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Pharmacology, Biochemistry and Behavior 83 (2006) 76 – 89
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Nicotine–ethanol interactions in flash-evoked potentials and behavior of Long-Evans rats

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Received 13 September 2005; received in revised form 8 December 2005; accepted 12 December 2005
Available online 23 January 2006

Abstract

Although nicotine and ethanol are often used together, little is known about their combined effects on visual system electrophysiology. This experiment examined the separate and combined effects of nicotine and ethanol on flash-evoked potentials (FEPs) recorded from both the visual cortex (VC) and superior colliculus (SC) of chronically implanted male Long-Evans rats. There were four treatment conditions administered on separate days: either saline or ethanol (2.0 g/kg, i.p.) was given 10 min before either saline or nicotine (1.0 mg/kg, s.c.). FEPs were recorded at 5, 20, and 40 min following the second injection. In the VC, ethanol significantly decreased the amplitude of most components, but increased P_{46}. Peaks P_{22} and N_{53} were unchanged. Nicotine enhanced most component amplitudes, but decreased N_{29} and P_{234}, while P_{22} and N_{139} were unchanged. In the SC, ethanol depressed the amplitude of all components studied. In contrast, nicotine significantly depressed only P_{27} and N_{48}. Latencies of most components in both structures were increased by ethanol, nicotine, and the combination treatment, although a nicotine-induced enhancement of the effects of ethanol on latencies was not typically observed. Each drug treatment also produced significant hypothermia, with the combination treatment resulting in the greatest hypothermia. Ethanol, either alone or in combination with nicotine, significantly reduced body movements during the FEP recording sessions. In subsequent open-field observations, ethanol, but not nicotine, significantly increased the number of squares crossed, while the combination treatment produced the greatest increase in movement. Nicotine significantly increased rearing behavior, but both ethanol and the combination treatment eliminated rearings. Overall, data suggesting that nicotine can counteract some of the effects of ethanol was demonstrated in varying degrees in the amplitude of VC components N_{39}, P_{46}, N_{53}, N_{65}, and P_{88}, the latency of VC component N_{53}, the amplitude of SC component N_{59}, and the latency of SC components N_{48} and N_{54}. In contrast, a nicotine-induced enhancement of the effects of ethanol was found for only the latency of VC components N_{39}, P_{88}, and P_{234}, body temperature, and open-field ambulation.

Keywords: Nicotine; Ethanol; Visual cortex; Superior colliculus; Flash-evoked potentials; Visual-evoked potentials; Rats; Hypothermia; Locomotion; Behavior

1. Introduction

The link between alcohol (i.e., ethanol) and nicotine use is compelling, with at least 70% of alcoholics also smoking heavily (Dawson, 2000; Hughes, 1999; Shiffman and Balabanis, 1995). In addition, alcoholics who smoke use more cigarettes per day than do non-alcoholic smokers (Dawson, 2000), and ethanol consumption has been shown experimentally to increase cigarette smoking (Mintz et al., 1985). What is less clear is why these two compounds are so closely associated. One possibility is that the actions of one counteract, at least in part, the actions of the other, while another possibility is that the actions of one may enhance the rewarding actions of the other (Alcohol Alert, 1998; Collins et al., 1988; Tizabi et al., 2002).

Nicotine is the primary active ingredient in tobacco, and it works as a nicotinic acetylcholine receptor (nAChR) agonist, producing a variety of effects in the central and peripheral nervous system. Peripherally, both sympathetic and parasympathetic ganglia are stimulated, resulting in a complex mix of sympathetic nervous system arousal combined with some physiological relaxation. Centrally, nicotine produces EEG activation and behavioral arousal or alerting (Levin, 1992; U.S. Department of Health and Human Services [USDHHS], 1988). With a wide distribution in the central nervous system (CNS), nAChRs are located not only on neuronal cell bodies and

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doi:10.1016/j.pbb.2005.12.009

dendrites, but also on axon endings, where they can modulate the release of numerous neurotransmitters, including dopamine, noradrenaline, serotonin, acetylcholine, glutamate, and GABA (MacDermott et al., 1999; Vizi and Lendvai, 1999; Wonnacott, 1997).

In contrast, ethanol is a CNS depressant which disrupts a variety of cognitive functions (Rezvani and Levin, 2003). Acute ethanol exposure influences a variety of neurotransmitter systems, both directly and indirectly, including increased availability of serotonin (Lovinger, 1997), enhanced action of GABA at GABA-A receptors (Proctor et al., 1992), interference with the excitatory effects of glutamate transmission at *N*-methyl-D-aspartate receptors (Hoffman and Tabakoff, 1993), and altered release of cortical acetylcholine (ACh; Stancampiano et al., 2004). Some recent interest has centered on the ethanol modulation of nAChRs (Narahashi et al., 1999). For example, ethanol significantly potentiates acetylcholine-induced currents in $\alpha4\beta2$ nAChRs expressed in human embryonic kidney cells (Zuo et al., 2004), while ethanol inhibits agonist-induced currents in $\alpha7$ homomers (Yu et al., 1996).

While there are an abundance of studies which have examined the cognitive and behavioral effects of ethanol and nicotine separately, relatively little work has examined the acute or chronic interactions between these two compounds (Dohrman and Reiter, 2003; Rezvani and Levin, 2003). This is especially true for sensory-evoked potential studies. Sensory-evoked potentials, which can be easily recorded from both cortical and subcortical sites in laboratory animals, are complex neural responses which are phase-locked to the stimulus presentation (Shah et al., 2004). The individual components of flash-evoked potentials (FEPs) are representations of neural pathways that are activated during the photic stimulation (Fox and Rosenfeld, 1972), and alterations in these components produced by exposure to pharmacological agents can indicate visual system dysfunction (Dyer, 1985).

In humans, the components of the cortical FEP can be broadly differentiated into early (peak latency less than about 100 ms) and late components. Early components are described as being involved in sensory processing, while later components are involved in cognitive processes (Porjesz et al., 2005). In the rat, the FEP can be separated into primary, secondary, and late components on the basis of latency. The primary components P1 and N1 are the most directly related to sensory processing, while later components are associated with behavioral and pharmacological manipulations (Bigler, 1977; Creel et al., 1974; Schwartzbaum et al., 1971).

Ethanol produces a distinct pattern of changes in the FEP recorded from the rat VC (Hetzler et al., 1981; Hetzler and Bednarek, 2001), and is also known to reduce the cortical release of acetylcholine (Carmichael and Israel, 1975; Stancampiano et al., 2004). To the extent that the ethanol-induced changes in the VC FEP result from diminished stimulation of nACh receptors, the administration of nicotine would be expected to counteract those changes. It is also of interest to determine if nicotine is more effective in reversing ethanol-induced changes in the primary (sensory) vs. later (cognitive) components. To examine the ability of nicotine to counteract ethanol-induced changes in cortical versus subcortical evoked activity, we also examined FEPs collected from the superior colliculus (SC), a subcortical structure involved in eye movements, attention, and orientation to sensory stimulation (Binns, 1999).

In addition to recording FEPs, body temperature was measured in the present study, since both ethanol and nicotine produce a dose-dependent hypothermia in rodents (Hetzler and Bednarek, 2001; Hetzler and Theinpeng, 2004; Luo et al., 1994; Rezvani and Levin, 2002), and such changes in body temperature may then result in secondary changes in evoked-potential parameters (Hetzler and Dyer, 1984; Hetzler et al., 1988). Finally, since both ethanol and nicotine alter behavior in rats (e.g., Jerome and Sanberg, 1987; June et al., 1998; Qiu et al., 1992), we monitored gross body movement during and after the evoked-potential recording sessions.

2. Materials and methods

2.1. Animals

Twenty-two adult, male Long-Evans hooded rats (Harlan, Indianapolis, IN), about 4 months old and weighing 372–420 g at the time of surgery, were used in the study. The rats were housed individually in standard cages in a room with a light/dark cycle (light from 07:00 to 19:00 h) and climate control (temperature about 22 °C and humidity about 50%). Purina Lab Chow (St. Louis, MO) and tap water were provided ad libitum.

2.2. Electrode implantation surgery

Recording electrodes were implanted in the VC and the SC. The rats first received an i.p. injection of atropine sulfate (0.06 mg) to minimize respiratory distress during anesthesia. They were then anesthetized with an i.p. injection of 50 mg of sodium pentobarbital per kilogram of body weight. The VC electrode ($0-80 \times 1/8$ in. stainless steel screw) was placed 6 mm posterior to bregma and 3 mm lateral to the right of the midline. Similar screw electrodes placed over the ipsilateral and the contralateral frontal cortex (anterior 3 mm, lateral 2 mm) provided for a recording reference and grounding, respectively. SC recordings were made from a twisted pair of nichrome wires (each 250 μm in diameter), insulated to the tip, with a vertical intertip distance of 1 mm. With the skull surface of the animal located in a horizontal plane (König and Klippel, 1963), the bipolar SC electrode was implanted 6.5 mm posterior to bregma and 1.5 mm lateral to the left of the midline and then lowered 4.8 mm below the surface of the skull. All electrodes were led to a 5-hole plastic cap (Wire Pro #223-1605), and the whole assembly was secured to the skull with additional screws and dental acrylic. After surgery, animals were handled briefly on a daily basis before testing.

At the conclusion of the experiment, placements of the SC electrodes were histologically verified (Hetzler et al., 1981). Results for the SC are reported for those 18 animals in which the lower member of the electrode pair penetrated the superficial layer of the SC (Dyer and Annau, 1977). VC

recordings from two animals were unusable because of problems with the cap.

2.3. Chemicals

Nicotine as the (−)nicotine tartrate salt (Sigma Product No. 5260, St. Louis, MO) was dissolved in 0.9% saline. The dose of 1.0 mg nicotine/kg body weight was based on the salt form of the drug. The ethanol dose employed was 2.0 g of ethanol per kilogram of body weight (20% ethanol, vol./vol.), diluted in saline. Drug dosages, injection intervals, and testing times following injections were based on prior research conducted in this and other laboratories (Levin, 1992; Hetzler and Theinpeng, 2004; Hetzler et al., 1982; Hetzler and Smith, 1984).

2.4. Procedure

Evoked potentials were amplified with Tektronix 122 preamplifiers (Tektronix, Beaverton, OR) with high and low filter settings of 1.0 kHz and 0.8 Hz for both the VC and the SC. Amplified waveforms were averaged (2000-Hz sample rate, 400 ms epoch, $n=100$) by an IBM PS/ValuePoint computer connected to a Modular Instruments M100 Mainframe containing the following modules: M202 Fast A/D, M210 Memory, and M214 Data Acquisition Timer. Fifty milliseconds of the epoch occurred before the application of the evoking stimulus. Evoking stimuli were presented with an interstimulus interval of 2 sec. Data collection was controlled with an S-215 Signal Averager program (Modular Instruments, West Chester, PA).

Recordings were obtained while animals were located inside a shielded recording chamber with dim background illumination of about 3 lx. The testing box, which was located inside the shielded chamber, measured $10\times10\times10$ in. and was constructed of white Plexiglas on three sides, the top, and the bottom, whereas the front panel was clear. The floor was made of clear Plexiglass rods, each 8 mm in diameter, with 10 mm space between the rods. Shielded Microdot cables (Microdot Connectors, South Pasadena, CA), which are designed to reduce artifacts associated with cable movements (Fox and Rosenfeld, 1972), were attached to the top of the chamber with a mercury swivel, allowing freedom of movement. Flash stimuli were presented by a Grass Model PS22C photostimulator (Grass-Telefactor, West Warwick, RI) with an intensity setting of 8. The flash lamp of the photostimulator was placed in a small sound-attenuating chamber to eliminate the auditory click present with each flash. The flash lamp was positioned outside the shielded recording chamber and was visible to the rat through a clear Plexiglas window.

At least 1 week after surgery, the animals were given 2 successive days of familiarization to the testing procedures, during which time they were tested as they would be in the experiment proper, except that only saline injections were given. This was followed by 1–2 days of rest before actual data collection. On each testing day, the animal first received one drop of 1% ophthalmic atropine in each eye to maintain constant pupil dilation (Gelatt, 1981). Fifteen minutes after the eyedrops were administered, the animal was injected intraperitoneally with either physiological saline (0.9% sodium chloride) or 2.0 g of ethanol per kilogram of body weight (20% ethanol (vol./vol.) diluted in saline and injected in a volume of 1.26 ml/100 g). Ten minutes later, the animal was injected s.c. in the dorsal surface of the neck with either physiological saline (0.9% sodium chloride, 1 ml/kg) or 1.0 mg of (−)nicotine tartrate per kilogram of body weight (1 ml/kg). The animal was placed in the testing chamber immediately after the second injection. After a 5-min waiting period, the data were collected. Thus, testing began 15 min after the first injection. Evoked potentials were collected simultaneously from the VC and the SC at 5, 20, and 40 min following placement in the testing chamber. The treatment order for each subject was counterbalanced across animals. Thus, each animal was tested using all four treatment conditions (see Table 1). Animals were given two days of rest between tests.

In addition to evoked potential collection, gross body movement was measured in the FEP recording chamber during FEP collection by intersecting photocell beams (S23-01; Coulbourn Instruments, Allentown, PA) that were positioned diagonally in the cage corners 3.5 cm above the floor of the cage. Electronic counters (R11-25; Coulbourn Instruments) tallied the number of beam interruptions. Immediately after testing, the animal was placed in a restraining tube, and a rectal thermistor probe (YSI No. 402; Yellow Springs Instruments, Yellow Springs, OH) was inserted 10 cm into the rectum. A rectal temperature reading (YSI 49TA Tele-Thermometer; Yellow Springs Instruments) was then taken. Ambient temperature (22.18 °C) was controlled by a wall thermostat, and was

Table 1
Experimental design

Treatment	Testing sequence					
	Eyedrops	1st injection	2nd injection	FEPs	Body temperature	Open field
Saline	Yes	Saline	Saline	5, 20, 40 min	Yes	Yes
Nicotine	Yes	Saline	Nicotine	5, 20, 40 min	Yes	Yes
Ethanol	Yes	Ethanol	Saline	5, 20, 40 min	Yes	Yes
Ethanol+Nicotine	Yes	Ethanol	Nicotine	5, 20, 40 min	Yes	Yes

Fifteen minutes after receiving eyedrops, the first injection (saline or ethanol) was given i.p. Ten minutes later, the second injection (saline or nicotine) was given s.c. Immediately after the second injection, the animal was placed in the testing chamber, and both flash evoked potentials (FEPs) and body movement were recorded at 5, 20, and 40 min after being placed in the chamber. Body temperature was recorded upon removal from the testing chamber, after which the animal was placed in an open field. Nicotine=1 mg/kg, Ethanol=2.0 g/kg.

recorded (along with body temperature) at the conclusion of each test.

Following body temperature measurement, each animal was observed for two minutes in a 0.92 m^2 open field, with walls 46 cm high. The field was constructed of standard plywood, and was painted gray. The Plexiglas floor of the apparatus was divided into 36 equal squares, 15 cm on each side. Overhead fluorescent lights provided illumination of approximately 635 lx. On each testing day, the animal was placed in the same middle square of the open field, and allowed to explore for 2 min. Both line crossings (movement of all four limbs of the rat across a line) and number of rearings (standing on hindlimbs, with forelimbs off the floor) were recorded. General qualitative observations were also made of each animal's behavior. All procedures were approved by the Lawrence University Animal Care and Use Committee.

2.5. FEP component identification

Fig. 1 presents group average FEP waveforms for the VC, while Fig. 4 presents group average FEP waveforms for the SC. Components studied in the present experiment are identified in the 5-min traces. Following the component identification procedure described in Hetzler and Theinpeng (2004), in the present study FEP components were designated by their polarity and by their latency from the onset of the light flash. Baseline-to-peak amplitudes and peak latencies were obtained for nine VC components (P_{22}, N_{29}, N_{39}, P_{46}, N_{53}, N_{65}, P_{88}, N_{139}, and P_{234}; see Fig. 1). Components P_{27}, P_{38}, N_{48}, N_{54}, and N_{59} were similarly analyzed in the SC waveforms (see Fig. 4). The baseline-to-peak amplitudes consisted of the difference between the mean voltage of 50 ms of prestimulus activity and the peak voltage. Peak latencies were measured from the onset of the evoking stimulus.

2.6. Data analysis

VC, SC, and photocell data were subjected to 2 (ethanol) × 2 (nicotine) × 3 (time) repeated measures analyses of variance. When a significant interaction was found, individual means were compared with the Dunnett test. The 5-min recording interval data and the saline–saline treatment served as the basis for comparisons in the Dunnett tests. That is, the 20- and 40-min data were compared to the 5-min data, while the saline–nicotine, ethanol–saline, and ethanol–nicotine treatments were compared to the saline–saline treatment. In addition, the ethanol–nicotine treatment was compared with the ethanol–saline condition with the Dunnett test. Changes in evoked potential amplitudes and latencies resulting from time-related factors were included in the analyses, but are not reported. Likewise, significant drug × time interactions are described only in relation to the main effects of the drug. Body temperature and open field line crossings data were subjected to 2 (ethanol) × 2 (nicotine) repeated measures analyses of variance. A significant interaction was followed by the Dunnett test. However, these tests were not appropriate for the open-field rearing data, since there was no variability in the ethanol–saline and the ethanol–nicotine conditions. Therefore, the nonparametric Friedman test was used, followed by multiple comparisons. In all analyses, statistical significance was assumed when $P<0.05$ for two-tailed comparisons. All 22 animals were included in the body temperature and movement data, even if they were removed from the evoked potential analyses (for technical reasons).

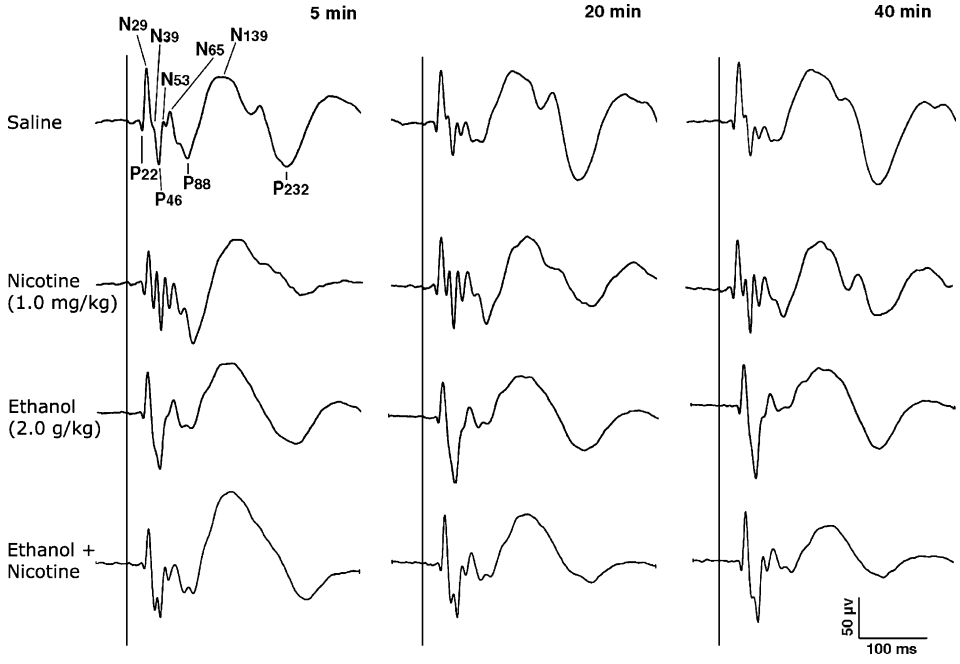

Fig. 1. Group average visual cortex (VC) flash-evoked potential (FEP) waveforms ($n=20$) for each treatment condition and each time interval. Vertical lines represent onset of the evoking stimulus. Individual FEP components are named by polarity and latency from the onset of the evoking stimulus. The effects of ethanol, nicotine, and coadministration of ethanol and nicotine varied with the component and time interval.

3. Results

3.1. Visual cortex: amplitude

The results are complicated and the statistical analyses complex. Group mean evoked potentials are displayed in Fig. 1, where it is apparent that both ethanol and nicotine altered the FEPs, and that the combination of ethanol and nicotine resulted in a pattern of results which varied with the component. Baseline-to-peak amplitude data are presented for each component in Fig. 2, while Table 2 provides a simplified overview of the pattern of results for the electrophysiology data.

For component P_{22}, there was a significant ethanol-induced reduction in amplitude at only the 5-min recording interval [ethanol: $F(1,19)=8.88$, $p=0.008$; ethanol × time: $F(2,38)=3.87$, $p=0.029$]. The amplitude of N_{29} was decreased by ethanol, nicotine, and the ethanol–nicotine combination in comparison to the saline–saline control, but overall there was no significant difference between ethanol–saline and ethanol–nicotine. In addition, nicotine caused a significant decrease in N_{29} amplitude at the 5-min interval in comparison to the amplitude without nicotine [ethanol: $F(1,19)=6.55$, $p=0.019$; ethanol × nicotine: $F(1,19)=18.96$, $p<0.001$; nicotine × time: $F(2,38)=5.85$, $p=0.006$].

N_{39} amplitude was drastically altered by the different drug treatments [ethanol: $F(1,19)=417.40$, $p<0.001$; nicotine: $F(1,19)=10.91$, $p=0.004$; ethanol × nicotine × time: $F(2,38)=6.29$, $p=0.004$], as evidenced by the obvious distortion of the evoked potential waveform (see Fig. 1). N_{39} amplitude was significantly depressed by both the ethanol–saline and the ethanol–nicotine treatments at all time intervals, but increased in amplitude at 5 and 20 min by nicotine. However, in comparison to ethanol–saline, the combination of ethanol and nicotine significantly increased N_{39} amplitude at 20 and 40 min.

All three drug treatments significantly enhanced P_{46} amplitude (although the effect of nicotine was not significant at the 5-min interval). The combination treatment resulted in an amplitude that was midway between that following ethanol alone and nicotine alone for the 20- and 40-min intervals, during which time P_{46} amplitude was significantly decreased in comparison to ethanol alone [ethanol: $F(1,19)=22.53$, $p<0.001$; ethanol × nicotine: $F(1,19)=12.72$, $p=0.002$; ethanol × time: $F(2,38)=13.83$, $p<0.001$; ethanol × nicotine × time: $F(2,38)=6.78$, $p=0.003$].

N_{53} amplitude was not significantly altered by ethanol, but nicotine increased the amplitude of this component at 20 and 40 min [nicotine: $F(1,19)=23.66$, $p<0.001$; nicotine × time: $F(2,38)=6.10$, $p=0.005$; ethanol × nicotine × time:

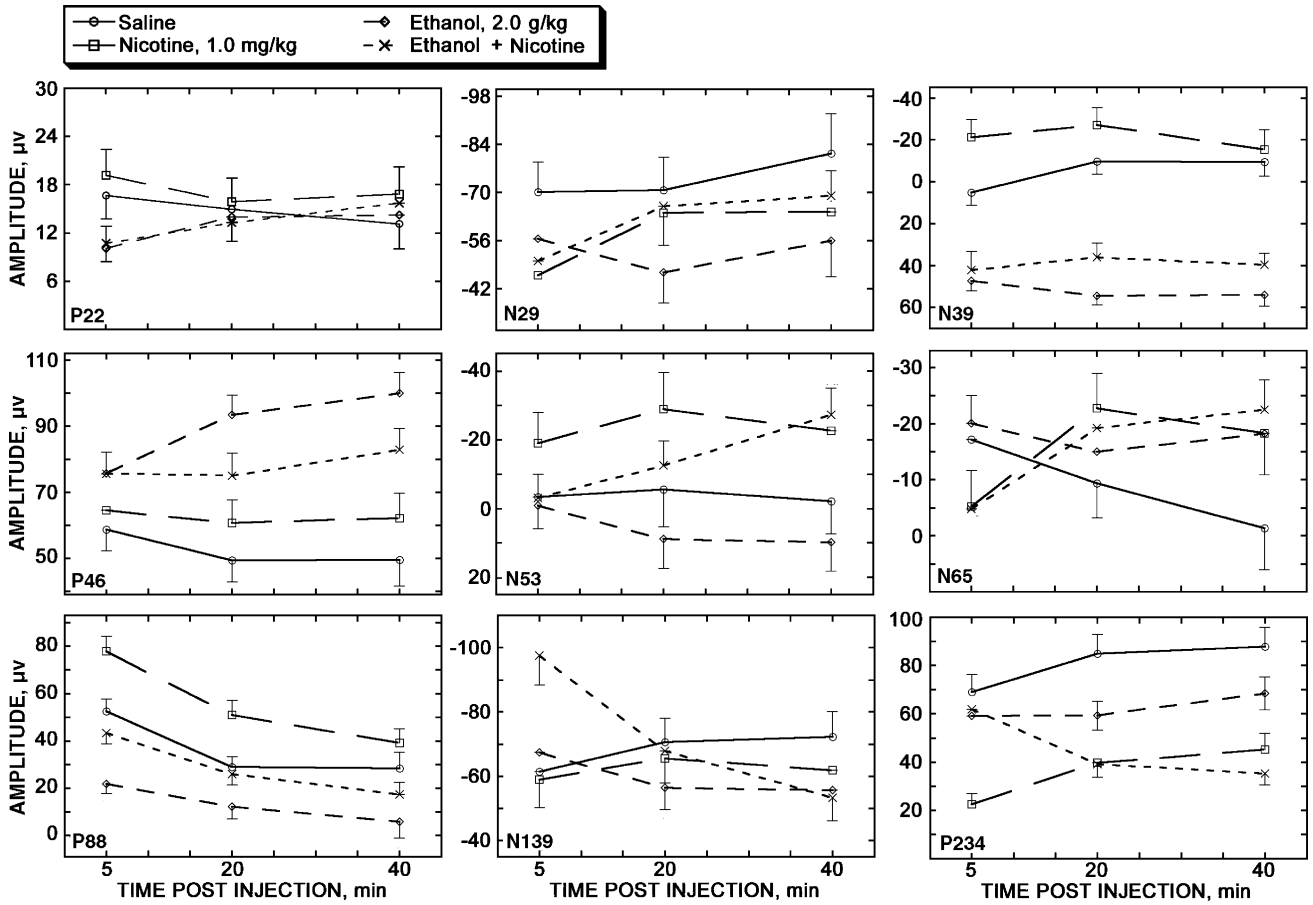

Fig. 2. Peak amplitudes of visual cortex (VC) flash-evoked potential (FEP) components ($n=20$) as a function of drug treatment and time interval. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above or below the mean values represent ±1 S.E.M.

Table 2
Simplified summary of flash evoked potential results

Component	Saline vs.			Ethanol vs.
	Nicotine	Ethanol	Ethanol+Niotine	Ethanol+Nicotine
VC amplitude				
P_{22}	–	↓(5)	↓(5)	–
N_{29}	↓	↓	↓	–
N_{39}	↑(5,20)	↓	↓	↑(20,40)
P_{46}	↑(20,40)	↑	↑	↓(20,40)
N_{53}	↑(20,40)	–	↑(40)	↑(20,40)
N_{65}	↓(5)↑(20,40)	↑(40)	↓(5)↑(20,40)	↓(5)
P_{88}	↑	↓	–	↑
N_{139}	–	↓(20,40)	↑(5)↓(40)	↑(5)
P_{234}	↓	↓(20,40)	↓(20,40)	↓(20,40)
VC latency				
P_{22}	↑	↑	↑	–
N_{29}	↑	↑	↑	–
N_{39}	↑	↑	↑	↑(20)
P_{46}	↑	↑	↑	–
N_{53}	↑(5,20)	↑	↑	↓
N_{65}	↑	↑	↑	–
P_{88}	↑	↑	↑	↑
N_{139}	↑	↑	↑	–
P_{234}	↑	↑(5)	↑(5)	–
SC amplitude				
P_{27}	↓	↓	↓	–
P_{38}	–	↓	–	–
N_{48}	↓	↓(5,20)	↓(5,20)	–
N_{54}	–	↓	↓	–
N_{59}	–	↓	–	↑
SC latency				
P_{27}	↑	↑	↑	–
P_{38}	↑	↑	↑	–
N_{48}	↑	↑	↑	↓(5)
N_{54}	↑	↑	↑	↓
N_{59}	↑	↑	↑	–

Each row presents a simplified summary of the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. The first 3 entries for each component show how the amplitude or latency of the component was altered by Nicotine, Ethanol, or the combination of Ethanol+Nicotine, in comparison to Saline. The final entry shows a comparison between the effects of Ethanol and the combination treatment of Ethanol+Nicotine. FEP=Flash Evoked Potential, VC=Visual Cortex, SC=Superior Colliculus, Nicotine=1 mg/kg, Ethanol=2.0 g/kg, ↑=increase, ↓=decrease, –=no change, (5,20,40)=change was observed only at this(these) recording interval(s). For details of the analyses, see Results, as well as Figs. 2, 3, 5, and 6.

$F(2,38)=3.46$, $p=0.042$]. When the combination treatment was given, there was no effect on N_{53} amplitude at the 5-min time interval. However, at 20 and 40 min the amplitude was significantly greater following the combination treatment than that following ethanol alone. At the 40-min interval, N_{53} amplitude was virtually identical to that following nicotine treatment, being significantly greater than either saline–saline or ethanol–saline.

For component N_{65}, only the ethanol × time, $F(2,38)=3.95$, $p=0.028$, and the nicotine × time, $F(2,38)=28.77$, $p<0.001$, interactions were significant. When ethanol was present, the average amplitude of N_{65} was significantly enhanced at 40 min; nicotine significantly decreased N_{65} amplitude at the 5-min interval, but increased this component's amplitude at both the 20 and 40 min intervals. In general, the combination dose closely resembled the effects of nicotine alone for this component.

A decrease in P_{88} amplitude resulting from ethanol administration was present at all time intervals [ethanol: $F(1,19)=152.34$, $p<0.001$; ethanol × time: $F(2,39)=4.08$, $p=0.025$], while an increased amplitude resulting from nicotine administration was also present at all time intervals [nicotine: $F(1,19)=33.50$, $p<0.001$; nicotine × time, $F(2,38)=4.51$, $p=0.018$]. As shown in Fig. 2, the combination dose produced an amplitude in-between that following either ethanol or nicotine alone, and therefore very similar to saline.

Results for the late wave N_{139} were complex, as reflected by the many significant interactions [ethanol × nicotine: $F(1,19)=9.09$, $p=0.007$; ethanol × time: $F(2,38)=29.12$, $p<0.001$; nicotine × time: $F(2,38)=10.81$, $p<0.001$; ethanol × nicotine × time: $F(2,38)=3.99$, $p=0.027$]. Although nicotine did not significantly alter the amplitude of this component, amplitude was significantly depressed by ethanol at 20 and 40 min, and by the combination treatment at 40 min. Surprisingly, there was also an enhancement of this component at 5 min, relative to both the saline and ethanol treatments, when the combination of ethanol–nicotine was given.

Finally, many of the analysis of variance results were significant for component P_{234} amplitude [nicotine: $F(1,19)=64.89$, $p<0.001$; ethanol × nicotine: $F(1,19)=23.58$, $p<0.001$; ethanol × time: $F(2,38)=17.42$, $p<0.001$; nicotine × time: $F(2,38)=4.10$, $p=0.024$; ethanol × nicotine × time: $F(2,38)=7.35$, $p=0.002$]. This component was significantly depressed by all three drug treatments, although only saline–nicotine resulted in a significant depression at the 5-min interval. At both the 20 and 40 min intervals, all three treatments significantly depressed P_{234} amplitude, with the combination treatment resembling the effects of saline–nicotine, and resulting in significantly greater reduction in amplitude than that produced by ethanol–saline.

3.2. Visual cortex: latency

The VC latency data are displayed in Fig. 3. In general, latencies of all of the components were increased by ethanol, nicotine, and the combination of ethanol and nicotine. P_{22} latency was significantly increased by nicotine, ethanol, and the combination treatment, but the latency following the combination treatment was not significantly different from that with the ethanol–saline treatment [ethanol: $F(1,19)=53.840$, $p<0.001$; nicotine: $F(1,19)=5.75$, $p=0.027$; ethanol × nicotine: $F(1,19)=24.74$, $p<0.001$; ethanol × time: $F(2,38)=16.24$, $p<0.001$; nicotine × time: $F(2,38)=8.03$, $p<0.001$]. The results for component N_{29} were similar to those just described for component P_{22} [ethanol: $F(1,19)=25.07$, $p<0.001$; ethanol × nicotine: $F(1,19)=10.19$, $p=0.005$], in that all 3 treatments significantly increased N_{29} latency, with ethanol producing the greatest increase, nicotine the least, and the combination dose in between. However, there was no significant

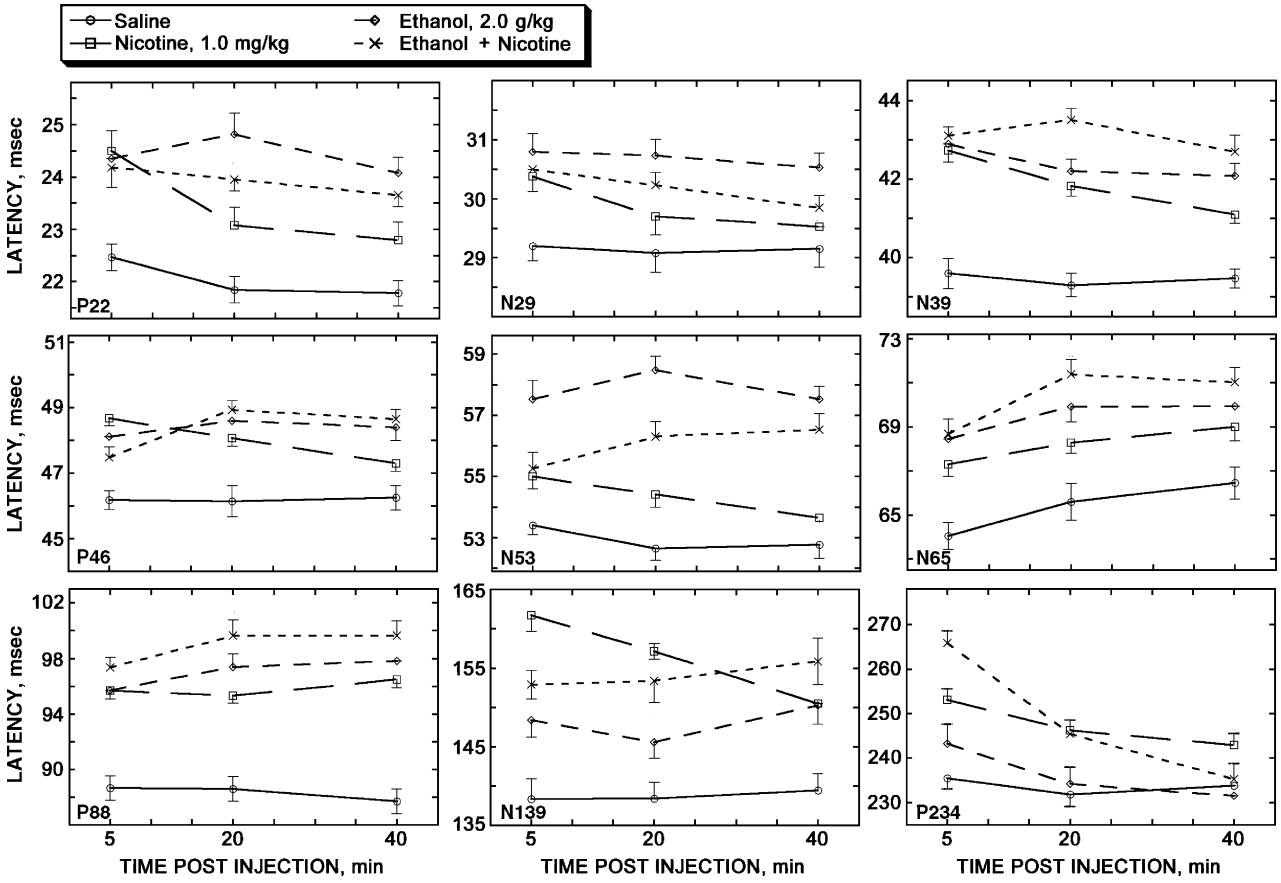

Fig. 3. Peak latencies of visual cortex (VC) flash-evoked potential (FEP) components ($n=20$) as a function of drug treatment and time interval. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above or below the mean values represent ±1 S.E.M.

difference between the latencies observed following ethanol–saline and ethanol–nicotine.

All 3 treatments again significantly increased the latency of component N_{39} [ethanol: $F(1,19)=134.03$, $p<0.001$; nicotine: $F(1,19)=25.71$, $p<0.001$; ethanol × nicotine: $F(1,19)=18.01$, $p<0.001$; nicotine × time: $F(2,38)=6.65$, $p=0.003$; ethanol × nicotine × time: $F(2,38)=9.16$, $p<0.001$], although in this instance the combination treatment produced the greatest increase, which was significantly greater than ethanol's effects at the 20-min reading. All three treatments significantly increased the latency of component P_{46} to approximately the same degree at all three time intervals [ethanol: $F(1,19)=35.14$, $p<0.001$; nicotine: $F(1,19)=13.27$, $p=0.002$; ethanol × nicotine: $F(1,19)=23.63$, $p<0.001$; ethanol × time: $F(2,38)=20.20$, $p<0.001$; ethanol × nicotine × time: $F(2,38)=9.48$, $p<0.001$].

The latency of component N_{53} was also significantly increased by all three treatments, but the ethanol–saline treatment caused the greatest increase, while the saline–nicotine treatment increased latency the least (in fact, following saline–nicotine the increase was not significant at the 40 min interval). The latency following ethanol–nicotine was significantly less than that following ethanol–saline at all time intervals [ethanol: $F(1,19)=144.29$, $p<0.001$; ethanol × nicotine: $F(1,19)=27.33$, $p<0.001$; ethanol × time: $F(2,38)=12.75$, $p<0.001$; ethanol × nicotine × time: $F(2,38)=6.18$, $p=0.005$].

All three treatments significantly increased the latency of component N_{65}. Although the greatest increase in latency was observed following ethanol–nicotine, it was not significantly greater than that observed following ethanol alone [ethanol: $F(1,19)=44.99$, $p<0.001$; nicotine: $F(1,19)=21.74$, $p<0.001$; ethanol × nicotine: $F(1,19)=7.18$, $p=0.015$]. Very similar results were observed for the latency of component P_{88}, in that all three treatments significantly increased component latency, with the ethanol–nicotine treatment resulting in the greatest increase (which in this case was significantly greater than that observed following ethanol–saline) [ethanol: $F(1,19)=69.27$, $p<0.001$; nicotine: $F(1,19)=36.75$, $p<0.001$; ethanol × nicotine: $F(1,19)=33.15$, $p<0.001$; ethanol × time: $F(2,38)=5.50$, $p=0.008$].

For component N_{139}, all three treatments significantly increased latency relative to saline–saline, but there was no significant difference between the ethanol–nicotine and the ethanol–saline conditions [nicotine: $F(1,19)=16.94$, $p=0.001$; ethanol × nicotine: $F(1,19)=8.83$, $p=0.008$: ethanol × time, $F(2,38)=4.91$, $p=0.013$; nicotine × time: $F(2,38)=5.10$, $p=0.011$]. Finally, for component P_{234}, the presence of nicotine significantly increased latency of this component at all time intervals, while the presence of ethanol increased component P_{234} latency at 5 min, but decreased it at 40 min [nicotine: $F(1,19)=125.68$, $p<0.001$; ethanol × time: $F(2,38)=30.43$, $p<0.001$; nicotine × time: $F(2,38)=15.40$, $p<0.001$].

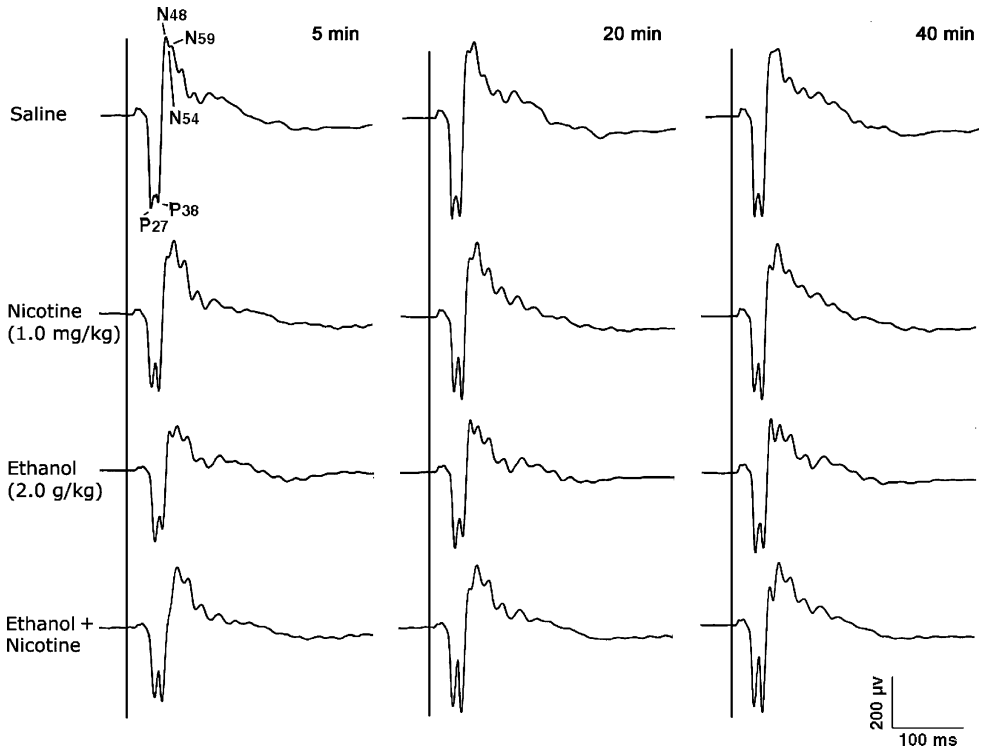

Fig. 4. Group average superior colliculus (SC) flash-evoked potential (FEP) waveforms ($n=18$) for each treatment condition and each time interval. Vertical lines represent onset of the evoking stimulus. Individual FEP components are named by polarity and latency from the onset of the evoking stimulus. The effects of ethanol, nicotine, and coadministration of ethanol and nicotine varied with the component and time interval.

3.3. Superior colliculus: amplitude

Group mean SC evoked potentials are displayed in Fig. 4. The components measured were P_{27} and P_{38} in the early positive complex, and N_{48}, N_{54}, and N_{59} on the subsequent negative portion of the waveform. Baseline-to-peak amplitude data for each of these components are presented in Fig. 5. In the early positive complex, component P_{27} was significantly depressed in amplitude to the same extent by all three treatments [ethanol: $F(1,17)=20.43$, $p<0.001$; nicotine: $F(1,17)=41.624$, $p<0.001$; ethanol × nicotine: $F(1,17)=7.50$, $p=0.014$]. In contrast, only ethanol significantly depressed the

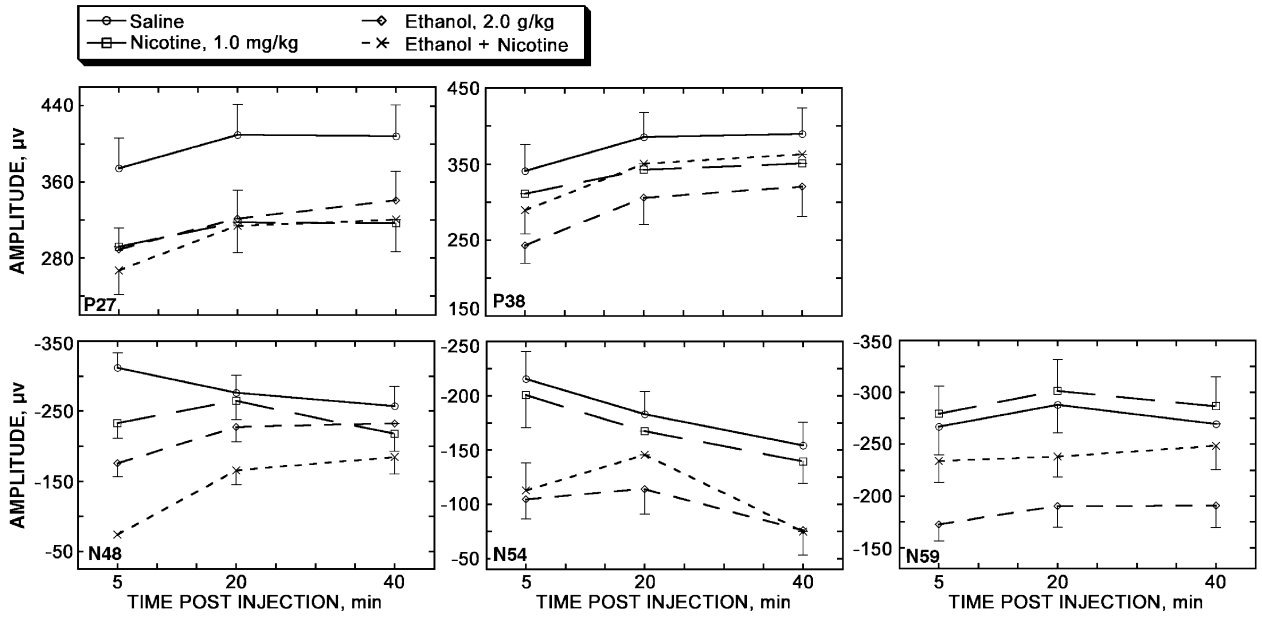

Fig. 5. Peak amplitudes of superior colliculus (SC) flash-evoked potential (FEP) components ($n=18$) as a function of drug treatment and time interval. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above or below the mean values represent ±1 S.E.M.

amplitude of component P_{38} in comparison to the saline–saline condition, but the ethanol–saline condition was not significantly different from the ethanol–nicotine condition [ethanol: $F(1,17)=9.65$, $p=0.006$; ethanol × nicotine: $F(1,17)=5.73$, $p=0.028$].

Component N_{48} was depressed at 5 and 20 min by the presence of ethanol, and at all time intervals by the presence of nicotine [ethanol: $F(1,17)=61.67$, $p<0.001$; nicotine: $F(1,17)=37.53$, $p<0.001$; ethanol × time: $F(2,34)=18.11$, $p<0.001$; nicotine × time: $F(2,34)=8.69$, $p=0.001$]. In contrast, only the presence of ethanol significantly depressed the amplitude of component N_{54} [ethanol: $F(1,17)=21.82$, $p<0.001$]. While ethanol also depressed the amplitude of N_{59}, nicotine counteracted this effect, since the combination of ethanol–nicotine produced an amplitude that was significantly larger than that resulting from the ethanol–saline treatment [ethanol: $F(1,17)=32.03$, $p<0.001$; nicotine: $F(1,17)=17.63$, $p=0.001$; ethanol × nicotine; $F(1,17)=4.30$, $p=0.05$].

3.4. Superior colliculus: latency

SC latency data are presented in Fig. 6. All three drug treatments generally increased the latency of all of the SC components measured. In addition, for all 5 components, the saline–nicotine treatment produced the smallest increase in latency. For component P_{27}, all three treatments significantly increased latency. However, there was no significant difference between the ethanol–saline condition and the ethanal–nicotine condition [ethanol: $F(1,17)=109.97$, $p<0.001$; nicotine, $F(1,17)=9.37$, $p=0.007$; ethanol × nicotine: $F(1,17)=13.04$, $p=0.002$]. Results for component P_{38} were virtually identical to those of P_{27} with all three treatments again increasing latency, but with no difference between ethanol–saline and ethanol–nicotine [ethanol: $F(1,17)=212.03$, $p<0.001$; nicotine, $F(1,17)=10.89$, $p=0.004$; ethanol × nicotine: $F(1,17)=33.92$, $p<0.001$; ethanol × time: $F(2,34)=5.91$, $p=0.006$; ethanol × nicotine × time: $F(2,34)=5.54$, $p=0.008$].

While all three treatments again produced significantly increased latencies at all three time intervals for component N_{48}, the ethanol–nicotine condition resulted in a significantly shorter latency than the ethanol–saline condition at the 5-min interval [ethanol: $F(1,17)=70.37$, $p<0.001$; ethanol × nicotine: $F(17)=17.29$, $p=0.001$; ethanol × time: $F(2,34)=6.31$, $p=0.005$; ethanol × nicotine × time: $F(2,34)=6.50$, $p=0.004$]. For component N_{54}, Dunnett test comparisons showed that all three treatments significantly increased the latency of this component, and that nicotine counteracted the effect of ethanol in that the latency following ethanol–nicotine treatment was significantly less than that following ethanol–saline treatment [ethanol: $F(1,17)=57.22$, $p<0.001$; ethanol × nicotine: $F(1,17)=20.76$, $p<0.001$; ethanol × time: $F(2,34)=7.44$, $p=0.002$].

Finally, the results for component N_{59} were very similar to those for the two primary positive components (P_{27} and P_{38}), with all three drug treatments significantly increasing the latency of this component, but no significant difference between the ethanol–saline condition and the ethanol–nicotine condition [ethanol: $F(1,17)=71.75$, $p<0.001$; nicotine: $F(1,17)=14.18$, $p=0.002$; ethanol × nicotine: $F(1,17)=27.61$, $p<0.001$].

3.5. Body temperature

Body temperature data are presented in Fig. 7. All three drug treatments produced significant hypothermia, with the combination of ethanol–nicotine resulting in significantly

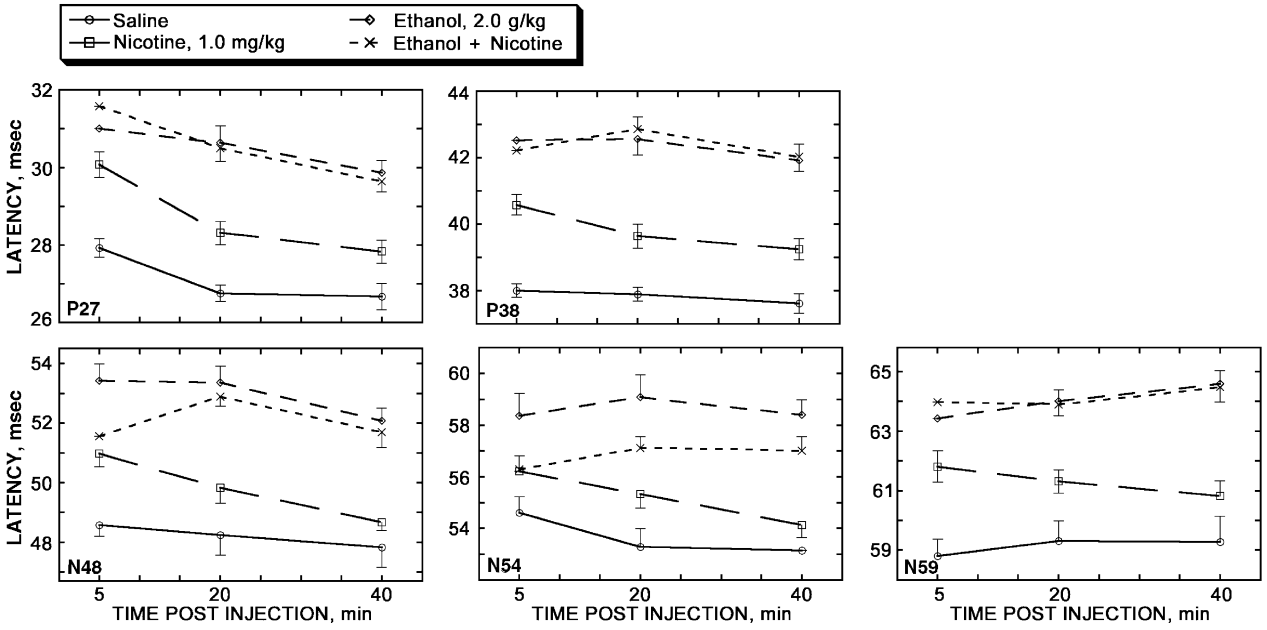

Fig. 6. Peak latencies of superior colliculus (SC) flash-evoked potential (FEP) components ($n=18$) as a function of drug treatment and time interval. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above or below the mean values represent ±1 S.E.M.

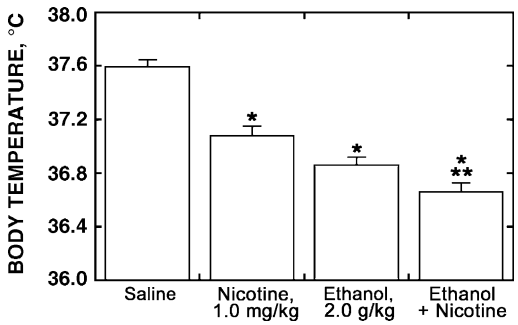

Fig. 7. Body temperature ($n=22$) as a function of drug treatment. Body temperature was measured immediately after FEP testing with a rectal thermistor probe. Vertical bars above the mean values represent +1 S.E.M. *$p<0.05$ when compared to the saline value, **$p<0.05$ when compared to the ethanol value, by a two factor repeated measures ANOVA followed by Dunnett test comparisons.

greater hypothermia than ethanol–saline [ethanol: $F(1,21)=79.39$, $p<0.001$; nicotine: $F(1,21)=39.17$, $p<0.001$; ethanol × nicotine: $F(1,21)=16.14$, $p=0.001$].

3.6. Body movement

Fig. 8 presents the mean number of photocell beam interruptions recorded during the 3.5-min of evoked potential recording for each recording session. The only significant effect was the main effect of ethanol, $F(1,21)=26.02$, $p<0.001$. Thus, the presence of ethanol greatly decreased the number of body movements in the recording chamber.

Following each recording session and temperature measurement, animals were observed for two minutes in an open field maze. There were significant differences between treatments in regard to both number of squares crossed (Fig. 9, left panel) and rearing behavior (Fig. 9, right panel). For the number of squares crossed, Dunnett tests revealed that ethanol, but not nicotine, significantly increased the number of squares crossed in the open field in comparison to the saline–saline condition. However, nicotine appeared to augment the ethanol-induced

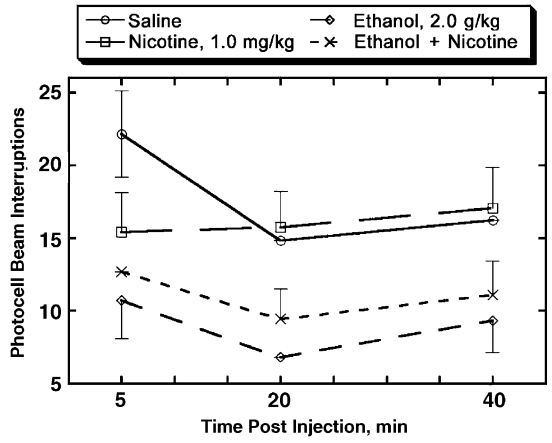

Fig. 8. Body movement ($n=22$) as measured by photocell beam interruptions during the 3.5-min evoked potential test sessions. Data are presented as a function of drug treatment and time interval. Vertical bars above or below the mean values represent ±1 S.E.M.

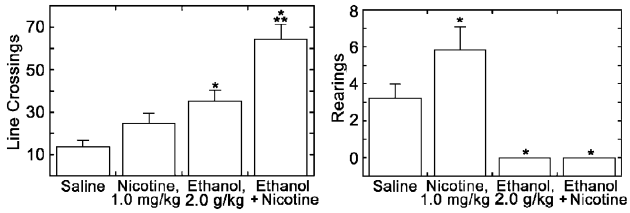

Fig. 9. Body movement during a 2-min open-field test session which followed evoked potential collection. Left panel: Mean line crossings ($n=22$). Right panel: Mean rearings ($n=22$). Data are presented as a function of drug treatment. Vertical bars above the mean values represent +1 S.E.M. *$p<0.05$ when compared to the saline value, **$p<0.05$ when compared to the ethanol value.

locomotion, since the combination dose produced the greatest increase in movement, significantly greater than that of ethanol alone [ethanol: $F(1,21)=43.09$, $p<0.001$; nicotine: $F(1,21)=28.36$, $p<0.001$; ethanol × nicotine: $F(1,21)=8.71$, $p=0.008$].

In regard to rearing behavior, results from the Friedman test were significant, $F(3,63)=57.93$, $p<0.001$. Subsequent treatment comparisons showed that while nicotine significantly increased this measure of behavior in comparison to saline, both ethanol and the combination treatment reduced (eliminated) rearings.

Behavioral observations were also made during this 2 min observational time. Following saline administration, the rats tended to go first to a corner, either immediately, or after a slight pause. This was sometimes followed by ambulation around the perimeter, or back and forth along one wall. They also spent much of the time in the corners—sniffing, sitting, rearing, or turning in circles. By the end of the 2 min, nearly all of the rats were sitting in a corner.

Not a single rat reared following ethanol administration. In this condition, rats ran immediately to a corner, and then ambulated around the perimeter, or back and forth along one wall. However, in contrast to the control condition, notable ataxia was observed, as evidenced by a tendency to fall to one side, or slip and slide as they ran. Pauses in a corner were often accompanied by head bobbing and swaying. There was also a greater tendency to cross the center of the maze than observed during the saline treatment.

Rearing behavior was notably augmented following nicotine administration. There were no obvious balance problems, nor did the animals fall to one side. There did seem to be a tendency for the animals to groom more frequently, but there was also a notable increase in sniffing, especially in corners where they turned in circles as well.

The greatest amount of locomotion was observed in the combination condition, with much of it occurring by running around the perimeter. As with the alcohol trials, these rats were more likely to run through the center of the maze than during the saline or nicotine trials. Balance was quite problematic for the animals, with a pronounced tendency to run into walls and ambulating in a less-than-straight line. They often paused in corners, sometimes changing directions. Also similar to ethanol trials, much head bobbing and swaying occurred.

4. Discussion

The results of the present study show that nicotine and ethanol produce a complicated pattern of functional interactions in the neuronal processes underlying the generation of FEPs recorded from both the VC and the SC. Ethanol depressed the amplitude of most VC FEP component amplitudes: N_{29}, N_{39}, P_{88}, N_{139}, and P_{234}. While there were minimal or no effects on components P_{22}, N_{53}, or N_{65}, ethanol enhanced P_{46} amplitude. These results are in general agreement with prior work from this laboratory (Hetzler and Bednarek, 2001; Hetzler et al., 1981, 1982). For example, Hetzler and Bednarek (2001) observed in male hooded rats an ethanol-induced depression of components N1 and N3, augmentation of P2, and no effect on P1 and N2 (analogous to N_{29}, N_{139}, P_{46}, P_{22}, and N_{65}, respectively).

Nicotine augmented VC components N_{39}, P_{46}, N_{53}, N_{65} (at 20 and 40 min) and P_{88} amplitudes, but depressed the amplitudes of components N_{29}, N_{65} (at 5 min), and P_{234}. Nicotine had little or no effect on the amplitude of components P_{22} and N_{139}. These results are in close agreement with a recently reported study involving the effects of a range of nicotine dosages on VC FEPs (Hetzler and Theinpeng, 2004). In that study, nicotine increased the amplitude of components N_{39}, N_{53}, N_{67}, and P_{88}, while decreasing the amplitude of components N_{30} and P_{235}.

In the present study, when given alone ethanol and nicotine had the most obvious opposing effects on the amplitudes of VC components N_{39}, N_{53}, and P_{88}. It is for these components that nicotine would most likely be expected to counteract the effects of ethanol. In contrast, ethanol and nicotine produced similar amplitude changes in components N_{29}, P_{46}, and P_{234}. But are such superficial similarities in component modification a reflection of similar underlying processes altered by both ethanol and nicotine? If so, then the combination of ethanol and nicotine would be expected to produce an effect greater than that observed from ethanol alone.

Coadminstration of nicotine and ethanol produced different effects on the amplitude of different VC FEP components, illustrating the unique pharmacological neurogenesis of each component. P_{22} and N_{29} are the two primary components of the rat VC FEP, with P_{22} the correlate of the presynaptic geniculate volley, while N_{29} represents a geniculocortical synaptic process produced by excitatory amino acid (EAA) release in Layer IV of the VC (Meeren et al., 1998; Siegel and Sisson, 1993). Nicotine neither enhanced nor counteracted the effects of ethanol on these sensory components.

However, for components N_{39}, P_{46}, N_{53}, and P_{88}, the combination treatment had an effect in between that produced by either drug alone, suggesting some type of counteracting interaction. Although N_{39} has not often been studied, the other components P_{46}, N_{53}, and P_{88} are the so-called secondary components which are thought to result from connections between the SC, brain stem, and diffuse thalamic projections (Creel et al., 1974). These components are not as influenced by stimulus manipulations as are the primary components (Creel et al., 1974), and presumably reflect more cognitive processing. The VC results therefore suggest that in the rat nicotine is more likely to counteract ethanol-induced impairments in measures of cognition than sensory abilities.

Of particular interest, however, was the finding that in no case did the combination of ethanol and nicotine result in an altered amplitude that resembled a nicotine-induced enhancement of the effects of ethanol. Such an effect would have been expected for component P_{46}, since it was increased by both ethanol and (to a lesser extent) nicotine. P_{46} represents a nongeniculate, non-EAA-mediated inhibitory postsynaptic potential on the pyramidal cells of cortical layers V and VI (Meeren et al., 1998; Siegel and Sisson, 1993). In part because drugs which block muscarinc AChRs augment this component, we speculated in earlier work that an ethanol-related reduction in cortical acetylcholine release, with a resulting decrease in stimulation of mAChRs, might explain an ethanol-induced increase in the amplitude of this component (Hetzler and Smith, 1984). Although it now appears that direct stimulation of nAChRs can also augment the amplitude of this component to some extent, nicotine may be able to counteract the greater effects of ethanol-induced augmentation by stimulating ACh release (Levin and Simon, 1998), producing secondary activation of mAChRs and lowering amplitude somewhat.

In any event, in the VC amplitude data we did not find support for the suggestion by Collins et al. (1988) that alcohol and nicotine are used together because they have similar effects on nAChRs. It is possible that our failure to note such an effect resulted from the relatively high doses of ethanol and nicotine employed in the present study. Tizabi et al. (2002) simultaneously administered systemic ethanol and microinjection of nicotine into the ventral tegmental area and found an additive effect on dopamine release in the nucleus accumbens only with low doses of ethanol and nicotine; high doses did not produce an additive effect, perhaps as a result of a ceiling effect.

Components N_{53} and P_{234} demonstrate interesting interactions between the effects of these two drugs, i.e., the combination dose matched ethanol's effects at 5 min, but closely resembled nicotine's effects at 40 min. Perhaps most unusual was the effect of the combined drugs on the amplitude of component N_{139}. At 5 min, the combination dose augmented this component, even though neither ethanol nor nicotine alone affected N_{139} amplitude at this recording interval. At the 20 min interval, the combination dose produced an amplitude that was approximately at the saline level, while by 40 min the combination depressed the amplitude of this component to the same degree as ethanol alone. Although neither drug has been shown to influence the metabolism of the other (Collins et al., 1988; Zacny, 1990), these time-dependent effects presumably are related to differing interactions between the 2 drugs as a function of differing stages of metabolism (Crooks and Dwoskin, 1997; Froehlich et al., 2001).

Components of the SC FEP are generated in the stratum griseum superficiale (Dyer and Annau, 1977), since that is where they show a polarity reversal. Ethanol significantly depressed the amplitude of all 5 components recorded from the SC. Past studies (Hetzler and Bednarek, 2001; Hetzler et al., 1981, 1982) have also shown the depressant effects of ethanol on SC

components, especially P3 and N4 (analogous to P_{38} and N_{48-59}). The effects of nicotine on SC component amplitudes were more selective than the effects of ethanol, depressing the amplitude of only components P_{27} and N_{48}. These results are similar to those reported in a prior study on the effects of nicotine (Hetzler and Theinpeng, 2004), which found that nicotine reduced the amplitude of components P_{27}, N_{48}, and N_{53}. In regard to the combined effects of ethanol and nicotine in the present study, nicotine selectively counteracted the depressant effect of ethanol on only N_{59} amplitude, and there was no statistically significant evidence for a nicotine-induced enhancement of the effects of ethanol. It therefore appears that individual components of cortical FEPs are the more labile in this regard.

All three drug treatments increased the latency of all nine VC components, with nicotine usually producing the least increment. Ethanol produced the greatest increase in latency for components P_{22}, N_{29}, and N_{53}, while the combination treatment resulted in the greatest increase in peak latency for components N_{39}, N_{65}, and P_{88}. That both ethanol and nicotine will increase VC component latencies is consistent with other rat FEP studies (Hetzler and Bednarek, 2001; Hetzler and Theinpeng, 2004; Hetzler et al., 1981, 1982), and the enhanced augmentation following the coadministration of ethanol and nicotine noted for components N_{39}, N_{65}, and P_{88} is consistent with the enhanced hypothermia likewise found (see discussion below). It was surprising, however, that for some components the combination treatment resulted in latencies which were in between that produced by the two individual drug treatments. Components P_{22}, N_{29}, N_{53}, and N_{139} show this effect.

Drug effects on FEP component latencies recorded from the SC were similar to their effects on VC component latencies, in that all three treatments increased SC FEP latencies, and nicotine invariably caused the least latency increase. Past research has shown similar results regarding the effects of both ethanol and nicotine on SC component latencies (Hetzler and Bednarek, 2001; Hetzler and Theinpeng, 2004; Hetzler et al., 1981, 1982). The effects of the combination dose typically were about the same as that for ethanol alone, although there was some suggestion of latencies in between that for either nicotine or ethanol alone for components N_{48} (5 min) and N_{54}.

Increases in peak latency may result from (1) decreased axonal conduction velocity, (2) increased synaptic delay, or (3) a combination of these 2 processes (Hetzler et al., 1988). In the present context, both the direct effects of the drugs and hypothermia may be contributing factors to the increased FEP peak latencies. That is, changes in body temperature can produce secondary changes in FEP peak latency (Hetzler et al., 1988). Both ethanol and nicotine produced significant hypothermia in the present study, with body temperature dropping 0.74 and 0.6 °C, respectively. Similar results have been reported by this, and other laboratories for both ethanol (Hetzler and Bednarek, 2001; Rezvani and Levin, 2002) and nicotine (de Fiebre et al., 1991; Hetzler and Theinpeng, 2004; Rezvani and Levin, 2002). In addition, the combination of ethanol and nicotine resulted in a greater hypothermia (1.0 °C) than that produced by ethanol alone, confirming the earlier work by Rezvani and Levin (2002).

Past research has demonstrated that a drop in body temperature results in a linear latency increase for FEP components recorded from the VC, but does not alter FEP amplitudes (Hetzler et al., 1988). For VC component P_{22}, the estimated latency increase from a 1.0 °C decrease in body temperature is about 1.4–1.7 ms, whereas for component N_{29} the expected increase in latency is about 1.6–2.2 ms. These estimates can account for nearly all of the observed ethanol- and nicotine-induced increases in latency observed for VC components P_{22} and N_{29}. However, these estimates cannot account for the findings that VC and SC component latencies following coadministration of ethanol and nicotine were rarely significantly greater than those caused by ethanol alone, even though there was a greater hypothermia following the combination treatment. Furthermore, on several occasions the combination treatment resulted in a component latency in between that observed for either ethanol or nicotine alone: VC P_{22}, N_{29}, N_{53}, N_{139}, SC N_{48}, N_{54}. There thus appear to be non-temperature-related processes altered by these two drugs which when activated simultaneously can counteract each other in regard to the rate of neuronal processing of information.

During the recording sessions in the testing chamber, ethanol depressed movement, a finding which is consistent with previous data (Hetzler and Bednarek, 2001). In the 2-min open field test, however, ethanol significantly increased locomotion, a result that is also supported by past research (e.g., Hilakivi et al., 1984). The ethanol-dependent behavioral difference between recording sessions and subsequent open field observations is most likely the result of differences in the testing chambers (Frye and Breese, 1981). This notion is supported by the results of a study by Päivärinta and Korpi (1993) using AA (ethanol-preferring) and ANA (ethanol-avoiding) male rats. Ethanol injections of 0.6 or 1.0 g/kg had no effect on locomoter activity in either the AA or ANA rats when the rats were tested in Macrolon size III cages (about 800 cm^2), but the 1.0 g/kg dose did increase activity (without a line difference) during the first minute of testing (12 min after injection) in a modified $110 \times 110 \times 35$ cm open field.

Nicotine did not significantly alter locomotion during either the FEP recording sessions, or later in the open field maze, although the number of rearings was significantly increased by nicotine, in contrast to their abolishment by ethanol. In prior research, we likewise did not observe increased locomotion in an open field following nicotine administration, but we did find increased photocell beam interruptions during the recording sessions (Hetzler and Theinpeng, 2004). The effects of nicotine on locomotor behavior in rats are complex, with many variables involved (see Jerome and Sanberg, 1987). In regard to ambulation, past researchers have reported increases (Benwell and Balfour, 1992), decreases (Erickson, 1971), and no overall effect (Paulus and Geyer, 1991). The effects of nicotine on rearing behavior in rats appear to be both dose- and time-dependent. Thus, Ksir (1994) found increased vertical movement following administration of 0.1 and 0.2 mg/kg (s.c.) doses of nicotine (calculated as nicotine base weight), but decreased vertical movement with a nicotine dose of 0.4 mg/kg, while Nagahara and Handa (1999) reported that a 0.75 mg/

kg (i.p.) dose of nicotine decreased rearing for 10 min, but later increased rearing.

The combination of ethanol and nicotine significantly reduced behavior during the recording sessions, although the results were not significantly different from that of ethanol alone. In contrast, in the open field, the combination treatment significantly increased the number of squares crossed when compared to both saline and ethanol alone. The combination of nicotine and ethanol has previously been shown to augment behavioral activity in the rat, though this has more commonly been in comparison to the stimulatory effects of nicotine alone (e.g., Schaefer and Michael, 1992). In contrast to the effects on locomotion, the combination treatment eliminated rearing activity, as was the case for ethanol alone. This failure to stand can most likely be attributed to ethanol-induced incoordination, which was not blocked by nicotine.

Overall, there are data suggesting that nicotine can counteract some of the effects of ethanol. This was demonstrated in varying degrees in the amplitude of VC components N_{39}, P_{46}, N_{53}, and P_{88}, the latency of VC component N_{53}, the amplitude of SC component N_{59}, and the latency of SC components N_{48} and N_{54}. With the possible exception of some of the VC latency data, and perhaps the amplitude of VC component P_{234}, the electrophysiological results generally did not support a nicotine-induced enhancement of the effects of ethanol. However, the body temperature and line crossings in an open-field did suggest that nicotine can enhance the effects of ethanol, illustrating the complexities involved in attempting to measure ethanol–nicotine interactions. Similarly, Collins (1990) concluded that the interactions between ethanol and nicotine found in human studies have been dose- and test-specific.

Such complexity is not surprising, given that nAChRs in the central nervous system modulate the release of a variety of neurotransmitters, including acetylcholine, dopamine, norepinephrine, serotonin, glutamate, and GABA (Levin and Simon, 1998; McGehee et al., 1995; Role and Berg, 1996; Zhu and Chiappinelli, 1999). Thus, nicotine can simultaneously affect the activity of many types of neurons (USDHHS, 1988), making it difficult to localize the origin of the effects reported here. Acute ethanol exposure also influences a variety of neurotransmitter systems (Narahashi et al., 1999), including an increased availability of serotonin (Lovinger, 1997), enhanced action of GABA at GABA-A receptors (Dalvi and Rodgers, 1996; Proctor et al., 1992), interference with the excitatory effects of transmission of glutamate at *N*-methyl-D-aspartate receptors (Hoffman and Tabakoff, 1993; Sepulveda et al., 1995), and altered release of Ach (Carmichael and Israel, 1975; Stancampiano et al., 2004). Further work on the underlying neuronal and pharmacological processes involved in FEP peak production/modulation will no doubt aid in understanding the effects reported here.

Acknowledgements

The present study was funded by Lawrence University. A preliminary report on portions of these data was presented at the 12th World Congress on Biomedical Alcohol Research, Heidelburg/Mannheim, Germany, 2004. The authors thank LeRoy Frahm for expert technical assistance, and both Matthew Ansfield and Joy Jordan for statistical assistance.

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