 24(3) 617–623, 1986 —To determine if behavioral arousal may be associated with ethanol preference, the effects of low to moderate doses of ethanol on spontaneous motor activity (SMA) were studied in the selectively bred alcohol-preferring (P) and -nonpreferring (NP) lines of rats as well as in the Maudsley Reactive (MR/N) and Nonreactive (MNR/N) strains Alcohol-naive rats had food and water available ad lib, but food was removed 24 hr before and during activity testing After an intraperitoneal injection of saline (5 ml) or ethanol (0 12 to 1 5 g/kg), SMA was monitored every three min for 30 min in an electronic activity monitor The P and MR/N rats exhibited increased SMA after doses of 0 12 and 0 25 g/kg Both the NP and MNR/N rats failed to show increased SMA at any ethanol dose Moderate doses of ethanol, 1 0 and 1 5 g/kg, consistently depressed SMA in all lines/strains In 24 hr-fasted rats, increased SMA occurred within 6–12 min after injection, but free-fed rats exhibited increased SMA 12–24 min after an ethanol dose of 0 25 g/kg Free-choice drinking scores (10% ethanol (v/v) versus water) for the P, MR/N, MNR/N and NP rats were 6 6±0 5, 4 9±0 8, 2 2±0 7 and 1 4±0 3 g ethanol/kg body wt/day (mean±SEM), respectively The data indicate a positive relationship between ethanol preference and ethanol-induced motor stimulation and suggest that hyperactivity may be an expression of the positive reinforcing effect of ethanol for alcohol-preferring rats

Ethanol Low-dose effects of ethanol Alcohol-preferring and -nonpreferring rats Spontaneous motor activity

WE have recently reported that the selectively-bred alcohol-preferring (P) and alcohol-nonpreferring (NP) lines of rats do not differ in initial sensitivity to the depressant action of ethanol, but the P rats acquire acute tolerance to the depressant effects of ethanol more rapidly than do the NP rats [31] Although the more rapid acquisition of tolerance may facilitate sustained high ethanol intake, it cannot adequately account for the ethanol preference of the P rats, because blood alcohol concentrations (BAC) that induce this kind of tolerance are considerably higher than those usually attained with voluntary oral consumption [15] Furthermore, we have found that when the BAC exceeds 50 mg%, the P animals begin to curtail their voluntary alcohol consumption [30] These results have suggested to us that the reinforcing properties of ethanol should be sought at low blood alcohol concentrations

Low doses of ethanol reportedly are excitatory and potentiate behaviors such as spontaneous motor activity (SMA) in rodents (for review see [22]) However, this action of ethanol appears to be task-specific and is more consistently seen in mice than in rats [22] Studies with inbred and selectively-bred mouse lines have demonstrated that the stimulatory effect of ethanol is genetically influenced [5, 10, 23, 24, 27]

One plausible hypothesis is that stimulation of SMA induced by low doses of alcohol is an expression of the positive reinforcing feature of ethanol Such a hypothesis is consistent with the notion that excitation effects (or euphoria) of drugs are important in their dependence liability and the possibility that this stimulating effect might facilitate alcohol ingestion in man [25] Accordingly, studies were performed with several lines of rats, including the Maudsley Reactive

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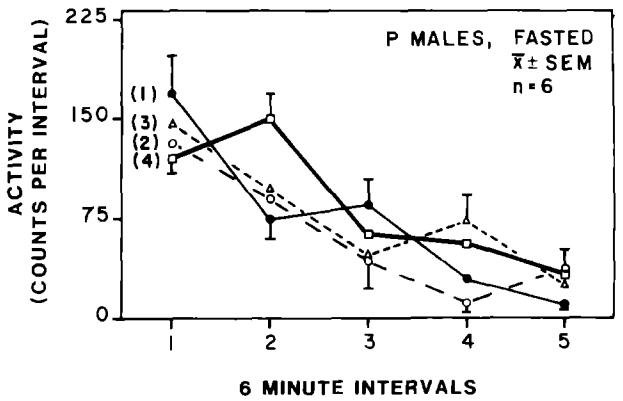

FIG 1 Effect of repeated saline injections on SMA of fasted (24 hr) male P rats At least four days elapsed between each injection Differences across trials at each 6 min interval were statistically evaluated with ANOVA

TABLE 1

SPONTANEOUS LOCOMOTOR ACTIVITY OF THE FOUR ANIMAL LINES DURING THE 30 MINUTES IMMEDIATELY FOLLOWING AN INTRAPERITONEAL INJECTION OF SALINE

	Activity Counts			
Sex	P	NP	MR/N	MNR/N
Male	811 ± 100	955 ± 122	801 ± 62	735 ± 120
Female	1141 ± 100*†	879 ± 94‡	664 ± 59	538 ± 74

Statistical significance across lines determined with ANOVA followed by Newman-Keuls test, within line gender differences evaluated with the independent t-test

*Significant difference from P males ($p<0.05$)

†Significant difference from NP MR/N and MNR/N females ($p<0.05$)

‡Significant difference from MNR/N females ($p<0.05$)

Mean ± SEM n=10

strain in which behavioral arousal has been reported [13] The purpose of the studies reported here was to determine whether there is an association between ethanol drinking preference and the stimulatory effect of low doses of ethanol

METHOD

Animals

Adult alcohol-preferring (P), -nonpreferring (NP), Maudsley Reactive (MR/N) and Maudsley Nonreactive (MNR/N) animals were used The P and NP lines were developed through selective breeding from a Wistar colony (Wrm WRC(WI)BR) at the Walter Reed Army Institute of Research [17] and maintained in our facilities The Maudsley rats were obtained from the National Institutes of Health Alcohol-naive male and female rats from each line were individually housed in a temperature- and humidity-controlled environment with a 12 hr day-night cycle (0700 hr lights on, 1900 hr lights off) At the start of the experiments, the weights of the animals were (male/female) P, 335–416/211–280 g, NP, 190–285/103–179 g, MR/N, 250–315/159–182 g, MNR/N, 164–253/131–176 g Except where indicated, standard laboratory chow (Wayne Lab-Blox, Allied Mills, Inc , Chicago, IL) and water were freely available in the home cages throughout the experiments An ethanol preference score was determined for each rat upon completion of all activity testing. The preference test procedure has been described previously in detail [17]

Apparatus

Spontaneous motor activity An electronic activity monitor (Model 31404, Stoelting Co , Chicago, IL) was used to measure spontaneous motor activity (locomotion) following an injection of ethanol or saline The gain was adjusted to record slow to moderate gross movement, such as locomotion, and not rapid movements such as grooming and scratching The validity of this gain setting was verified by visual observation of several animals not used in the study For subsequent experiments, a standard plastic cage (43×21×20 cm) was placed on the sensor unit and, after the rat was placed in the cage, covered with a nonmetallic lid The sensor and cage were located in a quiet, dimly lit room All testing in the activity monitor was done between 0900 and 1500 hr

Ethanol determination In some P and MR/N animals brain ethanol content and cerebral BAC were measured The brains were freeze-stopped by means of the copper tubing method of Sippel [26] Cerebral blood samples were collected from the blood that filled the cavity made upon withdrawal of the copper tubing The gas chromatography procedures for blood and tissue sample preparation and ethanol determination were the same as those described previously [18]

Statistical analyses Analyses of variance and post-hoc Newman-Keuls tests were used to evaluate statistical differences between SMA after different ethanol doses and after an injection of saline In experiments using a single ethanol dose, a paired t-test was employed to compare SMA after ethanol and saline injections The results of the activity testing are expressed as the mean (±SEM) counts per time interval above or below the saline control (saline=0) calculated from the individual ethanol-saline difference scores or as the mean (±SEM) actual counts per time interval An independent t-test was used to evaluate mean BAC values for statistically significant differences In all cases, $p<0.05$ was taken to indicate a significant difference between scores

Procedure

Experiment 1 The effect of repeated saline injections on SMA of fasted rats Fasted (24 hr) male P rats (n=6) and female MR/N rats (n=8) were given a series of four injections of 5 0 ml of sterile saline (0 9% NaCl in water) The injections were at least four days apart Immediately after an

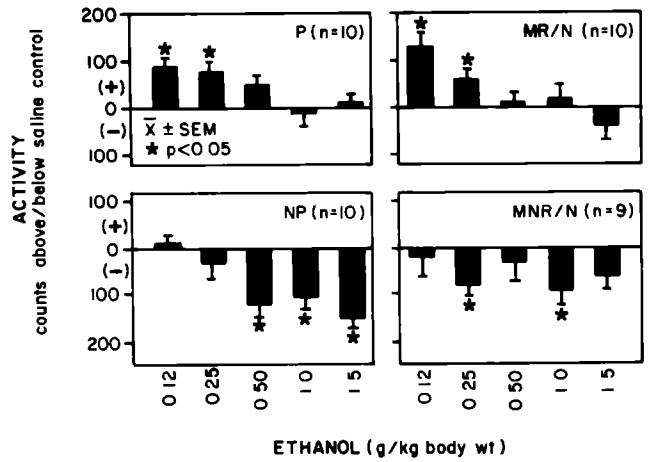

FIG 2 Effect of ethanol on spontaneous motor activity of fasted P, NP, MR/N and MNR/N male rats in the first six minutes postinjection The activity shown is relative to the saline control (saline=0) All injections were given intraperitoneally following a counterbalanced design At least four days elapsed between injections Differences were statistically evaluated with ANOVA followed by a Newman-Keuls test *Significant difference when compared with the saline value

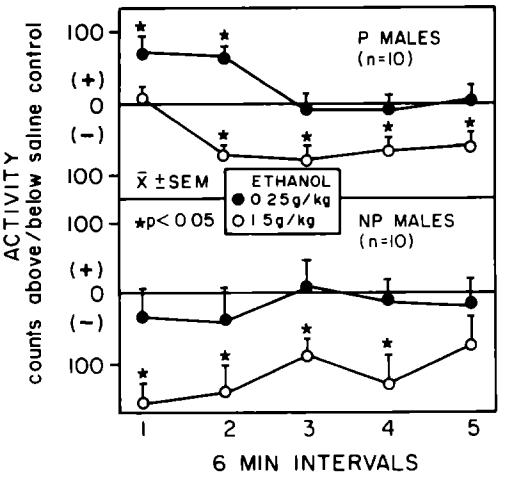

FIG 3 Effect of ethanol on spontaneous activity of fasted P and NP rats for successive six minute intervals postinjection (total time=30 minutes) for two selected doses of ethanol Differences were statistically evaluated with a paired *t*-test *Significant difference when compared with the saline value

injection, the SMA was monitored every three min for a total of 30 min in the activity monitor described above Prior to the series of saline injections, these animals were habituated by repeated exposure to the apparatus

Experiment 2 The effect of repeated counterbalanced injections of ethanol on SMA of fasted rats Since not all possible sequences of ethanol doses and saline could be tested, an incomplete counterbalancing technique was used to control for possible sequencing effects To the extent possible, each treatment appeared an equal number of times in each ordinal position and preceded and followed every other condition Before each activity-testing session, rats were food-deprived for 24 hr and then received an intraperitoneal injection of ethanol (0 12, 0 25, 0 50, 1 0 or 1 5 g/kg) or 5 0 ml of sterile saline This volume of saline was chosen because, with few exceptions, it equalled or exceeded the volume of the ethanol doses to be used (range 0 13 to 5 2 ml/animal) The ethanol for injection was a 12 0 g% solution prepared from 95% alcohol and sterile saline Each rat received an injection of each dose of ethanol or saline for a total of six injections The injections were at least four days apart Immediately following an injection, the SMA was monitored every three min for a total of 30 min in the activity monitor described above

BACs and brain alcohol concentrations were determined in fasted female P (n=6/dose) and male MR/N (n=5/dose) rats six min after the injection of either 0 12 or 1 5 g ethanol/kg in order to obtain information on the levels of ethanol which produced the observed behavioral stimulation or depression The animals used for these determinations had previously completed activity and ethanol preference testing

Experiment 3 The effect of repeated ethanol injections on SMA of free-fed and fasted rats In addition to determining if tolerance develops to repeated injections of ethanol, another objective of the present experiment was to ascertain if the ABA design could be used for future studies concerned with resolving neurochemical correlates of the low dose stimulatory effect of ethanol Free-fed animals were tested since they are more desirable to use than fasted animals for neurochemical studies Alcohol-naive male P rats were randomly assigned to two treatment groups (n=6/group) Following an ABA design, the animals received successive injections four days apart as follows Group 1 received saline (five injections), 0 25 g/kg ethanol (seven injections) and saline (three injections), Group 2 was similarly treated except the ethanol dose was 1 5 g/kg The volume of saline given to both groups was equivalent to the volume of ethanol given

However, in this experiment the time of the maximal effect of ethanol on SMA in Group 1 rats was delayed with respect to that observed with the counterbalanced design in Experiment 1 To assess whether the delay resulted from design features (ABA vs counterbalanced) or the feeding condition (free-fed vs fasted), a second phase of this experiment used 24 hr fasted animals in the ABA design After a minimum of two weeks without any injections, Group 1 rats were given successive injections four days apart after a 24 hr fast as follows saline (two injections), 0 25 g/kg ethanol (three injections) and saline (two injections) Other procedural details were similar to those described for the first phase The data from each phase of this experiment were analyzed separately with a within-subject design

In separate groups of stock Wistar animals, alcohol measurements were made as described above to determine whether the brain alcohol concentrations differed in free-fed and fasted rats after an intraperitoneal injection of 0 25 g/kg of ethanol

RESULTS

Experiment 1 The Effect of Repeated Saline Injections on SMA of Fasted Rats

Figure 1 shows the effect of individual successive injections of saline, given at least four days apart, in fasted habituated male P rats The data shown are for the entire 30 min test period An analysis of variance across trials for each 6 min interval failed to disclose any statistically significant

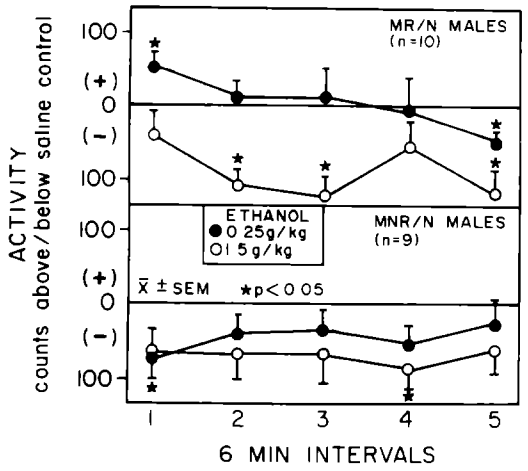

FIG 4 Effect of ethanol on spontaneous activity of fasted MR/N and MNR/N rats for successive six minute intervals postinjection (total time=30 minutes) for two selected doses of ethanol Differences were statistically evaluated with a paired *t*-test *Significant difference when compared with the saline value

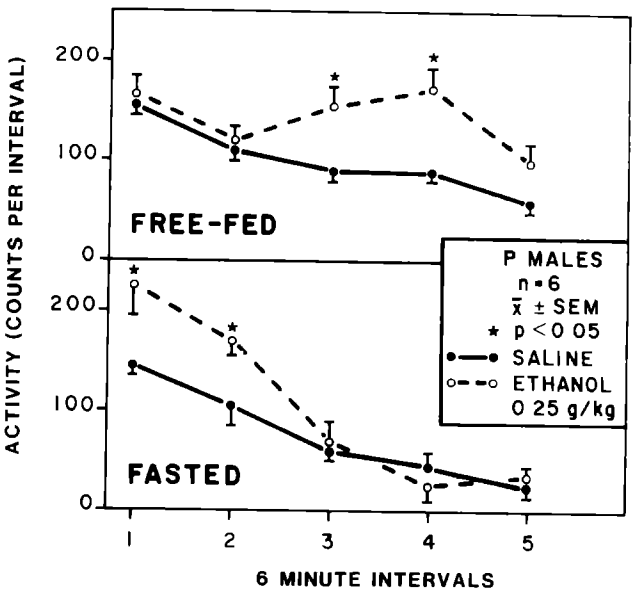

FIG 5 Effect of feeding condition on time of onset of ethanol-induced spontaneous motor activity of male P rats FREE-FED animals had access to food up to the time of testing FASTED animals were denied access to food for 24 hr before testing Differences were statistically evaluated with a paired *t*-test *Significant difference when compared with the saline value

intertrial differences in locomotor activity by these animals These results demonstrate the reproducibility of the monitored behavior Similar results were obtained with a group of fasted MR/N female animals (data not shown)

Experiment 2 The Effect of Repeated Counterbalanced Injections of Ethanol on SMA of Fasted Rats

The total activity during the 30 min period following an injection of saline in male and female animals from each line used in the study is presented in Table 1 The total SMA for the 30 min period for the male P, NP, MR/N and MNR/N groups were not significantly different from one another However, within the P line, females exhibited significantly more activity than did the males An analysis of variance across lines revealed the P females to be more active than females from the other lines and the NP females more active than MNR/N females

The effect of ethanol administered intraperitoneally on the SMA of fasted male P, NP, MR/N and MNR/N rats is shown in Fig 2 In the first six min after injection, low doses of ethanol (0 12 and 0 25 g/kg) significantly increased SMA in the P and MR/N rats, while doses of 0 5, 1 0 and 1 5 g ethanol/kg had no effect By contrast, activity of the NP and MNR/N rats was unaffected or depressed by ethanol (Fig 2) Similar results were observed in females of these four lines of rats (data not shown)

The SMA of the fasted P and NP male rats at ethanol doses of 0 25 and 1 5 g/kg over 6 min intervals of the entire 30 min test period is presented in Fig 3 The increase in activity exhibited by P rats after 0 25 g ethanol/kg occurred within the first six min and continued for 12 min postinjection Thereafter, the activity was similar to that seen following a saline injection The injection of 1 5 g ethanol/kg significantly decreased SMA of the P rats by the second 6 min interval this reduced activity lasted for the remainder of the 30 min test period The activity profile for NP rats was not significantly altered by a dose of 0 25 g ethanol/kg However, an early onset of depression of SMA of the NP group was induced by 1 5 g ethanol/kg which lasted throughout most of the 30 min test period (Fig 3)

A similar analysis of successive time blocks for the fasted male MR/N and MNR/N animals is shown in Fig 4 The MR/N rats were stimulated only in the first six min period by the 0 25 g/kg dose and were not significantly affected thereafter except for the last interval The dose of 1 5 g ethanol/kg had a generally depressant action on SMA by the MR/N male rats Both 0 25 and 1 5 g ethanol/kg tended to lower SMA in the MNR/N male animals (Fig 4)

The free-choice drinking scores, determined at the conclusion of activity testing, of the rats used in these experiments were 6 6±0 5, 4 9±0 8, 2 2±0 7 and 1 4±0 3 g ethanol/kg/day for P, MR/N, MNR/N and NP, respectively The number of animals tested for ethanol preference was 20/line or strain Since no sex differences were apparent in ethanol preference, the scores reported represent both male and female animals

In P female rats, the cerebral BAC and brain alcohol concentration during the period of maximum stimulation of motor activity were 15±0 8 mg% and 21±1 5 mg/100 g tissue, respectively, after the injection of 0 12 g ethanol/kg Similar values, 12±0 3 mg% and 16±1 1 mg/100 g tissue, were observed in MR/N males The depression of SMA induced by 1 5 g ethanol/kg at six min postinjection was associated with blood and brain alcohol levels of 223±4 0 mg% and 286±14 4 mg/100 g tissue, respectively, in P females and 214±11 0 mg% and 269±11 3 mg/100 g tissue, respectively, in the MR/N males All animals were in the fasted state

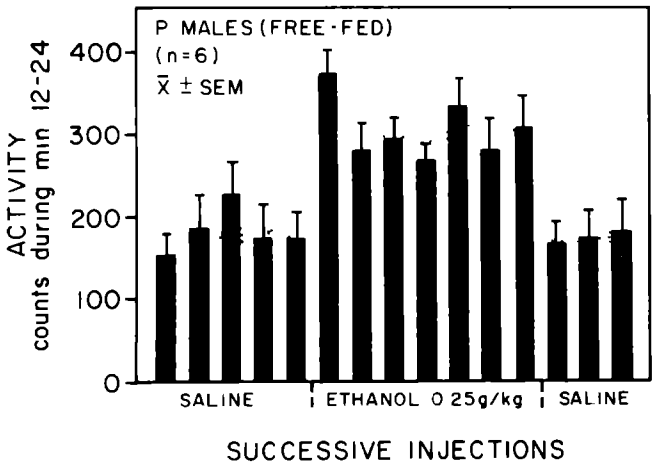

FIG 6 Effect of successive injections of saline and ethanol on spontaneous motor activity using an ABA design At least four days elapsed between each injection The stippled band indicates the mean ± SEM for each phase of the design The actual activity counts for minutes 12–24 are shown

Experiment 3 The Effect of Repeated Ethanol Injections on SMA of Free-Fed and Fasted Rats

In this study, six P animals received multiple injections of saline and ethanol (0 25 g/kg) following an ABA design The excitatory effect of 0 25 g ethanol/kg was consistently apparent in the first 12 min postinjection when the animals were fasted 24 hr prior to testing (Fig 5) The data shown are the mean of three ethanol injections administered at least four days apart There was no significant difference in SMA between the pre- and post-ethanol series of saline injections However, when these same animals had free access to food up to the time of activity testing, they exhibited a similar degree of excitation, but the maximal effect occurred during the middle third (minutes 12 to 24) of the test period (Fig 5) No difference between free-fed and fasted P rats in the onset of depression of SMA induced by 1 5 g ethanol/kg was observed (data not shown)

Figure 6 shows the results of individual successive injections of saline and ethanol (0 25 g/kg) using the ABA design These data further demonstrate that the monitored behavior was stable over three weeks There was no evidence of habituation to saline injections (after the first trial) or the development of tolerance to the excitatory effects of low dose ethanol, when given successively at least 4 days apart Successive injections of 1 5 g ethanol/kg, when given at least 4 days apart, also produced no indication of the development of tolerance to the depressive effects of high dose ethanol on SMA of free-fed animals (data not shown)

In male Wistar animals, brain ethanol concentrations were determined in both free-fed and fasted rats (n=6/group/interval) every six min up to 24 min after an injection of 0 25 g ethanol/kg These results are presented in Table 2 There were no significant differences between free-fed and fasted animals at any time point

TABLE 2

BRAIN ETHANOL CONCENTRATION IN FREE-FED AND FASTED (24 HR) MALE WISTAR RATS AT DIFFERENT TIMES FOLLOWING AN INTRAPERITONEAL INJECTION OF 0 25 g ETHANOL/kg

	mg ethanol/100 g tissue			
Feeding Condition	6	12	18	24 min
Free-fed	28 2 ± 1 3	20 9 ± 0 9	16 8 ± 0 7	15 6 ± 1 0
Fasted	28 9 ± 0 7	22 9 ± 0 7	18 0 ± 0 4	15 5 ± 0 4

Differences were statistically evaluated at each time point with an independent *t*-test

Mean ± SEM, n=6/condition/time point

DISCUSSION

Behavioral arousal occurring with low to moderate doses of ethanol, as evidenced by increased SMA, is a well-established phenomenon in mice [22] The intensity of stimulation differs in different inbred strains, indicating the existence of a genetic influence on this measure [4, 5, 6, 10, 23, 24, 27] Stimulation of SMA by ethanol has not been consistently observed in rats Whereas some investigators [1, 2, 14, 19] have seen an increase in SMA with low to moderate doses of ethanol, others [3, 8, 10, 12, 16] have not It is known that stimulation of SMA by ethanol can be affected by a number of variables including the concentration, dose, route and timing of ethanol administration, the sex and age of the rats, and the testing instrument itself [22] The studies reported here indicate in addition that, as with mice, the SMA response of rats to low-dose ethanol is strain or line specific (Fig 2) In rats, however, stimulation occurs within a narrower dose range and with lower doses than in mice These variables are likely also to contribute to some of the previous discrepancies in reported data with rats Moreover, depending upon whether rats are fasted or free-fed, the onset of stimulation may be immediate or delayed (Fig 5) Since the brain alcohol concentrations in free-fed and fasted rats do not differ within the first one-half hour of ethanol injection (Table 2), this difference is due to factors other than a change in ethanol pharmacokinetics

Stimulation of SMA was observed in the P and MR/N rats, animals which have high to moderate degrees of ethanol preference By contrast, the low preference NP and MNR/N rats were unaffected or depressed by ethanol in the low dose range It is unlikely that a pharmacokinetic difference can account for the different responses to ethanol We have previously reported that, within 2–3 min of an intraperitoneal injection of ethanol, the brain ethanol content is similar in P and NP rats [31] The blood and brain ethanol concentrations at which SMA stimulation occurs in the P and MR/N rats are in the range that P rats consistently attain on free-choice consumption of 10 percent ethanol and which do not negatively affect or curtail voluntary consumption [30] This positive relationship between SMA stimulation and alcohol preference in the rat suggests that the former may be an important measure in the study of reinforcing mechanisms of alcohol-seeking behavior As expected, all animals began to exhibit depression of SMA with 1 0 and 1 5 g ethanol/kg (Figs 2–4) There is, therefore, a dose-dependent, biphasic effect of ethanol on SMA

A relationship between ethanol preference and behavioral stimulation has been sought also in mice Some investigators [10, 23, 27] in the past were unable to demonstrate ethanol stimulation of SMA in the alcohol-preferring C57BL/6J strain of mice However, recent studies by Crabbe *et al* [6]

indicate that they do show alcohol-induced, dose-dependent increases in SMA, followed by depression Importantly, as with the P and Maudsley Reactive rats (Figs 2–3), stimulation in the C57BL/6J mice occurred early after alcohol injection (<4 min in the mice) and lasted for only a brief duration (<8 min) Thus, low-dose stimulation of SMA by ethanol is seen in both alcohol-preferring rats and mice

If low-dose alcohol stimulation of SMA is a reflection of the reinforcing properties of ethanol, one should expect no stimulation of SMA by alcohol in animals with low ethanol preference, such as the NP and Maudsley Nonreactive rats and DBA mice Indeed, we found a complete absence of alcohol stimulation of SMA in the NP and Maudsley Nonreactive rats (Figs 2–4) Only depression of SMA occurred with ethanol doses of 0 5 g/kg or higher On the other hand, the DBA mice have been shown to exhibit striking stimulation of SMA with ethanol [10,27] This reaction, however, occurs at all doses of ethanol that do not induce "sleep," i e , they do not show any depression of SMA, only stimulation, until the righting reflex is lost This unusual response to ethanol in the DBA mice may represent a uniquely different phenomenon in this strain that will require further investigation

In the experiments reported here, tolerance was not observed either to the depressant or to the stimulatory action of administered ethanol (Fig 6) This is likely due to the experimental design of spacing the test injections at least four days apart This ethanol-free interval was deliberately chosen to minimize carryover effects within the counterbalanced design It should be noted, however, whether tolerance develops to the low dose stimulatory effects is still unresolved Recent studies in mice have failed to detect tolerance to ethanol-stimulation or have observed "reverse tolerance," i e , increased stimulation [20,27] On the other hand an older study in rats [11] reported that tolerance develops to both the excitatory and to the depressant effects of ethanol Tabakoff and Kiianmaa [27] have suggested that differences in experimental design, with emphasis on the possibility of learned compensatory responses, could account for conflicting results Clearly, additional studies are needed both in mice and in rats to address this issue

If one assumes that the low-dose stimulatory effect of ethanol is a part or an expression of the positive reinforcing mechanism of ethanol and that the sedative-hypnotic effect of ethanol is aversive, an attractive hypothesis for ethanol-seeking behavior can be formulated, since tolerance to the sedative-hypnotic effects does develop with chronic ethanol intake [28] This combination of responses to ethanol can be envisioned to sustain and enhance drinking over time, until physical dependence becomes established It has been shown in rats that ethanol-dependence can induce the self-administration of large amounts of ethanol in animals that do not exhibit this behavior in the ethanol-naive state [7] However, as we have reported recently, the P rats will self-administer large amounts of ethanol, even by the intragastric route, without first having been forcibly made ethanol-dependent [29] Thus, the reinforcing actions of ethanol in nondependent animals will be clearly evident only when animals that have been selected for ethanol preference are employed for study

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