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Pharmacology, Biochemistry and Behavior 76 (2003) 179–186
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Nicotine and ethanol enhancements of acoustic startle reflex are mediated in part by dopamine in C57BL/6J mice

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Received 19 March 2003; received in revised form 16 July 2003; accepted 23 July 2003

Abstract

Nicotine has been shown to have additive as well as antagonistic effects on behavior stimulated by ethanol. Here, we examine the effects of nicotine, ethanol, and the coadministration of each drug on acoustic startle responding in C57BL/6J mice. Mice were tested at a range of decibel levels (80–115 dB, 5 dB increments), with administration of 0.031, 0.062, 0.125, and 0.25 mg/kg nicotine or 0.5, 1.0, 1.5, and 2.0 g/kg ethanol. Nicotine and ethanol each caused an increase in the acoustic startle response at the highest and lowest doses tested, respectively. Mecamylamine, a nicotinic receptor antagonist, administered in combination with nicotine or ethanol attenuated these increases in acoustic startle responding. Nicotine and ethanol, administered together, did not produce greater enhancement of startle than when administered alone. Haloperidol (1 mg/kg) was administered in combination with nicotine or ethanol to investigate if dopamine modulated nicotine or ethanol enhancement of acoustic startle. It was found that the increase in acoustic startle responses observed with ethanol or nicotine was attenuated by haloperidol. Thus, ethanol or nicotine may enhance the acoustic startle reflex through a common dopaminergic mechanism.

Keywords: Acetylcholine; GABA; Haloperidol; Mecamylamine; Alcohol; Startle; Dopamine; Nicotine

1. Introduction

The effects of nicotine and ethanol have been studied extensively in associative learning tasks including fear conditioning. Nicotine has stimulatory physiological effects and has been shown to enhance contextual fear conditioning and latent inhibition of cued fear conditioning in C57BL/6J mice (Gould et al., 2001; Gould, 2003b; Gould and Higgins, 2003; Gould and Wehner, 1999). Ethanol has biphasic behavioral effects in mice, with a stimulatory effect immediately after administration, followed by a depressive effect (Dudek et al., 1991; Kiianmaa and Tabakoff, 1983; Smoothy and Berry, 1985). Ethanol has been shown to interfere with contextual and cued fear conditioning tasks, as well as latent inhibition of cued fear conditioning in C57BL/6J mice (Gould, 2003a; Gould et al., 2001) and contextual fear conditioning in rats (Melia et al., 1996). Gould et al. (2001), however, found that coadministration of nicotine with ethanol in a latent inhibition paradigm ameliorated deficits produced by administration of ethanol alone. In addition, this ability of nicotine to ameliorate ethanol-associated deficits was also demonstrated for contextual fear conditioning (Gould and Lommock, 2003). It is unclear, however, if these findings are specific to learning-related processes or if they extend across other behaviors including nonassociative processes.

The acoustic startle response is a nonassociative reflex that occurs in the presence of a loud auditory stimulus (Davis, 1984). The "flinch" that is elicited immediately after the presentation of the auditory stimulus is the behavioral measure. Nicotine increases the acoustic startle response in mice and rats (Acri et al., 1991; Marks et al., 1983). Conversely, ethanol produces a decrease in the acoustic startle response in rats (Klosowicz et al., 1979; Pohorecky et al., 1976; Rassnick et al., 1992). To date, there has been little investigation of the effects of ethanol on the acoustic startle response in C57BL/6J mice.

The aim of the current experiment was to evaluate the effects of nicotine, ethanol, and the coadministration of nicotine and ethanol on the acoustic startle response in C57BL/6J mice and examine possible cellular substrates of

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doi:10.1016/S0091-3057(03)00213-2

these effects. Activation of the dopaminergic system can modulate the acoustic startle response. Previous studies have shown that drugs of abuse including cocaine and amphetamine, which are known to increase dopamine levels, increase the acoustic startle response (Harty and Davis, 1985; Swerdlow et al., 1990). It has also been shown that the dopamine D1 agonist SKF 38393 increases the acoustic startle response in rats (Zhang et al., 2000). Thus, pharmacological agents that enhance dopamine levels would be expected to enhance the startle reflex and both nicotine and ethanol enhance dopamine transmission. Previous research suggests that nicotine's rewarding effects are mediated, in part, by an increase in dopamine release (Di Chiara and Imperato, 1988; Imperato et al., 1986), and that presynaptic modulation of nicotinic acetylcholinergic receptors (nAChRs) can facilitate dopamine release (Grady et al., 2002). Likewise, increased dopamine transmission has been proposed to underlie some of the behavioral effects of ethanol (for a review, see Koob and Nestler, 1997). After ethanol administration, an initial stimulatory phase may be related in part to increased dopaminergic activity and a later sedative phase may be related to increased GABAergic inhibition (Boehm et al., 2002). Thus, because of the biphasic nature of ethanol, lower doses (but not higher doses) might be expected to enhance startle if the onset of the sedative effects of ethanol is earlier at higher doses than lower doses. In addition, nicotine and ethanol administered together may further facilitate dopamine activity. Nicotine and ethanol have been shown to have additive effects on dopamine transmission in the central nervous system at low doses of nicotine and ethanol, but at higher doses no additive effect was seen (Tizabi et al., 2002). Thus, it is possible that the ability of nicotine and ethanol to enhance dopamine activity could facilitate startle. It is also possible that ethanol-associated enhancement of GABAergic processes could inhibit the startle reflex. We investigated the dose dependent effects of nicotine and/or ethanol on the acoustic startle reflex and if dopamine was involved in these effects.

2. Method

2.1. Subjects

C57BL/6J mice ($n = 8–10$ per group; The Jackson Laboratory, Bar Harbor, ME) were tested at 2–4 months of age. Mice had ad libitum access to food and water. Mice were maintained on a 12/12-h light/dark cycle (lights on at 07:00 h), and all testing occurred between 09:00 and 16:00 h. Mice were only tested in one startle condition and not retested across startle conditions. All behavioral procedures were approved by the Temple University Institutional Animal Care and Use Committee. C57BL/6J mice were utilized in this study based on findings from Crawley et al. (1997). C57BL/6 mice are moderately responsive to a 120-dB acoustic startle stimulus, allowing for any increases or decreases in startle responding due to drug administration to be readily observable. In addition, C57BL/6J have also been used extensively in our laboratory to investigate the effects of nicotine and ethanol on associative tasks (Gould, 2003a,b; Gould et al., 2001; Gould and Higgins, 2003; Gould and Lommock, 2003; Gould and Wehner, 1999), which makes this strain appropriate for extending these findings to nonassociative tasks.

2.1.1. Apparatus

Testing occurred in two identical sound attenuating testing chambers ($65 \times 35 \times 25$ cm). Each chamber was equipped with a Radio Shack loudspeaker mounted 25 cm above the holding cylinder. Startle responses were recorded in a commercial startle reflex system (S-R Lab, San Diego Instruments, CA). Mice were placed in a Plexiglas holding cylinder mounted on a Plexiglas platform. A piezoelectric accelerometer located beneath the platform was used to transform startle responses into units based on force and latency of startle. Data were sampled at 250 samples/second, and the maximum voltage attained on each trial was used as the dependent variable.

2.2. Drugs

Saline (NaCl) and ethanol (0.5, 1.0, 1.5, and 2.0 g/kg; 20% v/v in saline) were administered by intraperitoneal injection 15 min prior to behavioral testing. Nicotine hydrogen tartrate salt (0.031, 0.062, 0.125, and 0.25 mg/kg, dose base on salt weight; Sigma, St. Louis, MO) was dissolved in saline and administered intraperitoneally, immediately prior to testing due to the half-life of nicotine in mice: approximately 6–10 min (Petersen et al., 1984). Mecamylamine (2 mg/kg; Sigma) was dissolved in saline and administered intraperitoneally 15 min before testing. For the haloperidol experiments, two separate experiments using two separate vehicles were conducted. Haloperidol (1 mg/kg; Sigma) was dissolved in either 3% tartaric acid or 20% DMSO and administered intraperitoneally 30 min prior to testing. Haloperidol-treated mice were compared with appropriate vehicle-treated mice (i.e., tartaric acid or DMSO-treated vehicle mice). The injection time points for all drugs were the same for the multiple injection procedures as for the other experiments.

2.2.1. Procedure

In Experiment 1, the effects of nicotine, ethanol, or saline on acoustic startle were assessed. Testing began with a 5-min acclimation period. During this time, only background noise (65 dB white noise) was present and no recording took place. After the 5-min acclimation, white noise bursts (80, 85, 90, 95, 100, 105, 110, and 115 dB) were presented in a pseudorandom order. Each of these bursts was 40 ms in duration. The procedure was repeated five times and the average response over five trials was computed. Each

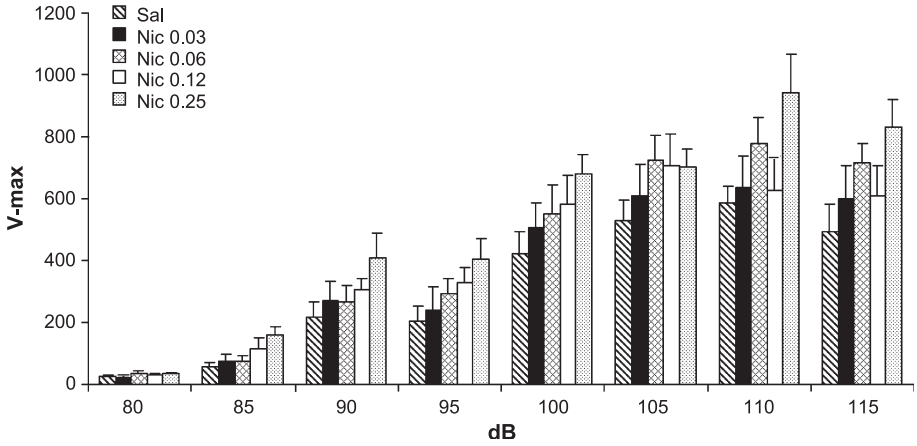

Fig. 1. Effects of nicotine on the acoustic startle response. The 0.25 mg/kg dose of nicotine significantly increased the acoustic startle response compared to saline controls at 85 dB and above. Note: The saline groups in Figs. 1 and 2 are the same groups. Nicotine and ethanol results were analyzed together but presented separately for clarity of presentation.

presentation was followed by an average ITI of 15 s. These intervals were randomly varied. Startle responses were recorded by computer as the maximum voltage difference detected. The duration of this test session was 15 min.

Experiment 2 extended the results of Experiment 1 by examining the effects of saline, 0.25 mg/kg nicotine, 0.5 g/kg ethanol, or coadministration of 0.25 mg/kg nicotine and 0.5 g/kg ethanol on the startle reflex. Although we had originally hypothesized that nicotine would ameliorate ethanol-associated deficits in startle, the results from Experiment 1 suggested that nicotine and ethanol coadministration might produce a greater enhancement of startle than either drug administered alone. The 100-dB startle pulse was used because of an apparent ceiling effect that emerged within the 105–115 dB levels (Figs. 1 and 2). The duration of this session was 6.5 min. The number of injections was equalized across all conditions.

Experiment 3 examined whether the nicotinic receptor antagonist mecamylamine would decrease the acoustic startle response and if mecamylamine would block the nicotine-associated enhancement and the ethanol-associated enhancement of the startle response. Saline, nicotine (0.25 mg/kg), ethanol (0.5 g/kg), or mecamylamine (2.0 mg/kg) was administered alone. Nicotine or ethanol was subsequently coadministered with mecamylamine. The procedure was the same as in Experiment 1. The number of injections was equalized across all conditions.

Experiment 4 examined if the nicotine-associated enhancement and ethanol-associated enhancement of startle were mediated in part by dopaminergic processes. Vehicle, nicotine (0.25 mg/kg), ethanol (0.5 g/kg), or haloperidol (1 mg/kg), subthreshold for disrupting the startle reflex, was administered alone. This dose of haloperidol has been shown previously to have no effect on the acoustic startle reflex in C57BL/6J mice (Ouagazzal et al., 2001). Nicotine or ethanol was subsequently coadministered with haloperidol. The 0.25 mg/kg dose of nicotine and the 0.5 g/kg dose of ethanol were utilized because these were the only doses

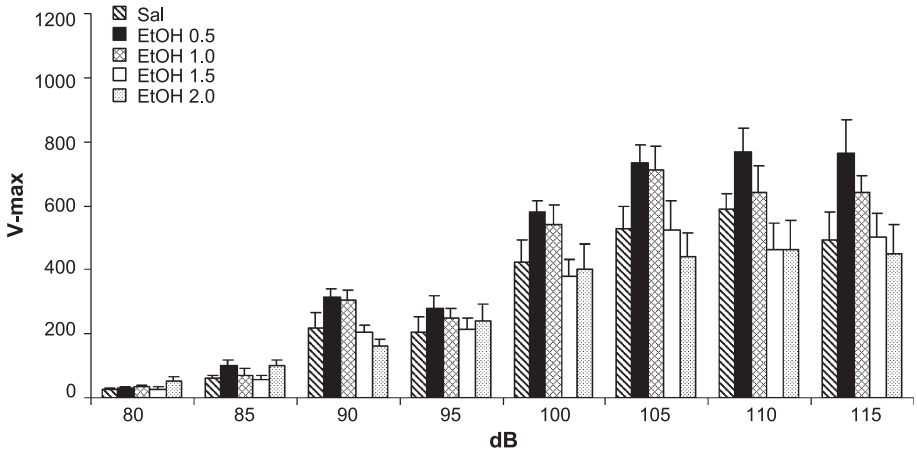

Fig. 2. Effects of ethanol on the acoustic startle response. The 0.5 g/kg dose of ethanol significantly increased the acoustic startle response at 100 dB and above. The 2.0 g/kg dose of ethanol significantly increased the startle response at the 80 dB level. No significant decreases in the startle reflex were found for the doses of ethanol tested.

that produced reliable increases in startle responding throughout Experiments 1–3. The procedure was the same as in Experiment 1. The number of injections was equalized across all conditions. Two experiments were run. For one, the vehicle was 20% DMSO and the other the vehicle was 3% tartaric acid.

2.2.2. Statistical analysis

A repeated measures analysis of variance on decibel level was performed on all data in Experiments 1, 3, and 4. Due to a significant interaction between decibel level and drug, the data were collapsed across decibel levels, and post hoc pairwise contrasts (Tukey corrected) were performed to detect differences at the $P < .05$ level for nicotine, ethanol, mecamylamine, and haloperidol. For drug doses found to be significantly different from saline, drug–saline comparisons were made at each decibel level. For Experiment 2, a one-way ANOVA was performed and a least-squares difference post hoc analysis was used to detect differences at the $P < .05$ level. Analyses were performed using SPSS version 11.5.

3. Results

3.1. Experiment 1: Effects of nicotine and ethanol

Experiment 1 examined the effects of nicotine, ethanol, or saline on the acoustic startle reflex. A repeated measures ANOVA revealed a significant overall effect of drug [$F(8,51) = 3.37$, $P < .001$] and decibel level [$F(7,357) = 251.42$, $P < .001$], as well as significant interaction between drug and decibel level on startle [$F(56,357) = 1.94$, $P < .001$]. Post hoc analysis for nicotine revealed a significant increase in the acoustic startle response for the 0.25 mg/kg dose compared to saline [$t(51) = 4.05$, $P < .05$] (Fig. 1). Specifically, nicotine enhanced the acoustic startle response at 85 dB and above. Post hoc analysis for ethanol revealed significant increases in acoustic startle responses for the 0.5 g/kg dose compared to saline [$t(51) = 2.24$, $P < .05$] (Fig. 2). Ethanol enhanced the acoustic startle response at 100 dB and above.

3.2. Experiment 2: Coadministration of nicotine and ethanol

Experiment 2 extended the results from Experiment 1 by examining the effects of coadministration of the 0.25 mg/kg dose of nicotine and the 0.5 g/kg dose of ethanol on the acoustic startle response; the most effective doses from Experiment 1. A one-way ANOVA revealed an overall effect of drug on startle [$F(3,31) = 3.23$, $P < .05$]. Post hoc analysis revealed an increase in the acoustic startle response for the 0.25 mg/kg nicotine group, for the 0.5 g/kg ethanol group, and for the coadministration of nicotine and ethanol group all compared to the vehicle group. However, no differences were detected between the coadministration group and either the 0.25 mg/kg nicotine group or the 0.5 g/kg ethanol group (Fig. 3). Thus, at the doses tested, no additive effect was found.

3.3. Experiment 3: Nicotinic receptor antagonism

Experiment 3 examined whether the nicotinic receptor antagonist mecamylamine would disrupt the acoustic startle response and if mecamylamine would disrupt the nicotine- and ethanol-associated enhancement of startle. A repeated measures ANOVA revealed an overall effect of drug [$F(5,41) = 7.77$, $P < .001$] and decibel level [$F(7,287) = 190.94$, $P < .001$], as well as a significant interaction between drug and decibel level on startle [$F(35,287) = 8.30$, $P < .001$]. Post Hoc analysis revealed a significant increase in the acoustic startle response in the 0.25 mg/kg nicotine alone condition [$t(41) = 3.47$, $P < .005$], as well as the 0.5 g/kg ethanol alone condition [$t(41) = 2.59$, $P < .05$] when compared to saline. Nicotine enhanced startle responding at 85,

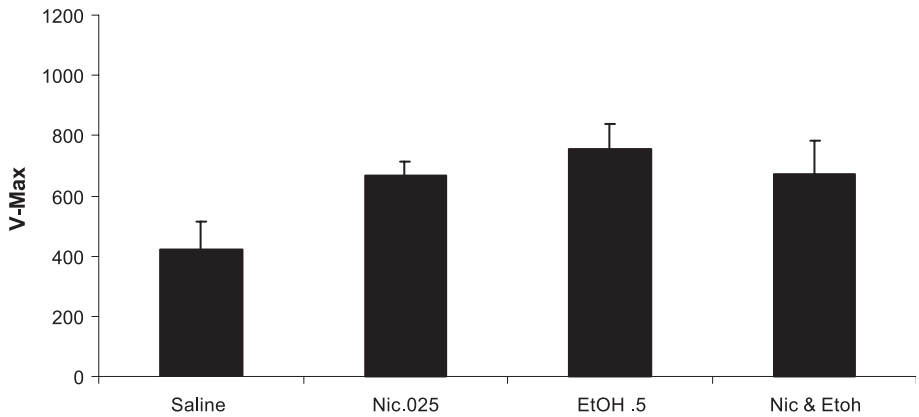

Fig. 3. The effects of nicotine (0.25 mg/kg), ethanol (0.5 g/kg), and the coadministration of both drugs on startle to a 100-dB stimulus. Both nicotine and ethanol significantly increased the acoustic startle response. The coadministration of both drugs significantly increased the startle response when compared to saline. However, the coadministration group was not different from the nicotine alone or ethanol alone groups indicating that the effects of the drugs were not additive at the decibel level and drug doses tested.

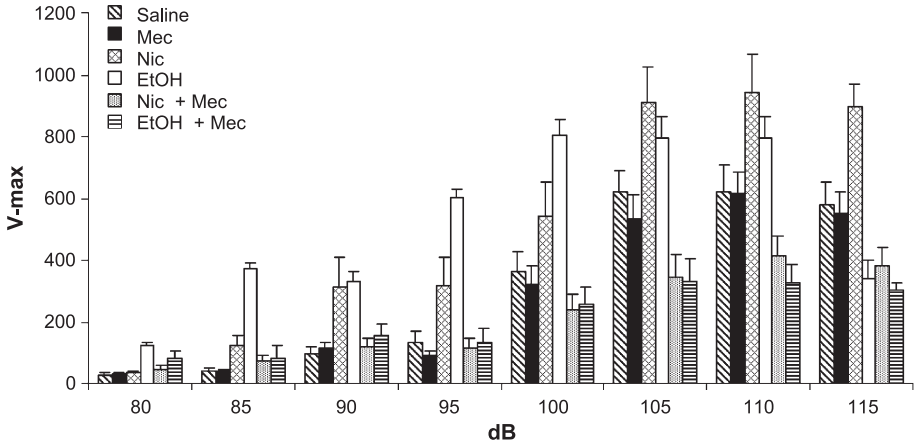

Fig. 4. The effects of mecamylamine (2.0 mg/kg) and nicotine (0.25 mg/kg) on the acoustic startle response. Mecamylamine had no significant effect on the startle reflex when administered alone. Nicotine increased the acoustic startle response at the 85, 90, 95, 105, 110, and 115 dB levels. Mecamylamine administered prior to nicotine administration blocked the nicotine-associated increase in startle at all decibel levels tested. In addition, the 0.5 g/kg dose of ethanol increased the acoustic startle response at the 90, 95, 100, and 115 dB levels. Mecamylamine administered prior to ethanol administration blocked the ethanol-associated increase in startle at all decibel levels tested.

90, 95, 105, 110, and 115 dB, and ethanol enhanced startle responding at 90, 95, 100, and 115 dB. These results are similar to the results from Experiment 1. Mecamylamine blocked the nicotine enhancement [$t(41) = 3.93$, $P < .001$] and ethanol enhancement [$t(41) = 4.13$, $P < .001$] of the startle response, and the startle response for both groups was lower than saline-treated mice. Mecamylamine administered alone, however, had no effect on acoustic startle compared to saline controls (Fig. 4).

3.4. Experiment 4: Effect of haloperidol

Experiment 4 examined if nicotine and ethanol enhanced startle through modulation of dopaminergic processes. If increased dopaminergic activity underlies the nicotine- and ethanol-associated increases in the startle reflex reported here, we would expect haloperidol to block the potentiation of startle when coadministered with nicotine or ethanol. A 1.0 mg/kg dose of haloperidol has been shown previously to be subthreshold for altering acoustic startle (Ouagazzal et al., 2001). For the experimental condition in which the vehicle for all groups was 3% tartaric acid, a repeated measures ANOVA revealed an overall effect of drug [$F(5,37) = 10.45$, $P < .001$] and decibel level [$F(7,259) = 82.02$, $P < .001$], as well as a significant interaction between drug and decibel level [$F(35,259) = 4.22$, $P < .001$]. Post hoc analysis revealed a significant increase in startle for the nicotine alone group when compared to vehicle group [$t(37) = 2.54$, $P < .05$]. This nicotine-associated enhancement was seen at the 95, 100, 105, and 110 dB levels. Post hoc analysis also revealed an increase in startle responding in the ethanol alone group when compared to vehicle group [$t(37) = 2.50$, $P < .05$]. This en-

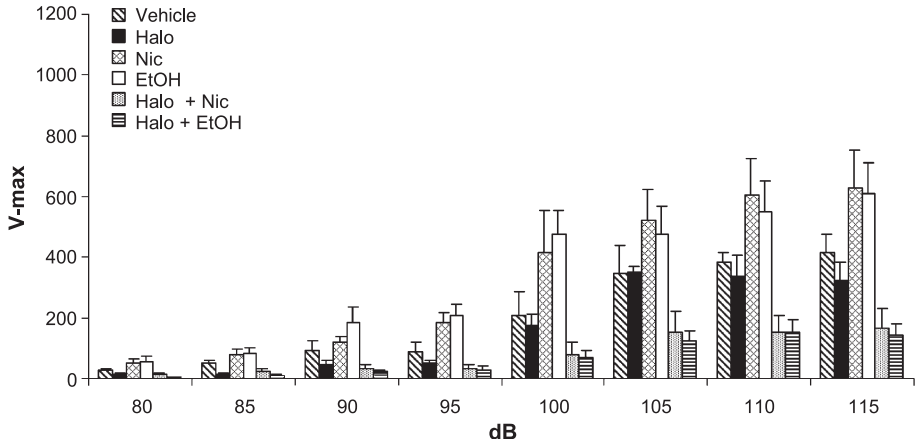

Fig. 5. The effects of haloperidol (1 mg/kg) on the acoustic startle response. Nicotine administered alone significantly increased the startle reflex at 95, 100, 105, and 110 dB. Ethanol significantly increased the acoustic startle response at 80, 90, 95, and 100 dB. Haloperidol administered alone had no overall effect on startle responding. Administration of haloperidol before nicotine or ethanol administration significantly reduced the nicotine- and ethanol-associated enhancement of startle at all decibel levels tested. This suggests that dopaminergic processes, in part, underlie the increase in the startle reflex associated with nicotine and ethanol administration. Vehicle for all groups was 3% tartaric acid.

hancement was seen at the 80, 90, 95, and 100 dB levels. Haloperidol blocked the nicotine-associated enhancement of startle [$t(37) = 4.80$, $P < .05$]. Haloperidol also blocked the ethanol-associated enhancement of startle [$t(37) = 5.28$, $P < .05$] (Fig. 5). Haloperidol administered alone had no significant effect on the startle response. This result is in accord with a previous finding that a 1.0 mg/kg dose of haloperidol had little effect on acoustic startle responding in C57BL/6J mice (Ouagazzal et al., 2001).

For the experimental condition in which the vehicle for all groups was 20% DMSO, a repeated measures ANOVA revealed an overall effect of drug [$F(5,41) = 15.60$, $P < .001$] and decibel level [$F(7,287) = 179.54$, $P < .001$], as well as a significant interaction between drug and decibel level [$F(35,287) = 7.92$, $P < .001$]. Post hoc analysis revealed an increase in startle responding in the nicotine alone condition [$t(41) = 3.67$, $P < .05$] as well as the ethanol alone condition [$t(41) = 6.70$, $P < .05$]. Haloperidol attenuated the nicotine-associated increase in startle [$t(41) = 3.27$, $P < .05$], as well as ethanol-associated increase [$t(41) = 4.99$, $P < .05$] at all decibel levels tested. It should be noted that even though DMSO had an overall effect on baseline startle, the results for Experiment 4 were replicated using two different vehicles. In the experiment with tartaric acid as the vehicle, haloperidol alone had no effect on startle. However, in the experiment with DMSO as the vehicle, haloperidol did reduce startle responses but only at 80 dB [$t(41) = 2.27$, $P < .05$] and 85 dB [$t(41) = 2.04$, $P < .05$], which were decibel levels where nicotine and ethanol effects were largely not seen.

4. Discussion

In the present study, we found that both ethanol and nicotine enhanced the acoustic startle response in C57BL/6J mice. The finding with nicotine supports previous research demonstrating an enhancement of the acoustic startle response with chronic nicotine treatment in rats (Acri et al., 1991) and with acute administration of nicotine in CH3 mice (Marks et al., 1983). However, it fails to support the findings of Marks et al. (1983) that nicotine administration in C57BL/6ibg mice produced no overall increase in the acoustic startle response. Three procedural differences could account for these conflicting results. First, Marks et al. (1983) administered (−)nicotine-free base, which may have different pharmacokinetics than the nicotine hydrogen tartrate salt utilized here. The possibility exists that equivalent doses of (−)nicotine-free base and the nicotine hydrogen tartrate salt do not result in equivalent brain nicotine concentrations at the same time points. Second, the mice tested in the Marks et al. (1983) study were C57BL/6ibg, a different substrain of C57BL/6 mice than the C57BL/6J mice used in the present study. Possible genetic drift between the strains could underlie the different effects of nicotine on startle between the studies, although this possibility is remote. Lastly, the most parsimonious explanation may be that differences in data analysis precluded detection of differences at a low dose of nicotine in the Marks et al. (1983) study. The acoustic startle response results presented in the Marks et al. (1983) study were analyzed in groups of non-, low, and high startle responses. No overall effect of nicotine on startle was found which precluded statistical analysis of individual doses. However, examination of individual doses of nicotine in that study reveals that C57BL/6ibg mice demonstrated increased startle at a low dose of nicotine. The low dose in the Marks et al. (1983) experiment may correspond more closely to the high dose in our experiment. The results presented here suggest that doses around 0.25 mg/kg nicotine may enhance the startle reflex. Furthermore, the Marks et al. (1983) study suggests that higher doses in C57BL/6 mice do not enhance the startle reflex. This is similar to what has been reported for contextual fear conditioning. Low doses but not high doses of nicotine enhanced contextual fear conditioning (Gould and Wehner, 1999).

The results of the current study also suggest that the effects of nicotine on startle responding are mediated by activation of nicotinic receptors because mecamylamine attenuated the nicotine-induced enhancement of startle. Mecamylamine did not inhibit the startle reflex when administered alone. The mecamylamine-associated attenuation of the nicotine-induced enhancement of startle observed here in C57BL/6J mice has previously been shown in DBA and C3H mice (Collins et al., 1986).

Ethanol administration increased the acoustic startle response at the lowest dose tested, 0.5 g/kg, but had no effect at 1.0, 1.5, or 2.0 g/kg. Previous studies have shown that both acute ethanol administration (1.0 g/kg) and chronic ethanol administration decreased the acoustic startle response in rats (Pohorecky et al., 1976; Rassnick et al., 1992). However, there has been little investigation on the effects of ethanol on startle responding in C57BL/6J mice. The ethanol-associated increase in the startle reflex observed in this study may be due, in part, to the well-established biphasic effects of ethanol (Dudek et al., 1991; Kiianmaa and Tabakoff, 1983). Initially, ethanol increases locomotor activity but this effect changes to sedation over time (Smoothy and Berry, 1985). Ethanol may initially stimulate dopamine release, increasing activity, but as the level of ethanol-stimulated GABAergic inhibition increases, sedation may occur (Engel, 1985; Boehm et al., 2002). The onset of the sedative effects of ethanol may increase with increasing concentrations of ethanol. For example, Cohen et al. (1997) observed increases in locomotor activity in CD1 mice at doses between 1 and 3 g/kg, with a marked decrease in activity at doses above 3 g/kg. These increases in locomotor activity were greatly attenuated when haloperidol, a D2/D3 antagonist, was coadministered with ethanol at the doses observed to increase activity. Dopamine agonists also increase the acoustic startle response (Harty and Davis, 1985; Swerdlow et al., 1990; Zhang et al., 2000). Thus, the

dose of ethanol observed to increase startle responding in this study, 0.5 g/kg, could stimulate dopamine release and enhance startle. The findings of Cohen et al. (1997) coupled with the findings here suggest that D2/D3 receptors mediate, in part, ethanol-induced increases in both locomotor activity and acoustic startle responding.

Mecamylamine attenuated the ethanol-induced increase in startle responding. This finding supports previous research suggesting that ethanol-induced increases in locomotor activity and dopamine efflux in the nucleus accumbens can be attenuated by mecamylamine (Larsson et al., 2002). Engel et al. (1999) have hypothesized that ethanol enhances dopamine release in the mesocorticolimbic system through enhancement of nAChR processes. The mechanism for this enhancement is unknown but several candidate mechanisms have been put forward. For instance, ethanol may stabilize the open state of nAChRs or increase the rate of nAChR channel opening (Dilger et al., 1994; Liu et al., 1994; Wu et al., 1994). Ethanol has also been demonstrated to increase acetylcholinergic receptor (AChR) responsiveness to agonists (De Fiebre et al., 1995; Forman et al., 1989). Additional studies further demonstrate that ethanol enhances nicotine-stimulated electrophysiological responses in select neural areas (Breese et al., 1993; Yang et al., 1999).

In the present study, coadministration of nicotine and ethanol did not enhance startle any more than administration of ethanol alone or nicotine alone. One possible reason for the absence of an additive effect at the doses tested is that nicotine and ethanol were affecting the same neural process. A candidate mechanism is ethanol enhancement and nicotine enhancement of dopamine release. Both drugs enhance dopamine release (Grady et al., 2002; Koob and Nestler, 1997) and dopamine agonists increase startle responding (Harty and Davis, 1985; Swerdlow et al., 1990; Zhang et al., 2000). The present study supports the idea that 0.25 mg/kg nicotine and 0.5 g/kg ethanol may enhance the acoustic startle reflex through dopaminergic processes. In this study, the dopamine receptor antagonist haloperidol had no overall significant effect on the startle reflex when administered alone. Haloperidol, however, attenuated the ethanol enhancement and the nicotine enhancement of acoustic startle.

In summary, both nicotine and ethanol increased the acoustic startle response in C57BL/6J mice at the 0.25 mg/kg and 0.5 g/kg doses, respectively. However, antagonism of nicotine receptors at the dose tested did not interfere with the startle reflex, suggesting that activation of nAChRs can modulate but may not be essential for startle. High doses of ethanol (1.0, 1.5, and 2.0 g/kg) had no effect on the startle reflex when compared to saline controls. The finding that ethanol enhanced startle at low doses but had no effect at higher doses may reflect differences in ethanol activation of dopaminergic and GABAergic processes. The observed increases in startle may be mediated, in part, by the actions of both drugs on dopamine release as evidenced by haloperidol attenuation of the nicotine- and ethanol-associated increase of startle.

Acknowledgements

The authors would like to acknowledge grant support from the American Federation for Aging Research (TG), the Pennsylvania Department of Health (TG), the Temple University (TG) and Kerra Constant for her assistance.

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