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The Startle Response in Rats: Effect of Ethanol[1]

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POHORECKY, L. A., M. CAGAN, J. BRICK AND L. S. JAFFE. *The startle response in rats: effect of ethanol.* PHARMAC. BIOCHEM. BEHAV. 4(3) 311–316, 1976. – The effects of acute and chronic ethanol intake on the startle response was examined in male rats. Ethanol given IP produced a dose-dependent decrease in the amplitude of the startle response measured 30 min later. With a dose of 1 g/kg, the effect was evident at 15 min and had recovered substantially by 60 min. The effect of ethanol on the startle response was potentiated by pretreatment of the animals with pimozide, haloperidol, and p-chlorophenylalanine but not by propranolol, phenoxybenzamine, α-methyltyrosine, or pargyline. After 3 weeks on an ethanol-containing diet, the startle response was greater than that shown by rats on the control iso-caloric, sucrose-containing diet. After ethanol withdrawal, the startle response was further increased, with a peak about 9 to 12 hr after discontinuation of ethanol; thereafter, the response declined. This time course of heightened startle response during ethanol withdrawal corresponds to the time course of the activation of noradrenergic neurons during withdrawal. It appears that dopaminergic and serotonergic neurons are involved in the mediation of the startle response in rats.

Ethanol Startle response Norepinephrine Dopamine Serotonin

RECENT evidence suggests that the startle response in rats involves brain monoamines [3, 4, 6]. As brain monoamines are affected by both acute and chronic ethanol intake [2, 9, 14, 19, 31], it is possible that ethanol affects the startle response. The intake of ethanol, in general, produces CNS depression and is believed to decrease the response of animals to a variety of external stimuli [11, 13, 15, 20]. Depression by ethanol of electrophysiological responses to auditory [16,21] and visual [1] stimuli have been reported. Connor *et al.* [3] showed that rats given p-chlorophenylalanine, (PCPA), an inhibitor of serotonin synthesis, showed heightened reactivity to startle stimuli. They suggested that brain serotonin plays an inhibitory role in this process. Animals with raphe lesions, which deplete brain serotonin levels, also have a heightened startle response [4]. Fechter [6] found that although PCPA had no effect on the startle response, selective replacement of serotonin with 5-hydroxytryptophan and a monoamine oxidase inhibitor after depletion by reserpine enhanced the startle response. He therefore suggested that serotonin has a facilitatory effect on the reflex. Furthermore, because monoamine oxidase inhibitors increase brain levels of catecholamines as well as of serotonin, he suggested that catecholamines might also be involved in the startle response [6].

The role, if any, of noradrenergic neurons in the startle response is unknown. It was our purpose in this study to examine the effects of ethanol on the startle response in normal rats and in rats with pharmacologically altered brain monoamines. The following questions were examined:

(1) Could the startle response be used as a behavioral correlate of the central effects of ethanol? In our search for a simple, sensitive quantitative measure for the central effects of ethanol we selected the startle response because one of the prominent effects of ethanol in man is the altered sensitivity and reactivity to sensory stimuli.

(2) Was the effect of ethanol on the startle response mediated by monoamines? As indicated above, the startle response possibly involves brain monoamines and ethanol is known to affect central monoaminergic neurons.

(3) Would the startle response represent a sensitive measure of the changes in CNS sensitivity during withdrawal? Would these changes correlate with the time course of changes in norepinephrine turnover observed previously during withdrawal? Again, our basis for asking these questions was the extreme hypersensitivity observed in animals undergoing withdrawal. The approximate time course for the hypersensitivity appeared to grossly correlate with the time course we found for the changes in norepinephrine turnover.

METHOD

Animals

The animals were Sprague-Dawley male rats (380–430 g at the time of testing) obtained from Holtzman (Madison, Wisconsin). The experimental groups consisted of 6 or 7 animals for each condition, as indicated in the pertinent tables. They were individually housed in a temperature-regulated room. Lights were on daily from 0700 to 1900 hours. Behavioral testing was carried out during the lights-on period. For all the acute studies, animals had free access to food and water before and throughout the experimental

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period, except when they were in the stabilimeter for testing.

For the chronic ethanol study, animals were placed on an all liquid diet consisting of chocolate flavored Nutrament (Mead Johnson) with 35–38% of the total calories represented by either added ethanol (ethyl alcohol, 95% v/v U.S.P.) or an equicaloric volume of a sucrose solution [7]. The liquid diet was supplemented with vitamins and minerals (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp., 3 g/liter). Animals were pair-fed with fresh diet daily, and water was available ad lib.

For the withdrawal experiments, the ethanol-treated rats were switched to a sucrose-containing liquid diet for varying periods of time.

In all of the experiments described, each animal was tested only once, to prevent complications arising from such factors as adaptation.

Apparatus

Startle response was measured with a stabilimeter. This was a small (12 × 10 × 9 in.), light-proof, animal chamber constructed from 1/8 in. aluminum which was painted black. The removable plastic floor was spring mounted in each corner to allow vertical movement. A cylindrical magnet mounted perpendicularly to the underside of the floor passed through a doughnut shaped coil mounted on the base of the apparatus. The magnet passing through the coil produced a change in the electromagnetic field which was amplified, transduced, and recorded on a Grass Model 5 polygraph. A buzzer attached to the center of the ceiling provided the startle stimulus. The stabilimeter was enclosed in a ventilated, sound-attenuated box (Lehigh Valley).

The amplitude of response was defined as the distance in millimeters from the baseline at the onset of the startle signal. Only the initial deflection was measured.

Behavioral Procedure

Tha animal was placed in the test chamber and allowed a 10-min adaptation period before the test session began. The animal then received a startle stimulus at an intensity of 108 db for 2 msec.

Drug Preparation and Administration

We examined the influence of different drugs on the startle response after a single dose of ethanol (1 g/kg, given 25 min before testing). We tested the effect of increased noradrenergic activity, produced by direct receptor stimulants or by increasing norepinephrine levels (by inhibiting monoamine oxidase), or of decreased noradrenergic activity, presumably resulting after depletion of norepinephrine levels after inhibiting its synthesis or by blocking noradrenergic receptors directly. We tested the alpha-receptor blocking drug phenoxybenzamine, since many of the central effects of norepinephrine are believed to be mediated through alpha-receptors. We included propranolol a beta-adrenergic blocker, because of its presumed alleviation of the behavioral symptoms accompanying alcohol intake in man [8]. The effects of most of these drugs on the startle response in rats is unknown: therefore we tested first the effects of the drugs and then secondly their interaction with ethanol.

The various drugs used were dissolved in sterile saline just before injection. All injections were given intraperitoneally. Pimozide (0.25 mg/kg) was first dissolved in glacial acetic acid, and then diluted with saline to the required volume. Haloperidol (0.5 mg/kg) was given 40 min prior to testing. Phenoxybenzamine (5 mg/kg) was injected 30 min before testing. Rats injected with propranolol (0.5 mg/kg) were tested 40 min later, and those treated with α-methyl tyrosine (100 mg/kg) were tested 30 min later. Pargyline (75 mg/kg) was injected 30 min prior to testing. p-Chlorophenylalanine (320 mg/kg) was given 72 hr before testing.

The drugs used in this study were generously supplied by Ayerst Laboratories (propranolol) and McNeil Laboratories (haloperidol and pimozide). p-Chlorophenylalanine methylester and α-methyltyrosine methylester HCl were purchased from Regis.

Determination of Brain Amines

Animals were sacrificed by decapitation; the brain was rapidly removed and dissected on ice, and the tissues rapidly frozen on dry ice. For the analysis, tissues were homogenized in 0.4 N perchloric acid; the homogenate was centrifuged at 10,000 × g for 15 min. The catecholamines present in the resulting supernatant fluid were then extracted by alumina chromatography [22]. Norepinephrine was determined by the fluorimetric procedure of von Euler and Lishajko [5] and dopamine by the procedure of Laverty and Taylor [10]. Brain serotonin was determined by the method of Maickel *et al.* [12].

Determination of Blood Ethanol Levels

Blood was collected from the tail vein into heparinized tubes. Blood ethanol levels were determined by gas chromatography, using a Packard Model 873 gas chromatograph equipped with a glass column (1/8 in. × 6 ft) packed with 50–80 mesh Poropak N (Applied Science Labs., State College, Pennsylvania). The column temperature was 160°C and the carrier gas flow (nitrogen gas, Matheson) was 50 ml/min. Retention time was 2 1/2 min. Ethanol concentrations were determined by direct integration of peak areas (Autolab digital integrator). Samples were run in triplicate, and acetone was used as an internal standard.

For statistical comparisons of treatment means, Student's *t*-test was used.

RESULTS

Animals Given a Single Dose of Ethanol

Dose response and time course. Initially we determined a dose-response curve of ethanol on the startle response of rats. Figure 1 shows the progressive decline in reactivity to the sound of the buzzer with increasing doses (0.5, 1, and 2.5 mg/kg) of ethanol; tests were made 30 min after drug administration. Even a small dose of ethanol (0.5 g/kg) produced a significant decrease in the startle response. Table 1 presents the blood ethanol levels for this study. As expected, they increased with increasing doses of ethanol.

Figure 2 illustrates the time course of this effect in animals given 1 g/kg ethanol. It can be seen that the effect of ethanol was rapid; almost maximal depression of the startle response was evident 15 min after ethanol injection. The response recovered considerably by 60 min, but was still somewhat depressed at 180 min. Table 2 presents the blood ethanol levels for the time-course studies. With the

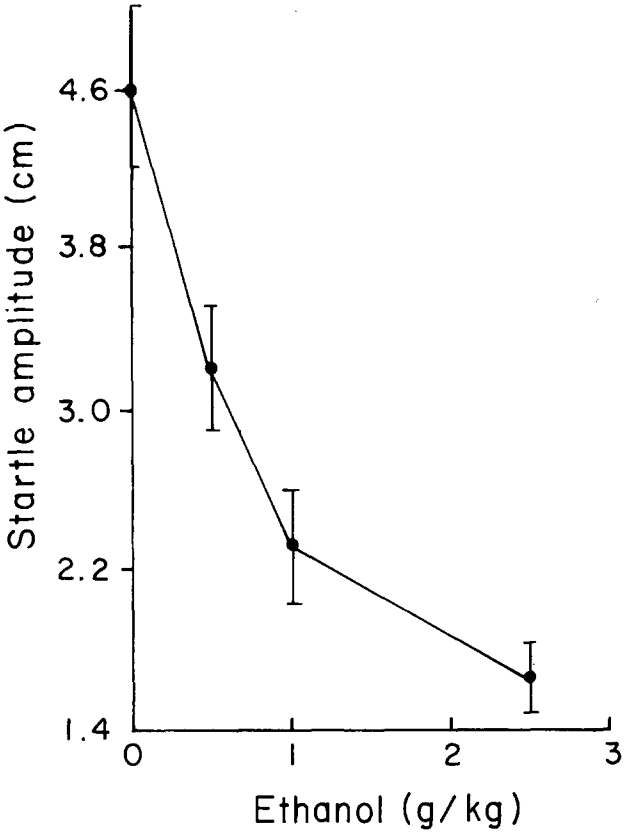

FIG. 1. Dose-response effect of ethanol on the startle response in rats. Groups of 6 rats were injected with 0.5, 1.0, or 2.5 g/kg of ethanol IP. The startle response was measured 30 min later. The control group received 1.5 ml of saline. The vertical bars represent the standard error of the mean.

TABLE 1

BLOOD ETHANOL LEVELS IN RATS INJECTED WITH ETHANOL

Ethanol (g/kg)	No. of Rats	Blood Ethanol (mg %)
0.5	6	47.4±1.05
1.0	6	130.1±2.02
2.5	6	333.9±2.89

Animals were injected with various doses of ethanol as indicated, and blood was collected 30 min later. Blood ethanol was determined by gas chromatography. Each group consisted of 6 rats. Results are expressed as means±standard errors of the mean (SEM).

1 g/kg dose given intraperitoneally, peak ethanol levels were achieved in 15 min. Blood ethanol declined rapidly and by 60 min it was 59.2% of the peak value and was no longer detectable by 180 min. It is not clear to us why blood ethanol levels for the 1 g/kg dose 30 min after ethanol injection was 110 mg/100 ml for the time-course study versus 130 mg/100 ml for the dose-response study. The animals had somewhat different startle responses also in these two separate studies. Several factors could have contributed to this. Among the most likely is the fact that animals for the two experiments originated from two different shipments and were housed in different animal rooms

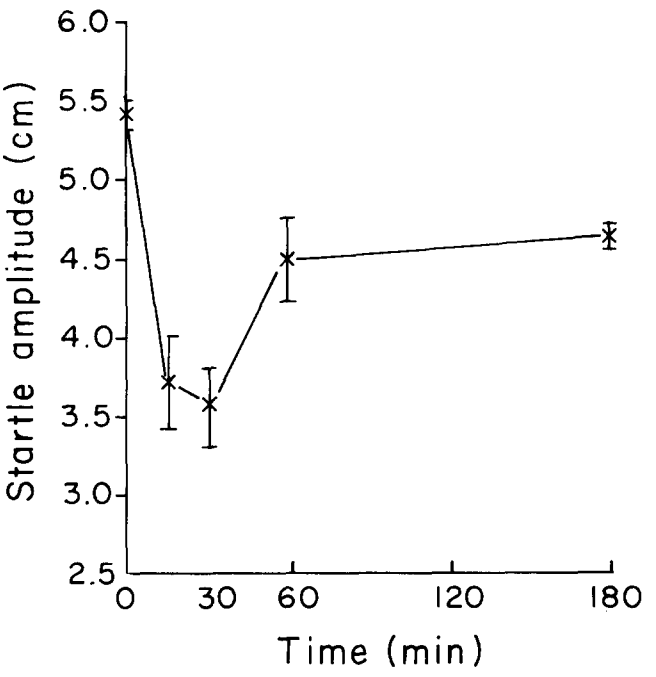

FIG. 2. Time course for the effect of a 1 g/kg dose of ethanol on the startle response. Five groups of 6 rats each were injected IP with 1 g/kg of ethanol; they were tested for their startle response at the indicated times after the drug treatment. Control rats received an injection of saline. The vertical bars represent the standard error of the mean.

TABLE 2

TIME COURSE FOR BLOOD ETHANOL LEVELS AFTER A 1 G/KG DOSE OF ETHANOL

Time (min)	Number of Rats	Blood Ethanol (mg%)
15	6	124.0±0.89
30	6	110.2±0.95
60	6	73.4±0.88
180	6	not detectable

Animals were injected with 1 g/kg of ethanol, and blood was collected at the indicated times. Blood ethanol was determined by gas chromatography. Each group consisted of 6 rats. Results are expressed as means ± SEM.

which differed slightly in background noise. We have observed that the amount of previous exposure to noise affects the animals' subsequent startle response. Differences in ethanol metabolism among animals are well known.

Effect of various drugs. Blockade of alpha-noradrenergic receptors did not affect the startle response of rats; propranolol, a beta-receptor blocker, decreased the response slightly (Table 3). These changes did not modify the startle response of ethanol-treated animals, nor did they alter brain norepinephrine levels. By contrast, blockade of dopamine receptors with pimozide or haloperidol potentiated the effect of ethanol by 35% and 50%, respectively. These drugs decreased the startle response slightly when given alone (significant at the 0.05 level). A decline in catecholamine levels (by 20%), produced by α-methyl-

TABLE 3

EFFECT OF ETHANOL AND VARIOUS DRUGS ON THE STARTLE RESPONSE IN RATS

Drug Treatment	Number of Rats	Startle Response (cm) Saline	Ethanol
Control	7	4.19±0.29	2.80±0.32*
Phenoxybenzamine	7	4.09±0.15	3.53±0.72
Propranolol	7	3.20±0.38*	2.47±0.45
a-Methyltyrosine	7	3.90±0.40	2.05±0.29
Haloperidol	7	3.87±0.58*	1.42±0.19†
Pimozide	7	3.19±0.50*	1.80±0.30†
Pargyline	7	3.28±0.49	2.09±0.30
p-Chlorophenylalanine	7	3.70±0.47	1.76±0.49†

The startle response was tested in groups of 7 animals pretreated with the following drugs: phenoxybenzamine (5 mg/kg), tested 30 min later; propranolol (0.5 mg/kg), tested 40 min later; a-methyltyrosine (100 mg/kg) tested at 30 min; haloperidol (0.5 mg/kg), tested at 40 min; pimozide (0.25 mg/kg), tested at 2 ½ hr; pargyline (75 mg/kg), tested at 30 min; and p-chlorophenylalanine (320 mg/kg), tested at 72 hr. Ethanol (1 g/kg) was administered 25 min before testing. All drugs were injected intraperitoneally. Results are expressed as means ± SEM.

*$p<0.05$ compared with saline control group.

†$p<0.05$ compared with ethanol control group.

tyrosine (AMT) treatment, or an increase (by 18%) resulting from the administration of pargyline, had no appreciable effect on the startle response. When given in combination with ethanol, these drugs potentiated slightly the change in startle response produced by ethanol, but the change did not reach statistical significance. Finally, PCPA, which blocks serotonin synthesis and resulted in an 80% decrease in serotonin levels, did not affect the startle response; however, it potentiated by 30% the depression of startle response produced by ethanol. Table 4 presents the corresponding amine levels for the various drug treatments.

Animals Given Chronic Ethanol Diet

In contrast to the animals given an acute dose of ethanol, those on the chronic ethanol diet showed no depression of the startle response. In fact, their startle response was slightly (but not significantly) higher than that of the animals on the control sucrose-containing diet (Table 5). At the time of testing, their average daily ethanol intake was 15–16 g, and the blood ethanol level was 250–300 mg%. At the time of testing, body weight of rats was 385 ± 15 g (ethanol group) and 405 ± 11 g (sucrose group).

To examine the startle response of animals undergoing withdrawal, other groups of animals (N = 6 each) had the ethanol in their diet replaced after 3 weeks by an equicaloric amount of sucrose. The removal of ethanol from the diet of the treated group was scattered in such a way that testing was always carried out during the daytime. Preliminary experiments has indicated that there was no significant difference in the startle response of animals tested in the morning versus in the afternoon. The various groups were tested for startle response at different time intervals during withdrawal. We found a time-dependent increase in the startle response with a peak occurring at 9–12 hr, at which time ethanol was no longer detectable in the blood. Thereafter, the magnitude of the startle response declined slowly; in about 4 days it reached a level comparable to that of rats on the sucrose-containing diet throughout.

TABLE 4

EFFECT OF ETHANOL IN COMBINATION WITH VARIOUS DRUGS ON MONOAMINE LEVELS IN THE BRAIN

	Saline	Ethanol
Norepinephrine		
Phenoxybenzamine	104.2±5.5	105.0±6.2
Propranolol	97.5±3.8	100.5±4.4
a-Methyltyrosine	80.0±8.5	77.3±9.1
Pargyline	118.1±4.2	116.1±3.6
Dopamine		
Haloperidol	90.1±3.9	123.8±3.0
Pimozide	94.0±2.8	103.0±1.1
Serotonin		
p-Chlorophenylalanine	30.1±1.1	35.3±1.9

For experimental details, see legend of Table 3. Results are expressed as percent of one control value (norepinephrine = 0.367±.025 μg/g, dopamine = 0.758±.033 μg/g, and serotonin = 0.590±.014 μg/g).

TABLE 5

STARTLE RESPONSE IN RATS ON AN ETHANOL-CONTAINING DIET

Treatment	Number of Rats	Startle Response (cm)
Sucrose	6	4.32±0.54
Ethanol	6	5.57±0.76
Withdrawal		
5 hr	6	8.39±0.47*
9 hr	6	8.84±0.36*
12 hr	6	8.68±0.67*
15 hr	6	8.00±0.52*
24 hr	6	7.29±0.34*
48 hr	6	6.03±0.42
72 hr	6	6.62±0.45
96 hr	6	5.32±0.63

Animals were maintained on the liquid diets for 3 weeks. At this time, the ethanol in the experimental diet was replaced with an equicaloric amount of sucrose. Startle response was tested at the indicated times after discontinuation of ethanol. Results are expressed as means ± SEM.

*$p<0.05$ or greater compared with the acute ethanol group.

DISCUSSION

In the present study, ethanol was found to have marked effects on the startle response of rats. Acute treatment produced rapid dose-dependent depression of the amplitude of the response, the effect becoming evident within 15 min after administration of ethanol (the earliest time examined) and persisting up to 3 hr later. Therefore, the effect of ethanol on the startle response lags slightly behind the blood ethanol levels. Since ethanol is believed to be a

general CNS depressant, it is not surprising to find a decreased startle response after its acute administration. Among the possible mechanisms one can advance for this effect of ethanol are: (1) Acute ethanol decreases the auditory stress, consequently the startle response is less. Several reports have indeed shown that ethanol decreases the electrophysiological response to auditory [11,20] and visual [1] stimuli. Similar effects have been obtained in man [9]. As shown by Perrin *et al.* [15], the evoked response to auditory stimuli is decreased in non-fasted cats intravenously injected with 1 g/kg 20% ethanol solution; the response recovers by 30 min. Therefore, at the time we tested our animals, their auditory sensitivity should have been relatively normal. (2) Ethanol impairs the motor activity of rats, so treated animals are less likely to move. Our previous studies have shown, however, that 1 g/kg ethanol, as used in the time-course study for instance, actually has a slight stimulatory effect on activity, while 2.5 g/kg depresses activity [17]. Both doses of ethanol produced depression of the startle response. (3) Ethanol directly impairs central neurons involved in the startle response rather than neurons responsible for the auditory perception.

Our experiments with drug treatments were designed to examine the possible involvement of noradrenergic neurons in the startle response. Recent evidence points to the involvement of monoamines in the effect of ethanol on the brain. Changes in levels and/or turnover of dopamine [2, 14, 19], norepinephrine [2, 9, 14, 19, 21], and serotonin [8, 18, 19] in the brain have been reported. We have recently found that ethanol produces a dose-dependent decline in the accumulation of labeled norepinephrine metabolities and norepinephrine synthesized from injected ^{3}H-tyrosine [17]. Therefore we tested the startle response in animals pretreated with drugs that modify the synthesis and metabolism of monoamines or block dopaminergic or noradrenergic receptors. Results indicate that the decrease in startle response produced by ethanol was potentiated by PCPA, haloperidol, and pimozide, and was not affected by AMT, pargyline, phenoxybenzamine, or propranolol. It thus appears that dopaminergic and serotonergic neurons could be involved in the mediation of the effect of ethanol on the startle response. It is possible, however, that this effect is characteristic of the benzodiazapine group of drugs; it would be of interest to test promethazine, which does not belong to this class of drugs.

During chronic ethanol intake, tolerance develops to the depressive effects of ethanol on the startle response. Thus, animals on such a diet actually have a startle response which is slightly higher than that of control animals. It is not known whether acoustically evoked potentials are affected during chronic ethanol intake. The greater startle response indicates a greater sensitivity of mechanism involved in the mediation of this response. It is quite evident from observation of animals undergoing withdrawal after a period of ethanol intake [16], and it is also well known in alcoholics under withdrawal, that the sensitivity to sensory stimulation is increased. It is not surprising then that the startle response was further increased during this phase in rats. This increase in reactivity of rats during withdrawal could be explained by the general hyperexcitability of the CNS that occurs during this state. Interestingly, the time course for the increase in startle response amplitude during withdrawal roughly corresponds to that for the stimulation of noradrenergic neurons in the brain during ethanol withdrawal [16]. Thus, during withdrawal an increase in norepinephrine turnover occurs in the brain with a peak about 9 hr after ethanol withdrawal; the increase in turnover persists for 24 hr.

In conclusion, it would appear from the studies reported here that ethanol affects markedly the startle response in rats. The mechanism of this effect is not clear; however, brain monoamines could possibly be involved.

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