Effects of acute ethanol and nicotine intake on REM sleep and its interaction with noradrenergic system in rats

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Abstract
Ethanol and nicotine are the two most widely used and abused substances in the world and often are co-consumed. This study examined the effect of ethanol and nicotine co-consumption on rapid-eye-movement (REM) sleep and the role of noradrenergic system, a critical component of REM sleep control, in mediating this effect. The sleep-wake parameters were recorded for 6 hours during day-time after intraperitoneal injections of ethanol (2g/kg and 3g/kg), nicotine (0.3 and 3mg/kg), a mixture of ethanol (3gm/Kg)-nicotine (3mg/kg), and prazosin, an alpha-1 receptor antagonist (0.01 mg/kg) followed by ethanol-nicotine. The findings suggest that: a) ethanol increased NREM sleep, but suppressed REM sleep; b) nicotine suppressed both NREM and REM sleep; c) ethanol-nicotine attenuated each other’s effect on NREM sleep, but REM sleep remained depressed; and d) prazosin attenuated REM sleep suppression observed after ethanol-nicotine treatment. These findings are consistent with known interactions between ethanol and nicotine in influencing REM sleep and a role of the noradrenergic system in mediating this effect.

Keywords: Ethanol; Nicotine; Non-rapid eye movement sleep; Rapid eye movement sleep; Wakefulness; Noradrenergic system

1. Introduction
Sleep is an essential behavioral state that is experienced across the animal kingdom, from fruit flies to humans. A healthy sleep is critical to the well-being whereas a chronic sleep disturbance has been associated with poor health including diseases like obesity, type-2 diabetes, cognitive decline and cardiovascular disease [1-3]. Sleep consists of two alternating and distinct stages called non-rapid eye movement (NREM) and REM sleep [1, 2, 4]. Normally, sleep onset occurs through NREM sleep, which accounts for 75-80% of sleep amount. NREM sleep is characterized by the onset of a relatively high amplitude and low-frequency electroencephalogram (EEG) waves, which slows down further to 0.5 –4 Hz delta waves as sleep deepens. Eye movements slows down or become absent, and muscle tone also diminishes progressively. NREM sleep progresses into periods of REM sleep, which is characterized by high frequency and low amplitude EEG waves, frequent eye movements, and loss of muscle tone. In humans, sleep is organized into a single nocturnal bout with ~90 min NREM-REM cycles, while in rodents, e.g., rats, sleep is polycyclic and organized into many short bouts of about 15 min NREM-REM cycles. Being nocturnal, rats spend more time in sleep during the lights-on phase. The sleep period consists of many switches back and forth from NREM to REM sleep, with occasional transitioning to waking.
Ethanol is taken for various reasons including as a relaxant and remedy for insomnia. This is due to the fact that after acute alcohol intake, initially a person falls asleep more rapidly and experience relatively sound sleep. The sleep-wake changes caused by acute alcohol consumption in healthy non-alcoholics during the first half of the night include a decrease in sleep latency, i.e., a rapid sleep onset; an increase in deep NREM sleep or sleep with slow wave activity, and a decreases in time spent in REM sleep [5-7]. However during the second half of the night, there are increases in instability of sleep stages, night-time waking, and also an increase in REM sleep. The findings from animal studies also confirm that an acute ethanol treatment reduces sleep latency, increases NREM sleep, and slow-wave activity [8-10]. The findings on REM sleep, however, are inconsistent ranging from a decrease to no change in REM sleep in the initial 3-6 hours of post-treatment phase [11-13]. On the other hand, both human and animals studies suggest that chronic exposure to ethanol causes sleep disturbance as manifested by increased sleep latency, decreased total sleep time, and more frequent
Nicotine is the principal pharmaco-active substance in tobacco smoke. It is one of the most consumed legal drugs in the world. In India, tobacco has been used for smoking since 2000BC and is mentioned in the Atharvaveda. According to the World Health Organization, there are approximately 120 million smokers in India, constituting nearly 12% of the world smoker’s population. Globally, about 35% of adults use tobacco. Both human and animal studies suggest that nicotine consumption is associated with sleep disturbances. The adverse effects of nicotine on sleep include increased sleep latency, i.e., it takes longer to fall asleep, sleep fragmentation as marked with increased sleep-wake shifting, and decreased NREM sleep. In these studies, nicotine’s effect on REM sleep seemed to be dose-dependent and opposite since lower doses of nicotine increased REM sleep, whereas, its higher dose decreased REM sleep time. Nicotine withdrawal also leads to insomnia complaints.

Ethanol and nicotine are the two most widely used and abused substances in the world and are often co-consumed [14]. About 85% of alcoholics smoke and nearly 30% of smokers are alcoholics [15]. However, the effects of co-consumption of ethanol and nicotine on REM sleep remains poorly understood. Therefore, in this study, we determined dose-dependent changes in sleep, especially REM sleep, after intraperitoneal injections of ethanol, nicotine, and both nicotine and ethanol given together. Also, in most of the earlier animal studies, ethanol was given in drinking water, liquid diet, or by gavage and therefore the amount of ethanol consumed and the amount that entered into the blood stream was not certain [16]. In this study, intraperitoneal injection (IP) was used for delivery of both ethanol and nicotine so that animals received the same amount of both drugs in any treatment condition. It is well established that noradrenergic system in the brain plays a key role in the regulation of REM sleep [17]. An inhibition of noradrenergic neurons in the locus coeruleus area of the brain is critical for REM sleep onset and maintenance [17, 18]. Although, both ethanol and nicotine affect REM sleep, the role of the noradrenergic system in ethanol and nicotine co-consumption-induced changes in REM sleep remains poorly understood. We determined if blocking noradrenergic transmission by a noradrenergic alpha-1 receptor antagonist, prazosin, affects ethanol-nicotine co-consumption-induced changes in REM sleep.

2. Materials and methods

Experiments were conducted on male Wistar rats weighing between 225-275 grams. These rats were maintained at 12 hour light and 12-hour dark cycles with lights on from 08:00 AM to 8:00 PM. The details of the experimental procedure were as follows:

2.1: Surgery: First, rats were prepared for recording their electroencephalogram (EEG), electromyogram or muscle tone (EMG), and electrooculogram (EOG) or eye movements to objectively quantify and characterize their sleep-wake patterns as used in earlier studies [19, 20]. The surgical procedure was done in aseptic condition, using sterilized instruments, and under isoflurane anesthesia. After rats became anesthetized, skin on the scalp was shaved and cleaned with alcohol and betadine. Then animal’s head was properly secured on the stereotaxic apparatus using ear and nose bars. The skull of the rat was exposed by making a longitudinal incision on the skin over the scalp. Two stainless steel screw electrodes were threaded through the skull (2 mm anterior to and 4 mm lateral to the bregma and midsagittal suture, respectively) for recording bipolar EEG. One screw electrode was also threaded on the midline over the frontal bone to serve as ground. Flexible insulated (except at the tip) wires were connected on either side to the dorsal cervical neck muscles and to the muscles near the external canthus of eyes for recording bipolar EMG or muscle tone and EOG or eye movements, respectively. Leads from EEG, EMG, and EOG electrodes were soldered individually to an electrical connector, which was fixed on the skull with dental cement. The wounds were sutured.

2.2 Acclimatization of animals: After surgical recovery for ten days, rats were acclimatized with the recording setup for 2-3 days.

2.3 Data Acquisition: On the day of the experiment the rat was weighed 30 min before light onset and placed in the recording cage with all the connections made at least 1 hour before the start of actual recording. The recording was done during lights-on phase or day time (8.00AM – 5.00PM). EEG, EMG, and EOG signals were recorded and displayed on the computer using the software “spike-2” and stored on computer disc for analysis. Before the experiment, baseline EEG, EMG and EOG were recorded for at least 60 minutes to check if rat showed a normal sleep-wake pattern. After baseline recording the following set of experiments were conducted:

Experiment 1: Acute effects of ethanol on REM sleep: After confirming that rats exhibited normal sleep-wake profiles, rats (n=6) were gently swaddled in a towel and slowly injected (IP) with 1 ml of saline and their sleep-wake parameters or EEG, EMG, and EOG signals were recorded for 6 hours as control. After control recording, rats were given a break of 3 days and then were injected with one of the two doses of ethanol (2g/kg or 3g/kg of the body weight in 1 ml of saline) and their sleep parameters were recorded for 6 hours. Again after three days of break, another dose of ethanol was injected and EEG, EMG, and EOG signals were recorded for 6 hours. The two doses of ethanol were selected on the basis of earlier studies [11, 21, 22]. The saline and two doses of ethanol injections were given in a random order.

Experiment-2: Acute effects of nicotine on REM sleep: In the second set of rats (n=6), the effects of 2 doses of nicotine on sleep-wake parameters were examined. The experimental procedure was the same as in experiment-1, except that instead of ethanol, two doses of nicotine, i.e., 0.3 and 3mg/kg of the body weight, were injected IP and their effects on sleep-wake parameters, i.e., EEG, EMG, and EOG changes were recorded for 6 hours after injection.

Experiment-3: Acute effects of ethanol-nicotine co-injection on REM sleep: In the third set of rats (n=6), the effects of a combination of both ethanol and nicotine on sleep-wake parameters were examined. The experimental protocol was the same as in earlier experiments, except that the effects of a mixture of ethanol (3g/kg) and nicotine (3 mg/kg) was injected IP and its effects on sleep parameters during 6 hours of the post-injection period were recorded.
Experiment-4: Effects of noradrenergic alpha-1 receptor antagonist on ethanol-nicotine induced effects on REM sleep: After examining the effects of ethanol-nicotine on EEG, EMG, and EOG in experiment-3, rats were given a break of 3 days. After three days of break, first, prazosin was injected (0.01 mg/kg, IP) and after 5 min the mixture of ethanol (3g/kg) + nicotine (3mg/kg) was injected, and their sleep-wake parameters were recorded for 6 hours. This dose of prazosin was chosen because at this dose prazosin does not affect REM sleep [23, 24].

At the end of the experiment, rats were euthanized by injecting pentobarbital @ 100mg/kg. The saline control in experiments 1 and 2 were pooled together and used as a control for experiments 3-4 because the experimental conditions were the same. The doses of both ethanol and nicotine were selected based on the available literature [11, 20].

2.4 Analysis: All analysis were performed off-line from the stored data files using “Spike 2” software for sleep-wake scoring and Sigmaplot software for statistical analysis.

Sleep-wake scoring: Sleep-wake states were scored manually in 10-second epochs on the basis of EEG, EMG, and EOG patterns using standard criteria [19]. Sleep-wake stages of rats were scored in terms of active-waking, quiet-waking, NREM sleep, and REM sleep (Figure-1). In brief, the criteria used were as follows:

a) Active-waking: EEG desynchronization or high frequency and low amplitude EEG waves, higher muscle tone with movements, and occasional eye movements.

b) Quiet-waking: EEG desynchronization with occasional synchronization, i.e., low frequency and high amplitude EEG waves (less than 25% time), higher muscle tone but without movements, and occasional eye movements, if any.

c) NREM sleep: EEG synchronization (>25% of the time bin, i.e., 10 sec analyzed), reduced muscle tone, and no eye movements.

d) REM sleep: EEG desynchronization, no muscle tone and frequent eye movements.

Statistical analysis: For analysis purposes, active- and quiet-waking were combined together as waking. Sleep-wake data during 2-hour intervals, i.e., 1-2 hours after treatment, 3-4 hours after treatment, and 5-6 hours after treatment were compared in different treatment groups. The statistical significance of the differences within the group and with multiple treatments were determined using One Way Repeated Measures ANOVA followed by a post hoc test, Holm-Sidak method. In cases of single treatment within the group the data was compared using paired t-test or between two groups using t-test.

3. Results

3.1: Effects of ethanol on REM sleep: The effects of saline and two doses of ethanol on sleep-wakefulness during the 6 hours of post-injection recording period during lights-on phase are shown in Figure-2. Being nocturnal and consistent with sleep-wake data in earlier studies [1, 2, 19], continuous recording without any treatment showed that during 8.00AM to 5.00PM rats spend significantly more time in NREM and REM sleep than in waking (data not shown). After saline injection (control) rats spent more time in NREM and REM sleep and less time in waking. During first 2 hour interval, waking was slightly but insignificantly higher due to the
handling of the animal and injection procedure. Ethanol injection in the same group of rats produced a dose-dependent effect on sleep-wake states including REM sleep (One Way Repeated Measures ANOVA, p <0.01).

The lower dose of ethanol (2g/kg) was marginally effective in increasing NREM sleep and decreasing both waking and REM sleep. However, the higher dose (3g/kg) increased NREM sleep and suppressed REM sleep significantly (p<0.01). It also reduced waking significantly (p<0.01). These effects were significant during the first 2 hours after injection. The REM sleep was higher during the 5-6 hours after injection period, but it did not reach significant levels. The sleep latency or the occurrence of the first episode of NREM sleep of at least 30 seconds in duration was shortened significantly (p<0.01), i.e., rats fell asleep significantly faster after ethanol treatment compared to saline control (Figure-3).

3.2: Effects of nicotine on REM sleep: The effects of saline and two doses of nicotine on sleep-wakefulness during the 6 hours of post-injection recording period during lights-on phase are shown in Figure-4. As compared to saline injection, nicotine injection produced a dose-dependent effect on sleep-wake states including REM sleep (One Way Repeated Measures ANOVA, p<0.01). The lower dose of nicotine (0.3mg/kg of body weight) produced no significant effect on
waking, NREM sleep, or REM sleep. However, a higher dose of nicotine (3mg/kg of body weight) significantly reduced both NREM and REM sleep and increased wakefulness. The latency to sleep-onset also increased significantly, i.e., it took longer for rats to fall asleep (Figure-3).

3.3: Effects of nicotine + ethanol on REM sleep: The effects of a mixture of the effective doses of ethanol (3g/kg) and nicotine (3mg/kg) on sleep-wakefulness during the 6 hours of the post injection period during lights-on phase are shown in Figure-5. When injected together, ethanol and nicotine significantly attenuated each other’s effects on waking and NREM sleep. As compared to saline injection, the mixture of ethanol-nicotine injection did not produce any significant effects on waking or NREM sleep. However, REM sleep amount remained significantly suppressed after ethanol-nicotine injection. As compared to saline, the latency to sleep-onset was not significantly different (Figure-3).

3.4: Effects of prazosin on nicotine + ethanol-induced effects on REM sleep: To examine if noradrenergic system is involved in ethanol-nicotine co-injection induced effects on REM sleep, rats were first treated with a dose of noradrenergic alpha-1 receptor antagonist, prazosin, on waking, NREM and REM sleep at every 2-hour intervals during the 6 hours of the post-injection recording period. After ethanol-nicotine treatment both waking and NREM sleep were insignificantly different as compared to saline injection. However, REM sleep was significantly suppressed. Prazosin significantly attenuated ethanol-nicotine induced REM sleep suppression. **, decrease as compared to saline treatment, p<0.01; +, increase as compared to ethanol-nicotine treatment, p<0.05.

5. Discussion
The findings of this study suggest that ethanol treatment acutely and in a dose-dependent manner reduced waking increased NREM sleep but suppressed REM sleep. On the other hand, nicotine dose-dependently increased waking and
not only suppressed NREM sleep, but it also suppressed REM sleep. When ethanol and nicotine were given together, they attenuated each other’s effects on waking and NREM sleep, so that the waking and NREM sleep became insignificantly different as compared to control. However, REM sleep remained depressed. A blockage of noradrenergic transmission by alpha-1 receptor antagonist did not influence the effects of ethanol + nicotine on waking and NREM sleep but reduced REM sleep suppression as seen after their individual or combination treatments.

The effects of ethanol, nicotine, their combination, or prazosin on sleep-wake states, in particular, REM sleep, as observed in this study were specific pharmacological effects and not non-specific or due to animal handling or injection procedure because: a) after saline injection, which was used as a control, rats spent almost similar amount of time in waking, NREM, and REM sleep as observed without any manipulation; b) the effects of ethanol and nicotine were opposite on waking and NREM sleep, while the effects were similar on REM sleep; c) the effects were time and dose-dependent; and d) similar effects of ethanol and nicotine were also observed in earlier studies using different routes of administration.

Our findings that higher dose of ethanol suppressed waking, reduced sleep latency and increased NREM sleep, but reduced REM sleep is consistent with earlier studies in human and animals (see Introduction). These studies suggest that irrespective of the time of administration, i.e., whether ethanol was given during the dark phase (when rats are mostly active or light-phase in humans) or light-phase (when rats predominantly sleep or dark-phase in humans), ethanol increased NREM sleep amount and slow wave activity. An initial suppression in REM sleep has been consistently reported in human after ethanol intake. However, there is some inconsistency in the findings of ethanol’s effect on REM sleep in rodents. Some studies found that ethanol only reduced the duration of REM sleep episode without much effect on total REM sleep amount, whereas others found a decrease in REM sleep amount. Our finding that ethanol acutely suppressed REM sleep amount, especially during the first 2 hours of the post-injection period, followed, in fact, by a REM sleep rebound is consistent with later studies. The ability of ethanol to reduce REM sleep in a dose-dependent manner suggest a direct effect of ethanol on REM sleep physiology.

In this study injection of nicotine significantly increased waking, increased sleep-latency, and reduced both NREM and REM sleep amounts. These findings are consistent with earlier human and animal studies indicating that nicotine intake increases sleep latency and decreases both NREM and REM sleep. As regards the underlying mechanism, recent studies suggest that nicotine activates wake-promoting neurons including those in the basal forebrain, which may contribute to nicotine’s wake-promoting and sleep-suppressing effects.

In this study, ethanol-nicotine co-injection attenuated each other’s effect on waking and NREM sleep or nicotine attenuated ethanol-induced suppression in waking, a decrease in sleep latency and increase in NREM sleep while the effects on REM sleep was not changed. These findings are partly consistent with an earlier study where the effects of ethanol were blocked by the injection of nicotine into the wake-promoting basal forebrain. Studies support that co-consumption of nicotine and ethanol are highly prevalent and the two drugs when taken together potentially create an effect that is different from the effects of either drug taken alone. The findings of this study suggest that nicotine co-consumption attenuates the sleep-promoting effects of ethanol and thus may increase the pleasure experience. Studies show that nicotine-treated animals or smokers consume more alcohol than control groups whereas, administration of nicotine receptor antagonist reduces alcohol consumption. Both ethanol and nicotine combination treatment did not produce an additive decrease in REM sleep. This could be due to the reason that nicotine and ethanol affect NREM sleep regulatory systems much strongly than those systems involved in REM sleep control.

The infusion of prazosin at a dose that does not produce any significant effect on sleep-wakefulness including REM sleep, prior to ethanol-nicotine treatment did not have much effect on waking and NREM sleep. However, prazosin moderately attenuated REM sleep suppression as seen after ethanol-nicotine combination treatment. This is consistent with a role of the monoaminergic system in REM sleep suppression and that prazosin inhibited the actions of the monoaminergic system by blocking its alpha-1 receptor. Earlier studies have shown that IP injection as well as injection of prazosin into the brain increases REM sleep. Prazosin has also been shown to increase total REM sleep time and average REM sleep episode duration in the human civilian population. It is possible that the effects of REM sleep suppression by both ethanol and nicotine involves noradrenergic systems and that is why its blockade reduced that suppression in REM sleep.

6. Conclusion

The findings of the present study suggest that ethanol treatment acutely decreased waking, increased NREM sleep, but decreased REM sleep. On the other hand, nicotine treatment acutely decreased both NREM and REM sleep and increased waking. The ethanol-nicotine co-treatment attenuated each other’s effect on waking and NREM sleep but did not produce any significant effect on REM sleep, which remained depressed. The blockade of the noradrenergic alpha-1 receptor by prazosin attenuated REM sleep suppression as observed after ethanol-nicotine treatment. These findings are consistent with current literature about the effects of ethanol and nicotine in influencing REM sleep and a role of the monoaminergic system in mediating this effect.

7. References

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