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The effects of chronic ethanol administration on amygdala neuronal firing and ethanol withdrawal seizures

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ABSTRACT

Physical dependence on ethanol results in an ethanol withdrawal (ETX) syndrome including susceptibility to audiogenic seizures (AGS) in rodents after abrupt cessation of ethanol. Chronic ethanol administration and ETX induce functional changes of neurons in several brain regions, including the amygdala. Amygdala neurons are requisite elements of the neuronal network subserving AGS propagation during ETX induced by a subacute "binge" ethanol administration protocol. However, the effects of chronic ethanol administration on amygdala neuronal firing and ETX seizure behaviors are unknown. In the present study ethanol (5 g/kg) was administered intragastrically in Sprague–Dawley rats once daily for 28 days [chronic intermittent ethanol (CIE) protocol]. One week later the rats began receiving ethanol intragastrically three times daily for 4 days (binge protocol). Microwire electrodes were implanted prior to CIE or on the day after CIE ended to record extracellular action potentials in lateral amygdala (LAMG) neurons. The first dose of ethanol administered in the binge protocol following CIE treatment did not alter LAMG neuronal firing, which contrasts with firing suppression seen previously in the binge protocol alone. These data indicate that CIE induces neuroadaptive changes in the ETX network which reduce LAMG response to ethanol. LAMG neuronal responses to acoustic stimuli prior to AGS were significantly decreased during ETX as compared to those before ethanol treatment. LAMG neurons fired tonically throughout the tonic convulsions during AGS. CIE plus binge treatment resulted in a significantly greater mean seizure duration and a significantly elevated incidence of death than was seen previously with the binge protocol alone, indicating an elevated seizure severity following chronic ethanol administration.

1. Introduction

Audiogenic seizures (AGS) can be induced by intense acoustic stimulation during ethanol withdrawal (ETX) after a "binge" ethanol administration protocol (Majchrowicz, 1975; Faingold and Riaz, 1994, 1995). The AGS in ETX rats are typically composed of a wild running episode, which is often followed by generalized clonic and/or tonic convulsions (Faingold and Riaz, 1994, 1995; Riaz and Faingold, 1994; Feng and Faingold, 2000; Feng et al., 2007). Previous studies demonstrated that several brainstem structures are involved in ETX seizure initiation and propagation (Faingold and Riaz, 1994, 1995; Faingold et al., 1998; Yang et al., 2003; Long et al., 2007). The amygdala is a limbic structure, which has been implicated in the ETX syndrome, based on metabolic, enzymatic, focal microinjection, immediate early gene, and behavioral evidence (Clemmesen et al., 1988; Rassnick et al., 1993; Knapp and Crews, 1999). We recently observed that amygdala neurons are also critically involved in the neuronal network subserving AGS propagation during ETX, since ETX seizures can be blocked by focal microinjection into amygdala, and amygdala neurons fire intensely during the later behavioral phases of AGS (Feng et al., 2007).

It has been well established that chronic ethanol administration leads to adaptive changes of neurotransmitter receptors in the mammalian brain (Faingold et al., 1998; Olsen et al., 2005; Weiner and Valenzuela, 2006). The experience of repeated withdrawals from chronic ethanol treatment leads to a kindling-like process (Gonzalez et al., 2001), and chronic ethanol treatment potentiates seizures evoked by several convulsants (Kokka et al., 1993; Becker et al., 1998). It has been shown that chronic ethanol administration leads to facilitated NMDA receptor function (Floyd et al., 2003; Roberto et al., 2004) and altered $GABA_A$ receptor pharmacology and subunit expression (Floyd et al., 2004) in the amygdala. These data suggest that amygdala neurons may have different responses to chronic ethanol than those resulting from subacute ethanol administration. However, the effects of chronic ethanol treatment on the amygdala neuronal firing and the AGS inducible during ETX in behaving animals have not been investigated.

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The present study used chronic single unit recording (microwire) techniques to examine the neuronal firing patterns and responses to acoustic stimuli in the lateral nucleus of the amygdala (LAMG). The animals in the present study were subjected to chronic intermittent ethanol (CIE) administration once daily for 28 days. Microwire chronic electrodes were implanted either before or after CIE. One week after CIE the rats were treated with the 4-day "binge" ethanol protocol (Majchrowicz, 1975). LAMG neuronal responses were evaluated during CIE, 1 h after the first dose of the binge ethanol, and during ETX in behaving animals. LAMG neuronal firing in response to acoustic stimuli and during AGS and its relationship with each behavioral component of ETX seizures were examined. An abstract on these data has been presented previously (Feng and Faingold, 2001).

2. Methods

Male Sprague–Dawley rats, weighing 300–400 g, were used in this study. The animals were housed in a temperature-controlled vivarium under a 12:12-h light/dark cycle with water and food available ad libitum. The experimental protocol was approved by the Laboratory Animal Care and Use Committee of Southern Illinois University School of Medicine. All efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to in vivo techniques.

Ethanol (95%) was diluted with water to form a 30% (v/v) ethanol solution, in which 5 ml of low-iron infant formula (Similac, Abbott Laboratories, Columbus, OH) was added to supplement nutrition. The rats were intubated intragastrically with 5 g/kg ethanol once daily for 28 days. The animals had free access to food and water during chronic intermittent ethanol (CIE) administration.

Microwire electrodes were implanted under anesthesia [ketamine/xylazine (85/3 mg/kg, i.p.)] prior to CIE or 1 day after CIE into the lateral nucleus of the amygdala (LAMG) at the following stereotaxic coordinates (−3.3 mm posterior to bregma, 5.3 mm medial/lateral, 7.6 mm vertical below the surface of the skull) (Paxinos and Watson, 1998). The microwire electrode assembly was composed of an amphenol connector (Dale Electronics, Yankton, SD), six 13-μm diameter insulated tungsten wires (California Fine Wire Company, Grover City, CA) and a harpoon. The microwires were bundled together using polyethylene glycol, which melted inside the brain, allowing the microwires a small degree of migration towards LAMG cell bodies (Faingold and Anderson, 1991). The animals were given tetracycline (1 g/l) in the drinking water to minimize postoperative infection during the 7-day recovery period (no ethanol solution was given during recovery).

One week after the surgery, the neuronal firing in the LAMG was recorded in a Plexiglass® cylinder (diameter: 44 cm) inside a sound-attenuating chamber. The acoustic stimulus was given in the form of tone bursts [12 kHz, 100 ms duration, 5 ms rise–fall, 50–100 dB SPL (re: 0.0002 dyne/cm^2), stimulus repetition rate of 0.5–4 Hz]. This involved 50 stimulus presentations at each of 4 stimulus intensities over a ~10 min period, and no evidence of tolerance or sensitivity was observed during the recordings. The neuronal firing patterns were recorded digitally. Ethanol dependence was induced by a subacute "binge" ethanol protocol (Majchrowicz, 1975) (see below). LAMG neuronal firing was recorded prior to and 1 h after the first dose of ethanol administration by this protocol and during ETX. LAMG neuronal firing simultaneous with seizure behaviors during ETX were videotaped with split-screen video techniques.

Ethanol dependence was induced in rats by intragastric intubation of ethanol solution (Majchrowicz, 1975). Initially, each animal was given a dose of 5 g/kg of ethanol in the form of ethanol solution (30% v/v), and each subsequent dose was based on the behavior to maintain the intoxication of the rats at the mild-to-moderate ataxic state (Majchrowicz, 1975). The rats were given the ethanol solution every 8 h for 4 days and ethanol was withdrawn at the end of the fourth day.

In order to examine the effect of ethanol on LAMG neuronal firing during the 4-week CIE treatment period, one group of rats was implanted with microwire electrodes, and the LAMG neuronal firing was recorded each week thereafter.

The data collected in the recordings were analyzed off-line. Single unit responses with a ratio of >3 to 1 to background activity were discriminated using the Digitizer Program (Plexon Inc., Dallas, TX) and the Offline Waveform Sorter (Plexon Inc.). One single unit was recorded from each animal. The resulting data were analyzed using poststimulus time histograms (PSTHs) (400 ms scan length, 1 ms bin width, 50 presentations) using the NEX program (Plexon/NEX Technologies, Dallas, TX).

The comparisons of LAMG neuronal firing in mean number of action potentials per PSTH after CIE treatment, 1 h after the first dose of binge ethanol and during ETX were evaluated using ANOVA of repeated measures with Tukey's test as post-hoc

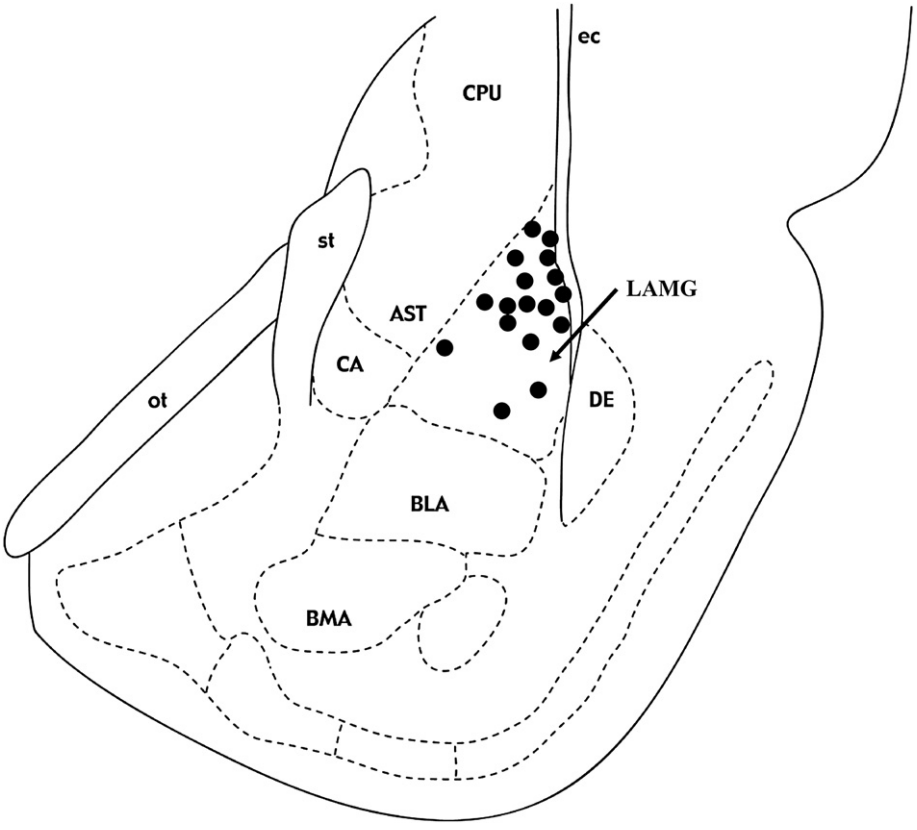

Fig. 1. This drawing shows recording sites of the microwire electrode assemblies in the lateral nucleus of amygdala (LAMG), according to the atlas of Paxinos and Watson (1998). AST, amygdalostriatal transition area; BLA, basolateral nucleus of amygdala; BMA, basomedial nucleus of amygdala; CA, central nucleus of amygdala; CPU, caudate putamen; DE, dorsal endopiriform nucleus; ec, external capsule; ot, optic tract; st, stria terminalis.