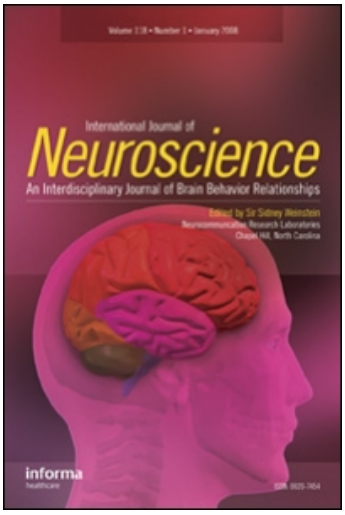

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Baclofen Does Not Counteract the Acute Effects of Ethanol on Flash-Evoked Potentials in Long-Evans Rats

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BACLOFEN DOES NOT COUNTERACT THE ACUTE EFFECTS OF ETHANOL ON FLASH-EVOKED POTENTIALS IN LONG-EVANS RATS

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This experiment examined the separate and combined effects of baclofen (5.0 mg/kg, i.p.), a $GABA_B$ receptor agonist, and ethanol (2.0 g/kg, i.p.) on flash-evoked potentials (FEPs) recorded from both the visual cortex (VC) and superior colliculus (SC) of chronically implanted male Long-Evans rats. In the VC, ethanol

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significantly decreased the amplitude of positive component P_{87}, but increased P_{37} and P_{47}. Other component amplitudes were not significantly altered. In contrast, baclofen reduced the amplitude of negative component N_{31} to such an extent that it became positive. Although P_{47} was also reduced by baclofen, the amplitude of most other components was increased. Only P_{24} and P_{87} were unchanged by baclofen. The combination of baclofen and ethanol resulted in amplitudes very similar to ethanol alone for secondary components P_{47}, N_{62}, and P_{87}, but very similar to baclofen alone for primary component N_{31} and late components N_{147} and P_{230}. In the SC, component amplitudes were generally decreased by ethanol, baclofen, and the combination treatment. Latencies of most components in both structures were increased by the drug treatments. Each drug treatment produced significant hypothermia. Locomotor behavior was also altered. These results demonstrate: (1) pharmacological differences between the primary and late components versus the secondary components of the cortical FEP, (2) that baclofen does not counteract significant effects of ethanol on cortical or collicular component amplitudes, and (3) that baclofen enhances N_{147}-P_{230} amplitude, suggesting reduced cortical arousal.

Keywords baclofen, ethanol, flash-evoked potentials, rats, superior colliculus, visual cortex

INTRODUCTION

Ethanol is a CNS depressant which influences a variety of neurotransmitter systems, both directly and indirectly, including increased availability of serotonin (Lovinger, 1997), enhanced action of γ-aminobutyric acid (GABA) at $GABA_A$ receptors (Proctor, Soldo, Allan, & Dunwiddie, 1992), interference with the excitatory effects of glutamate transmission at N-methyl-D-aspartate receptors (Hoffman & Tabakoff, 1993), altered release of cortical acetylcholine (Stancampiano et al., 2004), and altered functioning of nicotinic acetylcholine receptors (Aistrup, Marszalec, & Narahashi, 1999). In doing so, ethanol disrupts a variety of cognitive functions (Rezvani & Levin, 2002).

While most of the previous studies detailing ethanol's interaction with the GABA neurotransmitter system have emphasized the ionotropic $GABA_A$ receptor (e.g., Chester & Cunningham, 2002; Grobin, Matthews, Devaud, & Morrow, 1998), there is increasing interest in the effects of ethanol on the functioning of the metabotropic G-protein coupled $GABA_B$ receptors as well (e.g., Ariwodola & Weiner, 2004). In this regard, baclofen (beta-(4-chlorophenyl)-GABA), a lipophilic derivative of GABA that functions as a stereoselective $GABA_B$ receptor agonist (Addolorato et al., 2000; Harrison, Lange, & Barker, 1988) has recently received considerable attention. Baclofen has been used clinically since the 1970s as a centrally acting muscle relaxant and antispastic agent, suppressing both mono- and polysynaptic spinal reflexes

(Bowery & Enna, 2000). Recent interest has centered on its reported ability to reduce ethanol intake in rats (see Colombo et al., 2004; Czachowski, Legg, & Stansfield, 2006), reduce alcohol craving and intake, and alleviate alcohol withdrawal symptoms in human alcoholics (Addolorato et al., 2000; Flannery et al., 2004).

One method used to characterize the effects of drugs and toxic substances on the functioning of the nervous system utilizes sensory-evoked potentials (Dyer, 1985). Sensory-evoked potentials, which can be easily recorded from both cortical and subcortical sites in laboratory animals, are complex neural responses that 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 & Rosenfeld, 1972). Such evoked potentials are often used to assess the functional integrity of the brain (Dyer, 1985), because they provide an integrated view of neural activity and sensory processing (Sisson & Siegel, 1989). 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, Dustman, & Beck, 1974).

Ethanol produces a pattern of changes in the FEP recorded from the rat visual cortex (VC) (Hetzler & Bednarek, 2001; Hetzler & Martin, 2006). To the extent that activation of $GABA_B$ receptors is capable of modulating the effects of ethanol, pretreatment with baclofen would be expected to alter the ethanol-induced changes in FEPs. Given the different neurogenesis and functional significance of the primary, secondary, and late VC FEP components, it is also of interest to determine if baclofen is more effective in altering ethanol-induced changes in the primary (sensory) vs. later (cognitive) components. To examine subcortical interactions between baclofen and ethanol, 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 baclofen can produce hypothermia in rodents (Hetzler & Bednarek, 2001; Hetzler & Ondracek, 2007), and such changes in body temperature may then result in secondary changes in evoked-potential parameters (Hetzler, Boyes, Creason, & Dyer, 1988). Finally, since both ethanol and baclofen alter behavior in rats (e.g., Hetzler & Martin, 2006; Hetzler & Ondracek, 2007), we monitored gross body movement during and after the evoked-potential recording sessions.

METHODS

Animals

Twenty-eight adult, male Long-Evans hooded rats (Harlan, Indianapolis, IN), about 4-months old and weighing 386–420 g at the time of surgery, were used. The rats were housed individually in standard cages in a room with a light/dark cycle (light from 06:00 hr to 18:00 hr) and climate control (temperature about 22°C and humidity about 50%). Purina Lab Chow (St. Louis, MO) and tap water were provided ad libitum. All procedures were approved by the Lawrence University Animal Care and Use Committee.

Electrode Implantation Surgery

At least 1-week before adaptation, recording electrodes were implanted in the VC and the SC while the animals were under the effect of pentobarbital anesthesia. The rats first received an intraperitoneal (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/kg body weight. The VC electrode (0–80 × 1/8 inch 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 provided for a recording reference and grounding, respectively. SC recordings were made from a twisted pair of nichrome wires (each 250 microns 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 & 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 4.7 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, Heilbronner, Griffin, & Griffin, 1981). Results for the SC are reported for those 21 animals in which the lower member of the electrode pair penetrated the superficial layer of the SC (Dyer & Annau, 1977). VC recordings from three other animals were unusable because of problems with the cap or amplifiers.

Chemicals

(±)Baclofen (Sigma Product No. B-5399, St. Louis, MO) was dissolved in 0.9% saline to obtain a dose of 5.0 mg/kg, which was injected i.p. in a volume

of 2 ml/kg. 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 time following injections were based on prior research (Hetzler & Martin, 2006; Hetzler & Ondracek, 2007).

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 (2,000-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 s. 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 lux. The testing box, which was located inside the shielded chamber, measured 10 × 10 × 10 inches and was constructed of white Plexiglas on three sides, the top, and the bottom, whereas the front panel was clear. Shielded Microdot cables (Microdot Connectors, South Pasadena, CA), which are designed to reduce artifacts associated with cable movements (Fox & 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 Instrument Division, Astro-Med, West Warwick, RI) with an intensity setting of 8 (measured as 133 lux-s using a Model DR-1600 Photometer with a Model D-1500–2B Multiprobe; Gamma Scientific, San Diego, CA). 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.

The animals were given 2 days of familiarization to the testing procedures, followed by 1–3 days of rest before actual data collection. On each testing day, 5 min prior to the first injection, the animals received one drop of 1% ophthalmic atropine in each eye to maintain constant pupil dilation. The first i.p. injection (volume of 2.0 ml/kg) was either physiological saline (0.9% sodium chloride) or 5.0 mg of baclofen per kilogram of body weight. The second i.p. injection followed 20 min later, and was either saline or 2.0 g of ethanol/kg (volume of 1.26 ml/100 g). Baclofen was given prior to ethanol

Table 1. Experimental design

	Testing sequence					
Treatment	Eyedrops	1st injection	2nd injection	FEPs	Body temp.	Open field
Saline	Yes	Saline	Saline	5 min	Yes	Yes
Baclofen	Yes	Baclofen	Saline	5 min	Yes	Yes
Ethanol	Yes	Saline	Ethanol	5 min	Yes	Yes
Baclofen + Ethanol	Yes	Baclofen	Ethanol	5 min	Yes	Yes

Five minutes after receiving eye drops, the first injection (saline or baclofen) was given i.p. Twenty minutes later, the second injection (saline or ethanol) was given i.p. Ten minutes after the second injection, the animal was placed in the testing chamber, and both FEPs and body movement were recorded 5 min after being placed in the chamber.

since this is the sequence typically employed in assessing the effects of baclofen on ethanol self-administration in rats (e.g., Anstrom, Cromwell, Markowski, & Woodward, 2003; Czachowski et al., 2006). Ten minutes after the 2nd injection, the animal was placed in the testing chamber. After a 5-min wait, the data were collected. Thus, testing began 35 min after the first injection. Evoked potentials were simultaneously collected from both the VC and the SC. The treatment order for each subject was counterbalanced across animals, and the animals were given 2 days of rest between tests. Thus, each animal was tested using all four treatment conditions (see Table 1).

In addition to evoked potential collection, gross body movement was measured by two intersecting photocell sensors (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 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°C) was controlled by a wall thermostat, and was recorded at the conclusion of each test.

Following body temperature measurement, each animal was observed for 5 min 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 lux. On each testing day, the animal was placed in the same middle square of the open field, and was allowed

to explore for 5 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, and general qualitative observations were made of each animal's behavior.

FEP Component Identification

Figure 1 presents group average FEP waveforms for both the VC and the SC. 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_{24}, N_{31}, P_{37}, P_{47}, N_{62}, P_{87}, N_{147}, and P_{230}; see Figure 1). Components P_{29}, P_{38}, and N_{51} were similarly analyzed in the SC waveforms (see Figure 1). 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.

Data Analysis

VC, SC, photocell, body temperature, and open field line crossing data were subjected to 2 (baclofen) $\times$ 2 (ethanol) repeated measures analyses of variance. When a significant interaction was found, individual means were compared with the Dunnett test. That is, the baclofen-saline, saline-ethanol, and baclofen-ethanol treatments were compared to the saline-saline treatment. In addition, the baclofen-ethanol treatment was compared with the saline-ethanol condition with the Dunnett test. However, these tests were not appropriate for the open-field rearing data, since there was almost no variability in the saline-ethanol and the baclofen-ethanol conditions. Therefore, the nonparametric Friedman test was used, followed by multiple comparisons. In all analyses, statistical significance was assumed when $p < .05$ for two-tailed comparisons. All 28 animals were included in the body temperature and movement data, even if they were removed from the evoked potential analyses (for technical reasons).

RESULTS

Visual Cortex: Amplitude

Group mean evoked potentials for each treatment are displayed in the left side of Figure 1, while baseline-to-peak amplitude data are presented for each

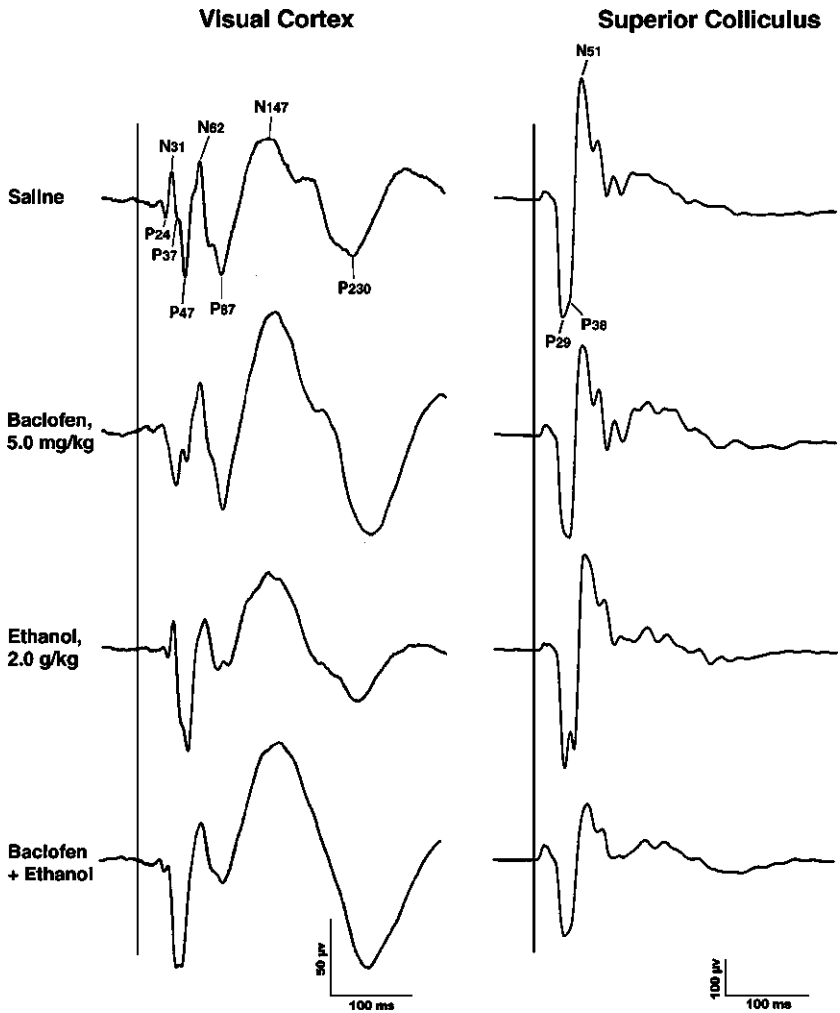

Figure 1. Group average visual cortex (VC) flash-evoked potential (FEP) waveforms (left side; $n = 25$) and superior colliculus (SC) FEP waveforms (right side; $n = 21$) for each treatment condition. Vertical lines represent onset of the evoking stimulus. Individual FEP components are named by polarity and latency from the onset of the evoking stimulus. The effects of ethanol, baclofen, and coadministration of baclofen and ethanol varied with the component.

component in Figure 2. It is apparent in these figures that both baclofen and ethanol altered the appearance of the FEPs recorded from the VC, and that the effects of both compounds varied with the component. In addition, for

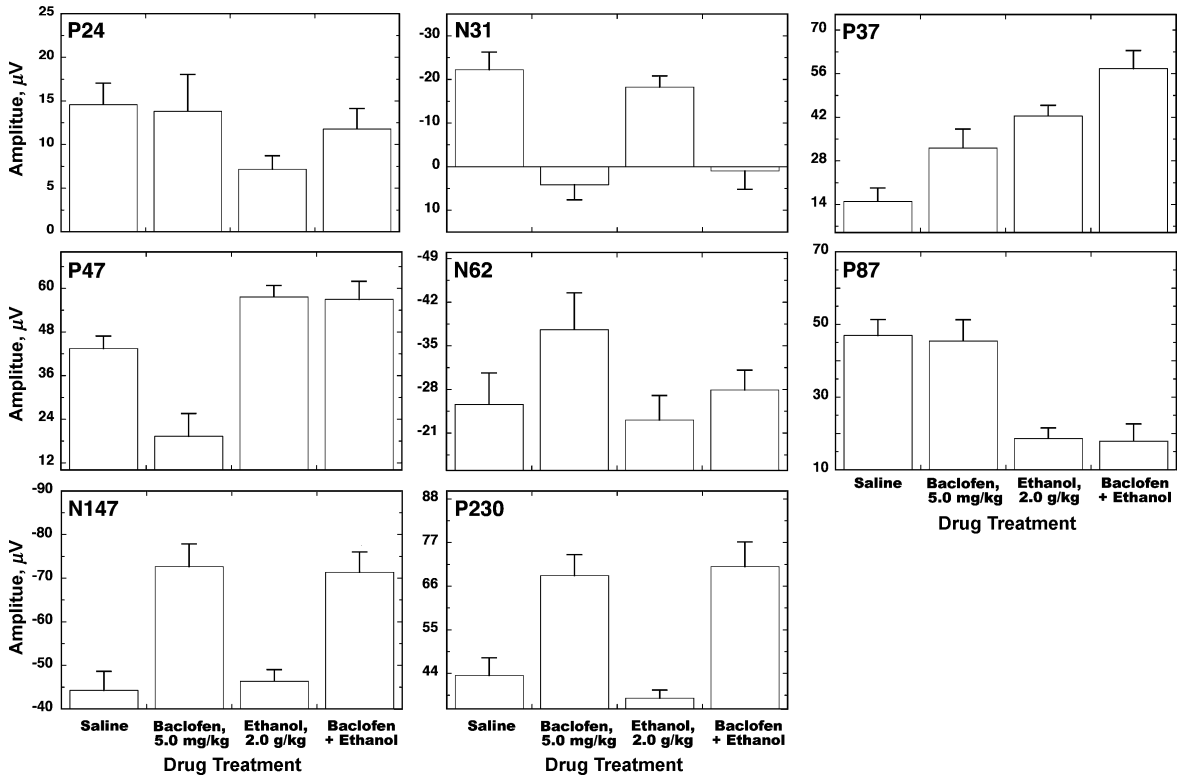

Figure 2. Peak amplitudes of visual cortex (VC) FEP components ($n = 25$) as a function of drug treatment. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above the mean values represent +1 S.E.M.

some components (N_{31}, N_{147}, P_{230}), the combination of baclofen and ethanol produced an amplitude very similar to that resulting from baclofen alone, while for other components (P_{47}, P_{87}) the combination treatment resulted in an amplitude similar to that caused by ethanol alone. In no case did it appear that baclofen counteracted a significant effect of ethanol.

Although component P_{24} was not significantly altered by any treatment (all p's $> .1$), the subsequent negative component N_{31} was virtually eliminated by baclofen, $F\,(1,24) = 65.06$, $p < .001$. Neither ethanol nor the baclofen $\times$ ethanol interaction were significant for N_{31} (both p's $> .10$). P_{37} (the small positive inflection between N_{31} and P_{47}) was enhanced by both baclofen, $F\,(1,24) = 10.20$, $p = .004$, and ethanol, $F\,(1,24) = 101.33$, $p < .001$, but the baclofen $\times$ ethanol interaction was not significant ($p = .53$). In marked contrast, P_{47} was altered in different directions by the compounds tested, with baclofen resulting in a significant decrease in amplitude, $F\,(1,24) = 28.63$, $p < .001$, while ethanol produced a significant enhancement, $F\,(1,24) = 25.05$, $p < .001$. A significant baclofen $\times$ ethanol interaction, $F\,(1,24) = 14.12$, $p = .001$, allowed for Dunnett test comparisons. Baclofen significantly reduced the amplitude of P_{47} in comparison to saline, while both ethanol and the combination of baclofen and ethanol produced significantly increased amplitudes, but there was no difference between the effects of the baclofen-saline and baclofen-ethanol treatments ($p > .05$).

Baclofen produced a significant increase in the amplitude of component N_{62}, $F\,(1,24) = 11.00$, $p = .003$, while the depressant effect of ethanol approached significance, $F\,(1,24) = 3.65$, $p = .068$. However, the baclofen $\times$ ethanol interaction was not significant ($p = .21$). P_{87} was not altered by the presence of baclofen ($p = .78$), but was reduced in amplitude by ethanol, $F\,(1,24) = 139.66$, $p < .001$. The baclofen $\times$ ethanol interaction was not significant ($p = .92$). Finally, both N_{147} and P_{230} were significantly enhanced by baclofen [N_{147}: $F\,(1,24) = 35.34$, $p < .001$; P_{230}: $F\,(1,24) = 48.74$, $p < .001$], but not significantly altered by ethanol, nor were the baclofen $\times$ ethanol interactions significant (all p's $> .29$)

Visual Cortex: Latency

The VC latency data are displayed in Figure 3. The latency of most of the VC components (all except N_{31}) was increased by baclofen and/or ethanol, but the baclofen $\times$ ethanol interaction was significant for only component P_{24}.

P_{24} latency was significantly increased by both baclofen, $F\,(1,24) = 20.21$, $p < .001$, and ethanol, $F\,(1,24) = 4.44$, $p = .046$, and the interaction was also

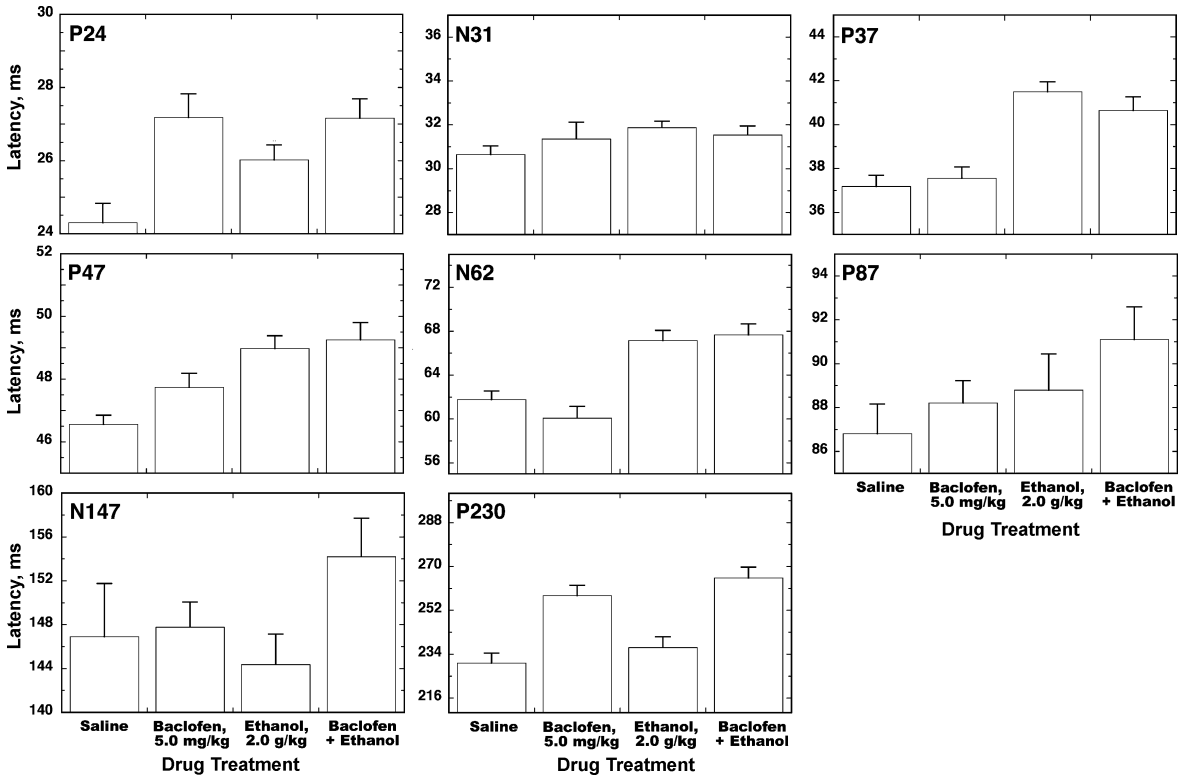

Figure 3. Peak latencies of visual cortex (VC) FEP components ($n = 25$) as a function of drug treatment. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above the mean values represent +1 S.E.M.

significant, F (1,24) = 4.83, p = .038. Dunnett test comparisons revealed that baclofen, ethanol, and baclofen in combination with ethanol, all produced P_{24} latencies significantly greater than those observed in the saline condition. However, the baclofen plus ethanol condition was not significantly different from the baclofen-saline condition.

Although the latency of N_{31} was not significantly altered by any of the treatments (all p's > .15), P_{37} latency was significantly increased when ethanol was given, F (1,24) = 76.94, $p < .001$. This was also the case for the latency of component P_{47}, F (1,24) = 15.45, p = .001, but for P_{47} the influence of baclofen approached significance, F (1,24) = 3.78, p = .064. N_{62} latency was enhanced only by ethanol, F (1,24) = 61.98, $p < .001$, but P_{87} latency was increased by both baclofen, F (1,24) = 5.79, p = .024, and ethanol, F (1,24) = 5.94, p = .023. Finally, the latencies of both components N_{147} and P_{230} were increased by only baclofen [N_{147}: F (1,24) = 4.74, p = .04; P_{230}: F (1,24) = 48.50, $p < .001$].

Superior Colliculus: Amplitude

Group mean SC evoked potentials are displayed in the right half of Figure 1, while baseline-to-peak amplitude data for each of these components are presented in the upper half of Figure 4. Both baclofen and ethanol had a depressant effect on SC FEPs, with ethanol significantly depressing the amplitude of all three components measured [P_{29}: F (1,20) = 25.46, $p < .001$; P_{38}: F (1,20) = 14.69, p = .001; N_{51}: F (1,20) = 79.85, $p < .001$], and baclofen depressing the amplitude of components P_{29}, F (1,20) = 11.39, p = .003, and N_{51}, F (1,20) = 22.46, $p < .001$. None of the baclofen × ethanol interactions were significant, all p's > .10.

Superior Colliculus: Latency

SC latency data are presented in the lower half of Figure 4. Ethanol significantly increased the latency of all three components measured [P_{29}: F (1,20) = 44.68, $p < .001$; P_{38}: F (1,20) = 142.14, $p < .001$; N_{51}: F (1,20) = 73.63, $p < .001$]. Although baclofen also significantly increased the latency of components P_{29}, F (1,20) = 10.45, p = .004, and N_{51}, F (1,20) = 5.34, p = .032, baclofen significantly decreased the latency of component P_{38}, F (1,20) = 7.43, p = .013. The baclofen × ethanol interaction was significant only for component N_{51}, F (1,20) = 6.63, p = .018. Dunnett test comparisons indicated that for component N_{51}, the latency following administration of baclofen-saline, saline-ethanol, and

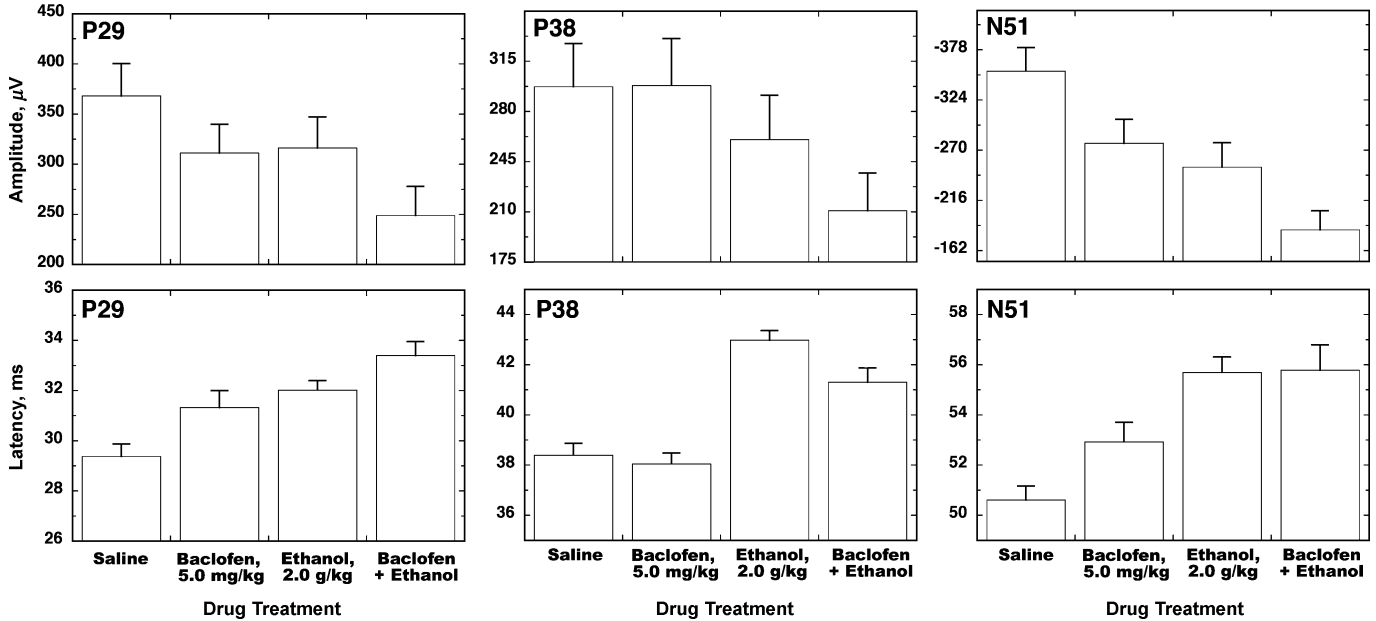

Figure 4. Peak amplitudes (upper half) and peak latencies (lower half) of superior colliculus (SC) FEP components ($n = 21$) as a function of drug treatment. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above the mean values represent +1 S.E.M.

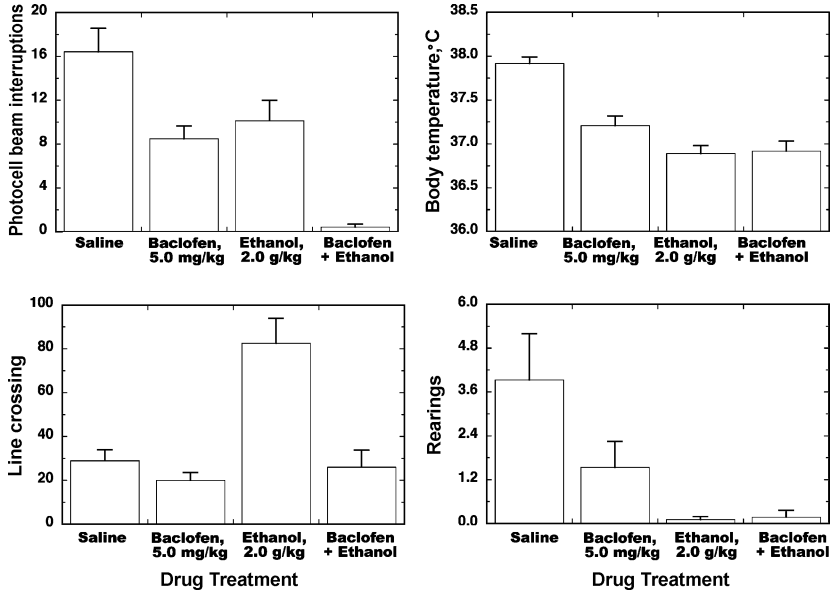

Figure 5. This figure illustrates the effects of the drug treatments on body movement and body temperature. In each figure, vertical bars above the mean values represent +1 S.E.M. Upper left panel: Body movement ($n = 28$) as measured by photocell beam interruptions during the 3.5-min evoked potential test sessions. Lower left panel: Mean line crossings ($n = 28$) during a 5-min open field test session that followed evoked potential collection. Lower right panel: Mean rearings ($n = 28$) during a 5-min open field test session that followed evoked potential collection. Upper right panel: Body temperature ($n = 28$). Body temperature was measured with a rectal thermistor probe immediately after FEP testing.

baclofen-ethanol was significantly greater than the latency observed with saline-saline. However, there was no significant difference between saline-ethanol and baclofen-ethanol.

Body Temperature

The upper right panel of Figure 5 presents body temperature data. Both baclofen, F (1,27) = 13.04, $p = .001$, and ethanol, F (1,27) = 63.06, $p < .001$, significantly decreased body temperature. The baclofen × ethanol interaction was also significant, F (1,27) = 19.53, $p < .001$. Dunnett test comparisons revealed that the baclofen-saline, saline-ethanol, and baclofen-ethanol treatments, all produced significant hypothermia in comparison to

saline-saline, but that there was no significant difference between the baclofen-ethanol and baclofen-saline conditions.

Body Movement

Figure 5 (upper left panel) presents the mean number of photocell beam interruptions recorded during 3.5 min of evoked potential recording for each recording session. Both baclofen, F (1,27) = 30.47, $p < .001$, and ethanol, F (1,27) = 23.26, $p < .001$, significantly reduced photocell beam interruptions. The drug × time interaction was not significant. The mean number of line crossings during the 5-min observation period following evoked potential testing is shown in the lower left panel of Figure 5. While the main effect of ethanol was to increase activity in the open field, F (1,27) = 14.98, $p = .001$, the main effect of baclofen was to decrease activity, F (1,27) = 31.93, $p < .001$. However, the baclofen × ethanol interaction was also significant, F (1,27) = 21.02, $p < .001$. Dunnett test comparisons revealed that only the saline-ethanol treatment was significantly different (increase) in comparison to saline-saline, and that baclofen-ethanol resulted in significantly fewer line crossings than saline-ethanol.

Rearing data are presented in the lower right panel of Figure 5. Results from the Friedman test were significant, F (3,81) = 11.26, $p < .01$. Subsequent treatment comparisons showed that baclofen, ethanol, and the combination of baclofen and ethanol, all significantly reduced rearings, but baclofen-ethanol was not significantly different from saline-ethanol.

Behavioral observations were also made during this 5-min observational time. Following saline administration, rats tended to ambulate around the perimeter, pausing briefly to turn circles, sniff, groom, or rear. By the end of 5 min, animals were typically sitting in a corner. When the rats received the 5.0 mg/kg dose of baclofen, they were noticeably wobbly on their feet. This behavior was characterized by slow and unsteady movement. Animals generally waddled around the perimeter pausing in the corners to groom, and then changed direction. Following ethanol administration, the rats slipped and scooted their way to the perimeter, then often walked back and forth along a single wall, circling, and changing directions. Pauses were accompanied by head bobbing, tipping over, and swaying. Only two animals reared. The ethanol-induced ataxia was notably augmented by the prior administration of baclofen. Not only were the animals slipping and wobbly, but most could not even support their own body weight and laid flat on their stomachs with their hind legs wiggling behind them. While most could push themselves either to

the perimeter or at least to a short distance, some were completely immobile, and only one rat reared.

DISCUSSION

Although a great deal of research indicates that activation of $GABA_A$ receptors play a prominent role in the cognitive and behavioral effects of ethanol (e.g., Chester & Cunningham, 2002; Grobin et al., 1998), the extent of $GABA_B$ receptor involvement is less clear. $GABA_B$ receptors are G-protein coupled, and are located both pre- and postsynaptically. The presynaptic receptors are apparently coupled to voltage-gated Ca^{+2} channels, with their activation leading to a reduction in Ca^{+2} conductance. In contrast, activation of the postsynaptic receptors produces a slow inhibitory postsynaptic potential as a function of the opening of K^+ channels and the resulting increase in K^+ conductance (Bowery, 1989; Kamatchi & Ticku, 1990). $GABA_B$ receptors thereby both reduce neurotransmitter release presynaptically, and decrease neuronal excitability postsynaptically. To the extent that activation of $GABA_B$ receptors is capable of modulating the effects of ethanol, pretreatment with baclofen (a $GABA_B$ agonist) was expected to alter the ethanol-induced changes in FEPs.

In the present study, the $GABA_B$ agonist baclofen altered FEPs recorded from the VC in a manner quite distinct from the changes observed following ethanol administration. The most striking effect of baclofen in the VC was in regard to component N_{31}, the initial excitatory postsynaptic potential (epsp) in cortical neurons following visual input (Meeren, Van Luijtelaar, & Coenen, 1998; Siegel & Sisson, 1993). Baclofen reduced the amplitude of this component to such an extent that it became positive. While P_{47} was also diminished by baclofen, components P_{37}, N_{62}, N_{147}, and P_{230} were all increased in amplitude by baclofen. Only components P_{24} and P_{87} were unaltered by baclofen. This pattern of results is in good agreement with an earlier study from this laboratory examining the dose-dependent effects of baclofen on FEPs (Hetzler & Ondracek, 2007).

By comparison, the effects of ethanol on the amplitude of VC FEP components were relatively modest. While early components P_{37} and P_{47} were increased by ethanol, late component P_{87} was significantly reduced in amplitude. This pattern of an increase in an early positive component, combined with a decrease in amplitude of one or more late components has been reported in prior animal studies (Hetzler & Bednarek, 2001; Hetzler & Martin, 2006).

The combination treatment of baclofen and ethanol produced different effects on the amplitude of different VC FEP components, but in no instance

did baclofen counteract the significant effects of ethanol. P_{24} is the correlate of the presynaptic geniculate volley (Siegel & Sisson, 1993), and neither baclofen nor ethanol altered the amplitude of this component. On the other hand, both baclofen and the baclofen-ethanol combination produced very similar reductions in amplitude of the next primary component, N_{31}. N_{31} is produced by a prominent sink in layer IV of area 17, and reflects the initial excitatory postsynaptic potential in pyramidal cells of layers V and VI following visual input (Brankačk, Schober, & Klingberg, 1990; Meeren et al., 1998; Siegel & Sisson, 1993). This component therefore appears to be more sensitive to the activation of $GABA_B$ receptors than it is to the myriad effects of ethanol. In contrast, the small component P_{37}, which is rarely examined in rat FEP studies, was enhanced in a similar fashion by both balcofen and ethanol.

The combined effect of baclofen and ethanol was most surprising for component P_{47}, since baclofen significantly decreased the amplitude of this component, while ethanol produced an amplitude increase. Nonetheless, the combination of baclofen-ethanol resulted in an amplitude almost identical to that observed in the saline-ethanol condition. P_{47}, corresponding to inhibitory postsynaptic potentials on the pyramidal cells of layers V and VI (Brankačk et al., 1990; Meeren et al., 1998), is one of the so-called secondary components (along with N_{62} and P_{87}), which presumably result from connections between the SC, brain stem, and diffuse thalamic projections (Creel, Dustman, & Beck, 1974). Interestingly, the effects of ethanol seem to dominate in the drug-induced alteration of these secondary components in comparison with the effects of baclofen. Thus, for all three components, the effects of the baclofen-ethanol treatment (i.e., P_{47}: increase; N_{62}: no change; P_{87}: decrease) were remarkably similar to the effects of ethanol alone, and did not resemble the effects of baclofen.

In marked contrast, both baclofen and baclofen in combination with ethanol produced an enhancement of both the late components N_{147} and P_{230}. It thus seems clear that the effects of baclofen dominate those components reflecting mainly thalamic input into the VC (N_{31}, N_{147}, P_{230}). Although the visual pathways use excitatory neurotransmitters (i.e., glutamate/aspartate) for transmitting information, at each excitatory synapse, local GABA-releasing cells gate and modify the responses produced in the postsynaptic cells (Somogyi, 1989). In the adult rat VC, GABA terminals are found in every layer, with the greatest density of synaptic contacts occurring in layer IV (Beaulieu, Kisvarday, Somogyi, Cynader, & Cowey, 1992), and electrophysiological studies have demonstrated that synaptic inhibition in the rat VC is mediated by both $GABA_A$ and $GABA_B$ receptors (e.g., Murakoshi, Guo, & Ichinose,

1993; Shao & Burkhalter, 1999). But even though excitatory thalamic input mainly contributes to the amplitudes of components N_{31} and N_{147}, the effects of baclofen are opposite (i.e,. decrease vs. increase), illustrating the differential circuitry involved in the production of these components. In fact, the increase in N_{147}-P_{230} amplitude could result from enhanced $GABA_B$ receptor activity in the thalamic recurrent inhibitory system, involving the thalamic reticular nucleus and/or thalamic interneurons and their inhibitory synapses onto the thalamocortical cells of the dorsal lateral geniculate (Bigler, 1977; Zhu & Lo, 1999). The secondary components P_{47}, N_{62}, and P_{87} are in contrast, not as affected by baclofen, with the ethanol-induced effects dominating, further separating the neural origin of these secondary components from both the primary and late components.

The mechanism(s) by which baclofen attenuates the intake of ethanol in rats are not well understood. One prominent view is that baclofen modulates dopamine transmission in the mesolimbic dopamine system originating in the ventral tegmental area (Colombo et al., 2004; Cousins, Roberts, & de Wit, 2002). More specifically, it has been speculated that baclofen activates $GABA_B$ receptors located on cell bodies in the ventral tegmental area, hyperpolarizing the membrane potential of these neurons, and thereby decreasing firing rate (Cousins et al., 2002). On the other hand, recent research utilizing rat hippocampal slices (Ariwodola & Weiner, 2004) indicates that ethanol does not affect postsynaptic $GABA_B$ receptor function, but ethanol does enhance presynaptic $GABA_B$ autoreceptor function at GABA synapses, thereby reducing the overall ethanol-induced potentiation of GABAergic transmission. In that study, pretreatment with baclofen blocked the ethanol-induced potentiation of $GABA_A$ inhibitory postsynaptic potentials. That is, ethanol-activation of presynaptic $GABA_B$ receptors may normally dampen activity at GABA synapses, and baclofen can increase this dampening, thereby effectively blocking ethanol's potentiation of $GABA_A$ produced inhibitory postsynaptic potentials.

Another perspective is that baclofen may reduce ethanol consumption by attenuating the corticothalamic activity necessary for sustaining behavioral activation states (Anstrom, Cromwell, Markowski, & Woodward,, 2003). In this regard, N_{147} in the VC FEP reflects secondary (or rebound) activation of cortical pyramidal cells (Brankačk et al., 1990; Meeren et al., 1998), and is generally viewed as the first component of the flash-evoked after-discharge (Bigler, 1977), which is produced by a thalamic recurrent inhibitory system involving both the dLGN and the thalamic reticular nucleus (see review by Bigler, 1977), the output of which is transmitted to the cortex. An increase in N_{147}-P_{230} amplitude

(which was produced by both baclofen and the combination of baclofen and ethanol in the present study) can reflect minimal sensory information processing (or reduced arousal) in the VC (Standage & Fleming, 1979), providing support for the Anstrom et al. (2003) proposal.

The SC is one of the areas of the brain with the highest concentration of GABA (Okada, Nitsch-Hassler, Kim, Bak, & Hassler, 1971). Within the SC, the highest level of GABA is found in the superficial gray layer (Okada, 1992). Likewise, the density of both $GABA_A$ and $GABA_B$ receptor subtypes is highest in the superficial gray layer, although in the rat SC there is a greater density of $GABA_B$ receptors than $GABA_A$ receptors (Mize, 1992). FEPs recorded from the SC were depressed by both baclofen and ethanol in the present study, with ethanol depressing all three components studied, and baclofen depressing components P_{29} and N_{51}. Interestingly, this is the second time we have reported that baclofen does not alter the amplitude of SC component P_{38} (see Hetzler & Ondracek, 2007), indicating a lack of involvement of $GABA_B$ receptors in the production of this component. Baclofen neither counteracted nor significantly enhanced the effects of ethanol on FEP amplitudes in the SC, suggesting that the two compounds work via separate mechanisms to alter these components.

Most component latencies were increased to some extent by baclofen and/or ethanol in both the VC and the SC. In the VC, baclofen increased the latency of components P_{24}, P_{87}, N_{147}, and P_{230}, while ethanol increased the latency of components P_{24}, P_{37}, P_{47}, N_{62}, and P_{87}. In the SC, baclofen increased the latency of components P_{29} and N_{51}, but decreased the latency of component P_{38}, while ethanol increased the latency of all three components. The baclofen-related decrease in P_{38} latency was noted in a prior study (Hetzler & Ondracek, 2007).

Given that a significant hypothermia was produced by both balcofen and ethanol, and that there is a clear relationship between reduced body temperature and increased FEP peak latency (Hetzler et al., 1988), it is likely that most of the observed increases in peak latency, were at least in part, produced by hypothermia. Other mechanisms by which baclofen and ethanol can directly alter component latencies include changes in synaptic delay as well as changes in axonal conduction velocity, thereby altering the time-course of synaptic temporal and spatial summation.

As mentioned above, both baclofen and ethanol produced hypothermia. Past studies in which baclofen is administered either s.c. or i.p. at doses at or slightly below 10 mg/kg typically produce hypothermia (see Hetzler & Ondracek, 2007). Likewise, there have been past reports of ethanol-dependent hypothermia (e.g., Hetzler & Martin, 2006; Rezvani & Levin, 2002).

During the FEP collection period, both baclofen and ethanol significantly decreased photocell beam interruptions. Similar findings have been previously reported for ethanol (Hetzler & Bednarek, 2001; Hetzler & Martin, 2006). Baclofen produces a dose-dependent depression of behavior in the rat, with effects beginning to be observed between 5 and 10 mg/kg (Grech & Balster, 1993; Hetzler & Ondracek, 2007). In the 5-min open field test, an ethanol-induced increase in locomotion was blocked by baclofen. This ethanol-induced increase in activity in the open field following decreased activity in the recording chamber has been reported previously (Hetzler & Martin, 2006) and was attributed to differences in the dimensions of the testing chambers. Rearing behavior was depressed by both baclofen and ethanol in the open field, findings of which have been previously reported (Hetzler & Martin, 2006; Hetzler & Ondracek, 2007).

Overall, the VC FEP amplitude data indicate that baclofen and ethanol most likely alter amplitudes by mainly separate mechanisms, and when given in combination produce results dominated by one or the other compound. Thus, the effects of baclofen dominate for components N_{31}, N_{147}, and P_{230}, while the effects of ethanol dominate for components P_{47}, N_{62}, and P_{87}. This provides further evidence for functional differences between the primary and late components versus the so-called "secondary" components. In contrast, in the SC, the effects of baclofen and ethanol were much more similar, and results of the combination treatment were suggestive of additive effects. In no instance did baclofen counteract significant FEP amplitude effects produced by ethanol, although baclofen was able to prevent the ethanol-induced increase in locomotion observed in the open field. However, the mechanism(s) by which baclofen and ethanol alter FEPs are not clear since both compounds can alter a variety of neurotransmitter systems. In particular, through the activation of $GABA_B$ receptors, baclofen can decrease the release of dopamine, serotonin, norepinephrine, glutamate, and GABA (Howe, Sutor, & Zieglgänsberger, 1987; Pirot. Godbout, Tassin, Glowinski, & Thierry, 1992; Santiago, Machado, & Cano, 1993; Wojcik & Holopainen, 1992). It will thus require further work on the underlying neuronal and pharmacological processes involved in FEP peak production/modulation to fully understand the effects reported here.

REFERENCES

Addolorato, G., Caputo, F., Capristo, E., Colombo, G., Gessa, G. L., & Gasbarrini, G. (2000). Ability of baclofen in reducing alcohol craving and intake: II-Preliminary clinical evidence. *Alcoholism: Clinical and Experimental Research*, *24*, 67–71.

Aistrup, G. L., Marszalec, W., & Narahashi, T. (1999). Ethanol modulation of nicotinic acetylcholine receptor currents in cultured cortical neurons. *Molecular Pharmacology*, *55*, 39–49.

Anstrom, K. K., Cromwell, H. C., Markowski, T., & Woodward, D. J. (2003). Effect of baclofen on alcohol and sucrose self-administration in rats. *Alcoholism: Clinical and Experimental Research*, *27*, 900–908.

Ariwodola, O. J., & Weiner, J. L. (2004). Ethanol potentiation of GABAergic synaptic transmission may be self-limiting: Role of presynaptic $GABA_B$ receptors. *The Journal of Neuroscience*, *24*, 10679–10686.

Beaulieu, C., Kisvarday, Z., Somogyi, P., Cynader, M., & Cowey, A. (1992). Quantitative distribution of GABA immunopositive and immunonegative neurons and synapses in the monkey visual cortex (area 17). *Cerebral Cortex*, *2*, 295–309.

Bigler, E. D. (1977). Neurophysiology, neuropharmacology and behavioral relationships of visual system evoked after-discharges: A review. *Biobehavioral Reviews*, *1*, 95–112.

Binns, K. E. (1999). The synaptic pharmacology underlying sensory processing in the superior colliculus. *Progess in Neurobiology*, *59*, 129–159.

Bowery, N. G. (1989). $GABA_B$ receptors and their significance in mammalian pharmacology. *Trends in Pharmacological Sciences*, *10*, 401–407.

Bowery, N. G., & Enna, S. J. (2000). γ-Aminobutyric $acid_B$ receptors: First of the functional metabotropic heterodimers. *The Journal of Pharmacology and Experimental Therapeutics*, *292*, 2–7.

Brankačk, J., Schober, W., & Klingberg, F. (1990) Different laminar distribution of flash evoked potentials in cortical areas 17 and 18b of freely moving rats. *Journal fur Hirnforschung*, *31*, 525–533.

Chester, J. A., & Cunningham, C. L. (2002). GABA(A) receptor modulation of the rewarding and aversive effects of ethanol. *Alcohol*, *26*, 131–143.

Colombo, G., Addolorato, G., Agabio, R., Carai, M. A. M., Pibiri, F., Serra, S., et al. (2004). Role of GABAB receptor in alcohol dependence: Reducing effect of baclofen on alcohol intake and alcohol motivational properties in rats and amelioration of alcohol withdrawal syndrome and alcohol craving in human alcoholics. *Neurotoxicity Research*, *6*, 403–414.

Cousins, M. S., Roberts, D. C. S., & de Wit, H. (2002). $GABA_B$ receptor agonists for the treatment of drug addiction: A review of recent findings. *Drug and Alcohol Dependence*, *65*, 209–220.

Creel, D., Dustman, R. E., & Beck, E. C. (1974). Intensity of flash illumination and the visually evoked potential of rats, guinea pigs and cats. *Vision Research*, *14*, 725–729.

Czachowski, C. L., Legg, B. H., & Stansfield, K. H. (2006). Ethanol and sucrose seeking and consumption following repeated administration of the $GABA_B$ agonist baclofen in rats. *Alcoholism: Clinical and Experimental Research*, *30*, 812–818.

Dyer, R. S. (1985). The use of sensory evoked potentials in toxicology. *Fundamental and Applied Toxicology*, *5*, 24–40.

Dyer, R. S., & Annau, Z. (1977). Flash evoked potentials from rat superior colliculus. *Pharmacology Biochemistry and Behavior*, *6*, 453–459.

Flannery, B. A., Garbutt, J. C., Cody, M. W., Renn, W., Grace, K., Osborne, M., et al. (2004). Baclofen for alcohol dependence: A preliminary open-label study. *Alcoholism: Clinical and Experimental Research*, *28*, 1517–1523.

Fox, S. S., & Rosenfeld, J. P. (1972). Recording evoked potentials. In R. D. Myers (Ed.), *Methods in Psychobiology, Vol. 2, Specialized Laboratory Techniques in Neuropsychology and Neurobiology* (pp. 345–369). New York: Academic Press.

Grech, D. M., & Balster, R. L. (1993). Pentobarbital-like discriminative stimulus effects of direct GABA agonists in rats. *Psychopharmacology*, *110*, 295–301.

Grobin, A. C., Matthews, D. B., Devaud, L. L., & Morrow, A. L. (1998). The role of $GABA_A$ receptors in the acute and chronic effects of ethanol. *Psychopharmacology*, *139*, 2–19.

Harrison, N. L., Lange, G. D., & Barker, J. L. (1988). (-)-Baclofen activates presynaptic GABA-B receptors on GABAergic inhibitory neurons from embryonic rat hippocampus. *Neuroscience Letters*, *85*, 105–109.

Hetzler, B. E., & Bednarek, E. M. (2001). Effects of ethanol on flash-evoked potentials of rats: Lack of antagonism by naltrexone. *Alcohol*, *25*, 21–30.

Hetzler, B. E., Boyes, W. K., Creason, J. P., & Dyer, R. S. (1988). Temperature-dependent changes in visual evoked potentials of rats. *Electroencephalography and Clinical Neurophysiology*, *70*, 137–154.

Hetzler, B. E., Heilbronner, R. L., Griffin, J., & Griffin, G. (1981). Acute effects of alcohol on evoked potentials in visual cortex and superior colliculus of the rat. *Electroencephalography and Clinical Neurophysiology*, *51*, 69–79.

Hetzler, B. E., & Martin, E. I. (2006). Nicotine-ethanol interactions in flash-evoked potentials and behavior of Long-Evans rats. *Pharmacology Biochemistry and Behavior*, *83*, 76–89.

Hetzler, B. E., & Ondracek, J. M. (2007). Baclofen alters flash-evoked potentials in Long-Evans rats. *Pharmacology Biochemistry and Behavior*, *86*, 727–740.

Hoffman, P. L., & Tabakoff, B. (1993). Ethanol, sedative hypnotics, and glutamate receptor function in brain and cultured cells. *Alcohol and Alcoholism*, *Supplement 2*, 345–351.

Howe, J. R., Sutor, B., & Zieglgänsberger, W. (1987). Baclofen reduces post-synaptic potentials of rat cortical neurons by an action other than its hyperpolarizing action. *The Journal of Physiology*, *384*, 539–569.

Kamatchi, G. L., & Ticku, M. K. (1990). Functional coupling of presynaptic GABA-B receptors with voltage-gated Ca2+ channel: Regulation by protein kinases A and C in cultured spinal cord neurons. *Molecular Pharmacology*, *38*, 342–347.

König, J. F. R., & Klippel, R. A. (1963). *The Rat Brain, A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. New York: Krieger.

Lovinger, D. M. (1997). Serotonin's role in alcohol's effects on the brain. *Alcohol Health & Research World*, *21*, 114–120.

Meeren, H. K. M., Van Luijtelaar, E. L. J. M., & Coenen, A. M. L. (1998). Cortical and thalamic visual evoked potentials during sleep-wake states and spike-wave discharges in the rat. *Electroencephalography and Clinical Neurophysiology*, *108*, 306–319.

Mize, R. R. (1992). The organization of GABAergic neurons in the mammalian superior colliculus. In R. R. Mize, R. E. Marc, & A. M. Sillito (eds.), *GABA in the retina and central visual system. Progress in Brain Research, Vol 90*, Amsterdam: Elsevier, 219–248.

Murakoshi, T., Guo, J. Z., & Ichinose, T. (1993). Electrophysiological identification of horizontal synaptic connections in rat visual cortex in vitro. *Neuroscience Letters*, *163*, 211–214.

Okada, Y. (1992). The distribution and function of gamma-aminobutyric acid (GABA) in the superior colliculus. In R. R. Mize, R. E. Marc, & A. M. Sillito (eds.), *GABA in the retina and central visual system. Progress in Brain Research, Vol 90*, Amsterdam: Elsevier, 249–262.

Okada, Y., Nitsch-Hassler, C., Kim, J. S., Bak, I. J., & Hassler, R. (1971). Role of gamma-aminobutyric acid (GABA) in the extrapyramidal motor system. 1. Regional distribution of GABA in rabbit, rat, guinea pig and baboon CNS. *Experimental Brain Research*, *13*, 514–518.

Pirot, S., Godbout, R., Tassin, J. P., Glowinski, J., & Thierry, A. M. (1992). Inhibitory effects of ventral tegmental area stimulation on the activity of prefrontal cortical neurons: Evidence for the involvement of both dopaminergic and GABAergic components. *Neuroscience*, *49*, 857–865.

Proctor, W. R., Soldo, B. L., Allan, A. M., & Dunwiddie, T. V. (1992). Ethanol enhances synaptically evoked GABA-A receptor-mediated responses in cerebral cortical neurons in rat brain slices. *Brain Research*, *595*, 220–227.

Rezvani, A. H., & Levin, E. D. (2002). Nicotine-alcohol interactions and cognitive function in rats. *Pharmacology Biochemistry and Behavior*, *72*, 865–872.

Santiago, M., Machado, A., & Cano, J. (1993). In vivo release of dopamine from rat striatum, substantia nigra and prefrontal cortex: Differential modulation by baclofen. *British Journal of Pharmacology*, *109*, 814–818.

Shao, Z., & Burkhalter, A. (1999). Role of $GABA_B$ receptor-mediated inhibition in reciprocal interareal pathways of rat visual cortex. *Journal of Neurophysiology, 81*, 1014–1024.

Shah, A. S., Bressler, S. L., Knuth, K. H., Ding, M., Mehta, A. D., Ulbert, I., et al. (2004). Neural dynamics and the fundamental mechanisms of event-related brain potentials. *Cerebral Cortex*, *14*, 476–483.

Siegel, J., & Sisson, D. F. (1993). Evoked field potentials—Beyond correlates of behavior: An approach to determining the neural mechanism of behavior. In W.

Haschke, A. I. Roitbak, & E.-J. Speckmann (eds.), *Slow potential changes in the brain*, Boston: Birkhauser, 151–165.

Sisson, D. F., & Siegel, J. (1989). Chloral hydrate anesthesia: EEG power spectrum analysis and effects on VEPs in the rat. *Neurotoxicology and Teratology*, *11*, 51–56.

Somogyi, P. (1989). Synaptic organization of GABAergic neurons and GABAA receptors in the lateral geniculate nucleus and visual cortex. In D. Man-Kit Lam, & C. D. Gilbert (eds.), *Neural Mechanisms of Visual Perception. The Second Retina Research Foundation Symposium*, Houston: Portfolio Publishing Company, 35–62.

Stancampiano, R., Carta, M., Cocco, S., Curreli, R., Rossetti, Z. L., & Fadda, F. (2004). Biphasic effects of ethanol on acetylcholine release in the rat prefrontal cortex. *Brain Research*, *997*, 128–132.

Standage, G. P., & Fleming, D. E. (1979). Visual cortical hypersynchronous bursting as an index of shifts in behavioral state. *Physiology & Behavior*, *23*, 35–38.

Wojcik, W. J., & Holopainen, I. (1992). Role of central $GABA_B$ receptors in physiology and pathology. *Neuropsychopharmacology*, *6*, 201–214.

Zhu, J. J., & Lo, F.-S. (1999). Three GABA receptor-mediated postsynaptic potentials in interneurons in the rat lateral geniculate nucleus. *The Journal of Neuroscience*, *19*, 5721–5730.