



Published in final edited form as:

Drug Alcohol Depend. 2015 November 1; 156: 126–132. doi:10.1016/j.drugalcdep.2015.09.002.

Varenicline decreases nicotine but not alcohol self-administration in genetically selected Marchigian Sardinian alcohol-preferring (msP) rats

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Abstract

Background—Alcohol and nicotine are largely co-abused. Here, we investigated whether concurrent exposure to both addictive drugs influences each other's consumption and whether varenicline attenuates alcohol consumption in the presence of nicotine.

Methods—Marchigian Sardinian alcohol-preferring (msP) rats trained to simultaneously self-administer oral alcohol (10% v/v) and intravenous nicotine (30 µg/kg/inf.) were used. Additional groups of rats were trained to self-administer either alcohol or nicotine. Further, msP rats were also trained to self-administer nicotine followed by 22-h/day access to alcohol and water in a two bottle free choice paradigm or water alone. The effects of varenicline (0.0, 0.3, 1.0, 3.0 mg/kg, p.o.) on alcohol and nicotine consumption was tested.

Results—In a self-administration paradigm, msP rats showed a significantly high levels of alcohol and nicotine intake when the drugs were administered alone. However, when access to both drugs occurred concomitantly, the number of nicotine infusions self-administered was significantly decreased. Nicotine self-administration was markedly reduced by varenicline regardless of whether it was self-administered alone or concurrently with alcohol. In a two bottle choice test, varenicline significantly decreased nicotine self-administration but had no influence on alcohol consumption.

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Author contributions

All of the authors contributed to the experimental design and data analyses. G.S. and A.C. were responsible for the study concept and design. G.S. conducted the experiments and wrote the manuscript. A.C. wrote the manuscript and assisted with the interpretation of findings. M.U., L.T. and R.C. provided critical revision of the manuscript for important intellectual content. All authors critically reviewed the content and approved the final version for publication.

Conflict of interest

No conflict declared.

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Conclusion—Varenicline is highly efficacious in decreasing nicotine self-administration either alone or in combination with alcohol. However, varenicline failed to influence both operant responding for alcohol and home-cage alcohol drinking in msP animals. Taken together, our findings suggest that the effects of varenicline could be specific to nicotine under conditions where excessive alcohol drinking is facilitated by genetic factors as in msP rats.

Keywords

ethanol; addiction; drug-dependence; tobacco smoking; co-abuse

1. INTRODUCTION

Alcohol and nicotine are largely co-abused. Epidemiological data indicate that most alcoholics are also smokers and that smokers have an increased risk of developing alcohol use disorders (DiFranza and Guerrera, 1990; Miller and Gold, 1998). Nicotine dependence appears **to be** more severe in smokers with a history of alcohol dependence and it is more difficult to quit tobacco use when the smoking habit is associated with alcohol drinking (Romberger and Grant, 2004). In addition, the co-occurrence of nicotine and alcohol dependence leads to increased morbidity and mortality, with tobacco-related disease being the leading cause of death in recovering alcohol-dependent patients (Hurt et al., 1996). This strong association observed between alcohol and nicotine addiction is due to a variety of factors. First, psychosocial factors, as childhood maltreatment, are robust and common risk factors for the occurrence of the two disorders (Mingione et al., 2012). Second, in most countries alcohol and tobacco consumption are both legally accepted, hence making them widely available to the population (Funk et al., 2006). Lastly, common molecular and cellular mechanisms (Doyon et al., 2013; Hendrickson et al., 2013) may subserve their use, shaping the risks of co-abuse of these two highly addictive drugs. For example, both alcohol and nicotine activate the mesolimbic dopamine (DA) reward pathway and combining the two might yield additive or synergistic motivational effects (Tizabi et al., 2002).

While nicotine primarily acts via nicotinic acetylcholine receptors (nAChRs), alcohol acts upon a wide range of receptors and molecular substrates, including nAChRs. Specifically, it has been demonstrated that development of nicotine dependence may result from initial activation of DA neurons via nAChRs containing $\alpha 4$ and $\beta 2$ subunits (Picciotto et al., 1998; Tapper et al., 2004). On the other hand, alcohol can also increase acetylcholine release into the ventral tegmental area (Larsson et al., 2005), driving activation of DA neurons through nAChRs. In this regard, a role of $\alpha 4$ nAChR subunits in alcohol reward has been proposed (Liu et al., 2013). It has also been shown that nicotine may increase alcohol's reinforcing properties via upregulation of nAChRs, thus setting the condition for enhanced vulnerability to consume alcohol (Hendrickson et al., 2013). Recently, the $\alpha 6$ nAChR subunit has also been indicated as an important player in the response to nicotine and alcohol co-exposure in neurons of the ventral tegmental area (Engle et al., 2015). Based on these findings, nAChRs have been proposed to be targets for the development of novel and possibly more effective pharmacological interventions not only for the treatment of nicotine, but also alcohol addiction (Chatterjee and Bartlett, 2010; Hendrickson et al., 2013).

Varenicline (Chantix) is currently the most effective pharmacotherapy for promoting smoking cessation (Jorenby et al., 2006). Although hypothesized to reduce smoking via partial activation of the $\alpha 4\beta 2$ nAChRs, varenicline also shows partial or full agonist activity at other nAChR subtypes including those containing $\alpha 6$, $\alpha 3\beta 4$ and $\alpha 7$ subunits (Chatterjee et al., 2011; Cippitelli et al., 2015c; Mihalak et al., 2006; Rollema et al., 2007). This complex pharmacological profile reflects complex actions of varenicline *in vivo*. If, on the one hand, it reduces nicotine consumption, on the other hand, varenicline acts as a primary reinforcer and elicits reinstatement of nicotine seeking in rats (Cippitelli et al., 2015c). Pre-clinical studies have demonstrated that varenicline decreases both operant and home cage alcohol self-administration in rodents (Kamens et al., 2010; Steensland et al., 2007) and, recently, clinical evidence indicating reduced drinking in a population of heavy-drinking smokers or primary alcoholic patients treated with varenicline have started to emerge (Childs et al., 2012; Fucito et al., 2011; Litten et al., 2013; McKee et al., 2009; Mitchell et al., 2012). These well-documented anti-alcohol properties of varenicline seem to be dependent on the activation of $\alpha 4$ nAChRs (Hendrickson et al., 2010). In contrast, $\alpha 7$ (Kuzmin et al., 2009) and $\alpha 3\beta 4$ nAChRs (Carnicella et al., 2010; Cippitelli et al., 2015b) may not be involved in alcohol reinforcement. Consistent with this literature, we have recently demonstrated the efficacy of varenicline in decreasing alcohol self-administration at doses that also decreased nicotine self-administration in an operant co-administration paradigm (Cippitelli et al., 2015c). A similar response was observed when the effects of varenicline were tested on alcohol and food-maintained responding in a similar concurrent access procedure (Ginsburg and Lamb, 2013).

Genetic factors are known to contribute to alcohol and nicotine co-abuse (Dani and Harris, 2005; Tyndale, 2003). Evidence of common genetic vulnerability traits for nicotine and alcohol dependence in humans have been documented (Enoch and Goldman, 2001). For instance, data obtained in P rats, a line genetically selected for high alcohol consumption, showed that these animals were more sensitive to nicotine (Gordon et al., 1993), consumed greater amounts of nicotine intravenously and showed increased vulnerability to alcohol relapse (Le et al., 2006) than alcohol non-preferring (NP) rats. Here, we used genetically selected marchigian sardinian alcohol preferring (msP) rats, an established animal model of excessive alcohol drinking and preference (Ciccocioppo et al., 2006), to investigate whether concurrent exposure to alcohol and nicotine influences the intake of either drug. We also investigated whether varenicline alters the consumption of alcohol and nicotine in msP animals when both drugs are made simultaneously available under operant self-administration conditions or when nicotine self-administration is followed by home-cage access to ethanol. The present study extends our previous investigation in Sprague-Dawley rats (Cippitelli et al., 2015c) to evaluate if varenicline may represent a suitable pharmacological treatment for alcohol drinking when consumption occurs in conjunction with nicotine in animals with innate predisposition to consume high amounts of alcohol. The results of this study may be useful to predict if a subpopulation of smokers with a genetic predisposition to alcohol abuse may differ in their response to varenicline.

2. MATERIALS AND METHODS

2.1 Animals

Male genetically selected alcohol-preferring msP rats were used. MsP rats were bred at the University of Camerino and housed in groups of two except where specified, in rooms with a reverse 12 h light-dark cycle (lights off at 08:30 AM) at a constant temperature of $20 \pm 2^\circ\text{C}$ and relative humidity of 45–55%, with free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). Experiments were conducted during the dark phase of the light/dark cycle. Animals were handled three times before the onset of each experiment. All procedures followed the EU Directive for Care and Use of Laboratory Animals.

2.2 Drugs

Varenicline or its vehicle (distilled water) were given orally (p.o.), by gavage. Varenicline was prepared from 1-mg tablets (Chantix, Pfizer, Italy). Tablets were crushed in a laboratory ceramic mortar. Powder was transferred into 20 ml test tubes where it was suspended in distilled water. Test tubes were vortexed before filling the syringe for oral administration. Injection volume was 2 ml/kg. Nicotine (Sigma, Milan, Italy) was dissolved in 0.9% saline (30 $\mu\text{g}/\text{kg}/0.1$ ml). The solution was neutralized to an isotonic pH (7.4) with NaOH 5M and given intravenously (i.v.). Nicotine self-administration doses are reported as free base concentrations. Alcohol 10% (v/v) was prepared by dilution of 95% alcohol (F.L. Carsetti s.n.c., Camerino, Italy) in water.

2.3 Intravenous surgery

Catheter implantation in the rat jugular vein was performed as previously described (de Guglielmo et al., 2013). In brief, animals were anesthetized by intramuscular injection of 100–150 μl of a solution containing tiletamine chloridrate (58.17mg/ml) and zolazepam chloridrate (57.5 mg/ml). Incisions were made to expose the right jugular vein and a catheter made from silicon tubing (I.D. =0.020 inches, O.D. =0.037 inches) was subcutaneously positioned. After insertion into the vein, the proximal end of the catheter was anchored to the muscles underlying the vein with surgical silk. The distal end of the catheter was attached to a stainless-steel cannula bent at a 90° angle, inserted in a support made by dental cement and covered with a plastic cap. The cannula was connected to the fluid swivel system during nicotine self-administration. Catheter patency was confirmed with an injection of 0.2–0.3 ml of thiopental sodium (250 mg/ml) solution. Patency of the catheter was assumed if there was an immediate loss of reflexes. Self-administration experiments began 1 week after surgery. Rats self-administering only alcohol were subjected to a sham surgery that involved ligation of the right jugular vein.

2.4 Operant self-administration apparatus

Self-administration of only nicotine or alcohol as well as drug co-administration experiments were carried out in operant conditioning chambers (Med Associate, Inc., St. Albans, VT) enclosed in light and sound-attenuating, ventilated environmental cubicles. The front door and the back wall of the chamber were made of transparent plastic and the other walls were opaque metal. Each chamber was equipped with two retractable levers located in

the front panel of the chamber to the sides of a drinking receptacle. Levers were extended at the beginning of the self-administration sessions. The chambers were also equipped with a white house light located near the top of the chamber opposite the lever and white cue lights located above the levers. Infusions occurred by means of syringe pumps (Med Associates, Inc., St. Albans, VT) and liquid swivels (Instech Solomon, Plymouth Meeting, PA), connected to plastic tubing protected by a flexible metal sheath for attachment to the external catheter terminus. In the case of self-administration of one reinforcer alone, an infusion pump was activated by responses on the right (active) lever, while responses on the left (inactive) lever were recorded but did not result in any programmed consequences. Activation of the pump resulted in a delivery of 0.1 ml of nicotine or alcohol. During co-administration of intravenous nicotine and oral alcohol, chambers were equipped with two active levers and two infusion pumps, one that delivered iv nicotine (0.1 ml over 5 seconds) and one that delivered alcohol (0.1 ml over 1 second) into the drinking receptacle. Thus, appropriate responding on the right lever resulted in activation of the pump containing nicotine while responding on the left lever resulted in activation of the pump that released alcohol, which was connected to the drinking receptacle. A microcomputer controlled the delivery of reinforcers, presentation of auditory and visual stimuli and recording of the behavioral data. Self-administration sessions were performed starting at 9.30 a.m. which is 1 hour after the onset of the dark cycle.

2.5 Two-bottle free choice drinking paradigm

Two groups of msP rats were used for this study. Animals were single-housed to provide accurate record of home-cage drinking (Ayanwuyi et al., 2013). One group (alcohol-exposed, N=12) was offered concurrent, continuous access to 10% alcohol solution, water, and food pellets. The other group (non-exposed, N=12) had only water and food available. Fluids were presented in graduated plastic tubes equipped with stainless-steel drinking spouts inserted through two grommets in front of the cage. Alcohol and water tubes were refilled daily, 60 min into the dark period of the light/dark cycle. The placement of the alcohol bottle was alternated daily to control for side preference. Both groups (exposed vs non-exposed) were subjected to a daily 2-h nicotine self-administration session, 5 days a week. This procedure was carried out for the whole duration of the experiment (training and drug testing). Data are presented as daily alcohol intake (g/kg).

2.6 Self-administration of nicotine alone, alcohol alone or their co-administration in msP rats: Effect of varenicline

Three groups of msP rats were used: one group (N=8) was trained to co-administer 10% (v/v) alcohol and nicotine (30 µg/kg/inf.) during the same operant session. A second group of rats (N=9) was trained to self-administer nicotine alone and the last group (N=10) was trained to self-administer alcohol alone. Co-administration of nicotine and alcohol was conducted as previously described (Cippitelli et al., 2015c; Le et al., 2010). Following each nicotine infusion, a 20-sec time out (TO) period occurred, during which responses at the lever that delivered nicotine (right lever) did not lead to programmed consequences. Nicotine reinforcements were accompanied by concurrent illumination of the cue light above the nicotine-associated lever to signal delivery of nicotine. Alcohol reinforcements were accompanied by a flashing house light (0.5 sec on, 0.5 sec off) with a TO period of 10

sec during which responses at the lever that delivered alcohol (left lever) did not lead to programmed consequences. An intermittent tone (7 kHz, 70 dB) was sounded throughout the 60-min session. Self-administration of only nicotine or only alcohol used identical conditions as co-administration sessions but only one lever was active. All self-administration sessions were conducted under a fixed ratio 1 (FR-1) reinforcement schedule. Sessions lasted 60 min except where otherwise specified. Nicotine, alcohol and their simultaneous self-administration was maintained for 19 days. Following this training period, the effect of varenicline (0.3, 1 and 3 mg/kg/2ml) or its vehicle (0 mg/kg/2ml) on operant self-administration was tested. Experiments were conducted according to a within-subject Latin square counterbalanced design. Varenicline was administered p.o., by gavage, 30 minutes before the beginning of operant sessions. Testing sessions were conducted every 3–4 days. The day after the experiment, rats were allowed 1 day off and then a new baseline was established over the following 1 or 2 days (Cipitelli et al., 2015b). Data are expressed as mean \pm S.E.M. number of infusions or rewards for nicotine and alcohol, respectively.

2.7 Nicotine self-administration in msP rats with or without access to alcohol in the two bottle choice paradigm: Effect of varenicline

Rats were trained to self-administer nicotine (60 μ g/kg/inf.) in daily 2-h sessions. Half of the rats were exposed to alcohol daily for 22h, in the two-bottle choice paradigm. The other half had water and food but no alcohol available in their home-cages. Nicotine sessions were conducted on a FR-1 reinforcement schedule. As described above, each nicotine infusion was followed by a 20-sec TO period during which presses on the active lever did not lead to any programmed consequences. TO was accompanied by illumination of a cue light located above the active lever to signal delivery of the positive reinforcement, while an intermittent tone was presented throughout the 2-h session. After 17 days of intravenous nicotine self-administration followed by alcohol exposure, the effect of varenicline was investigated. Rats were treated with varenicline (0.0, 0.3, 1, 3 mg/kg) in a within-subject counterbalanced Latin square order 30 min before the start of the nicotine operant session. Immediately after the end of the session, rats were returned to their home-cages where alcohol, water and food intake was monitored at 2 and 24 h following the onset of alcohol exposure. Experiments were performed every three days. The day after drug testing, animals were left undisturbed in their home-cages and on the following day a nicotine self-administration baseline was re-established.

2.8 Statistical analysis

Nicotine or alcohol self-administration alone versus lever pressing on drug co-administration were analyzed by a two-way ANOVA with “co-administration” used as the between-subject factor and “day” as the within-subject factor. A t-test analysis was used to compare the baseline levels of lever presses over the last three training days. Effects of varenicline on nicotine alone and co-administration, and alcohol alone and co-administration were analysed by a two-way ANOVA with “co-administration” used as the between-subject factor and “treatment” as the within-subject factor. The same approach was used to analyze the effect of varenicline on nicotine self-administration of msP rats in the presence or absence of alcohol. In this two-way ANOVA analysis “alcohol exposure” was the between-subjects factor and “treatment” was the within-subjects factor. Data from the two-bottle free

choice experiment were analysed by a two-way ANOVA that used “treatment” and “time” as the within-subject factors. The level of significance was set at $p < 0.05$. When appropriate, analyses were followed up by Fisher’s least significant difference (LSD) post-hoc test. Prior to performing ANOVA’s all data were analyzed to confirm that the assumption of sphericity had not been violated using Mauchly’s test of sphericity.

3. RESULTS

3.1 Self-administration of nicotine alone, alcohol alone or their co-administration in mSP rats: Effect of varenicline

Rats self-administering nicotine alone showed a greater increase in the number of infusions self-administered compared to rats co-administering both drugs (Figure 1A). Overall, the ANOVA revealed a main effect of administration condition [$F_{(1,17)}=6.0$, $p < 0.05$] with pairwise comparisons showing differences between the nicotine alone and the co-administration group particularly over the last few operant sessions. The analysis also showed an effect of day [$F_{(18,306)}=3.04$, $p < 0.001$], while the interaction between day and administration condition was not statistically significant [$F_{(18,306)}=0.71$, $p = \text{NS}$]. A t-test analysis conducted on the mean number of infusions over the last three training days confirmed a significant difference between the mean number of nicotine infusions earned by the two groups ($[t_{(17)}=2.145$, $p < 0.05$], Figure 1B). The overall ANOVA revealed no differences [$F_{(1,18)}=2.9$, NS] in number of lever presses for alcohol in the co-administration group compared to the group of animals self-administering only alcohol (Figure 1C). This result was confirmed by a t-test conducted on the mean number of alcohol rewards earned during the last three operant sessions ($[t_{(18)}=0.95$, NS], Figure 1D). The ANOVA also showed an effect of day [$F_{(18,324)}=19.05$, $p < 0.001$], but there was no interaction between day and co-administration [$F_{(18,324)}=1.3$, $p = \text{NS}$].

As expected, varenicline significantly decreased nicotine intake independently from alcohol availability (Figure 2). Overall, the ANOVA revealed a significant main effect of treatment [$F_{(3,45)}=12.0$, $p < 0.001$] that was not accompanied by a main effect of administration condition [$F_{(1,15)}=2.6$, NS] or an interaction between the two factors [$F_{(3,45)}=0.2$, NS]. Post-hoc comparisons showed significant decrease in the number of nicotine infusions self-administered following treatment with varenicline at 1.0 and 3.0 mg/kg ($p < 0.05$ and $p < 0.001$, respectively). In rats subjected to both alcohol-self-administration alone and in the co-administration group, varenicline did not modify the number of alcohol doses earned (main effect of treatment: [$F_{(3,48)}=0.6$, NS], interaction [$F_{(3,48)}=1.9$, NS], Figure 3). Overall, the ANOVA also revealed a significant main effect of the “co-administration” factor [$F_{(1,16)}=7.9$, $p < 0.05$], suggesting that in the presence of nicotine, alcohol self-administration was reduced. This finding is in contrast with data reported in Figs 1C and 1D in which no difference in alcohol self-administration was observed between the co-administration and alcohol alone groups. This difference may be due to the fact that varenicline itself is a reinforcer and the presence of three drugs may result in a reduction in alcohol consumption.

3.2 Nicotine self-administration in msP rats with or without access to alcohol in the two-bottle choice paradigm: Effect of varenicline

Overall, the ANOVA showed a robust main effect of varenicline treatment [$F_{(3,66)}=22.0$, $p<0.001$] on nicotine self-administration. No main effect of alcohol exposure [$F_{(1,22)}=0.0$, NS] or interactions between varenicline treatment and alcohol exposure [$F_{(3,66)}=0.8$, NS] were observed. Post-hoc analysis showed a significant reduction in nicotine self-administration at all the three doses tested ($p<0.001$, Figure 4). When the effect of varenicline was analysed against home-cage alcohol drinking (Figure 5) following operant nicotine self-administration sessions, the ANOVA revealed no effect of varenicline at both 2 hours ($[F_{(3,33)}=0.6$, NS] and 24 hours ($[F_{(3,33)}=2.5$, NS]) post treatment. Food intake was also not affected by varenicline ($[F_{(3,33)}=1.41$, NS]; data not shown).

4. DISCUSSION

In the present study, we examined the effect of varenicline, a nAChRs ligand, on the concurrent administration of nicotine and alcohol in marchigian sardinian alcohol-preferring rats. We found that while msP rats readily self-administered both alcohol and nicotine, there was an attenuation in nicotine self-administration in the presence of alcohol. Varenicline decreased operant nicotine self-administration as anticipated, but failed to decrease both operant and home-cage alcohol intake under co-administration conditions.

4.1 Nicotine and alcohol co-administration

To mimic the co-abuse of alcohol and nicotine that often occurs in humans (DiFranza and Guerrero, 1990; Miller and Gold, 1998), we used two distinct paradigms of alcohol and nicotine co-administration in this study. Firstly, both intravenous nicotine and oral alcohol were made simultaneously available in 60-min daily operant sessions, as originally described by Le et al (2010). Secondly, rats were trained to self-administer nicotine in daily 120-min operant sessions, which was followed by access to 10% alcohol in the home-cage.

Results from our operant co-administration study indicate that msP rats readily self-administered nicotine and alcohol concurrently. Interestingly, nicotine intake was significantly lowered in rats co-administering alcohol compared to those self-administering nicotine alone. This effect was limited to operant training sessions and became less robust during testing, perhaps due to the presence of varenicline on board along with alcohol and nicotine. Our findings are consistent, at least in part, with the studies of Le et al. where the authors evaluated the effect of concurrent access to nicotine and alcohol in heterogenous Wistar rats. While it is plausible that a decrease in nicotine self-administration in the presence of alcohol availability is due to the sedative or motor-impairing properties of alcohol as hypothesized by Le et al., a decrease in nicotine self-administration is also observed with concurrent access to cocaine (Manzardo et al., 2002). Thus, at least for nicotine, it appears that polydrug availability may lead to a decrement in the operant self-administration of a single reinforcer. This hypothesis is generally in agreement with the theory of competition between multiple reinforcers, according to which the availability of an alternate reward may attenuate the demand for the primary reinforcer. For instance,

concurrent availability of a drug of abuse and a sweetened drink reduces demand for the drug (Carroll et al., 1991; Lenoir and Ahmed, 2008).

Contrary to the finding discussed above, results from co-administration studies where msP rats were allowed *ad libitum* access to 10% v/v ethanol in the home-cage following operant nicotine self-administration sessions indicate no changes in nicotine intake. This is likely due to procedural differences as rats in the two-bottle choice experiment did not consume alcohol and nicotine simultaneously.

4.2 Effect of varenicline on nicotine and alcohol co-administration

Varenicline is known to reduce nicotine and alcohol intake in both humans and rodents (Cippitelli et al., 2015c; Jorenby et al., 2006; Kamens et al., 2010; Litten et al., 2013; Steensland et al., 2007). Results from the present study demonstrate a robust reduction in nicotine consumption in accordance with previous preclinical and clinical evidence (Jorenby et al., 2006; Rollema et al., 2007). This decrease was observed regardless of concurrent consumption of alcohol. Surprisingly, we observed a lack of varenicline effect on alcohol self-administration in msP rats. While this finding differs from previous studies showing a reduction of alcohol consumption both in rodents and humans following varenicline administration (Fucito et al., 2011; Kamens et al., 2010; McKee et al., 2009; Mitchell et al., 2012; Steensland et al., 2007), a lack of efficacy of varenicline on alcohol drinking has also been previously reported in both rodents and humans (Ginsburg and Lamb, 2013; Plebani et al., 2013; Randall et al., 2015). Moreover, in a recent place conditioning study varenicline was unable to prevent the expression of alcohol place preference, indicating a lack of drug efficacy in attenuating the motivational effects of alcohol-associated cues (Gubner et al., 2015). For further confidence in our results, we also examined the effect of varenicline using a two-bottle free choice drinking procedure. Similar to the lack of effect of varenicline on operant alcohol intake, we found no effect of the drug on two-bottle free choice drinking of alcohol, excluding the possibility of a paradigm-dependent effect.

A possible explanation for the lack of effect of varenicline on alcohol intake is that this drug is not adequately bioavailable to bind to nACh receptors. However, this is unlikely since it has been documented that brain and plasma unbound concentrations of varenicline remain stable for at least 6 hours after oral administration (Rollema et al., 2010). In addition, the effect of varenicline on ethanol consumption in non-preferring rats is maintained for up to 6 hours (Steensland et al., 2007). We also recently found that in heterogeneous Sprague-Dawley rats, varenicline reduced both nicotine and alcohol self-administration when tested in an operant co-administration paradigm identical to the one used here (Cippitelli et al., 2015c). Thus, it is unlikely that the lack of effect of varenicline on alcohol intake is due to bioavailability issues. However, it is seemingly likely that the effect of varenicline is dependent upon the rat strain and that msP rats may be less sensitive to the actions of the drug on alcohol intake.

The msP rat is an established animal model of excessive alcohol drinking, and has been hypothesized to represent a “phenocopy” of post-dependent animals (Ciccocioppo et al. 2006). MsP rats share similar neuroadaptations with heterogeneous rats following a history of alcohol intoxication. For instance, both animal models are characterized by dysregulation

of stress-mechanisms, upregulation of corticotropin releasing factor 1 receptors (CRF1 receptor), altered response to the anxiolytic peptide nociception/orphanin FQ and enhanced emotional reactivity (Hansson et al., 2006; Heilig and Koob, 2007; Economidou et al., 2008). Based upon these considerations it is tempting to speculate that altered expression, composition or function of nAChRs could contribute to shape the post-dependent state and possibly alter the innate predisposition to excessive drinking in msP rats, while simultaneously causing reduced sensitivity to the effect of varenicline in these animals. Previous work has shown that during selection for excessive drinking a few genetic traits have been segregated in msP rats. For instance it has been demonstrated that msP rats carry a mutation at the CRF₁ receptor locus that confers increased sensitivity to the effects of CRF1 receptor antagonists (Ayanwuyi et al., 2013; Cippitelli et al., 2015a). Similar mechanisms might be at play in case of nAChRs, thus modulating the neurochemical, and subsequently behavioral response to drugs acting at these receptors. Future work aimed at determining the expression and function of nAChRs in reward-related brain structures in msP rats might provide an insight into this hypothesis. While the effects of varenicline have been examined in heavy drinking smokers or primary alcoholics (Erwin and Slaton, 2014) in clinical studies, patients were not selected based upon their genetic predisposition to alcohol abuse. Our results suggest that the efficacy of varenicline on alcohol drinking may be highly dependent on the genetics of the patient population treated.

4.3 Conclusion

We propose that rats genetically selected for high alcohol intake and preference, such as those from the msP line, are a useful tool to study the physiopathology of nicotine and alcohol co-abuse. Our results in msP rats demonstrate that treatment with varenicline reduces nicotine self-administration but does not alter alcohol consumption in these animals. While further studies are warranted to determine the receptor mechanisms underlying lack of effect of varenicline on alcohol intake, the present results may have important clinical implications and may raise important pharmacogenetic considerations on whether the efficacy of varenicline on alcohol abuse is limited only to a sub-set and does not extend to all patient populations.

Acknowledgments

Role of Funding Source

Nothing declared.

We wish to thank Dr. Sunila Nair for critical comments on the manuscript. This study was supported by grants NIH AA014351 and AA017447. We are thankful to Alfredo Fiorelli for technical support as well as Rina Righi and Mariangela Fiorelli for animal care.

References

Ayanwuyi LO, Carvajal F, Lerma-Cabrera JM, Domi E, Bjork K, Ubaldi M, Heilig M, Roberto M, Ciccocioppo R, Cippitelli A. Role of a genetic polymorphism in the corticotropin-releasing factor receptor 1 gene in alcohol drinking and seeking behaviors of marchigian sardinian alcohol-preferring rats. *Front Psychiatry*. 2013; 4:23. [PubMed: 23630503]

- Carnicella S, He DY, Yowell QV, Glick SD, Ron D. Noribogaine, but not 18-MC, exhibits similar actions as ibogaine on GDNF expression and ethanol self-administration. *Addict Biol.* 2010; 15:424–433. [PubMed: 21040239]
- Carroll ME, Carmona GG, May SA. Modifying drug-reinforced behavior by altering the economic conditions of the drug and a nondrug reinforcer. *J Exp Anal Behav.* 1991; 56:361–376. [PubMed: 1955822]
- Chatterjee S, Bartlett SE. Neuronal nicotinic acetylcholine receptors as pharmacotherapeutic targets for the treatment of alcohol use disorders. *CNS Neurol Disord Drug Targets.* 2010; 9:60–76. [PubMed: 20201817]
- Chatterjee S, Steensland P, Simms JA, Holgate J, Coe JW, Hurst RS, Shaffer CL, Lowe J, Rollema H, Bartlett SE. Partial agonists of the alpha3beta4* neuronal nicotinic acetylcholine receptor reduce ethanol consumption and seeking in rats. *Neuropsychopharmacology.* 2011; 36:603–615. [PubMed: 21048701]
- Childs E, Roche DJ, King AC, de Wit H. Varenicline potentiates alcohol-induced negative subjective responses and offsets impaired eye movements. *Alcohol Clin Exp Res.* 2012; 36:906–914. [PubMed: 22339626]
- Ciccocioppo R, Economidou D, Cippitelli A, Cucculelli M, Ubaldi M, Soverchia L, Lourdasamy A, Massi M. Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats: an animal model to study the neurobiology of alcoholism. *Addict Biol.* 2006; 11:339–355. [PubMed: 16961763]
- Cippitelli A, Ayanwuyi LO, Barbier E, Domi E, Lerma-Cabrera JM, Carvajal F, Scuppa G, Li H, Ubaldi M, Heilig M, Roberto M, Ciccocioppo R. Polymorphism in the corticotropin-releasing factor receptor 1 (CRF1-R) gene plays a role in shaping the high anxious phenotype of Marchigian Sardinian alcohol-preferring (msP) rats. *Psychopharmacology.* 2015a; 232:1083–1093. [PubMed: 25260340]
- Cippitelli A, Brunori G, Gaiolini KA, Zaveri NT, Toll L. Pharmacological stress is required for the anti-alcohol effect of the alpha3beta4* nAChR partial agonist AT-1001. *Neuropharmacology.* 2015b; 93C:229–236. [PubMed: 25689019]
- Cippitelli A, Wu J, Gaiolini KA, Mercatelli D, Schoch J, Gorman M, Ramirez A, Ciccocioppo R, Khroyan TV, Yasuda D, Zaveri NT, Pascual C, Xie XS, Toll L. AT-1001: a high-affinity alpha3beta4 nAChR ligand with novel nicotine-suppressive pharmacology. *Br J Pharmacol.* 2015c; 172:1834–1845. [PubMed: 25440006]
- Dani JA, Harris RA. Nicotine addiction and comorbidity with alcohol abuse and mental illness. *Nat Neurosci.* 2005; 8:1465–1470.
- de Guglielmo G, Cippitelli A, Somaini L, Gerra G, Li H, Stopponi S, Ubaldi M, Kallupi M, Ciccocioppo R. Pregabalin reduces cocaine self-administration and relapse to cocaine seeking in the rat. *Addict Biol.* 2013; 18:644–653. [PubMed: 22734646]
- DiFranza JR, Guerrera MP. Alcoholism and smoking. *J Stud Alcohol.* 1990; 51:130–135. [PubMed: 2308350]
- Doyon WM, Thomas AM, Ostroumov A, Dong Y, Dani JA. Potential substrates for nicotine and alcohol interactions: a focus on the mesocorticolimbic dopamine system. *Biochem Pharmacol.* 2013; 86:1181–1193. [PubMed: 23876345]
- Economidou D, Hansson AC, Weiss F, Terasmaa A, Sommer WH, Cippitelli A, Fedeli A, Martin-Fardon R, Massi M, Ciccocioppo R, Heilig M. Dysregulation of nociceptin/orphanin FQ activity in the amygdala is linked to excessive alcohol drinking in the rat. *Biol Psychiatry.* 2008; 64:211–218. [PubMed: 18367152]
- Engle SE, McIntosh JM, Drenan RM. Nicotine and ethanol cooperate to enhance ventral tegmental area AMPA receptor function via alpha6-containing nicotinic receptors. *Neuropharmacology.* 2015; 91:13–22. [PubMed: 25484253]
- Enoch MA, Goldman D. The genetics of alcoholism and alcohol abuse. *Curr Psychiatry Rep.* 2001; 3:144–151. [PubMed: 11276410]
- Erwin BL, Slaton RM. Varenicline in the treatment of alcohol use disorders. *Ann Pharmacotherapy.* 2014; 48:1445–1455.

- Fucito LM, Toll BA, Wu R, Romano DM, Tek E, O'Malley SS. A preliminary investigation of varenicline for heavy drinking smokers. *Psychopharmacology*. 2011; 215:655–663. [PubMed: 21221531]
- Funk D, Marinelli PW, Le AD. Biological processes underlying co-use of alcohol and nicotine: neuronal mechanisms, cross-tolerance, and genetic factors. *Alcohol Res Health*. 2006; 29:186–192. [PubMed: 17373407]
- Ginsburg BC, Lamb RJ. Effects of varenicline on ethanol- and food-maintained responding in a concurrent access procedure. *Alcohol Clin Exp Res*. 2013; 37:1228–1233. [PubMed: 23413834]
- Gordon TL, Meehan SM, Schechter MD. P and NP rats respond differently to the discriminative stimulus effects of nicotine. *Pharmacol Biochem Behav*. 1993; 45:305–308. [PubMed: 8327536]
- Gubner NR, Cunningham CL, Phillips TJ. Nicotine enhances the locomotor stimulating but not the conditioned rewarding effect of ethanol in DBA/2J mice. *Alcohol Clin Exp Res*. 2015; 39:64–72. [PubMed: 25623407]
- Hansson AC, Cippitelli A, Sommer WH, Fedeli A, Bjork K, Soverchia L, Terasmaa A, Massi M, Heilig M, Ciccocioppo R. Variation at the rat *Crhr1* locus and sensitivity to relapse into alcohol seeking induced by environmental stress. *Proc Natl Acad Sci U S A*. 2006; 103:15236–15241. [PubMed: 17015825]
- Heilig M, Koob GF. A key role for corticotropin-releasing factor in alcohol dependence. *Trends Neurosci*. 2007; 30:399–406. [PubMed: 17629579]
- Hendrickson LM, Guildford MJ, Tapper AR. Neuronal nicotinic acetylcholine receptors: common molecular substrates of nicotine and alcohol dependence. *Front Psychiatry*. 2013; 4:29. [PubMed: 23641218]
- Hendrickson LM, Zhao-Shea R, Pang X, Gardner PD, Tapper AR. Activation of alpha4* nAChRs is necessary and sufficient for varenicline-induced reduction of alcohol consumption. *J Neurosci*. 2010; 30:10169–10176. [PubMed: 20668200]
- Hull LC, Gabra BH, Bailey CP, Henderson G, Dewey WL. Reversal of morphine analgesic tolerance by ethanol in the mouse. *J Pharmacol Exp Therapeutics*. 2013; 345:512–519.
- Hurt RD, Offord KP, Croghan IT, Gomez-Dahl L, Kottke TE, Morse RM, Melton LJ 3rd. Mortality following inpatient addictions treatment. Role of tobacco use in a community-based cohort. *JAMA*. 1996; 275:1097–1103. [PubMed: 8601929]
- Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR. Varenicline Phase 3 Study G. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *JAMA*. 2006; 296:56–63. [PubMed: 16820547]
- Kamens HM, Andersen J, Picciotto MR. Modulation of ethanol consumption by genetic and pharmacological manipulation of nicotinic acetylcholine receptors in mice. *Psychopharmacology*. 2010; 208:613–626. [PubMed: 20072781]
- Kuzmin A, Jerlhag E, Liljequist S, Engel J. Effects of subunit selective nACh receptors on operant ethanol self-administration and relapse-like ethanol-drinking behavior. *Psychopharmacology*. 2009; 203:99–108. [PubMed: 18987848]
- Larsson A, Edstrom L, Svensson L, Soderpalm B, Engel JA. Voluntary ethanol intake increases extracellular acetylcholine levels in the ventral tegmental area in the rat. *Alcohol Alcohol*. 2005; 40:349–358. [PubMed: 16043436]
- Le AD, Li Z, Funk D, Shram M, Li TK, Shaham Y. Increased vulnerability to nicotine self-administration and relapse in alcohol-naive offspring of rats selectively bred for high alcohol intake. *J Neurosci*. 2006; 26:1872–1879. [PubMed: 16467536]
- Le AD, Lo S, Harding S, Juzysch W, Marinelli PW, Funk D. Coadministration of intravenous nicotine and oral alcohol in rats. *Psychopharmacology*. 2010; 208:475–486. [PubMed: 20013113]
- Lenoir M, Ahmed SH. Supply of a nondrug substitute reduces escalated heroin consumption. *Neuropsychopharmacology*. 2008; 33:2272–2282. [PubMed: 17971831]
- Litten RZ, Ryan ML, Fertig JB, Falk DE, Johnson B, Dunn KE, Green AI, Pettinati HM, Ciraulo DA, Sarid-Segal O, Kampman K, Brunette MF, Strain EC, Tiouririne NA, Ransom J, Scott C, Stout R, Group NS. A double-blind, placebo-controlled trial assessing the efficacy of varenicline tartrate for alcohol dependence. *J Addict Med*. 2013; 7:277–286. [PubMed: 23728065]

- Liu L, Hendrickson LM, Guildford MJ, Zhao-Shea R, Gardner PD, Tapper AR. Nicotinic acetylcholine receptors containing the alpha4 subunit modulate alcohol reward. *Biol Psychiatry*. 2013; 73:738–746. [PubMed: 23141806]
- Manzardo AM, Stein L, Belluzzi JD. Rats prefer cocaine over nicotine in a two-lever self-administration choice test. *Brain Res*. 2002; 924:10–19. [PubMed: 11743990]
- McKee SA, Harrison EL, O'Malley SS, Krishnan-Sarin S, Shi J, Tetraault JM, Picciotto MR, Petrakis IL, Estevez N, Balchunas E. Varenicline reduces alcohol self-administration in heavy-drinking smokers. *Biol Psychiatry*. 2009; 66:185–190. [PubMed: 19249750]
- Mello NK, Fivel PA, Kohut SJ, Carroll FI. Effects of chronic varenicline treatment on nicotine, cocaine, and concurrent nicotine+cocaine self-administration. *Neuropsychopharmacology*. 2014; 39:1222–1231. [PubMed: 24304823]
- Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at alpha4beta2 and a full agonist at alpha7 neuronal nicotinic receptors. *Mol Pharmacol*. 2006; 70:801–805. [PubMed: 16766716]
- Miller NS, Gold MS. Comorbid cigarette and alcohol addiction: epidemiology and treatment. *J Addict Di*. 1998; 17:55–66.
- Mingione CJ, Heffner JL, Blom TJ, Anthenelli RM. Childhood adversity, serotonin transporter (5-HTTLPR) genotype, and risk for cigarette smoking and nicotine dependence in alcohol dependent adults. *Drug Alcohol Depend*. 2012; 123:201–206. [PubMed: 22172222]
- Mitchell JM, Teague CH, Kayser AS, Bartlett SE, Fields HL. Varenicline decreases alcohol consumption in heavy-drinking smokers. *Psychopharmacology*. 2012; 223:299–306. [PubMed: 22547331]
- Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature*. 1998; 391:173–177. [PubMed: 9428762]
- Plebani JG, Lynch KG, Rennert L, Pettinati HM, O'Brien CP, Kampman KM. Results from a pilot clinical trial of varenicline for the treatment of alcohol dependence. *Drug Alcohol Depend*. 2013; 133:754–758. [PubMed: 23916324]
- Randall PA, Jaramillo AA, Frisbee S, Besheer J. The role of varenicline on alcohol-primed self-administration and seeking behavior in rats. *Psychopharmacology*. 2015
- Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, Sands SB, Schaeffer E, Schulz DW, Tingley FD 3rd, Williams KE. Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology*. 2007; 52:985–994. [PubMed: 17157884]
- Romberger DJ, Grant K. Alcohol consumption and smoking status: the role of smoking cessation. *Biomed Pharmacother*. 2004; 58:77–83. [PubMed: 14992787]
- Ruttenber AJ, Kalter HD, Santinga P. The role of ethanol abuse in the etiology of heroin-related death. *J Forensic Sci*. 1990; 35:891–900. [PubMed: 2391481]
- Steenland P, Simms JA, Holgate J, Richards JK, Bartlett SE. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proc Natl Acad Sci USA*. 2007; 104:12518–12523. [PubMed: 17626178]
- Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. *Science*. 2004; 306:1029–1032. [PubMed: 15528443]
- Tizabi Y, Copeland RL Jr, Louis VA, Taylor RE. Effects of combined systemic alcohol and central nicotine administration into ventral tegmental area on dopamine release in the nucleus accumbens. *Alcohol Clin Exp Res*. 2002; 26:394–399. [PubMed: 11923594]
- Tyndale RF. Genetics of alcohol and tobacco use in humans. *Ann Med*. 2003; 35:94–121. [PubMed: 12795339]

Highlights

- Alcohol and nicotine are largely co-abused
- Varenicline is a first-line treatment option for nicotine dependence
- Varenicline attenuates nicotine self-administration in alcohol-preferring animals
- Varenicline fails to reduce ethanol intake in alcohol-preferring animals
- When alcohol and nicotine are co-used, varenicline's effect is restricted to nicotine

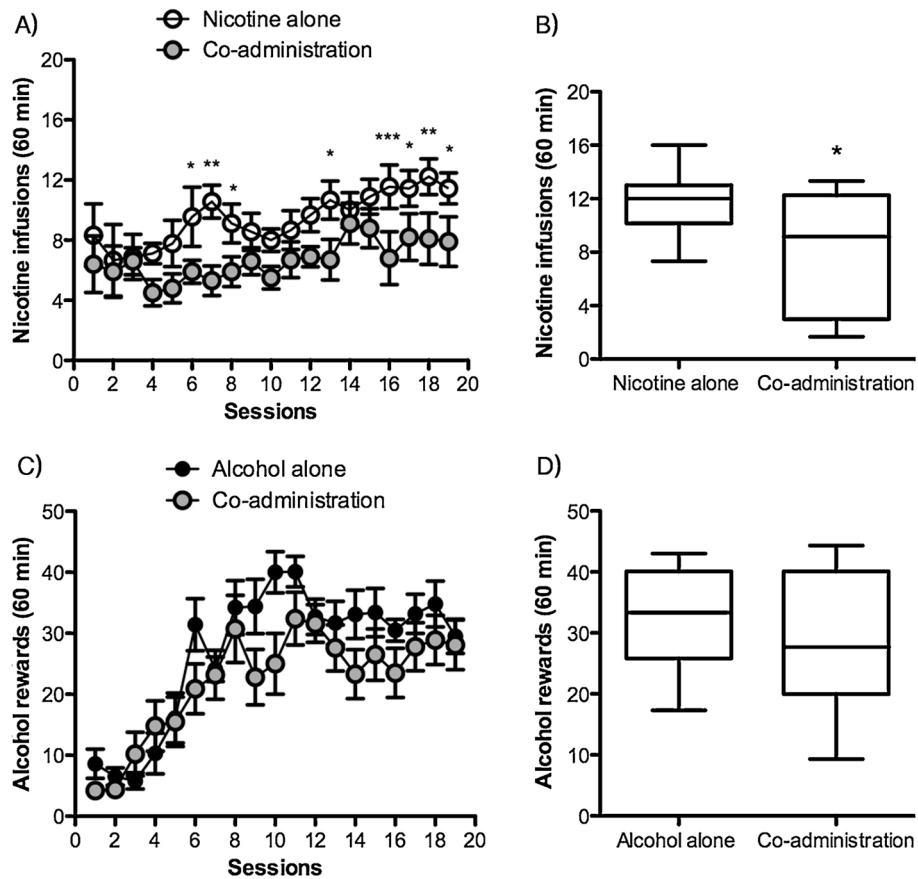


Figure 1. Self-administration of 10% (v/v) alcohol and 30 $\mu\text{g}/\text{kg}/\text{inf}$ nicotine in msP rats trained to co-self-administer both drugs during the same operant session or trained to lever press for only nicotine or only alcohol. **(A)** Nicotine lever pressing is higher in the msP group with access to only nicotine (N=9) than the group with access to both reinforcers (N=8). **(B)** Nicotine infusions during the last 3 sessions differed between the only nicotine and the co-administration group (** $p < 0.001$). **(C)** Alcohol self-administration did not significantly differ between the group pressing for alcohol alone (N=10) and the co-administration group (N=8). **(D)** Alcohol rewards during the last 3 sessions were not altered between the only alcohol and the co-administration group. Data are expressed as the mean (\pm SEM) number of infusions (nicotine) or rewards (alcohol) earned by rats during the last three 60-min self-administration sessions. For detailed statistics see “Results”.

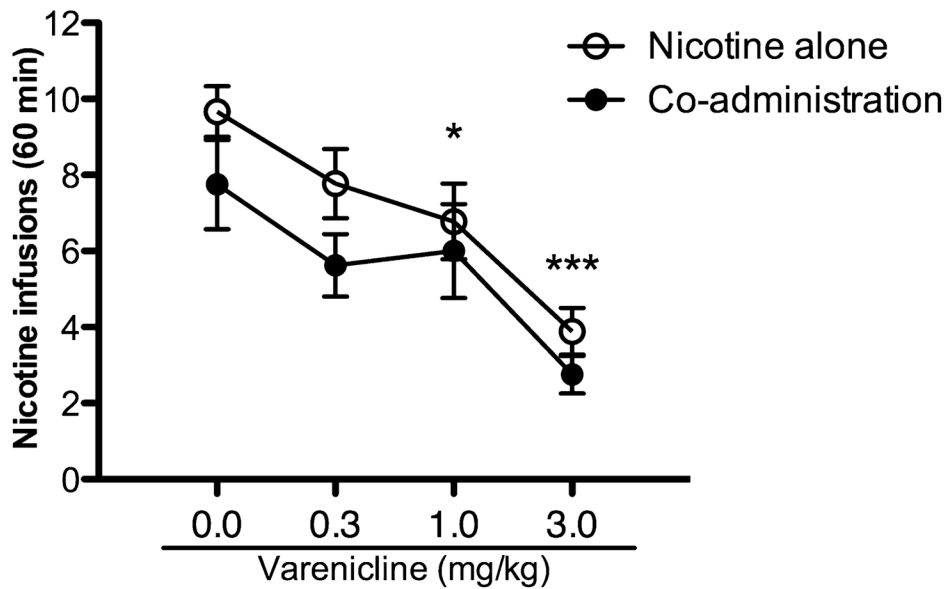


Figure 2. Effect of varenicline (0.0, 0.3, 1.0 and 3.0 mg/kg) on nicotine (30 $\mu\text{g}/\text{kg}/\text{inf}$) taking-behavior in msP rats subjected to nicotine/alcohol co-administration (N=8) and nicotine self-administration only (N=9). After a stable pattern of alcohol and/or nicotine self-administration was reached, animals were treated with varenicline, according to a latin square design. Varenicline was administered p.o. 30 minutes before the beginning of test sessions. The day after the test, animals were given a day off and self-administration baseline was re-established the following day. Results showed that varenicline doses of 1.0 and 3.0 mg/kg significantly reduced nicotine lever pressing in both groups. Results are expressed as the mean (\pm SEM) number of infusions in 60 min. * $p < 0.05$, *** $p < 0.001$ difference from vehicle-treated rats (0.0 mg/kg). For detailed statistics see “Results”.

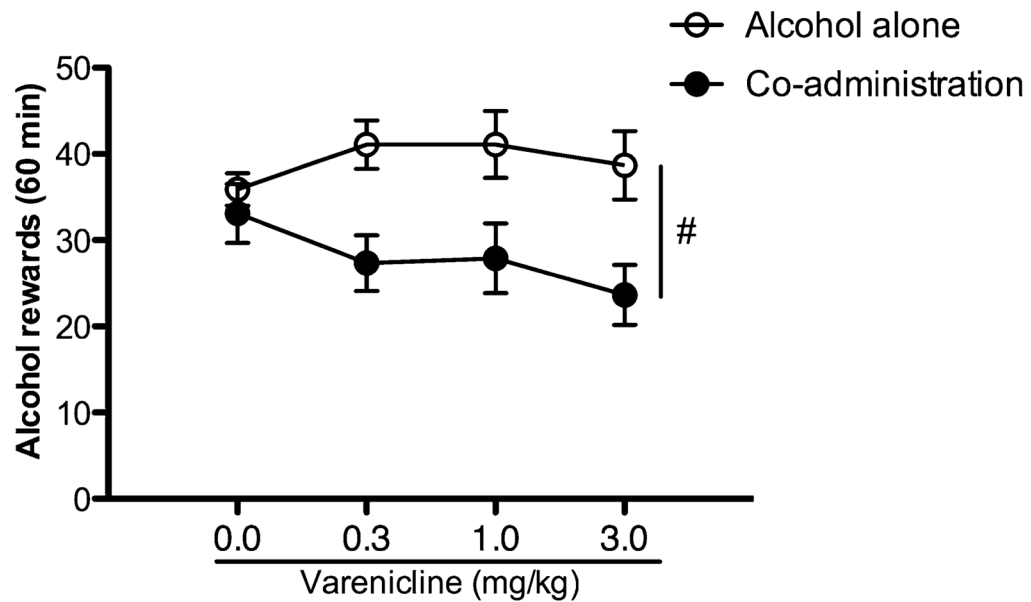


Figure 3. Effect of varenicline (0.0, 0.3, 1.0 and 3.0 mg/kg) on 10% alcohol-maintained lever pressing in msP rats subjected to nicotine/alcohol co-administration (N=8) and alcohol self-administration alone (N=10). Briefly, after a stable pattern of nicotine and/or alcohol self-administration was reached, animals were treated with varenicline, according to a within subject design. Varenicline was administered p.o. 30 minutes before the beginning of test sessions. The day after the test, animals were given a day off and self-administration baseline was reestablished the following day. Statistical analysis of the number of alcohol rewards demonstrated that varenicline failed to alter alcohol self-administration. Results are expressed as the mean (\pm SEM) number of rewards in 60 min. For detailed statistics see “Results”.

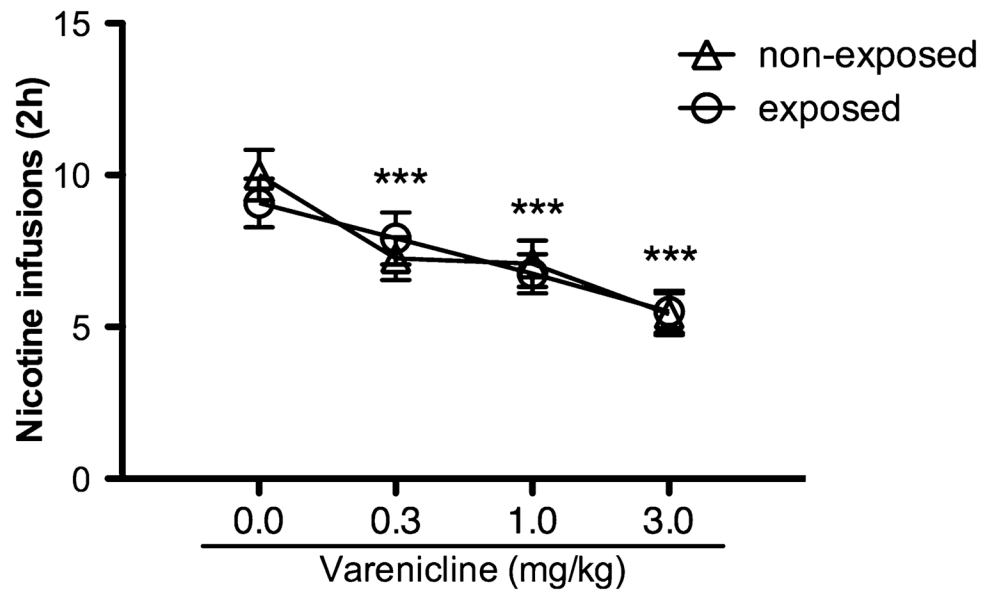


Figure 4. Effect of varenicline (0.0, 0.3, 1.0 and 3.0 mg/kg) on nicotine self-administration in msP rats exposed (N=12) and non-exposed (N=12) to 22-h 10% (v/v) alcohol access in their home-cages (two bottle choice paradigm). Varenicline, given 30 minutes before the operant nicotine 2-hours sessions, at all three doses tested significantly reduced nicotine lever pressing in alcohol exposed as well as non-exposed groups. Values represent the mean (\pm S.E.M) number of nicotine infusions. *** $p < 0.001$ difference from vehicle-treated animals (0.0 mg/kg). For detailed statistics see “Results”.

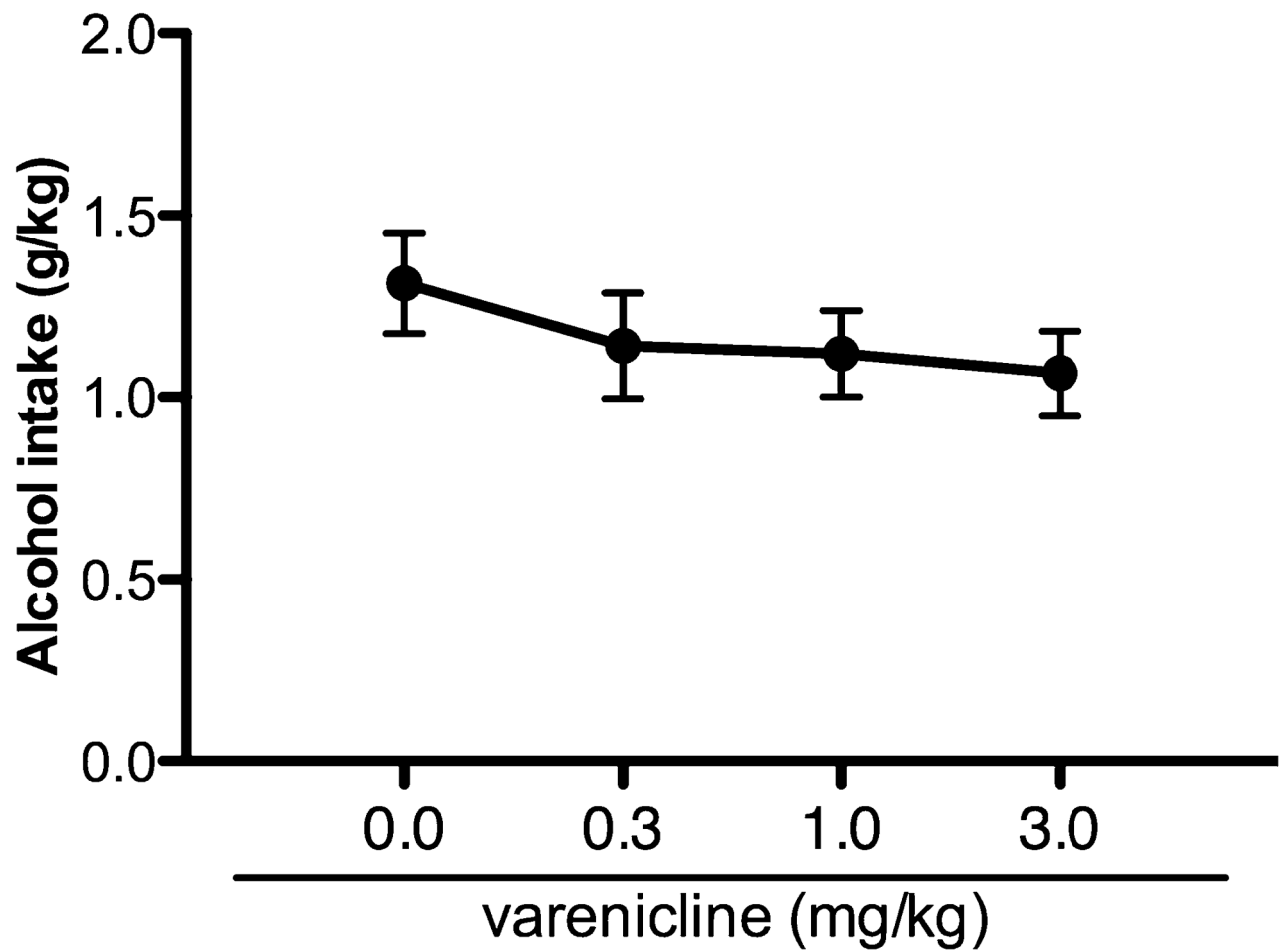


Figure 5. Effect of varenicline (0.0, 0.3, 1.0 and 3.0 mg/kg) on 10% (v/v) alcohol drinking assessed by the two-bottle free choice drinking paradigm (N=12). Drinking of msP animals was not affected by any dose of varenicline at 2-h from the beginning of alcohol exposure. Results represent the mean (\pm S.E.M) alcohol intake expressed as g/kg. For detailed statistics see "Results".