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Site-Specific Enhancement of γ-Aminobutyric Acid-Mediated Inhibition of Neural Activity by Ethanol in the Rat Medial Septal Area[1]

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Abstract

Because of uncertainty concerning the interaction of ethanol with γ-aminobutyric acid (GABA) receptor-mediated events, the present work was designed to investigate the effect of ethanol on GABA transmission in the rat septal area using behavioral and electrophysiological techniques. Microinjection of the $GABA_A$ agonist muscimol into the medial septal area (MSA) enhanced, and bicuculline administration antagonized, ethanol-induced impairment of the aerial righting reflex. Microinjection of these drugs into the lateral septum (LSi) did not influence this measure of ethanol-induced sedation. Furthermore, intraseptal injections of muscimol or bicuculline in saline-treated rats had no effect on the aerial righting reflex. These data suggest that the MSA plays a critical modulatory role in the sedative actions of ethanol. To assess the effect of ethanol on muscimol responses in the MSA and LSi at the cellular level, GABA was applied by iontophoresis to rhythmically bursting neurons of the MSA and to cells in the LSi. The magnitude of the resultant inhibition by GABA on these cells was assessed before and after systemic administration of ethanol. Ethanol enhanced GABA-mediated inhibition of MSA neural activity, but did not alter GABA-mediated inhibition of cellular activity in the LSi. In contrast, the inhibition of cellular activity in the MSA, caused by a maximally effective concentration of the benzodiazepine flurazepam, was not altered by ethanol. Other work in the MSA demonstrated that electrical stimulation of the fimbria caused an inhibition of ongoing single unit activity that was reduced by concurrent application of bicuculline. The duration of this electrically elicited inhibition in the MSA was enhanced after ethanol injection and then recovered to base-line levels. In addition, ethanol (1.5 mg/kg) caused an enhancement of the inhibition induced by nipecotic acid, a GABA uptake inhibitor. These findings demonstrate that GABA-mediated neural inhibition is enhanced by ethanol in the MSA but not the LSi, indicating that the actions of ethanol on GABA-induced inhibition can be site specific. It is proposed that the cellular action of ethanol may depend upon a specific molecular composition of the GABA receptor complex which may vary at selected sites in the brain.

Early behavioral studies demonstrated that peripherally administered GABAmimetic drugs exacerbate ethanol intoxication whereas GABA antagonists reduce the effects of ethanol (Frye and Breese, 1982; Liljequist and Engel, 1982; Martz *et al.,* 1983). This work led to the hypothesis that acute ethanol intoxication may result from an enhancement of the action of GABA (see Hunt, 1983; Allan and Harris, 1987 for reviews). Recent biochemical studies

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using *in vitro* techniques have provided further evidence that ethanol influences GABA function. For example, ethanol has been demonstrated to enhance GABA-stimulated Cl^- flux across biological membranes from a variety of sources: microsacs (Allan and Harris, 1986, 1987), synaptoneurosomes (Suzdak *et al.,* 1986a,b) and primary cultured spinal cord neurons (Ticku *et al.,* 1986).

In contrast to the behavioral and biochemical studies, electrophysiological investigations have provided mixed findings in regard to the hypothesis that ethanol facilitates GABA transmission. In support of this view, Nesteros (1980) applied GABA iontophoretically onto cerebral cortical cells in the cat and found that the resultant inhibition was potentiated by very low doses of ethanol. This effect appeared to be specific to GABA as there was no potentiation of glycine-mediated inhibition. Recently, the electrophysiological effect of ethanol on GABA-mediated Cl^- currents has been assessed directly. In cultured chick spinal cord neurons, ethanol potentiated the whole cell Cl^- currents associated with pressure-ejected GABA, but not glutamate (Celantano *et al.,* 1988). In dorsal root ganglion cells in primary culture, Nishio and Narahashi (1988) examined the effects of ethanol on GABA-induced currents under voltage clamp conditions. In this work, GABA caused a biphasic response and ethanol was found to enhance the initial peak current by up to 30% without affecting the sustained component. In contrast to the data supportive that ethanol enhances GABA transmission, other electrophysiological studies have yielded data that do not affirm the view that GABA transmission is enhanced by ethanol. For example, Mancillas *et al.* (1986) observed an approximate 20% enhancement when GABA was applied iontophoretically to CA1 pyramidal cells after ethanol exposure, but they also observed this response after saline treatment and concluded that the change was due to a “warm up” effect. Similarly, ethanol did not enhance GABA-induced responses from CA1 pyramidal cells in hippocampal slices from adult rats (Carlen *et al.,* 1982; Siggins *et al.,* 1987) and did not enhance GABA responses in cultured embryonic rat hippocampal neurons (Harrison *et al.,* 1987) or GABA-activated current in locus coeruleus neurons (Shefner, 1989).

Several investigations have provided evidence that alterations in neural mechanisms in the MSA can have a profound influence on the acute sedative properties of ethanol (Breese *et al.,* 1984; McCown *et al.,* 1986) and other sedative hypnotic drugs (Brunello and Cheney, 1981; Kalivas and Horita, 1980). One of the most consistent findings concerning the organization and regulation of the MSA is the critical involvement of the neurotransmitter GABA (Costa *et al.,* 1983; McLennan and Miller, 1974; Allen and Crawford, 1984; Dutar *et al.,* 1985; Segal, 1986; Onteniente *et al.,* 1987; Leranth and Frotscher, 1987). In regard to ethanol-induced sedation, microinjection of the GABA antagonist bicuculline into the MSA, at a dose that has no effect alone, antagonizes the impairment of the righting reflex and the reduced activity levels that accompany ethanol administration (Breese *et al.,* 1984). Conversely, when the GABA agonist muscimol is microinjected into the MSA, the duration of ethanol-induced narcosis is prolonged (McCown *et al.,* 1986). Hence, the MSA provides a site in brain that can influence the sedative action of ethanol *via* a GABA mechanism.

Given the controversy from electrophysiological data surrounding the hypothesis that ethanol enhances GABA neurotransmission, the major purpose of the present study was to extend investigations in the MSA concerning the role of GABA mechanisms in the action of ethanol. Initial experiments evaluated the effects of bicuculline and muscimol administered into the MSA on the sedative effects of ethanol. Subsequent experiments determined the effects of ethanol on GABA-mediated inhibition of neural activity in the MSA. The neuroanatomical specificity of the neural changes produced by ethanol as related to GABA function in the MSA was evaluated by conducting identical behavioral and electrophysiological investigations in the lateral septum. The electrophysiological experiments demonstrate that GABA-mediated inhibition in the MSA is enhanced by

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ethanol administration and that this interaction between ethanol and GABA is not observed in the lateral septum.

Methods

Animals—Male Sprague-Dawley rats weighing 300 to 400 g were used in all experiments. The animals were group housed in a room that was maintained at 25°C with a light/dark cycle of 12/12 hr. Food and water were available *ad libitum.*

Aerial righting reflex—The sedative effects of ethanol were assessed using the "aerial righting reflex," a test procedure described by Frye and Breese (1982). The only equipment required for the test was a meter stick mounted vertically behind a 10-cm thick foam rubber pad. Rats were held by the back of the neck and the base of the tail in an inverted position at specific heights above the pad and released. A successful righting required that the rat land with all four feet on the foam rubber pad on two of three consecutive releases. Ethanol was administered i.p. in a 10% solution over a 1-min period at a dose of 1.5 g/kg immediately after the microinjection; other rats received saline. Animals were tested for aerial righting ability at time 0 and 10, 20, 30, 40, 50, 60, 90, 120, 150 and 180 min after injection. Each righting test began at 5 cm, and if the animal did not successfully right after two releases, the height was increased by 5 cm and the procedure repeated. Rats given saline generally required 5 cm for a successful landing. Animals were never released from heights greater than 55 cm. The minimum height required for successful righting was used as an index of sedation. In addition to measuring aerial righting, body temperature was recorded with a rectal thermometer (Yellow Springs Instrument Co., Yellow Springs, OH) at 30-min intervals throughout the experiment. Tail blood (20 μl) was drawn at 60 min from animals that received ethanol to determine blood ethanol levels.

Drug microinjection—To assess the role of the septum in the action of drugs known to influence ethanol-induced sedation, rate were implanted with a guide cannula directed toward the septal area so that drugs could be microinjected into the lateral as well as the medial septum. Animals were anesthetized with pentobarbital (40 mg/kg), placed in a stereotaxic apparatus and a 10-mm guide cannula made of 26-gauge stainless-steel hypodermic tubing was implanted. The guide cannula angled 15° toward midline, entered the brain 1.5 mm lateral and 0.6 mm anterior to bregma, was lowered 3.75 mm below the surface of the brain and then was fixed in place with dental acrylic. After a 7-day recovery period, rats were tested in the aerial righting procedure. Drug was infused into the MSA or unilaterally into the LSi through a 33-gauge stainless-steel injector (11.5 mm for LSi and 13 mm for MSA) that was inserted into the guide cannula. Rats received one of three solutions over a 5-min period in a 0.5 μl volume: 1) muscimol (30 ng); 2) saline vehicle; or 3) bicuculline (150 ng). The injector was left in place for an additional 1 min. Immediately after the microinjection procedure, rats were administered either ethanol or saline. The solutions injected were coded so that the experimenter was unaware of the drug condition. Not more than five injections were made in any rat, with at least 3 days allowed between tests. The order in which rats were assigned to the 12 possible experimental conditions was determined using a crossed-over counterbalanced design. The placement of the guide cannula was determined histologically for each animal. Sky blue or fast green dye injections were made through the two injectors into each rat to allow identification of the injection sites. Brains were processed as described in the histology section.

Electrophysiological Recording Studies

General—Previous studies have demonstrated that the response of MSA neurons to ethanol in rats anesthetized with urethane is similar to the response observed in free-moving rats

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(Givens and Breese, 1990). Therefore, rats used in the electrophysiological studies were anesthetized with urethane (1.5 g/kg i.p.) and placed into a David Kopf stereotaxic apparatus. The scalp was retracted and burr holes placed in the skull above the septal area and above the fimbria. For microiontophoresis studies, five barrel pipettes were pulled and their tips broken back to 7 to 10 microns. A single barrel recording pipette was glued with epoxy glue to the side of the multiple barrel iontophoresis pipette such that it extended 20 μm beyond the five barrel pipette. The recording electrode complex was advanced into the septal area by means of a hydraulic microdrive (Trent Wells, Inc.). Single unit activity of MSA neurons was recorded through single barrel pipettes with a 1 to 2 micron tip diameter and an impedance of 4 to 8 megohms. The recording pipette was filled with a 2 M NaCl solution saturated with fast green dye. Recording electrodes were lowered into the MSA region at a 15° angle. The coordinates for the point of entry into the brain were 0.6 mm anterior to bregma and 1.5 mm lateral to the midsaggital suture. Extracellular electrode potentials were amplified via a Grass P15 high impedance preamplifier and a secondary amplifier, and monitored with a Tektronix oscilloscope and audiomoniter. The raw signals were filtered by the preamplifier (300 Hz 1/2 amplitude low and a 10 kHz 1/2 amplitude high filter cutoffs). Action potentials isolated from background activity with at least a 3-to-1 signal-to-noise ratio and a constant duration and configuration were defined as from a single neuron. Individual spikes were digitized by a window comparator with the digital (TTL) pulse output fed into an IBM PC XT which generated ratemeter, interspike interval and peristimulus interval histograms.

For MSA recordings, unit activity was sampled from cells having a rhythmically bursting pattern of activity. Bursts were defined as groups of 2 to 18 spikes, with a duration of 50 to 200 msec that occurred at a frequency of 3 to 6 Hz. Lateral septal neurons were identified by their location and irregular firing rate. MSA neurons were generally found 6.0 to 7.0 mm ventral, and lateral septal neurons 4.5 to 5.5 mm ventral, to the dural surface of the brain. After encountering an active cell, the spontaneous firing rate was monitored for at least a 20-min base-line period before initiating the various manipulations. Body temperature of the rat was monitored continuously with a rectal probe and was maintained at 37 ± 0.5°C with a hot water bottle.

Microiontophoresis—The protocol for experiments using iontophoresis was as follows: 1) a spontaneously active septal neuron was isolated; 2) an iontophoretic current/neuronal response curve was generated; 3) ethanol at one of three doses (0.75, 1.5 or 3.0 g/kg; *i.e.,* depending on the experiment) or saline was administered systemically to the animal; and 4a) current/response curves were redetermined or 4b) a constant current (*i.e.,* to elicit a 30% inhibition) was applied at regular intervals to the cell. Appropriate pH, current and vehicle controls were performed. Current/response curves were generated by determining the neuronal response to a variety of current levels (1–100 nA) applied to the pipette. A minimum of 2 min was allowed between iontophoretic drug applications. Only one cell was recorded from an animal at a given dose of systemically administered ethanol and only one dose was given to an animal.

Electrical stimulation—To assess afferent influences on the activity of MSA neurons, bipolar electrodes were inserted unilaterally into the fimbria (McLennan and Miller, 1974). The coordinates used for implantation were 1.8 mm caudal to bregma, 2.0 mm lateral to midline and 3.7 mm ventral to the dural surface. Electrical stimulation consisted of a 0.2-msec monophasic square-wave with a current ranging from 0.05 to 1.0 mA. A Grass 588 stimulator was used to send the pulses to a Grass PSIU6 stimulus isolation unit that delivered a constant current to the stimulating electrode. The analog raw signal, a voice channel and stimulus synchronization pulses were recorded on magnetic tape by a dual channel tape recorder for off-line analysis. The protocol for the electrical stimulation

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experiments involved applying a single monophasic pulse to the stimulating electrode in the fimbria and recording the response of the MSA neuron. The stimulation current was varied over a wide range (0.05–1.0 mA) to determine the current intensity that elicited a half-maximal response. Once determined, this half-maximal current intensity was used to stimulate the fimbria at 0.5 Hz. A base-line peristimulus histogram was then accumulated over 100 stimulations. Bicuculline, at a current which blocked a dose of GABA that inhibited cell activity by 50%, was iontophoresed onto the cell and a 100 stimulation peristimulus histogram was generated in order to establish the involvement of GABA in the inhibition. Ethanol (1.5 g/kg) or vehicle (at a volume equal to the ethanol dose) was then administered i. p., and a series of 100 stimulation peristimulus time histograms were recorded over a 2-hr period.

In a separate set of experiments, the effect of ethanol on stimulus-induced inhibition was examined in unanesthetized free-moving rats. In this case, a threaded base was fixed (A, +0.6; L, 1.5) onto the skull at a 15° angle of rats anesthetized with sodium pentobarbital (45 mg/kg). A stainless-steel reference wire was lowered into the frontal lobe of the cortex and was connected to a three pin connector secured to the skull with dental acrylic. These animals were tested in a large circular container (52 × 34 cm). During recording, rats were required to walk on a wire-covered cylinder (13 × 7.5 cm diameter) which rotated on a rod passed through the walls of the container at a rate of 0.3 revolutions per min. The rats were trained before surgery to perform this simple procedure. Recordings in these free-moving rats were made with the use of a head mounted microdrive (Deadwyler *et al.,* 1979). The stimulus parameters and alcohol dose (1.5 g/kg) were the same as those used for the experiments in the anesthetized rats.

Histology—For histological verification of the recording site, an anodal d.c. current of 20 μA was passed for 45 sec though the recording pipette that contained a 2 M NaCl solution saturated with fast green dye. A cathodal 20 μA current was applied to the stimulation electrode for 45 sec. The animal was then perfused with a 10% potassium ferrocyanide-formalin solution. Potassium ferrocyanide reacts with the metal ions that are deposited during current passage to the stimulation electrodes to form a blue spot. The brain was removed, stored in formalin for 3 days and then freeze-mounted onto a microtome chuck. With the use of a cryostat and microtome (Damon/IEG Division), 50-μm slices were sectioned, mounted onto slides and stained with cresyl violet (Fisher Scientific Co., Springfield, NJ). Brains from rats used for microinjection studies were processed in a similar manner. The location of each recording site or guide cannula and dye injection sites was logged onto a rat brain atlas.

Blood ethanol measurement—The concentration of ethanol in venous blood was determined for each animal that received ethanol according to the method of Frye *et al* (1981). Blood samples (20 μl) were collected from the tail vein and diluted immediately with 180 μl of ice-cold distilled water containing 0.2 μg/ml *tert.*-butanol as an internal standard. The samples were centrifuged (10,000 × g for 10 min) and aliquots (10 μl) of the supernatant were injected into a gas chromatograph (Varian Associates, Palo Alto, CA) that used flame-ionization detection. A glass column packed with 0.2% Carbowax was used to separate ethanol and *tert.*-butanol. The concentration of ethanol in tail blood was measured in all rats that received ethanol 30, 60 or 90 min after ethanol administration. In the various studies, blood ethanol levels were not different between groups of animals receiving similar doses of ethanol ($P > .1$; see data for tables 1 and 2, “Results”).

Statistical analysis—An analysis of variance with repeated measures was used to analyze the behavioral data; analysis of variance with a post-hoc test was used to assess the effect of ethanol on firing rate; and analysis of variance and linear regression were used to

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compare current/response curves. The *a priori* selected significance level was $P < .05$. A Student's *t* test was used to evaluate any changes pre- and postethanol in the period of inhibition to fornix stimulation.

Drugs—A 10% ethanol (AAPER Alcohol & Chemical Co., Shelbyvile, KY) solution was prepared in physiological saline. Urethane (1.5 mg/kg i.p.; Sigma Chemical Co., St. Louis, MO), the anesthetic used, was prepared in physiological saline. Drugs used for microiontophoretic application were dissolved in sterile distilled water. These drugs included: GABA (Sigma Chemical Co.), 0.2 M, pH 6; bicuculline methiodide (Sigma), 5 mM, pH 5; and flurazepam HCl (Hoffmann-La Roche; Nutley, NJ) 0.1 M, pH 4. All doses are presented as the salt of the drug.

Results

Behavioral analysis of GABA-ethanol interactions in the septal area

Rats that received a 1.5 g/kg dose of ethanol rapidly developed an impairment in aerial righting ability by 10 min that remained near maximal for 20 min and then gradually recovered over the next 60 min (fig. 1). Rats that received saline were unimpaired, consistently righting at a height of 6.0 ± 0.9 cm. Both muscimol and bicuculline had potent effects on the ethanol-induced righting impairment after microinjection into the MSA. Microinjection of muscimol (30 ng in 0.5 μl) increased the height of ethanol-impaired aerial righting relative to saline microinjection, an effect that persisted for 150 min postinjection (fig. 1). Conversely, microinjection of bicuculline (150 ng in 0.5 μl) reduced the height of aerial righting and accelerated the recovery rate from the deficit, such that animals returned to control levels by 45 mm postinjection (fig. 1). Neither muscimol nor bicuculline, when microinjected into the MSA of saline-treated control rats, had an effect on the aerial righting reflex ($P > .1$; data not shown). Thus, the enhancement of the reflex impairment by muscimol and the partial reversal of the impairment by bicuculline appear to be due to an interaction in the MSA between these drugs influencing GABA receptor function and the action of ethanol to impair aerial righting.

In order to assess the site specificity of the behavioral effects of microinjecting muscimol and bicuculline into the MSA, studies were repeated with these drugs at a second injection site, the intermediate zone of the LSi. This site was chosen for three reasons: 1) it is adjacent to the MSA at a site closer to the lateral ventricle; 2) it contains neurons that provide one of the major sources of afferents to the MSA; and 3) it has a high density of GABA receptors. In contrast to the results obtained in the MSA, the righting reflex impairment was not altered by any of the treatment conditions in the LSi (fig. 2). The difference in the effect of muscimol on aerial righting between the MSA and LSi groups was not attributable to ethanol metabolism, as comparisons between the groups yielded no differences in body weight or blood ethanol levels (table 1). Although microinjection of muscimol or bicuculline into the LSi did not alter ethanol-induced impairment of the aerial righting reflex, it did bring about another significant change in the animal. Microinjection of muscimol into the LSi caused a 0.9°C decrease in rectal temperature, whereas an identical microinjection in the MSA caused a 1.3°C increase in rectal temperature.

Electrophysiological Analysis of Ethanol-GABA Interactions in Medial Septal Area

GABA iontophoresis—Rhythmically bursting cells located in the MSA (*i.e.,* the medial septal nucleus and the nucleus of the vertical limb of the diagonal band (fig. 3) were chosen for investigation because of their sensitivity to ethanol (Givens and Breese, 1988,1990). Effect of ethanol on firing rate of cells in the MSA over time is presented in table 2. GABA applied iontophoretically to MSA neurons reliably inhibited the spontaneous activity in a

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dose-dependent manner (fig. 4). This inhibition produced by GABA was antagonized consistently by iontophoretically applied bicuculline (data not shown). A current-response curve was generated for iontophoretically applied GABA pulses and then repeated after a 1.5-g/kg injection of ethanol. The firing rate inhibition to pulses of GABA was enhanced after exposure to ethanol, but only at those currents that produced less than 50% suppression of activity (fig. 4). Because the greatest enhancement by ethanol occurred at currents that produced a 30% suppression of spontaneous activity, this degree of suppression was used in all subsequent experiments.

Figure 5 illustrates the inhibition produced by constant current pulses applied at regular intervals to a GABA-containing pipette of a single cell before and after ethanol administration (1.5 g/kg). Systemic administration of ethanol resulted in a dose dependent enhancement of GABA-induced inhibition when measured 60-mm postinjection of 43 ± 6.4% at 0.75 g/kg and 56 ± 3.5% at 1.5 g/kg. The enhancement of GABAergic inhibition by ethanol expressed as percentage of change typically was present for 90 to 120 mm, whereas no change in the inhibition produced by GABA was observed after systemic administration of saline (table 3). The absolute firing rates produced by GABA after ethanol also are presented in table 3.

Fimbria stimulation—This experiment evaluated whether the response of MSA cells to synaptically induced inhibition is influenced by ethanol. As illustrated in figure 6, stimulation of the fimbria elicited an inhibition in the spontaneous activity of the majority of MSA neurons (*i.e.,* 87% of cells analyzed). In the other neurons (3 of 24), fimbria stimulation evoked an excitation-inhibition sequence. In three cells, antidromic activation was observed when the stimulation current was at an intensity that caused maximal inhibition. However, at the currents used for 50% maximal inhibition, antidromic spikes were never observed. In addition to inhibiting the ongoing activity, fimbria stimulation in all cases reset the rhythmic pattern of activity of MSA cells. In order to assure that the stimulus-evoked inhibition involved GABA, as has been proposed (McLennan and Miller, 1974; Dutar *et al.,* 1987), bicuculline was iontophoresed onto MSA neurons while the fimbria was electrically stimulated. The duration of fimbria stimulation-induced inhibition was reduced or abolished by bicuculline in 25 of 31 cells analyzed (fig. 6). Bicuculline antagonized the late phase (>50 msec) of the inhibition in all cells, but did not always block the early phase (<50 msec) of the fimbria-evoked inhibition. Only those cells in which bicuculline blocked the late inhibition induced by fimbria stimulation were used to evaluate ethanol's effect on this response. Every unit tested was inhibited by iontophoretically applied GABA and this response was consistently reversed by bicuculline.

Once the characteristics of the response to fimbria stimulation were established, a response profile of MSA neurons to fimbria stimulation before and after ethanol administration was generated. In MSA neurons with a bicuculline-sensitive inhibitory response to fimbria stimulation, ethanol (1.5 g/kg) enhanced the duration of the response at 30 and 60 min after ethanol administration (table 4). To ensure that this was a true facilitation of synaptic inhibition, a series of responses to increasing stimulus strengths to the fimbria were obtained before and after ethanol. Ethanol (1.5 g/kg) significantly shifted leftward the current-response curve for fimbria stimulation-evoked inhibition (fig. 7). Thus, ethanol enhanced the inhibition due to electrical stimulation of the fimbria, an inhibition that appears to be a GABA-mediated response. It is of interest that in four of six cells that were insensitive to bicuculline, ethanol did not enhance the electrically elicited inhibition (data not shown; $P > .1$). Thus, these data are consistent with the results obtained in the experiments using iontophoresis, reinforcing the hypothesis that there is an interaction between ethanol and GABA-mediated inhibition in the MSA.

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In order to rule out the possible confounding influence of anesthesia on the observed enhancement by ethanol of fimbria stimulation-induced inhibition, the stimulation experiment was repeated in the free-moving rat. The spontaneous activity of rhythmically bursting neurons in the MSA in the free-moving rat was similar to that in the urethane-anesthetized rat except that both the bursting frequency and the firing rate of the neurons were greater (data not shown; see Givens and Breese, 1990). Under these conditions, stimulation of the fimbria produced an inhibition of spontaneous activity of rhythmically bursting MSA cells just as was observed in anesthetized rats. In 16 cells from 9 rats, the current necessary to inhibit the cell by 50% of maximum was applied repeatedly to the stimulating electrode before and after ethanol was administered i.p. to the rat. The stimulation-induced inhibition was enhanced 30 min after ethanol administration, but then returned to base-line levels by 90 min postinjection (fig. 8).

Endogenous GABA inhibition with nipecotic acid—The GABAergic projection onto MSA neurons appears to be tonically active in that neurons of the MSA were readily excited after synaptic blockade of GABA transmission with bicuculline (Givens and Breese, 1986). Based upon these data with bicuculline, the consequences of applying the GABA uptake inhibitor nipecotic acid by iontophoresis onto MSA cells was assessed. Nipecotic acid produced a reliable inhibition of activity of MSA cells (table 3), providing further evidence of a tonically active GABA mechanism in the MSA. Subsequently, the nipecotic acid was used to evaluate the effects of ethanol on a response related to ongoing GABA function in the MSA. As shown in table 5, this inhibition produced by nipecotic acid was enhanced significantly by a 1.5-g/kg dose of ethanol, but not saline, whether presented as percentage of change or as absolute firing rate. The duration of the enhancement of nipecotic acid was approximately 90 min (table 5).

Inhibition of cellular activity with flurazepam—The GABA receptor complex is known to incorporate a benzodiazepine binding site (Olsen, 1981). Application of the benzodiazepine flurazepam onto MSA cells produced an inhibition, but the maximal effect that could be elicited by flurazepam was a 30% inhibition of these MSA cells (fig. 9). In contrast to the enhancement of GABA inhibition produced by ethanol, ethanol had no effect on the inhibition produced by a near maximal dose of flurazepam (fig. 10). The inhibition of firing rate by flurazepam during the course of the depression caused by ethanol is presented in table 6. These data are particularly important because the percentage of inhibition calculated from base line remains constant for flurazepam. This contrasts with the increased inhibition observed for GABA after ethanol (table 3). In addition to this determination, flurazepam as well as GABA were applied alternately by iontophoresis onto cells. In the latter case, the flurazepam inhibitory response between 0 and 60 min was not altered (pre-ethanol = 34.2 ± 1.8% inhibition; n = 10 replicates; postethanol = 35.2 ± 1.4% inhibition; n = 20 replicates; $P > .1$), whereas the GABA response was enhanced (pre-ethanol = 30.6 ± 2.6% inhibition; n = 10 replicates; postethanol = 53.9 ± 1.9%; n = 20 replicates; $P > .05$). These latter data provide additional evidence for an interaction between GABA and ethanol.

Electrophysiological Analysis of Ethanol-GABA Interactions in the Lateral Septum

The aerial righting experiments suggested that muscimol in the lateral septum did not enhance the depressant effects of ethanol (see fig. 2). Therefore, GABA was iontophoresed onto neurons of the lateral septum in the absence and then the presence of systemically administered ethanol to explore whether an interaction of GABA and ethanol could be demonstrated at the cellular level in the lateral septum. In order to make a direct comparison with the results obtained in the MSA, the experimental parameters determined in the MSA were used in the experiments evaluating the lateral septum, (*i.e.,* rats were anesthetized with urethane, EC_{30} currents were used for GABA iontophoresis and a dose of 1.5 g/kg of

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ethanol was used). As in the MSA, iontophoretic pulses of GABA produced a reliable inhibition of the ongoing activity of LSi neurons and these inhibitions routinely were shown to be antagonized by iontophoretically applied bicuculline (data not shown). The current for injecting GABA that gave a 30% suppression of activity was determined and then used to repeatedly pulse GABA onto cells in the LSi before and after administration of 1.5 g/kg of ethanol. Ethanol did not alter the inhibition in LSi neural activity produced by iontophoretically applied GABA (table 2; fig. 11). Likewise, peripheral administration of saline had no effect on GABA-mediated inhibition in the LSi. In order to rule out quantitative differences in the amount of GABA liberated from the iontophoretic pipette accounting for the differences between the results in the MSA and LSi, the currents from the two sets of experiments were compared. The currents that were required to cause a 30% inhibition in the MSA were not statistically different from those used in the LSi (8 ± 2.1 nA *vs*. 10 ± 1.2 nA; $P > .1$).

Discussion

The aerial righting reflex proved to be a reliable behavioral measure of the sedation induced by low doses of ethanol. The behavioral sedation measured by this test was significantly affected by $GABA_A$ receptor drugs when microinjected into the MSA. For example, microinjection of muscimol into the MSA caused a potentiation of ethanol-induced sedation, whereas bicuculline treatment antagonized the sedation. When given alone into the MSA, muscimol and bicuculline had no inherent effects on aerial righting. These findings extend previous work that demonstrated the potentiation by intraseptal muscimol of sleep time induced by a large dose of ethanol (McCown *et al.,*1986) and the observation that bicuculline will antagonize ethanol-induced impairment of locomotor activity (Breese *et al.,* 1984). These results support the hypothesis that changes in GABA transmission in the MSA can alter the sedation produced by ethanol and confirm previous behavioral studies that implicated GABA in the sedative properties of ethanol (Frye and Breese, 1982; Liljequist and Engel, 1982). That the MSA is an important site in brain for influencing ethanol-induced sedation is supported further by other studies demonstrating that microinjection of TRH into the MSA will antagonize the sedation produced after ethanol administration (Breese *et al.,* 1984; McCown *et al.,* 1986).

The most parsimonious explanation for the potentiation of ethanol-induced sedation by muscimol is a direct interaction between muscimol and ethanol within the MSA. Alternatively, microinjected muscimol might have diffused into the lateral ventricle, and been transported to another site in the brain in which the interaction with ethanol could have occurred. However, diffusion from the site and transport to another site in brain seems unlikely because an identical microinjection of these drugs into the intermediate zone of the LSi, a site closer to the lateral ventricle, did not produce a change in the height of the righting reflex. In addition, dye injected into the MSA was confined to the MSA (see under “Methods”). Although the change in body temperature caused by muscimol could be a contributing factor to the increased sedation observed by ethanol after muscimol, a comparison of results obtained with TRH with those obtained with muscimol on ethanol-induced sedation argues against this possibility. TRH which prevents the fall in body temperature after ethanol administration reduces ethanol-induced central nervous system depression (Cott *et al.,* 1976). In the present paper, the increase in body temperature is associated with an increase in ethanol-induced impairment of aerial righting suggesting that this factor alone could not be responsible for the change in function after microinjection of muscimol into the MSA.

The electrophysiological investigations explored the hypothesis that ethanol enhances GABA transmission in the MSA. Several electrophysiological observations suggest that the

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GABAergic input onto MSA cells is tonically active. For example, bicuculline, a GABA antagonist, applied ionotophoretically to MSA cells, causes a rapid and dose-dependent increase in neural activity (Givens and Breese, 1986). In the present study, nipecotic acid, a GABA uptake inhibitor, reduced neural activity of MSA cells when applied iontophoretically. When ethanol was administered systemically, the response of MSA cells to iontophoretic pulses of GABA and to inhibition by fimbria stimulation were enhanced. In addition, the suppression of cellular activity by nipecotic acid also was enhanced by ethanol. These results support the hypothesis that ethanol can facilitate GABA transmission in the MSA.

It is of interest that this facilitation of GABA by ethanol was lost at a time when ethanol concentration in blood remained elevated, suggestive of an acute tolerance. An acute tolerance also develops to the depression of firing rate in the medial septum caused by ethanol (table 2; Givens and Breese, 1990). Because the depression of firing rate by ethanol appears to return before the enhancement of GABA by ethanol recovers to base-line, these two examples of acute tolerance may represent distinct mechanisms. Nevertheless, further study will be required before it is clarified whether the acute tolerance to ethanol and the recovery of GABA inhibition can indeed be dissociated.

The rhythmic bursting of MSA cells continued to be reset after fimbria stimulation, but the delay to the first burst was increased by ethanol and the subsequent bursts were equally right shifted. Interestingly, after ethanol administration the spontaneous rhythmicity of MSA neurons is lost (Givens and Breese, 1990), but rhythmic bursts are still present immediately after fimbria stimulation. The trigger mechanism for the resetting of the rhythmic activity after fimbria stimulation appears to be unaffected by ethanol, but the mechanism that causes the delay to the first burst is affected by ethanol and may involve GABA. From anatomical data that indicates that all afferent and local GABA terminals in the MSA originate from afferent sources (Leranth and Frotscher, 1987), the possibility exists that two separate processes are occurring to bring about stimulation-induced inhibition: an extrinsic GABAergic effect directly from afferents and an intrinsic effect, through a synaptic network within the MSA, that does not use GABA as a transmitter. The initial (0–50 msec) inhibition may cause the resetting of the rhythmic activity and result from an intrinsic (population) effect that is resistant to ethanol, whereas the latter (50–120 msec) inhibition results from extrinsic GABA afferent input that is sensitive to ethanol.

Ethanol has been demonstrated previously to enhance GABA function in electrophysiological studies in the cortex (Nestoros, 1980); the cerebellum (Lee *et al.,* 1987; Gruol, 1983); and the spinal cord (Celantano *et al.,* 1988). However, not all electro-physiological investigations have observed a facilitation of GABA function after ethanol administration. In this regard, the inhibition of cellular activity by GABA in the lateral septum was not enhanced by ethanol in the present investigation. Mancillas *et al.*, (1986) recording in the hippocampus did not find an enhancement of GABA applied to CA1 neurons after ethanol. Others who have investigated the effect of ethanol on GABA function in hippocampal preparations also did not find an enhancement of iontophoretically applied GABA (Carlen *et al.,* 1982; Siggins *et al.,* 1987; Harrison *et at.,* 1987). From these data, it would appear that brain site is an important contributing factor to changes in GABA sensitivity after ethanol administration. Thus, regionally specific changes in sensitivity to GABA after ethanol may account for apparent discrepancies in the literature. Identification of brain regions in which ethanol either selectively affects GABA function or does not alter GABA responses could provide important clues about how ethanol influences specific neural systems in brain to bring about the various behavioral effects of ethanol.

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The effect of ethanol was greatest when currents of GABA that caused a 30% inhibition in firing rate were used. In fact, the ethanol-induced enhancement was not effective if currents of GABA producing 50% or greater inhibition were applied. The effect of ethanol on GABA-induced Cl^- currents *in vitro* are also greatest at low concentrations of GABA, with no enhancement seen at high concentrations (Celentano *et al.,* 1988). Similarly, in Cl^- flux experiments using cultured spinal cord neurons, Mehta and Ticku (1988) reported that ethanol (20 mM) does not affect the maximal response to GABA but produces a leftward shift in the GABA dose-response curve that is greatest at low GABA concentrations. If ethanol acts directly on the GABA receptor complex, then these results suggest that ethanol does not enhance the number of binding sites or the maximal Cl^- ion flux, but may increase the binding affinity, the efficiency of coupling of the GABA receptor to the Cl^- channel or the kinetics of the channel.

Although ethanol caused a reliable enhancement of GABA-induced inhibition in the MSA, the inhibitory action of flurazepam on these cells was not altered by ethanol. Benzodiazepines are well known for their ability to facilitate GABA without having any significant effects of their own (Tailman and Gallager, 1985). Flurazepam was applied iontophoretically to produce an approximate 30% suppression of activity, the maximal response of MSA neurons to this drug. The observation that ethanol did not alter flurazepam responses indicates clearly that the action of ethanol to enhance the inhibitory action of GABA is not due to a nonspecific membrane action. However, one must question why ethanol did not enhance the action of flurazepam on neural activity given that the action of the benzodiazepines are dependent upon GABA (Taliman and Gallager, 1985). In this experimental protocol, the current used to apply flurazepam to the cell allowed comparable inhibition to that for GABA, but this inhibition was maximal for flurazepam. Therefore, the lack of an effect of ethanol on flurazepam inhibition of cellular activity may represent a maximal effect for its interaction with endogenous GABA in the synaptic cleft. Under such conditions, ethanol might not be expected to enhance the action of flurazepam. Another possibility for the lack of ethanol enhancement of flurazepam induced inhibition may be that ethanol acts like a benzodiazepine to enhance the GABA response, *i.e*., ethanol saturates the mechanism by which flurazepam acts to enhance GABA-mediated inhibition (Breese *et al.,* 1979). This view is consistent with studies that have demonstrated that RO-15–4513, a benzodiazepine receptor inverse agonist, will antagonize the sedative actions of ethanol (Suzdak *et al.,* 1986a; Lister, 1988) and work demonstrating cross-tolerance between ethanol and chlordiazepoxide (Criswell and Breese, 1989). Additional studies will be required to resolve these possibilities concerning the action of ethanol on benzodiazepine inhibition of cellular activity.

Previous results published concerning the presence of muscimol and benzodiazepine binding sites in the medial and lateral septum may allow a logical explanation for the differential action of ethanol at these sites (Givens and Breese, 1990) when given in combination with iontophoretically applied GABA or when muscimol is microinjected into these sites. Within the septal nuclei, the high affinity benzodiazepine binding sites are exclusively and heavily concentrated in the MSA, whereas the LSi is devoid of such benzodiazepine receptor binding (Marcel *et al.,* 1986). $GABA_A$ receptor sites are found in both nuclei. Interestingly, unlike MSA neurons, iontophoretic applications of GABA to the lateral septal neurons caused no change in inhibition after ethanol and microinjection of muscimol into this site did not enhance the behavioral sedation induced by ethanol. If the different responses observed in the medial and lateral septum are related to the presence of benzodiazepine receptors, the *alpha* subunit of the $GABA_A$ receptor complex appears to be a domain where ethanol could interact, but the *gamma* subunit that directs benzodiazepine binding to the receptor complex also could contribute to the selectivity of ethanol on GABA receptor mechanisms (see Shivers *et al.,* 1989).

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In summary, although functional studies provide unequivocal evidence that changes in GABA receptor function can influence the degree of ethanol sedation (Frye and Breese, 1982), electrophysiological studies concerning the interaction between ethanol and GABA function have provided a confusing picture (Nesteros, 1980; Celantano *et al.,* 1988; Nishio and Narahashi, 1988; Mancillas *et al.,* 1986; Carlen *et al.,* 1982; Siggins *et al.,* 1987). In the present studies, two adjacent brain regions were differentially sensitive to the facilitative effects of ethanol on the behavioral action of muscimol and GABA-mediated inhibition. Such site-specific sensitivity differences in the GABA-chloride channel complex to ethanol may explain how ethanol can interact with specific systems in the brain to bring about selective behavioral and cognitive changes in central nervous system function. Possible differences in the molecular composition of the protein subunits of the GABA receptor complex are proposed to account for the apparent discrepancy in the effects of ethanol on GABA inhibition at the two sites in brain. Such structural differences in the GABA receptor complex could explain the confusing electrophysiological data concerning the interaction between GABA and ethanol inasmuch as several different sites in brain have been chosen to make this assessment. Therefore, the present studies support the view that it is critical to investigate the action of ethanol at the brain site(s) involved in a specific function altered by ethanol before drawing conclusions about the involvement of a given neurotransmitter in ethanol's actions (Breese *et al.,* 1988).

Acknowledgments

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ABBREVIATIONS

GABA	γ-aminobutyric acid
MSA	medial septal area
LSi	lateral septal area
TRH	thyrotropin-releasing hormone

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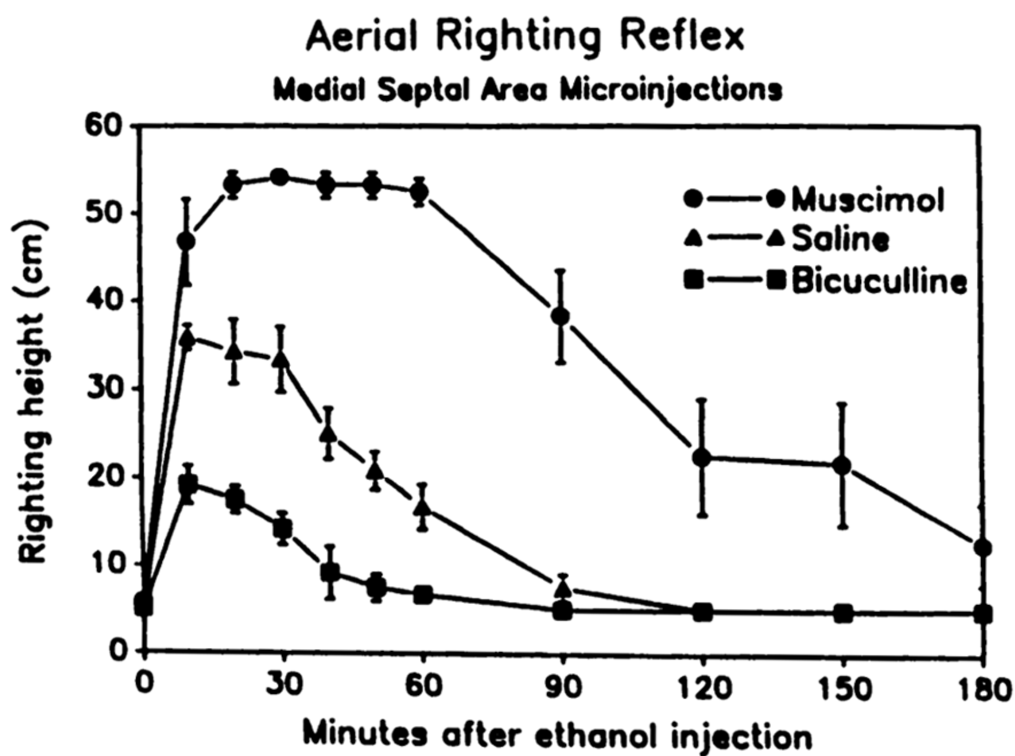

Fig. 1.

Effect of direct microinjection of muscimol (30 ng) and bicuculline (150 ng) into the MSA on the height of aerial righting in ethanol (1.5 g/ kg)-treated rats. There are six rats in each treatment group.*$P < .01$ when compared to the saline treatment.

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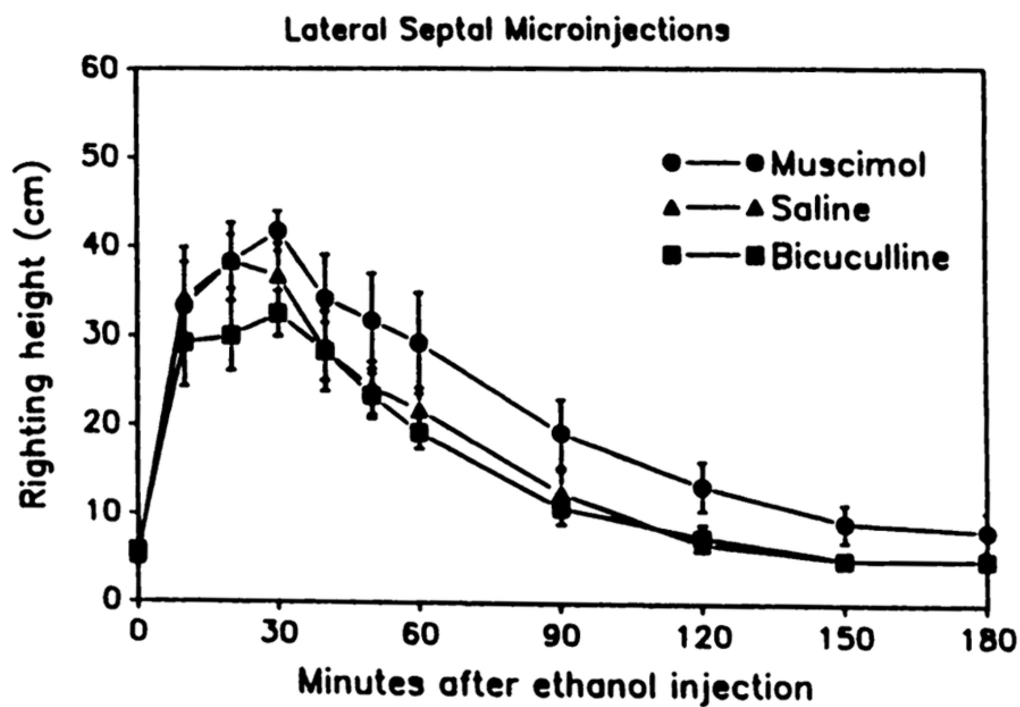

Fig. 2.

Effect of direct microinjection of muscimol (30 ng) and bicuculline (150 ng) into the LSi on the height of aerial righting in rats after 1.5 g/kg of ethanol. There are six rats in each condition. There are no significant differences across the group ($P > .1$).

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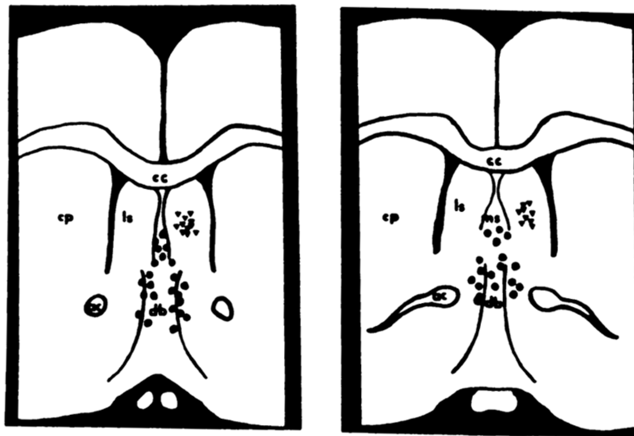

Fig. 3.
Histological location of the tips of electrodes that recorded rhythmically bursting MSA neurons (●) and irregularly firing LSi neurons (▼). Abbreviations are: cc, corpus callosum; cp, caudate-putamen; Is, lateral septum; ac, anterior commissure; ms, medial septum; db, diagonal band (vertical limb).

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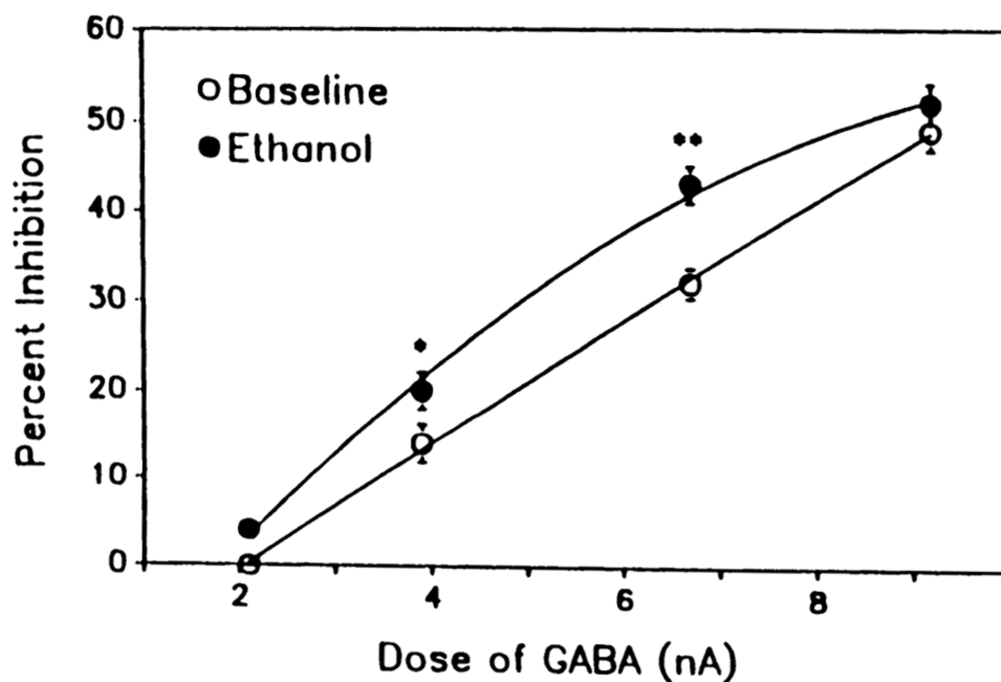

Fig. 4.

Current-response relationship between MSA cells and iontophoretic GABA pulses before and 60mm after the administration of 1.5 g/kg of ethanol in urethane-anesthetized rats. There are at least six determinations for each value recorded. *P <.05; **P<.01when compared to baseline.

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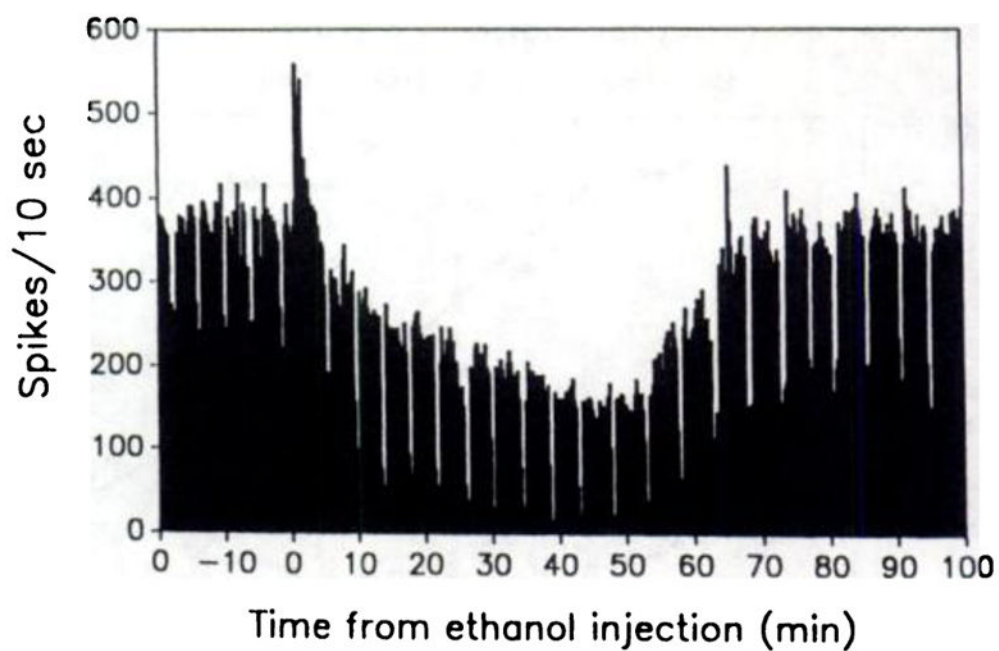

Fig. 5.

Rate histogram demonstrating the effect of systemically administered ethanol (1.5 g/kg) on responses of MSA neurons to iontophoretically applied GABA in urethane-anesthetized rats. The acute firing rate decreases in the histogram occurred immediately upon the application of GABA to the neuron.

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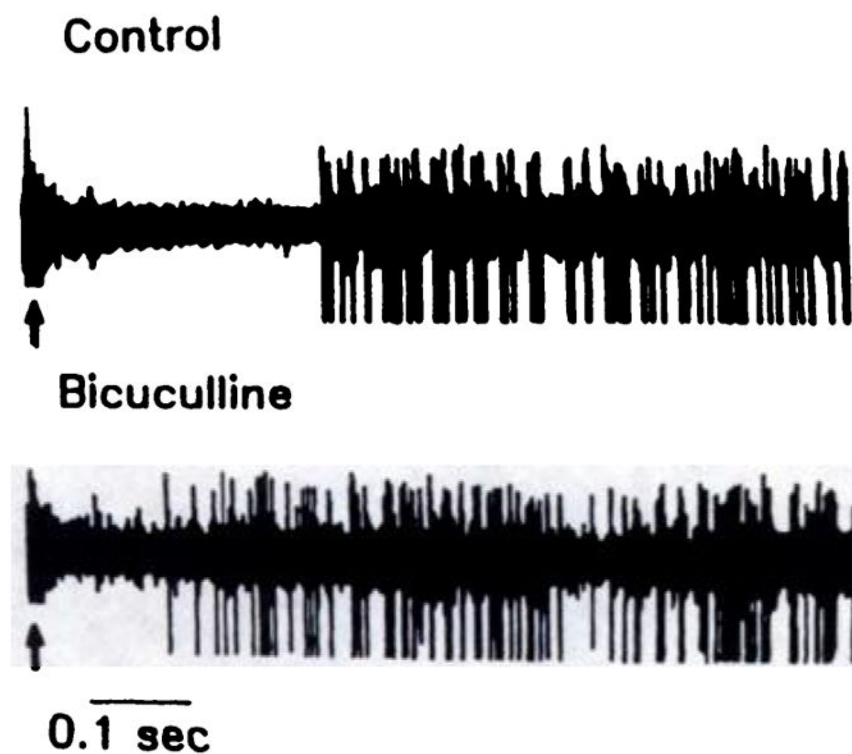

Fig. 6.

Oscilloscope traces of the response of a single rhythmically bursting MSA neuron to fimbria stimulation. The upper trace illustrates the long-lasting inhibition of unit activity after a single 0.2-msec electrical stimulation. The effect of bicuculline on the stimulus-evoked inhibition can be seen in the lower trace.

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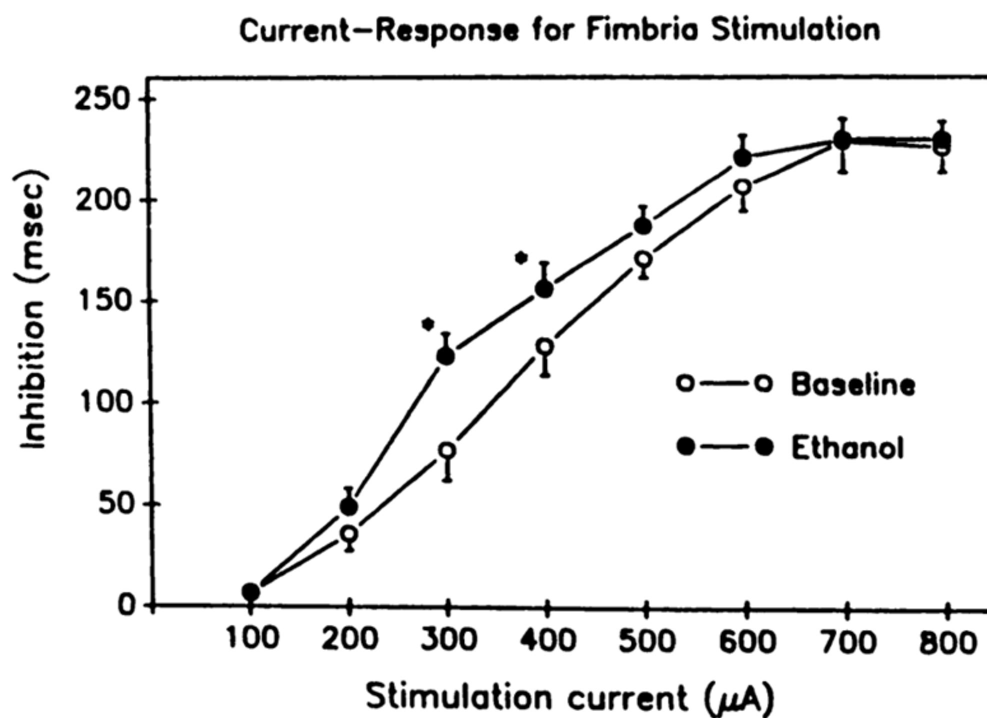

Fig. 7.

Response of MSA neurons to currents of increasing strengths through an electrode placed in the fimbria before and 30 min after i.p. administration of 1.5 g/kg of ethanol. Values are based on 11 determinations. For the analysis of variance analysis, $F = 6.31$ (1,174). $^*P < .05$ when compare to base-line.

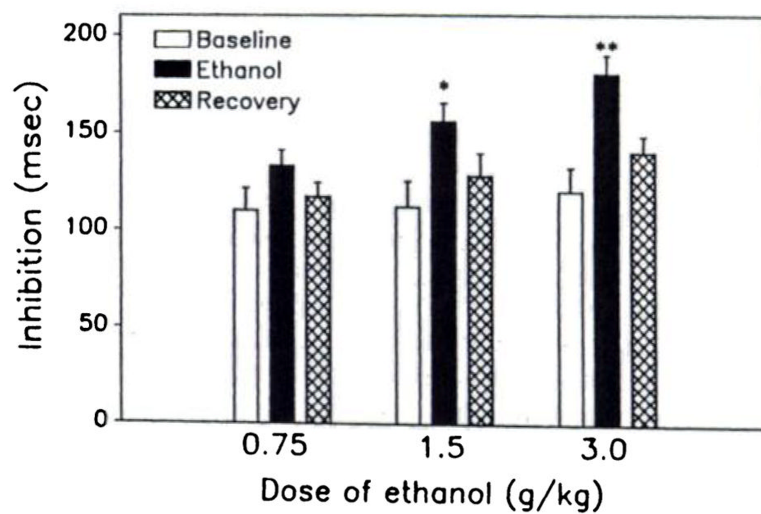

Fig. 8.
Inhibition of MSA neural activity after fimbria stimulation in the free-moving rat before (base-line) and 30 min (ethanol) or 90 min (recovery) after systemically administered ethanol at one of three doses. There are six determinations for each ethanol dose. *$P < .05$ when compared to base-line.

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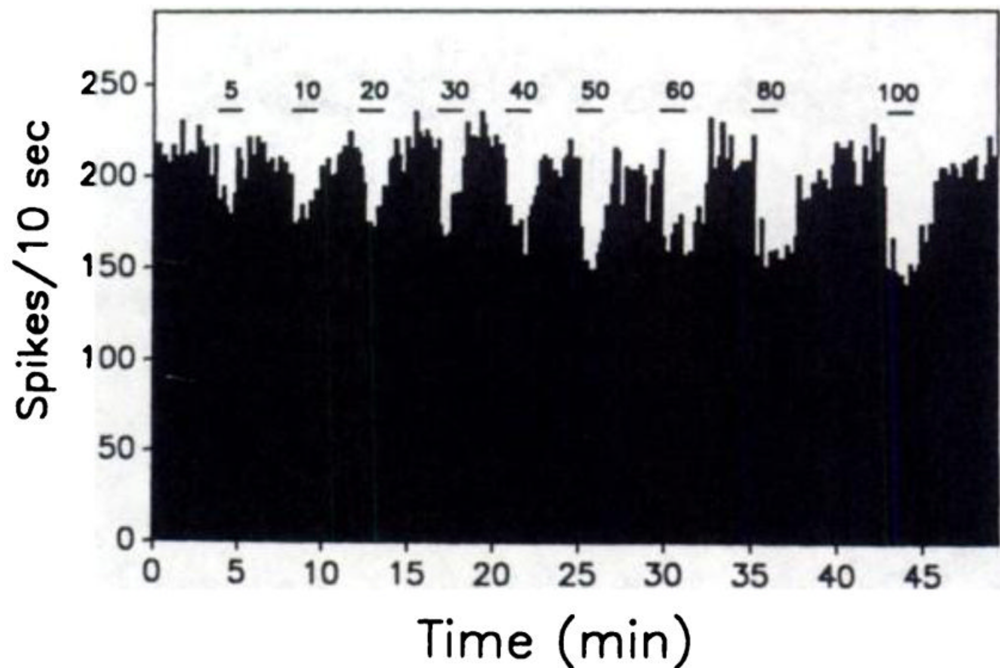

Fig. 9.

Current-response relationship of MSA neurons and iontophoretically applied flurazepam. Note that the maximal response is an approximate 30% suppression of activity. The bars above the histogram indicate when the designated current was applied to the flurazapam-containing pipette.

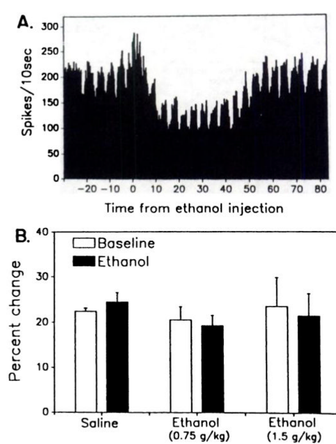

Fig. 10.

The response of MSA neurons to iontophoretically applied flurazepam 60 min after ethanol. A, rate histogram illustrating the inhibition in firing of an MSA neuron by flurazepam before and after systemic administration of ethanol (1.5 g/kg). Inhibition of firing rate in the histogram is due to the application of flurazepam to the neuron. B, summary of the effect of two different doses of ethanol on the flurazepam-elicited inhibition. No significant differences between ethanol and saline were observed.

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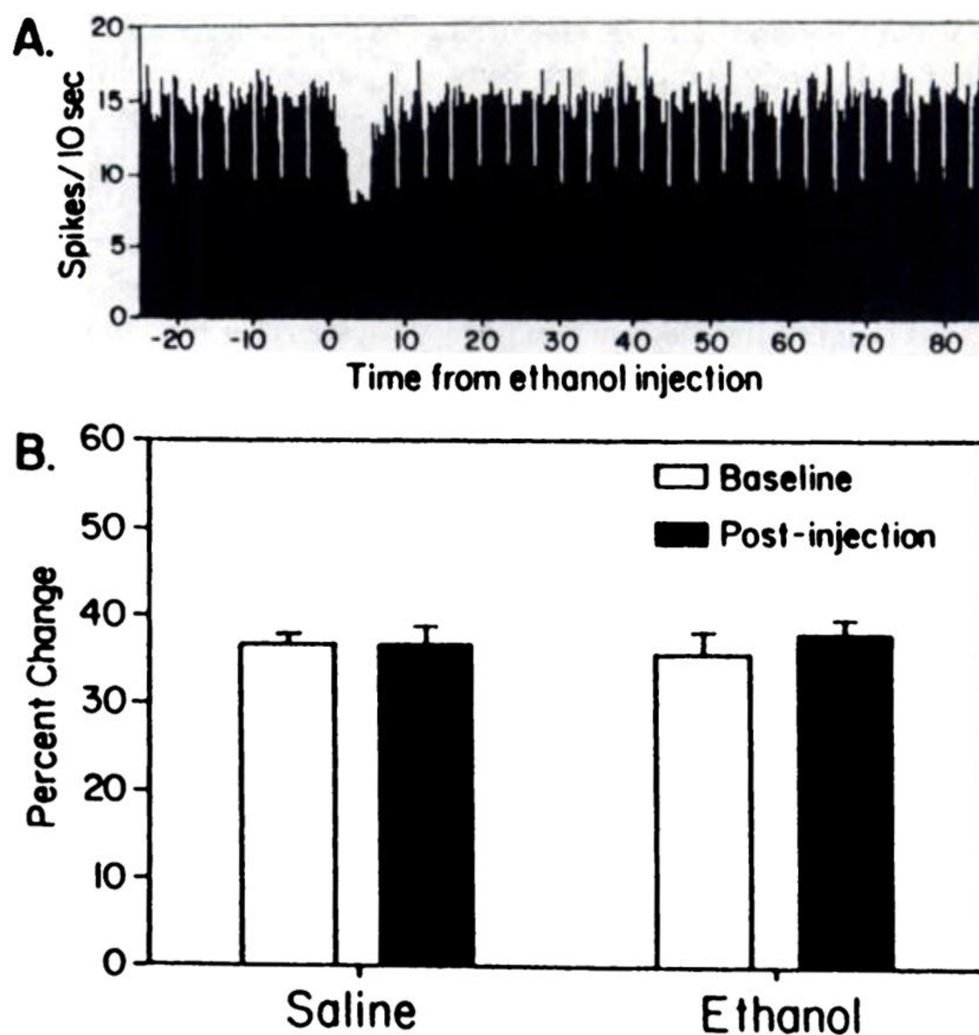

Fig. 11.

The response of LSi neurons to iontophoretically applied GABA. A, rate histogram illustrating the inhibition in firing of a LSi neuron by GABA before and after systemic administration of ethanol (1.5 g/kg). The acute interruptions in the histogram are the points at which GABA was applied iontophoretically to the neuron. The inhibition that occurred during the first few minutes after ethanol is in response to the ethanol administration. B, summary of the effect of 1.5 g/kg of ethanol on GABA-elicited inhibition in the LSI measured 30 min postinjection. Ethanol produced no significant change in the neural response to GABA ($P > .1$).

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TABLE 1

Blood ethanol concentration (BEC) and body weights In groups of rats treated peripherally with saline or ethanol (1.5 g/kg) after central microinjection of bicuculline, muscimol or saline Into the MSA or LSi[a]

Treatment[b]	Saline (Body wt.)		Ethanol (Body wt.)		BEC[c]	
	MSA	LSi	MSA	LSi	MSA	LSi
	g		*g*		*mg%*	
Muscimol	328 ± 9.6	337 ± 8.9	333 ± 10.9	347 + 13.7	156 ± 8.1	153 ± 11.7
Saline	338 ± 10.0	342 ± 8.8	332 ± 8.1	343 ± 7.1	153 ± 5.6	158 ± 12.9
Bicuculline	331 ± 14.3	342 ± 9.0	345 ± 9.1	341 + 5.9	143 ± 6.0	150 ± 5.5

[a] There are six determinations for each of the 12 conditions; there were no significant differences among the various treatment groups. Ethanol or saline (15 ml/kg) was administered i.p. as described under "Methods."

[b] Saline a dose of 30 ng of muscimol 140 ng of bicuculline was microinjected in a volume of 0.5 μl over 5 mm.

[c] BEC was determined 60 mm after administration.

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TABLE 2

Neuronal firing rates and blood ethanol concentrations (BEC) for iontophoresis experiments[a]

Time from Injection	Saline	Ethanol (1.5.g/kg)	BEC *mg%*
Experiments on LSi Neurons (n = 10)[b]			
−15	29.4 ± 6.4	30.5 ± 6.4	
15	32.4 ± 8.0	15.7* ± 5.0	
30	26.4 ± 7.4	10.7* ± 6.9	153 ± 4.2
45	28.5 ± 6.2	14.2* ± 4.5	
60	29.9 ± 6.1	19.4 ± 5.8	141 ± 6.8
90	26.4 ± 5.9	25.0 ± 6.8	131 ± 8.2
120	27.7 ± 5.8	26.9 ± 7.0	124 ± 7.4
Experiments on LSi Neurons (n = 10)			
−15	13.4 ± 4.6	15.6 ± 5.0	
15	14.3 ± 4.7	15.3 ± 3.8	
30	12.5 ± 6.2	14.9 ± 2.9	167 ± 8.5
45	14.1 ± 4.7	14.3 ± 3.2	
60	13.8 ± 5.1	14.3 ± 4.6	156 ± 6.3
90	13.2 ± 5.9	15.2 ± 7.1	148 ± 4.9
120	14.6 ± 6.0	13.8 ± 4.5	121 ± 6.9

[a]The firing rates are the mean number of spikes/second ± S.E.M. and the BEC is the mean milligram percentage of ethanol in venous blood ± S.E.M.

[b]Group data for experiments using iontophoretic GABA, nipecotic acid or flurazepam were not significantly different from one another and were pooled for this analysis.

*P < .01 when compared to saline.

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TABLE 3

Time course of the effect of systemically administered ethanol on GABA inhibition of MSA neurons[a]

Minutes from Injection	Saline	Ethanol (0.75 g/kg)	Ethanol (1.5 g/kg)
% Inhibition of Firing Rate[b]			
−15	33.4 ± 2.3	34.4 ± 3.2	29.2 ± 2.9
15	36.6 ± 4.5	41.1 ± 4.6	37.6 ± 5.4
30	34.8 ± 0.9	55.3* ± 5.3	64.1* ± 8.6
45	32.4 ± 2.4	58.6* ± 6.9	51.8* ± 7.6
60	38.4 ± 2.6	42.6* ± 6.4	55.9* ± 3.5
90	33.1 ± 4.1	46.4* ± 7.1	56.5* ± 6.8
120	35.3 ± 3.5	39.7 ± 8.3	45.4* ± 7.2
Absolute Firing Rate During GABA Application[c]			
−15	19.4 ± 1.2	18.3 ± 0.4	20.3 ± 0.9
15	18.9 ± 0.8	12.5* ± 1.2	11.9* ± 1.0
30	17.5 ± 0.9	7.5* ± 1.1	2.8* ± 2.6
45	18.5 ± 1.6	10.7* ± 1.7	8.3* ± 2.1
60	18.7 ± 2.1	13.9* ± 1.2	8.5* ± 2.5
90	16.8 ± 0.8	15.2 ± 1.3	11.8* ± 1.6
120	19.6 ± 1.3	16.6 ± 1.1	15.4 ± 1.7

[a]GABA inhibition was achieved by direct iontophoresis onto single cells in the MSA.

[b]Values presented as the mean percentage of change in firing rate ± S.E.M. from the immediately preceeding base-line.

[c]Values presented as the mean absolute firing rate ± S.E.M. (spikes/second) during the application of GABA.

*P < .01 when compared to saline.

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TABLE 4

Inhibition of rhythmically bursting MSA neurons to electrical stimulation of the fimbria after systemically administered ethanol in the urethane-anesthetized rat[a]

Minutes from Injection	Ethanol (0.75 g/kg)	Ethanol (1.5 g/kg)
−15	89 ± 8.1	95 ± 10.2
30	124* ± 19.3	140* ± 23.4
60	112 ± 12.6	126* ± 17.6
90	97 ± 9.9	112 ± 7.8

[a]Values represent the mean duration of inhibition ± S.E.M. in milliseconds for four to six cells in each condition.

*P < .01 when compared to −15 min preinjection value.

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TABLE 5

Response of rhythmically bursting MSA neurons to iontophoretlc application of nipecotic acid after systemically administered saline and ethanol

Minutes from Injection	Saline	Ethanol (1.5 g/kg)
% Change in Firing Rate[a]		
−15	31.1 ± 1.8	33.4 ± 3.2
30	34.1 ± 2.8	55.0* ± 8.7
60	32.7 ± 5.1	47.3* ± 2.8
90	37.3 ± 3.7	43.9* ± 7.6
120	33.4 ± 4.6	38.4 ± 6.6
Absolute Firing Rate[b]		
−15	17.9 ± 0.9	16.8 ± 1.2
30	16.3 ± 1.1	4.4* ± 0.8
60	19.2 ± 1.3	7.7* ± 0.9
90	15.3 ± 1.9	12.3* ± 0.8
120	17.8 ± 1.6	14.7 ± 1.0

[a] Values presented as the mean percentage of change in firing rate ± S.E.M. from the immediately preceeding base line after iontophoretic application of nipecotic acid.

[b] Values presented as the mean absolute firing rate ± S.E.M. (spikes/second) during iontophoretic application of nipecotic acid.

* $P < .01$ when compared to saline.

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TABLE 6

Time course of the effect of systemically administered ethanol on flurazepam inhibition of MSA neurons[a]

Minutes from injection	Saline	Ethanol (1.5 g/kg)
% Inhibition of Firing Rate[b, *]		
−15	24.6 ± 1.1	27.1 ±0.9
15	26.8 ± 2.6	28.1 ± 1.7
30	26.3 ± 2.8	27.4 ± 2.0
45	27.4 ±2.8	25.5 ± 1.5
60	27.2 ± 2.1	25.8 ± 1.3
90	25.3 ± 2.2	26.2± 1.3
120	29.0 ± 2.0	30.6 ± 2.5

[a]Flurazepam inhibition was achieved by direct ionotophoresis onto a single cell in the MSA.

[b]Values presented as the mean percentage change in firing rate ± S.E.M. from the immediately proceeding base-line. There are 4 to 11 determinations from two cells in the saline group and 13 to 40 determinations from six cells in the ethanol treatment group.

[*]$P > .1$ when compared to saline or to the inhibition before ethanol.