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Inhibitory influence of mecamylamine on the development and the expression of ethanol-induced locomotor sensitization in mice

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ABSTRACT

Several evidences have indicated the involvement of neuronal nicotinic acetylcholine receptors (nAChR) in behavioral effects of drugs of abuse, including ethanol. nAChRs are implicated in ethanol-induced behaviors as well as neurochemical responses to ethanol. Recently, it is demonstrated that mecamylamine, a nAChR antagonist blocks cocaine-, *d*-amphetamine-, ephedrine-, nicotine-, and methylphenidate-induced psychomotor sensitization. However, no reports are available on its role in ethanol-induced psychomotor sensitization. Therefore, an attempt was made to evaluate its effect on ethanol-induced locomotor sensitization using a model previously described by us. The results revealed that acute administration of mecamylamine (1 and 2 mg/kg, i.p.) blocked the acute stimulant effect of ethanol (2.0 g/kg, i.p.). In addition, treatment with mecamylamine (0.5–2.0 mg/kg, i.p.), 30 min prior to the challenge dose of ethanol (2.0 g/kg, i.p.) dose dependently attenuated expression of sensitization to locomotor stimulant effect of ethanol. Moreover, administration of mecamylamine (1 and 2 mg/kg, i.p.) during development (prior to each ethanol injection on days 1, 4, 7, and 10) blocked acquisition as well as expression (day 15) of sensitization to locomotor stimulant effect of ethanol. Mecamylamine *per se* did not affect locomotor activity. Further, it also did not influence blood ethanol levels and rotarod performance in mice. These results support the hypothesis that neuroadaptive changes in nAChRs may participate in the development and the expression of ethanol-induced locomotor sensitization.

1. Introduction

Chronic administration of drugs of abuse is reported to induce long-term neuronal and molecular adaptations in brain (Robinson and Kolb, 2004; Koob, 2008). Chronic and intermittent administration of such agents may result in an increase in its behavioral stimulant effect; a process termed as sensitization (Kalivas and Stewart, 1991). In case of ethanol abuse, sensitization is reported to persist at least for weeks to month even after its intake is stopped (Henry and White, 1995; Fish et al., 2002). This phenomenon and relapse to drug-seeking behavior after long periods of abstinence are highly correlated (Segal and Schuckit, 1983; Hunt and Lands, 1992; Robinson and Berridge, 1993; Lessov and Phillips, 1998; Koob, 2008). Numerous studies have demonstrated locomotor sensitization in mice on repeated administration of ethanol (Lister, 1987; Phillips et al., 1995; Goeldner et al., 2005; Umathe et al., 2009), which is thought to initiate in the ventral tegmental area (VTA) and then express in the nucleus accumbens (NAc), presumably through enhancement of dopamine responses (Kalivas and Duffy, 1990). Furthermore, ethanol-induced psychomotor sensitization is influenced by different neurotransmitters and neuropeptide system modulators, such as dopamine (Palmer et al., 2003; Broadbent et al., 2005), serotonin (Goeldner et al., 2005; Ferraz and Boerngen-Lacerda, 2008; Umathe et al., 2009), gamma-aminobutyric acid (Broadbent and Harless, 1999), glutamate (Broadbent et al., 2003; Kotlinska et al., 2006), nitric oxide (Itzhak and Martin, 2000), corticotrophin-releasing hormone (Fee et al., 2007), gonadotrophin-releasing hormone (Umathe et al., 2008), etc.

Ethanol consumption and tobacco smoking are highly correlated behaviors. More than 90% of alcoholics are smokers (Walton, 1972; Bobo et al., 1987), and approximately 35% of alcoholics have nicotine co-dependence (Grant et al., 2004). The high prevalence of co-dependence on ethanol and nicotine is suggestive of a shared neurobiological mechanism(s) mediating reinforcement and reward (Little, 2000; Funk et al., 2006). In recent years, neuronal nicotinic acetylcholine receptors (nAChRs) have received particular attention for their involvement in alcoholism. Several researchers have demonstrated an interaction between nAChR and ethanol using

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electrophysiological and behavioral studies (Narahashi et al., 1999; Larsson and Engel, 2004; Ford et al., 2009; Bhutada et al., 2010b). High to moderate densities of nAChRs are reported to be present in mesolimbic dopamine-innervated areas (Clarke et al., 1984; Klink et al., 2001; Wonnacott et al., 2005; Champtiaux et al., 2006). Chronic ethanol intake was found to produce changes in the binding characteristics (B_{max}) of radiolabeled nicotine in different regions of the rat brain (Yoshida et al., 1982). nAChRs in VTA and NAc are reported to be involved in ethanol intake and preference as well as ethanol-induced stimulation of the mesolimbic dopaminergic system and locomotor activity (Nadal et al., 1998; Le et al., 2000; Soderpalm et al., 2000; Blomqvist et al., 2002; Larsson et al., 2002, 2004; Chi and de Wit, 2003; Larsson and Engel, 2004; Young et al., 2005). Moreover, mecamylamine, a nAChR antagonist is reported to inhibit sensitization to locomotor stimulant effect of cocaine, *d*-amphetamine, nicotine, ephedrine and methylphenidate (Karler et al., 1996; Kempsill and Pratt, 2000; Schoffelmeer et al., 2002; Miller and Segert, 2005; Wooters and Bardo, 2009). All these evidences suggest that nAChR may have some role to play in the process of ethanol-induced psychomotor sensitization.

Thus, in view of 1) high prevalence of ethanol and nicotine co-dependence; 2) presence of nAChR in brain areas responsible for the development and the expression of sensitization to ethanol; 3) ability of mecamylamine, a nAChR antagonist to inhibit sensitization to drugs of abuse; we hypothesized that nAChR antagonists may inhibit the process of ethanol-induced psychomotor sensitization, and tested the influence of mecamylamine treatment on acquisition and expression of ethanol-induced locomotor sensitization in mice.

2. Materials and methods

2.1. Subjects

Inbred Swiss albino male mice (22–26 g), born and reared in the Animal House of the Agnihotri College of Pharmacy, Wardha, from a stock originally purchased from Shree Farm, Bhandara, India, were used as subjects. Mice were group housed (four per cage) in opaque polypropylene cages ($28\times21\times14$ cm) and maintained at 23 ± 2 °C under 12:12 h light/dark cycle (light cycle: 0800–2000 h), with free access to standard rodent diet and tap water. Animals were naive to the drug treatment and experimentation at the beginning of all the experiments. Each experimental group comprised of six to nine mice. Behavioral studies were carried out between 09.00 and 14.00 h to minimize circadian influences. Testing was carried out in a counter-balanced order with respect to the treatment conditions, in a noise free room.

The present investigations employed Swiss mice as ethanol (2.0 g/kg) is known to induce a locomotor stimulant effect in these mice, which becomes more pronounced with repeated exposure (Masur and Boerngen, 1980; Camarini et al., 1995). Further, only males were used, as different phases of estrus cycle in females are reported to modulate the response to ethanol (Biggio et al., 2007).

All experiments were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.2. Drugs and solutions

Mecamylamine hydrochloride (Sigma-Aldrich Co, St. Louis, MO) and ethanol (Changshu Yangyuan Chemical, China) were dissolved in 0.9% saline. Mecamylamine was administered at a dose 0.5–2.0 mg/kg, i.p., in a volume of 10 ml/kg body weight, on the basis of previous reports (Larsson et al., 2002; Kamens and Phillips, 2008) and our preliminary studies. Ethanol was administered at dose of 2 g/kg, i.p. in a volume of 12.5 ml/kg body weight, as this dose was previously demonstrated to induce sensitization in mice (Masur and Boerngen, 1980; Boerngen-Lacerda and Souza-Formigoni, 2000). All other chemicals employed in the present investigation were of analytical grade and purchased from SISCO Research Laboratories, Mumbai, India.

2.3. Locomotor activity

Locomotor activity was assessed in an actophotometer (VJ Instruments, Amravati, India). It consists of an enclosed circular arena of 40 cm, fitted with four infrared beam cells on the circular wall (2 cm above the floor). The light beam interruptions due to movement of animals are recorded by a digital counter. The tests were performed in sound-attenuated chamber with low, indirect incandescent lighting (40 lx on the testing floor). Locomotor activity was expressed in terms of total number of light beam interruptions in 30 min.

On the test day, mice were weighed and moved in their home cages to the testing room 60 min before drug administration to adapt with the testing environment. Mice were treated with either mecamylamine or vehicle (pretreatment), and returned to their home cages. Thirty minutes after the pretreatment, ethanol or saline (post-treatment) was administered, and the animals were again returned to their home cages. Five minutes thereafter individual mouse was placed in actophotometer and the locomotor activity was recorded for 30 min (Bellot et al., 1996; de Araujo et al., 2009).

2.4. Accelerated rotarod test

The ataxic behavior of the mice was evaluated by accelerating rotarod test (VJ Instruments, Amravati, India) using methods described previously with slight modifications (Rustay et al., 2003; Boyce-Rustay et al., 2006). The apparatus had a 25 cm fall height and a 6 cm diameter dowel, which was lined on the surface to facilitate grasping (Bhutada et al., 2010a). The latency to fall from dowel was automatically recorded, with a cut off at 5 min. Each mouse was individually placed on the slowly (5 rpm) rotating rod, which was accelerated from 5 to 40 rpm at a rate of 7 rpm/min. Each mouse was first given 4 consecutive training trials on the accelerating rotarod with 30 s rest between trials. After the fourth trial, each mouse was administered with vehicle or mecamylamine (pretreatment) and 30 min thereafter treated with saline or ethanol (post-treatment), and returned to the home cage; 20 min later, a test trial was performed. This time point (mid-point of locomotor activity session) was selected for the test as we have observed the locomotor activity for 30 min.

2.5. Blood ethanol concentrations

Blood ethanol concentration was determined by alcohol dehydrogenase assay as described by us earlier (Umathe et al., 2009). In brief, blood samples (40 µl) were obtained from the retro-orbital sinus immediately after locomotor activity experiments (35 min post-ethanol administration). 3% perchloric acid (160 µl) was added, it was vortexed and then centrifuged at $10{,}000\times g$ in a cooling centrifuge. The supernatant was stored at 4 °C until analysis.

For the assay, 60 µl of supernatant was incubated for 40 min at room temperature in 3 ml of 0.5 M Tris–Cl buffer (pH 8.8) containing 5.5 µg/ml of alcohol dehydrogenase and 1.5 mM β-nicotinamide adenine dinucleotide (β-NAD). Thereafter, accumulation of β-NADH was measured by reading absorbance at 340 nm. The ethanol concentration in the samples was estimated by using a standard calibration curve.

2.6. Effect of mecamylamine treatment on the locomotor stimulant effects of acute ethanol administration

Mice ($n=6$–8) were randomly assigned to treatment conditions and mecamylamine (0.25, 0.5, 1.0, or 2.0 mg/kg, i.p., in volume 10 ml/kg body weight) or vehicle (0.9 % saline, 10 ml/kg body weight, i.p.) was administered. Thirty minutes thereafter mice received either ethanol (2.0 g/kg, i.p., in volume 12.5 ml/kg body weight) or 0.9 % saline (12.5 ml/kg body weight, i.p.). Five minutes after the last treatment, locomotor activity was assessed for 30 min using actophotometer. After completion of the locomotor session, blood ethanol levels were assessed in ethanol treated group by the method described above.

2.7. Acquisition and expression of sensitization to locomotor stimulant effect of ethanol

Sensitization to locomotor stimulant effect of ethanol was developed as per the method described earlier (Kotlinska et al., 2007; Umathe et al., 2009) with slight modifications. Mice were administered with ethanol (2.0 g/kg, i.p., in volume 12.5 ml/kg body weight) or saline (12.5 ml/kg body weight, i.p.) on day 1, 4, 7, and 10. Five minutes after each ethanol injection, locomotor activity of each mouse was assessed for 30 min as described above. The locomotor count was plotted against time to ascertain the acquisition of sensitization.

To assess the expression of sensitization to locomotor activity, challenge dose of ethanol (2.0 g/kg, i.p., in volume 12.5 ml/kg) was given to ethanol and saline treated groups on day 15, and locomotor activity was assessed for 30 min as described above. The group chronically treated with saline received saline on the challenge day (day 15), and served as respective control group.

2.8. Influence of mecamylamine acute treatment on the expression of sensitization to locomotor stimulant effect of ethanol

In order to study the effect of mecamylamine on the expression of sensitization to locomotor stimulant effect of ethanol, separate groups of mice ($n=6$–8) were sensitized to ethanol as described above, and on day 15, 30 min prior to the challenge dose of ethanol, mice were treated with mecamylamine (0.25, 0.5, 1.0, or 2.0 mg/kg, i.p., in volume 10 ml/kg body weight) or vehicle (0.9 % saline, 10 ml/kg body weight, i.p.). Five minutes after the injection of ethanol, locomotor activity was assessed for 30 min as described above, and after the completion of the locomotor activity test session, the blood ethanol levels were assessed by the method described above.

2.9. Influence of mecamylamine chronic treatment on the acquisition and the expression of sensitization to the locomotor stimulant effect of ethanol

Separate groups of mice ($n=6$–9) were treated with mecamylamine (0.5, 1.0, or 2.0 mg/kg, i.p., in volume 10 ml/kg body weight) or vehicle (0.9 % saline, 10 ml/kg body weight, i.p.), 30 min prior to each ethanol (2.0 g/kg, i.p., in volume 12.5 ml/kg body weight) or saline (0.9 % saline, 12.5 ml/kg body weight, i.p.) injection on days 1, 4, 7, and 10 (acquisition). On day 15, all mice received only a challenge dose of ethanol. Five minutes after the challenge, locomotor activity was assessed for 30 min as described above (expression), and after the completion of the locomotor session on day 15, the blood ethanol levels were assessed.

2.10. Influence of mecamylamine and ethanol treatment on motor coordination in mice

In order to confirm the specificity of inhibitory influence of mecamylamine on ethanol-induced locomotor sensitization, studies were carried out in mice treated with both ethanol and mecamylamine using accelerating rotarod apparatus. This was thought necessary to eliminate any non-specific effects of mecamylamine on the ability of the mice to stimulate to ethanol.

Mice were randomly assigned to treatment conditions ($n=6$) in which mecamylamine (0.25, 0.5, 1.0, or 2.0 mg/kg, i.p., in volume 10 ml/kg body weight) or vehicle (0.9 % saline, 10 ml/kg body weight, i.p.) was administered, and 30 min later ethanol (2.0 g/kg, i.p., in volume 12.5 ml/kg body weight) was injected. Twenty minutes after the last treatment, mice were subjected to accelerating rotarod test (test trial) and latency to fall was recorded as described above.

2.11. Data analysis

The results are expressed as a mean ± S.E.M. Data were analyzed using either one-way analysis of variance (ANOVA) followed by Tukey's/Dunnett's multiple comparison test or two-way ANOVA/repeat measure ANOVA followed by Bonferroni test for multiple comparisons. $P<0.05$ was considered statistically significant in all the cases.

3. Results

3.1. Effect of mecamylamine on locomotor activity of saline or ethanol treated mice

Fig. 1 depicts the influence of acute mecamylamine treatment on the locomotor activity of saline or ethanol treated mice. Two-way ANOVA revealed a significant locomotor stimulant effect of ethanol (2.0 g/kg, i.p.) in mice [F (1, 61) = 42.71, $P<0.0001$]. Similarly, mecamylamine treatment also had a significant influence on the locomotor activity of mice [F (4, 61) = 5.27, $P=0.001$]. However, mecamylamine had different effects in saline and ethanol treated mice {Ethanol × Mecamylamine [F (4, 61) = 3.225, $P=0.0182$]}.

Post hoc Bonferroni test indicated that mecamylamine (1.0 and 2.0 mg/kg) significantly ($P<0.001$) reduced the acute locomotor stimulant effect of ethanol, whereas the lower doses were ineffective ($P>0.05$). Further, mecamylamine pretreatment had no influence on the locomotor activity of saline treated mice ($P>0.05$).

Acute administration of mecamylamine (1.0 and 2.0 mg/kg, i.p.) had no influence on the blood ethanol levels ($P>0.05$) (Table 1).

3.2. Acquisition and expression of sensitization to locomotor stimulant effect of ethanol

The data from that single experiment have been divided as acquisition and expression of sensitization to ethanol, and represented in the two figures (Figs. 2 and 3). The data on acquisition and the expression of sensitization to locomotor stimulant effect of ethanol is

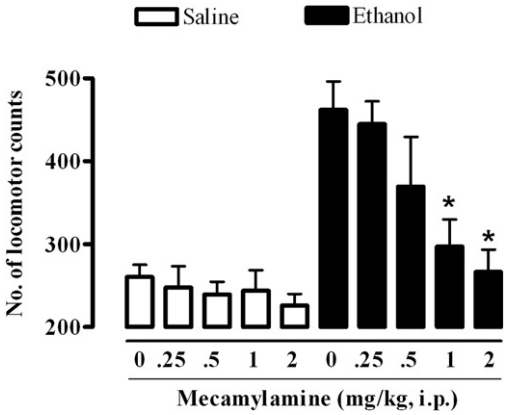

Fig. 1. Effect of mecamylamine on locomotor activity of saline or ethanol treated mice. Fig. 1 shows effects of mecamylamine on locomotor responses to saline and to acute ethanol. Each bar represents the mean ± S.E.M. of 6–8 mice per group. *$P<0.001$ vs. ethanol control group (Two-way ANOVA followed by Bonferroni test).

Table 1
Effect of mecamylamine on blood ethanol concentrations.

Treatments	Dose		Blood ethanol concentration (mg/dl) Mean ± SEM
	Mecamylamine (mg/kg, i.p.)	Ethanol (g/kg, i.p.)	
Effect of mecamylamine on the locomotor stimulant effect of acute ethanol			
Saline (a) + EtOH (a)	–	2.0	100.61 ± 13.19
Mecamylamine (a) + EtOH (a)	1.0	2.0	99.00 ± 6.30
	2.0	2.0	93.92 ± 7.90
Influence of mecamylamine acute treatment on expression of sensitization to locomotor stimulant effect of ethanol			
Saline (ch) + saline (a) + EtOH (a)	–	2.0	113.56 ± 5.03
EtOH (ch) + saline (a) + EtOH (a)	–	2.0 ± 2.0	110.16 ± 5.75
EtOH (ch) + mecamylamine (a) + EtOH (a)	0.25	2.0 ± 2.0	109.79 ± 5.43
	0.50	2.0 ± 2.0	97.42 ± 11.34
	1.0	2.0 ± 2.0	99.67 ± 8.24
	2.0	2.0 ± 2.0	96.82 ± 6.53
Influence of mecamylamine chronic treatment on acquisition and expression of sensitization to locomotor stimulant effect of ethanol			
Saline (ch) + EtOH (ch) + EtOH (a)	–	2.0 ± 2.0	108.54 ± 8.54
Mecamylamine (ch) + EtOH (ch) + EtOH (a)	1.0	2.0 ± 2.0	109.44 ± 8.25
	2.0	2.0 ± 2.0	95.89 ± 9.06

a, acute; ch, chronic; EtOH, Ethanol in g/kg, i.p.; mecamylamine, mg/kg, i.p.; Saline ml/kg, i.p.

shown in Fig. 2. Ethanol administration (2.0 g/kg) on days 1, 4, 7, and 10 progressively increased the locomotor activity indicating acquisition of sensitization. Two-way repeat measures ANOVA revealed a significant influence of ethanol treatment [F (2, 54) = 147.7, P<0.0001] and duration of ethanol treatment [F (3, 54) = 26.86, P<0.0001] on locomotor activity of mice, and this effect was different on each day {Ethanol treatment × Time [F (6, 54) = 27.75, P<0.0001]}.

The post hoc Bonferroni test indicated a significant influence of ethanol chronic treatment on the locomotor activity of mice on day 1 (P<0.01), days 4, 7, and 10 (P<0.001).

On day 15, challenge with ethanol produced a large increase in the locomotor activity of mice chronically treated with ethanol indicating expression of sensitization.

One-way ANOVA indicated a significant effect of ethanol treatment on locomotor activity [F (2, 20) = 122.4, P<0.0001]. The post hoc Tukey's test further indicated that the locomotor activity in chronically ethanol treated group was significantly (P<0.001) different as compared to acute ethanol treated as well as saline treated mice (Fig. 2).

3.3. Influence of mecamylamine acute treatment on expression of sensitization to locomotor stimulant effect of ethanol

Influence of mecamylamine acute treatment on expression of sensitization to locomotor stimulant effect of ethanol is depicted in Fig. 3. One-way ANOVA revealed a significant influence of mecamylamine acute treatment on expression of sensitization to locomotor stimulant effect of ethanol [F (4, 34) = 19.98, P<0.0001]. Dunnett's post hoc test further indicated that mecamylamine (0.5, 1, and 2 mg/kg) significantly (P<0.05, 0.01, 0.01) reduced the locomotor activity as compared to vehicle treated mice, whereas it had no effect at 0.25 mg/kg (P>0.05) [Fig. 3]. The data of control group in Fig. 3 is same as represented in Fig. 2.

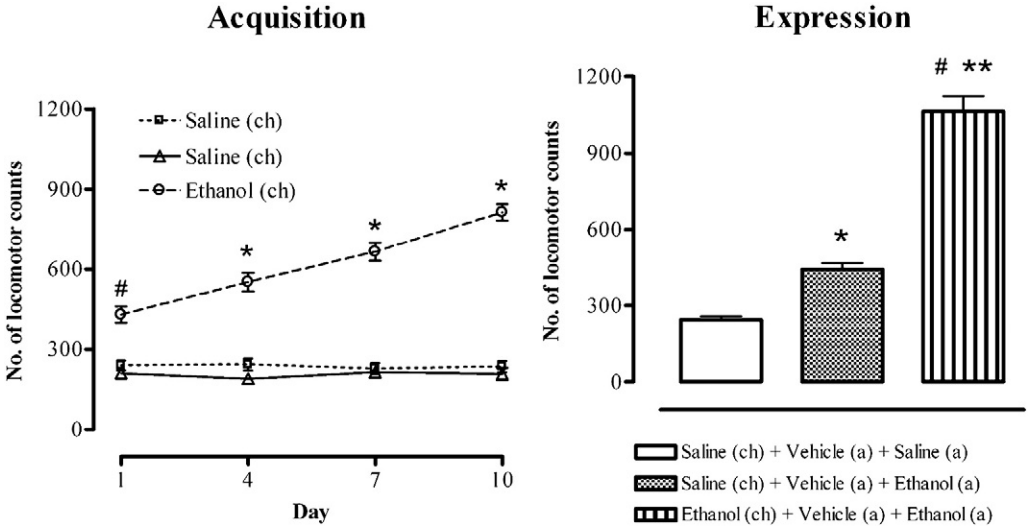

Fig. 2. Acquisition and expression of sensitization to locomotor stimulant effect of ethanol. Each bar/value represents the mean ± S.E.M. of 6–8 mice per group. #P<0.01, *P<0.001 vs. saline treatment in acquisition experiment. *P<0.01, **P<0.001 vs. [saline + saline + saline] treated mice, #P<0.001 vs. [saline + saline + ethanol] treated mice in expression experiment (day 15). (Two-way repeat measures ANOVA followed by Bonferroni test for acquisition and One-way ANOVA followed by Tukey's multiple comparison test for expression experiment). ch: chronic; a: acute.

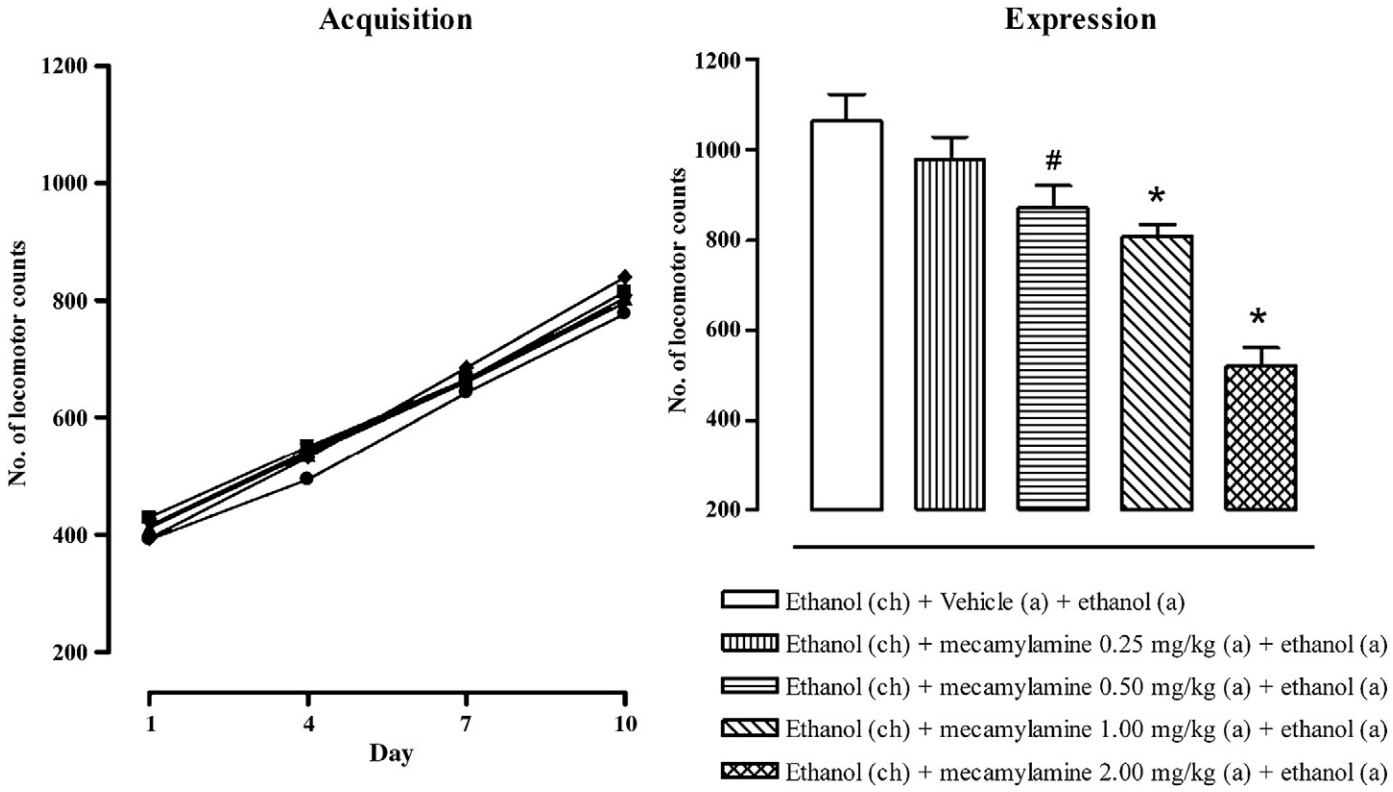

Fig. 3. Influence of acute mecamylamine treatment in mice chronically treated with ethanol. Each value/bar represents the mean ± S.E.M. ($n = 6–8$). Mice were administered with ethanol (2.0 g/kg, i.p.) or saline (12.5 ml/kg, i.p.) on days 1, 4, 7 and 10. On day 15, mice were acutely treated with mecamylamine (0.25, 0.5, 1.0 or 2.0 mg/kg, i.p.) or vehicle 30 min prior to challenge dose of ethanol. Five minutes after ethanol injection, locomotor activity of each mouse was assessed for 30 min. $^{\#}P<0.05$, $^{*}P<0.01$ vs. [ethanol (ch) + vehicle (a) + ethanol (a)] treated group on day 15 (One-way ANOVA followed by Dunnett's test). ch: chronic; a: acute.

One-way ANOVA further revealed that acute administration of mecamylamine (0.25–2.0 mg/kg, i.p.) in sensitized animals did not influence blood ethanol levels [F (5, 35) = 0.7083, $P = 0.6212$] (Table 1).

3.4. Influence of mecamylamine chronic treatment on acquisition and expression of sensitization to locomotor stimulant effect of ethanol

When the groups treated with mecamylamine 1.0 mg/kg were considered, the repeated-measures two-way ANOVA detected significant differences for the chronic treatment factor [F (3, 75) = 120.7, $P<0.0001$] and the test condition factor [F (3, 75) = 51.47, $P<0.0001$], and a significant interaction between the two factors [F (9, 75) = 16.53, $P<0.0001$] (Fig. 4).

When the groups treated with mecamylamine 2.0 mg/kg were considered, the repeated-measures two-way ANOVA detected significant differences for the chronic treatment factor [F (3, 75) = 224.2, $P<0.0001$] and the test condition factor [F (3, 75) = 19.49, $P<0.0001$], and a significant interaction between the two factors [F (9, 75) = 24.58, $P<0.0001$] (Fig. 4).

The Bonferrroni post hoc test indicated that mecamylamine (1.0 mg/kg) significantly reduced the acquisition of sensitization on days 7 and 10 ($P<0.01$), whereas mecamylamine (2.0 mg/kg) reduced the acquisition of sensitization on all days ($P<0.001$), and it had no effect at 0.5 mg/kg. Similarly, mecamylamine chronic treatment at all tested doses had no influence on the locomotor activity compared to vehicle treated mice ($P>0.05$) (Fig. 4).

On day 15, challenge with ethanol produced a large increase in the locomotor activity of mice chronically treated with ethanol indicating expression of sensitization. One-way ANOVA indicated a significant effect of mecamylamine treatment on ethanol-induced increase in locomotor activity [F (7, 52) = 42.99, $P<0.0001$]. Post hoc Tukey's test indicated that mice treated with mecamylamine (1 and 2 mg/kg) on days 1, 4, 7, and 10 exhibited a significant ($P<0.001$) decrease in the locomotor activity after ethanol challenge dose on day 15 as compared to vehicle treated group (Fig. 4). However, it had no influence at 0.5 mg/kg ($P>0.05$). Further, chronic treatment of mecamylamine *per se* (days 1, 4, 7, and 10) at these doses had no influence on locomotor activity after ethanol challenge dose on day 15 as compared to vehicle treated mice. Further, chronic mecamylamine (1.0 or 2.0 mg/kg, i.p.) treatment did not alter blood ethanol levels ($P>0.05$) (Table 1).

3.5. Influence of mecamylamine and ethanol treatment on motor coordination in mice

One-way ANOVA revealed no significant influence of mecamylamine pretreatment (0.5–2.0 mg/kg, i.p.) on the rotarod performance of ethanol treated mice [F (4, 29) = 0.398, $P = 0.8081$] (Fig. 5).

4. Discussion

In order to examine our hypothesis that changes in nACh may relate to ethanol-induced locomotor sensitization, we studied the influence of mecamylamine, a nAChR antagonist on ethanol-induced locomotor sensitization. The results revealed that mecamylamine blocked the expression and prevented the development of sensitization to locomotor stimulant effect of ethanol in mice.

Behavioral sensitization is defined as the long-lasting and progressive enhancement of locomotor and motivational responses to a drug following repeated and intermittent administration (Kalivas and Stewart, 1991), and is suggested to be analogous to the characteristic behavior of drug addiction (Segal and Schuckit 1983; Robinson and Berridge, 1993). Generally, sensitization may be

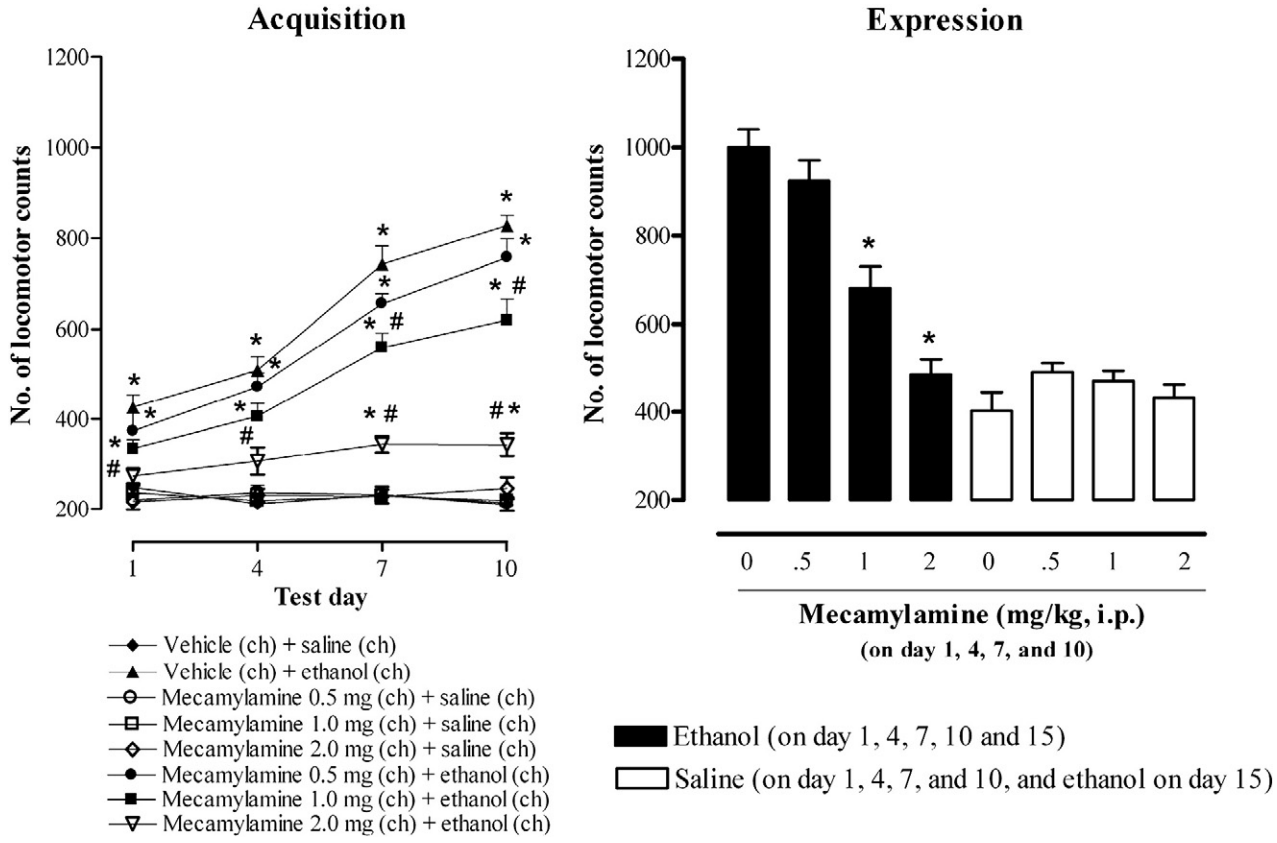

Fig. 4. Influence of mecamylamine chronic treatment on acquisition and expression of sensitization to locomotor stimulant effect of ethanol. Each value/bar represents the mean ± S.E.M. of 6–9 mice. Mice were treated with mecamylamine (0.5, 1.0 or 2.0 mg/kg, i.p.) on days 1, 4, 7, and 10, 30 min prior to ethanol, and on day 15, only challenge dose of ethanol was administered. *P<0.05 vs. [vehicle (ch) + saline (ch)]; #P<0.01 vs. [vehicle (ch) + ethanol (ch)] treated group on respective day in acquisition experiment (Two-way repeat measures ANOVA followed by Bonferroni test). *P<0.001 vs. vehicle treatment in ethanol treated group (One-way ANOVA followed by Tukey's multiple comparison test for expression experiment). ch: chronic.

involved in reinstating drug-seeking behavior (De Vries et al., 1998), which suggests that the treatments that prevent the expression of sensitization may reduce the probability of relapse. In addition, detailed investigations on the process of behavioral sensitization have revealed a host of cellular neuroadaptations that are likely to contribute to the neural mechanisms of addictive behavior (Nestler and Aghajanian 1997; White and Kalivas, 1998). Hence, the present investigations employed the behavioral sensitization paradigm to study the role of nAChR in alcohol abuse and screen the influence of mecamylamine on the same.

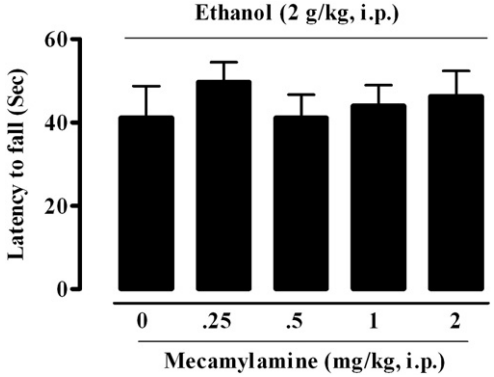

Fig. 5. Influence of mecamylamine and ethanol treatment on motor coordination in mice. Each bar represents the mean ± S.E.M. of 6 mice per group. (One-way ANOVA followed by Tukey's multiple comparison test).

Locomotor sensitization study was carried out by using earlier reported methods (Kotlinska et al., 2007; Umathe et al., 2008; 2009) with slight modifications. It was observed that the locomotor stimulant effect of ethanol (2.0 g/kg, i.p.) progressively increased with each administration on days 1, 4, 7, and 10, and the challenge dose of ethanol on day 15, produced higher locomotor stimulation, which indicated the expression of sensitization. These observations indicate the successful induction of ethanol sensitization in mice. Our observations further revealed that mecamylamine not only attenuated locomotor stimulant effect of ethanol but also blocked the development and the expression of sensitization to ethanol. These effects of mecamylamine were not associated with changes in blood ethanol concentration, suggesting that the effects of the mecamylamine were not secondary to alterations of ethanol metabolism. The results raise a possibility that changes in ethanol-induced locomotor activity produced by mecamylamine pretreatment might be due to non-specific locomotor depression and/or sedative effects. But, the observation that mice treated with mecamylamine (0.25–2.0 mg/kg) alone exhibited no change in locomotor activity, and earlier reports also shown that mecamylamine *per se* did not influence locomotor activity (Larsson et al., 2002; Miller and segert, 2005; Kamens and Phillips, 2008; Wooters and Bardo, 2009), which further supports non-involvement of any non-specific locomotor depression, ataxia and/or sedative effects in the influence of mecamylamine on effects of ethanol. In addition, mecamylamine is reported to attenuate the acute locomotor stimulant effect of ethanol in different strains of the mice in the doses used in the present study (Larsson et al., 2002; Kamens and Phillips, 2008). However, the dose of ethanol chosen for rotarod testing was the same as that used for measurement of sensitization.

This dose of ethanol is higher than that used by many investigators for ataxia testing in rotarod test, and the latencies to fall from the rod were extremely short. Thus, whether there was any floor effect such that even shorter latencies could have been seen, remains unknown.

Development of psychomotor sensitization involves plastic changes in dopaminergic cell bodies of the VTA, whereas the expression involves changes in dopamine transmission in axon terminal fields of the NAc (Kalivas and Stewart, 1991; Cador et al., 1995). Earlier studies revealed that ethanol augments nAChR activity depending on the subtype of nicotinic receptors (Forman and Zhou, 2000; Zhou et al., 2000; Zuo et al., 2002). Moreover, systemic ethanol is reported to increase acetylcholine concentrations in VTA, presumably through activation of nAChRs (Ericson et al., 2003). In addition, nAChRs have reported to partially mediate the reinforcing properties of ethanol (Soderpalm et al., 2000). Interestingly, earlier studies have shown that mecamylamine delivered systemically or directly into the VTA blocks elevation of ethanol mediated dopamine release in the NAc (Blomqvist et al., 1993, 1997). Recently, it is also reported that mecamylamine significantly reduces the number of DAergic neurons in the VTA that were activated by repeated exposure to ethanol (Hendrickson et al., 2009). These reports and our investigations support the contention that plastic changes associated with dopaminergic cell bodies in VTA by ethanol may be subsequent to nAChR activation.

Ethanol is also known to act at both N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors (Faingold et al., 1998) that play an important role in the expression of sensitization to psycho-stimulants including ethanol (Vanderschuren and Kalivas, 2000; Broadbent et al., 2003). Interestingly, mecamylamine is reported to noncompetitively inhibit NMDA receptor function (O'Dell and Christensen, 1988; Fu et al., 2008). It is also indicated that nAChR antagonists including mecamylamine block the NMDA-induced behavioral effects and exhibit NMDA antagonist-like effect (McDonough and Shih, 1995). Moreover, it is well established that certain types of NMDA receptor antagonists (channel blockers) potently bind to nicotinic receptors and may act as nicotinic receptor antagonist (for reviews see, Zakharova et al., 2005). Therefore, the involvement of NMDA in addition to nAChR in the effects of mecamylamine cannot be completely eliminated. In addition, reports indicate that mecamylamine blocks behavioral sensitization of cocaine, *d*-amphetamine, ephedrine, nicotine and methylphenidate (Karler et al., 1996; Schoffelmeer et al., 2002; Miller and Segert, 2005; Wooters and Bardo, 2009). Therefore, results obtained in present study raise the possibility that mecamylamine blocked the expression and development of ethanol-induced locomotor sensitization by acting on nACh receptors.

However, our results clearly indicate that the doses of mecamylamine (1.0 and 2.0 mg/kg) suppressing the acute stimulant effects of ethanol are the doses that suppress acquisition and expression of sensitization. Hence, mecamylamine does not appear to be having non-specific effects on sensitization by producing ataxia, its effects on sensitization seem to be directly linked to suppression of acute ethanol stimulation. The paradigm validity suggests that stronger evidence of a specific effect of mecamylamine on sensitization would require suppression of acquisition and expression of sensitization in the absence of acute effects on ethanol stimulation. However, NMDA receptor antagonists and acamprosate, a clinically used drug in alcoholism also blocked the expression of sensitization to ethanol at the doses which also blocked acute locomotor stimulation (Kotlinska et al., 2006).

Thus, the present findings demonstrate that chronic effects of ethanol on locomotor activity in mice are complex and can be specifically modulated by nAChR. From a clinical point of view, if our experimental design had prevented the development and reversed the established ethanol-induced sensitization similarly to that depicted for other drugs of abuse, then it would be remarkable for future research on human alcoholism treatment. In conclusion, the current data add to the growing literature implicating brain nACh receptor signaling modulates the development and the expression of ethanol-induced locomotor sensitization in mice.

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