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Pharmacologically targeting the P2rx4 gene on maintenance and reinstatement of alcohol self-administration in rats

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ABSTRACT

Genetic studies indicate that alcohol consumption associates with expression of the P2rx4 gene, a gene that codes for the $P2X_4$ receptor. This receptor is a subtype in the purinergic system of ligand-gated ion channels that when activated exerts excitatory effects in CNS. $P2X_4$ function is inhibited by alcohol and $P2X_4$ receptors are modulated positively by the antiparasitic agent, ivermectin. Two experiments were performed to test the ability of ivermectin to alter the behavioral effects of alcohol in rats. After alcohol exposure was achieved via the "drinking in the dark" procedure, separate groups of Sprague–Dawley rats were trained to lever press for either alcohol (10% ethanol/2% sucrose) or sucrose (3%) solutions in operant chambers. Rats were tested for maintenance of operant self-administration under a progressive ratio condition (Experiment 1) and for reinstatement of extinguished responding induced by solution presentation (Experiment 2) after ivermectin (0; 1–10 mg/kg; IP) administration. Ivermectin decreased the amount of work that the animal performed to obtain reinforcers in the maintenance study, particularly in the group reinforced with alcohol, and tended to decrease reinstated lever press responding. Conditioned approach behavior (head entries) was significantly reduced by ivermectin in both experiments. Reduction in motor activity was seen during the longer maintenance sessions but not in the shorter reinstatement sessions. Results suggest some support for ivermectin-like drugs as potential treatment agents for alcohol dependence. Caution is warranted due to modest specificity on behavior reinforced by alcohol, some reduction in general activity levels, and the lack of dose–response effects.

1. Introduction

Alcohol dependence is a serious problem in the United States affecting about 10–13% of the population (Regier et al., 1990). It is a chronic relapsing disorder in which the individual has difficulty controlling his or her drinking, develops tolerance to its effects, suffers from withdrawal symptoms, and typically has social, financial, or personal problems directly related to excessive drinking (Schuckit et al., 2005). Preventing relapse to drinking is a major challenge in alcohol abuse treatment. After obtaining abstinence, exposure to cues associated with drinking or to alcohol itself can promote the return to excessive drinking. Thus, an important treatment goal is to either attenuate the ability of alcohol or its cues to provoke the re-initiation of drinking or, once drinking has begun, to reduce the amount of drinking. Pharmacological treatments may help obtain this goal (Anton, 2001; Swift, 1999).

Part of the process of developing potential pharmacotherapies for addiction is the use of animal models of alcohol drinking, craving, and relapse. No one model can emulate the whole spectrum of addiction; rather, different procedures can be used to probe mechanisms of or test manipulations on specific phases of the addiction process (Koob et al., 2009). For example, maintenance of operant self-administration of alcohol models the binge–intoxication phase of drinking and the reinstatement of this behavior after extinction models the preoccupation–anticipation phase (Koob et al., 2009). Pharmacological agents to be tested in these models can be identified based on their ability to target genes that are associated with alcohol consumption or are differentially expressed between strains that vary in alcohol preference such as the alcohol-preferring (P) and alcohol-non-preferring (NP) rats. P and NP rats were selectively bred for divergence in alcohol preference under free-choice access conditions and are commonly used in alcohol research (Lumeng et al., 1982; McBride and Li, 1998).

One candidate to target pharmacologically is the P2rx4 gene. P rats show lower functional expression of this gene compared to NP rats (Kimpel et al., 2007). Further support is provided by a study that used a genetical genomic approach in conjunction with phenotypic analysis of alcohol drinking among 23 recombinant inbred rat strains (Tabakoff et al., 2009). In this study, the P2rx4 gene showed a strongly significant negative correlation with alcohol drinking. The P2rx4 gene codes for the $P2X_4$ receptor, one of seven distinct P2X subtypes of the purinergic system (Koles et al., 2007; North, 1996). P2X receptors are ligand-gated ion channels that can be activated by extracellular adenosine 5′-triphosphate (ATP). When activated, P2X receptors exert excitatory actions at various regions of the central nervous system (Lalo et al.,

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2007; Le et al., 1998; Sim et al., 2006). $P2X_4$ function is inhibited by alcohol (Asatryan et al., 2010; Davies et al., 2005; Li et al., 2000; Xiao et al., 2008) perhaps due to its ability to decrease its agonist affinity (Weight et al., 1999). This suggests that pharmacological agents that affect $P2X_4$ receptor function may alter the behavioral effects of alcohol.

$P2X_4$ receptors show excellent specificity to the allosteric modulator effects of ivermectin (Khakh et al., 1999; Priel and Silberberg, 2004), an antiparasitic drug used widely in veterinary medicine (Campbell, 1989). Ivermectin is also prescribed for humans as an antiparasitic agent (NCBI, 2008) and thus, if promising results are seen in animals, research can be readily translated into human populations. The present report describes the results of two experiments aimed at testing the ability of ivermectin to alter the behavioral effects of alcohol in rats. First, we examined the effects of ivermectin on maintenance of operant self-administration of alcohol in outbred, Sprague–Dawley rats. We utilized a progressive ratio (PR) procedure in which the response requirement increases over the session and this is thought to measure the motivation to work to obtain alcohol (or other reinforcer) delivery (Hodos, 1961; Le and Shaham, 2002; Richardson and Roberts, 1996). Next, we investigated the effects of ivermectin on reinstatement of extinguished operant responding induced by exposure to alcohol along with the cues associated with its delivery. To assess the specificity of the effects of ivermectin on maintenance and reinstatement of responding for alcohol, we performed parallel studies in rats trained to lever press for sucrose solution as we did previously (Vosler et al., 2001). In our prior study, levels of reinstated responding induced by alcohol were low. We attempted to increase response levels in rats in the present study by exposing them to alcohol prior to initial self-administration training although all tests were conducted several weeks after termination of the alcohol exposure procedure.

2. Materials and methods

2.1. Subjects and setting

Adult (70–90 days at the start of the study), male Sprague–Dawley rats (Harlan Sprague–Dawley Inc., Indianapolis, IN) were housed individually in polypropylene cages in a temperature- and humidity-controlled room maintained on a reverse 12:12 light/dark cycle (lights on at 19:00). Food and water were available *ad libitum* except as noted under experimental procedures. Protocols were approved by the Institutional Animal Care and Use Committees and followed the "Principles of Laboratory Animal Care" (NIH publication No. 85–23, revised 1996).

2.2. Alcohol dependence induction procedure

In our previous reinstatement study (Vosler et al., 2001), the levels of reinstated active lever press responding were not very high (about 9 presses). We reasoned that we might be able to increase this level of responding by exposing the rats to alcohol prior to initial operant self-administration training. Rats ($n = 38$) were initially exposed to a modified "drinking in the dark" procedure (Bell and McBride, 2009). In this procedure, rats were maintained on a multiple scheduled access to alcohol solution (5% w/v) concurrently with water. Initially, two bottles, one per solution type, were available 24-h a day during Week 1, 16-h a day during Week 2, 8-h per day during Week 3, and 4-h a day during Week 4 on Monday–Friday. Drinking periods of 8-h or less occurred during the dark phase only and most of the 16-h drinking period occurred during this phase. Thereafter, access was limited to three, 1-h periods per day each separated by a 2-h non-access break on Monday–Friday. Rats were provided *ad libitum* access to water on weekends. These schedules were in effect for 6-wks after which rats were returned to *ad libitum* water access and began training for operant self-administration. Note that several weeks of self-administration training were conducted prior to test sessions and thus rats would not be considered alcohol dependent or in withdrawal when tests were performed.

The 24 rats that drank the greatest proportion of alcohol out of the total intake during the last week of the drinking in the dark procedure were entered into the experiments. All rats drank more than half of their daily fluid intake as alcohol with mean ($\pm$S.E.M.) of $75 \pm 3\%$. Half of these rats were assigned to the alcohol responding group (EtOH) and half to the sucrose responding group (Suc). Assignments were made so that groups did not differ in alcohol preference. One rat from the EtOH group failed to show stable lever press behavior and was not entered into either study. Some rats from each group were run in both the maintenance and reinstatement experiments and some rats were run in only one experiment.

2.3. Operant self-administration training

Sessions were conducted in standard operant chambers housed within sound-attenuating cubicles equipped with fans (Coulbourn Instruments, Whitehall, PA). Each chamber was outfitted with a house light on one side of the cage and two levers on the opposite wall. Above each lever was a triple cue light and in between the levers was an access area through which a dipper could protrude. The dipper was immersed in a solution reservoir and could be activated to present 0.1 ml of solution through the access area within the chamber. This access area was equipped with a light and with infrared sensors that were used to detect head entries into the area. Another infrared detector was mounted on the ceiling and used to tabulate the animal's body movements. Tabulation of all data as well as programming of stimulus parameters was performed with a Coulbourn Instruments based hardware and software (Graphic State Notation) system.

Illumination of the house light signaled the beginning of the sessions that were 30-min in length. Rats were initially trained to press either lever and this would activate the dipper that was immersed within a reservoir of 10% sucrose (w/v) solution. Depression of a lever also illuminated the triple cue light above the lever and the light within the dipper access area for the duration of the dipper presentation. As training progressed, several parameters were changed gradually until test conditions were met. These included decreasing the duration of the dipper presentation from 15-s to 3-s to 1-s and decreasing the concentration of the sucrose solution for the Suc group to 5% and then 3%. The concentration of sucrose for the EtOH group was also decreased as alcohol was added using a modified sucrose fading procedure (Samson et al., 1998; Vosler et al., 2001). The solution modification procedure for the EtOH group began at 0% EtOH/10% sucrose and proceeded to 5% EtOH/10% Sucrose, then to 10% EtOH/5% sucrose, then to 10% EtOH/3% sucrose, and finally to 10% ethanol/2% sucrose. Thus, lever presses were ultimately reinforced by 10% ethanol/2% sucrose (w/v) for the EtOH group and by 3% sucrose (w/v) in the Suc group. These two solutions at these concentrations support equivalent levels of operant responding under the conditions used in this study (Czachowski et al., 1999; Samson et al., 1998; Vosler et al., 2001). Finally, the response requirements were altered so only one lever was active and the fixed ratio was raised to FR2. Number of presses on the other lever (inactive) were tabulated but had no programmed consequences.

2.4. Drugs

Ivermectin (22,23-Dihydroavermectin B1; Sigma Chemicals, St. Louis, MO) was prepared in isotonic saline and a concentration of 1 mg/ml and administered IP at one of three doses (1, 3, or 10 mg/kg) 30-min prior to sessions. Alcohol (ethyl alcohol, 109 Proof, organic grain alcohol, USP grade; Pharmco-Aaper, Brookfield, CT) was diluted with water and sucrose (EMD Chemicals, Whitehouse Station, NJ) at various concentrations as described above.

2.5. Experiment 1: maintenance of operant self-administration

2.5.1. Groups

Rats were assigned to either the alcohol responding group (EtOH; n = 9) or to the sucrose responding group (Suc; n = 11).

2.5.2. Progressive ratio tests

Once operant responding for the assigned solution was stable under the FR2 schedule of reinforcement (<20% variability of active lever presses emitted over 2 days), test sessions using a progressive ratio (PR) schedule were initiated twice per week with training sessions continuing on alternate days. The response requirements proceeded using the following schedule: 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 7, 7, 9, 9, 11, 11,13, 13, 15, 15, 18, 18, 21, 21, 24, 24, etc. based on Walker and Koob (2007). That is, to earn each successive reinforcer, the rat was required to respond at each level in sequence. The length of the test session was increased to 3-h. All other stimulus parameters were not altered. Rats were injected (IP) with ivermectin or vehicle (isotonic saline) 30-min prior to the initiation of test sessions. Ivermectin doses were presented in a non-systematic order and were each tested twice. The means of the data collected on the two test sessions per dose were used in the analyses.

2.5.3. Data analysis

Measures assessed included the final ratio requirement completed as well as numbers of reinforcers earned, head entries into the dipper access area, and body movements made during the session. Data were analyzed with 2 × 4 ANOVA representing the between group factor of Group (EtOH vs. Suc) with repeated measures on Dose. Significance was set at P = 0.05 and trends towards significance at P = 0.10. Data are presented as mean ± standard error of the mean (S.E.M.).

2.6. Experiment 2: reinstatement of operant responding

2.6.1. Groups

Rats were assigned to either the alcohol responding group (EtOH; n = 9) or to the sucrose responding group (Suc; n = 7).

2.6.2. Reinstatement tests

Once lever press response levels were stable under the FR2 schedule of reinforcement (<20% variability of active lever presses emitted over 2 days), extinction sessions begun. In these sessions, no solutions were available for delivery although all other stimulus parameters remained constant. Lever press responding decreased over sessions to minimal levels. Once a rat emitted 5 or fewer active lever presses for two consecutive days, a reinstatement session was performed based on Bienkowski et al. (2000). First, 15 non-contingent solution (alcohol or sucrose depending upon group assignment) presentations were made. The house light was illuminated for 12-s during which the dipper delivered the solution to which the animal was trained for 8-s followed by a 4-s timeout. Next, a 30-min session in which lever press responding was not followed by dipper presentations commenced. All other stimulus parameters were the same as in training sessions. Rats were injected (IP) with ivermectin or vehicle (isotonic saline) 30-min prior to the initiation of test sessions. Ivermectin doses were presented in a non-systematic order and were each tested twice. The means of the data collected on the two test sessions per dose were used in the analyses.

2.6.3. Data analysis

Measures assessed included numbers of active and inactive lever presses, head entries into dipper area, and body movements made during the session. Data were analyzed with 2 × 4 ANOVA representing the between group factor of Group (EtOH vs. Suc) with repeated measures on Dose. Significance was set at P = 0.05 and trends towards significance at P = 0.10. Data are presented as mean ± standard error of the mean (S.E.M.).

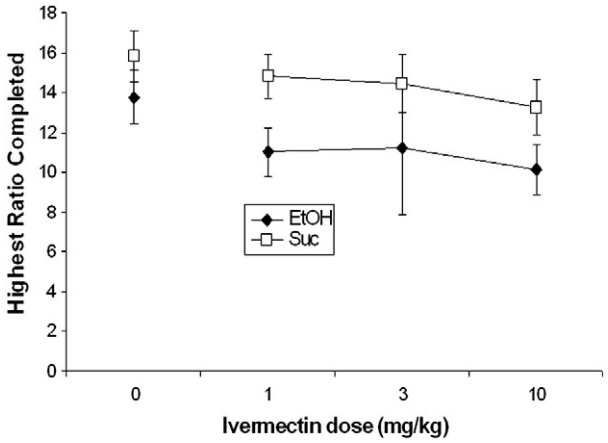

Fig. 1. Ivermectin attenuates the level of work that rats will engender to obtain solution reinforcers in the maintenance study conducted under a progressive ratio (PR) schedule. Mean (±S.E.M.) last ratio requirement completed over the 3-h progressive ratio tests sessions is shown by ivermectin dose. Each data point is based on the mean of two sessions performed per dose and per rat. Ivermectin produced a dose-related decrease in the final ratio completed. Final ratios completed were lower in the group responding for alcohol solution (EtOH group; closed symbols) compared to the group responding for sucrose solution (Suc group; open symbols). See text for statistics.

3. Results

3.1. Experiment 1: maintenance of operant self-administration

The effects of ivermectin on maintenance of operant responding for alcohol or sucrose were assessed under a progressive ratio (PR) schedule during 3-h test sessions. Data on last ratio completed under these conditions with ivermectin pretreatment are shown in Fig. 1. Ivermectin lowered the ratio the animal would complete as supported by the significant Dose effect, $F(3,54) = 2.92$; $P < 0.05$. Final ratios completed were lower in rats from the EtOH group compared to rats from the Suc group as seen in Fig. 1. This statement is supported by the significant Group effect, $F(1,18) = 5.19$; $P < 0.05$, although the Group × Dose effect failed to reach significance ($P > 0.10$).

Data on active lever presses emitted during the ivermectin test sessions are presented in Table 1. Ivermectin decreased active lever press responses as supported by the significant Dose effect, $F(3,54) = 2.76$; $P = 0.05$. The EtOH group tended to show lower levels of active lever press responding compared to the Suc group as supported by the trends towards significance for the Group effect, $F(1,18) = 3.74$; $P < 0.07$. The Group × Dose interaction was not significant ($P > 0.10$). Numbers of inactive lever presses were minimal (means across Group and Dose were less than 4) and did not differ across doses or between groups (P's > 0.10; data not shown).

Numbers of earned reinforcers decreased significantly with ivermectin pretreatment and were lower in the EtOH compared to the Suc group as seen in Table 1. These conclusions are supported by the

Table 1
Numbers of active lever presses, earned reinforcers, and head entries into dipper access area shown during maintenance test sessions conducted under a progressive ratio schedule are presented by ivermectin dose and solution reinforcer group.

Dose (mg/kg)	Active presses[a]		Earned reinforcers[b,c]		Head entries[b]	
	EtOH	Suc	EtOH	Suc	EtOH	Suc
0	123.6 ± 19.7	150.4 ± 18.0	17.9 ± 1.3	19.4 ± 1.0	41.8 ± 5.7	36.4 ± 1.8
1	93.9 ± 15.8	137.9 ± 16.9	15.4 ± 1.5	18.5 ± 1.1	30.3 ± 3.7	33.1 ± 2.8
3	91.8 ± 13.8	125.6 ± 16.8	15.6 ± 1.2	18.0 ± 1.1	27.8 ± 3.4	27.7 ± 1.3
10	84.7 ± 15.9	116.4 ± 16.2	14.7 ± 1.3	17.1 ± 1.3	30.8 ± 7.1	26.6 ± 2.1

[a] Significant Group effect, P = 0.05 and trends towards significance for Dose, P < 0.10.
[b] Significant Dose effect, P's < 0.05.
[c] Significant Group effect, P < 0.05.

significant Dose, $F(3,54) = 3.02$; $P<0.05$; and Group $F(1,18) = 4.77$; $P<0.05$, effects although the interaction failed to reach significance ($P>0.10$). The number of head entries made into the dipper access area were decreased significantly by ivermectin administration, a statement supported by the significant Dose effect, $F(3,54) = 5.09$; $P<0.005$. These data are also presented in Table 1. The Group effect and its interaction with Dose were not significant (P's>0.10) for head entries. Finally, ivermectin decreased the number of body movements made during the sessions in rats of both groups. This is supported by the significant Dose effect, $F(3,54) = 8.61$; $P<0.001$ and these data are presented in Fig. 2. The Group effect and its interaction with Dose were not significant (P's>0.10).

3.2. Experiment 2: reinstatement of operant responding

The average numbers of active lever presses emitted during the two FR2 training trials prior to implementing extinction sessions did not differ between groups (EtOH: 68.9 ± 9.0; Suc: 66.3 ± 8.4; $p>0.10$). On the last extinction session prior to the first reinstatement, the mean (±S.E.M.) number of active lever presses did not differ between groups (EtOH: 2.2 ± 0.6; Suc: 3.3 ± 0.5; $p<0.10$) and were significantly less than the number of active lever presses emitted during the first reinstatement session, $F(1,16) = 11.19$; $P<0.005$.

The effect of ivermectin on reinstatement of operant responding for alcohol or sucrose induced by solution presentation was assessed. Data on active lever presses emitted during the reinstatement sessions are shown in Fig. 3. As seen in Fig. 3, ivermectin tended to decrease responding as supported by the trend towards significance of the Dose effect, $F(3,42) = 2.43$; $P<0.08$. The Suc group tended to show lower levels of active lever press responding compared to the EtOH group as supported by the trends towards significance for the Group effect, $F(1,14) = 3.18$; $P<0.10$. The Group × Dose interaction was not significant ($P>0.10$).

Ivermectin significantly decreased the numbers of head entries made into the dipper access area as shown in Fig. 4. This statement is supported by the significant Dose effect, $F(3,42) = 3.34$; $P<0.05$. The Group effect and its interaction with Dose were not significant (P's>0.10). There was no effect of ivermectin on numbers of inactive lever presses emitted or on numbers of body movements made during sessions (P's>0.10). These data are presented in Table 2.

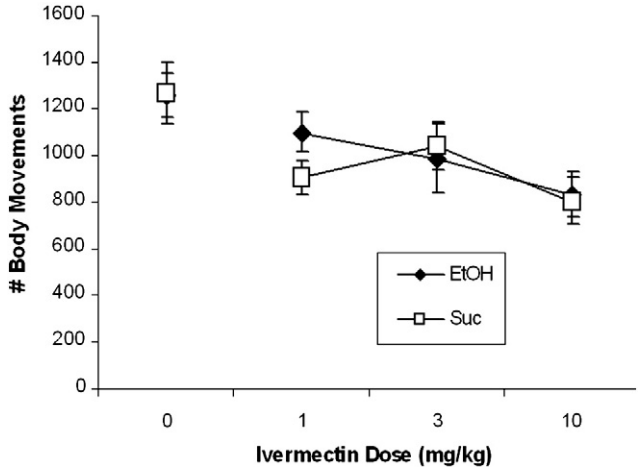

Fig. 2. Ivermectin decreases general activity levels during the maintenance of operant self-administration study. Mean (±S.E.M.) number of body movements recorded during the 3-h tests is shown by ivermectin dose. Each data point is based on the mean of two sessions performed per dose and per rat. Ivermectin decreased body movements in the EtOH (closed symbols) and Suc (open symbols) groups. See text for statistics.

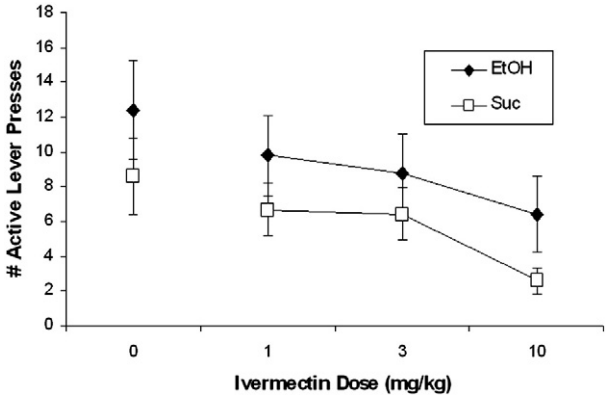

Fig. 3. Ivermectin tends to decrease reinstatement of extinguished lever press responding. Mean (±S.E.M) number of active lever presses is shown for the EtOH (closed symbols) and Suc (open symbols) groups by ivermectin dose. Each data point is based on the mean of two sessions performed per dose and per rat. Ivermectin tends to decrease the numbers of active lever presses evoked by solution presentation during the reinstatement tests in the EtOH (closed symbols) and Suc (open symbols) groups. See text for statistics.

4. Discussion

The results of the present study show that ivermectin decreased operant responding for alcohol and sucrose in rats. Ivermectin also tended to attenuate reinstatement of extinguished responding for alcohol and sucrose. The specificity of the effects of ivermectin to reduce the reinforcing effects of alcohol is modest. Reinstatement of lever press behavior originally reinforced by sucrose shows a tendency to be attenuated more by ivermectin than behavior originally reinforced by alcohol. Although the Group × Dose interaction was not significant, data shown in Fig. 3 suggest that most of the trend towards significance of the ivermectin dose effect was carried by the Suc group. In contrast, examination of the data from the maintenance study shown in Fig. 1 and Table 1 suggests that most of the significance of the ivermectin dose effect was carried by the EtOH group. And, although the Group × Dose interaction failed to reach significance, the Group effect was significant for the maintenance study. Ivermectin administration did decrease general activity levels (e.g., number of body movements) under maintenance conditions although this effect was not seen during reinstatement tests. The differential effects of ivermectin on movements between the two session types likely reflect the different session lengths (3-h for PR tests vs. 30-min for reinstatement tests). Nonetheless, the data are consistent with the notion that pharmacological manipulations

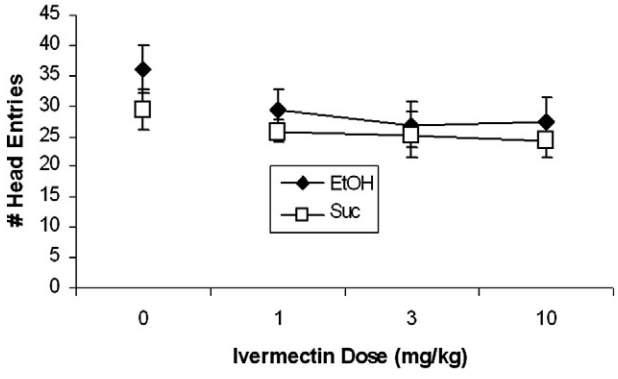

Fig. 4. Ivermectin decreases conditioned approach during reinstatement test sessions. Mean (±S.E.M) number of head entries into the dipper access area is shown for the EtOH group (closed symbols) and Suc group (open symbols) by ivermectin dose test session. Each data point is based on the mean of two sessions performed per dose and per rat. Ivermectin decreased the number of head entries during reinstatement tests. See text for statistics.

Table 2
Numbers of inactive lever presses emitted and body movements made during reinstatement test sessions are presented by ivermectin dose and solution reinforcer group.

Dose (mg/kg)	Inactive presses		Body movements	
	EtOH	Suc	EtOH	Suc
0	0.2 ± 0.1	1.1 ± 1.2	344.7 ± 40.9	364.1 ± 43.9
1	0.6 ± 0	0 ± 0	348.7 ± 41.2	290.5 ± 21.1
3	0.1 ± 0.1	0 ± 0	343.3 ± 31.3	363.9 ± 45.2
10	0 ± 0	0 ± 0	342.4 ± 34.5	339.3 ± 42.2

of $P2X_4$ receptor activity affect behaviors maintained by alcohol or sucrose reinforcement.

In addition to assessments of lever press responding for alcohol, we also measured numbers of head entries into the solution access area during maintenance and reinstatement tests. Head entries showed stronger statistical effects of ivermectin compared to active lever presses in both the maintenance and reinstatement tests. Rats emitted about twice as many head entries than earned reinforcers during the maintenance tests. This can be seen by comparing data on earned reinforcers to head entries in Table 1. Although it is obviously necessary for the animal to protrude its head into this access area to obtain the solution delivery, during the reinstatement tests when no solution is available for delivery, the ratio of head entries to active lever presses was even greater. Rats emit about three times as many head entries as lever presses (i.e., compare Figs. 3 and 4). A head entry can be considered a conditioned appetitive response because it had been associated with the solution delivery and, like lever pressing in the absence of reinforcer delivery, may be a measure of "craving".

A reduction in craving should assist maintaining abstinence from alcohol in alcohol dependent persons. The challenge for treating alcohol dependence is to prevent relapse to drinking or, if drinking is initiated, attenuate the potential escalation into excessive drinking. Ivermectin may assist treatment for alcohol dependence on both fronts. Craving or head entries under conditions of alcohol delivery or no alcohol delivery is decreased by ivermectin. Ivermectin likely would decrease the chances of escalating alcohol drinking once initiated. This statement is based on the fact that ivermectin reduced responding under the progressive ratio condition in which the response requirement increases over the session.

This study shows support for the approach of testing a pharmacological agent that was chosen based on information gleaned from genetic studies. The idea to test the effects of ivermectin on behaviors supported by alcohol was based on the following. First, the P2rx4 gene was identified as a candidate gene related to alcohol drinking in recombinant inbred rats (Tabakoff et al., 2009) and this gene is differentially expressed in selectively bred P and NP rats (Kimpel et al., 2007). Both studies showed that higher levels of alcohol consumption were associated with lower levels of the gene. The P2rx4 gene codes for the $P2X_4$ receptor, part of the purinergic system of ion channels activated by ATP (Koles et al., 2007; North, 1996). When activated, P2X receptors exert excitatory effects in various neural regions. Alcohol interacts with the $P2X_4$ receptor system by inhibiting its excitatory effects (Asatryan et al., 2010; Davies et al., 2005; Li et al., 2000; Xiao et al., 2008). This system can be enhanced in the presence of ivermectin because it acts as a specific positive allosteric modulator of $P2X_4$ receptors (Khakh et al., 1999; Priel et al., 2004). For example, deletion of the P2rx4 gene in mice reduces long-term potentiation (LTP) in hippocampal Schaffer collateral synapses and ivermectin enhances LTP in wild-type but not in P2rx4 knock-out mice (Sim et al., 2006). Ivermectin also increases the amplitude and frequency of spontaneous P2X-mediated EPSCs in cortical slices (Lalo et al., 2007). Thus, it is possible that ivermectin altered, in part, the behavioral effects of alcohol seen in the present study through $P2X_4$ mechanisms. However, other actions of ivermectin have been reported, such as effects on GABA, glycine, and nicotinic systems (Adelsberger et al., 2000; Dawson et al., 2000; Hugel and Schlichter, 2000; Krause et al., 1998; Shan et al., 2001). Any of these systems may have contributed to the results reported herein either directly or indirectly (Haile et al., 2008; Vengeliene et al., 2008; Xiao et al., 2008).

One possible scenario to explain the effects of ivermectin on alcohol self-administration behavior is as follows. Under typical conditions, activation of $P2X_4$ exerts excitatory effects on the inhibitory GABAergic neurons in the ventral tegmental area (VTA). GABAergic neurons inhibit dopamine (DA) cells in nucleus accumbens (NAc) and DA levels in NAc are linked to the rewarding effects of various drugs of abuse (DiChiara and Imperato, 1988; Haile et al., 2008). Alcohol inhibits GABAergic transmission to DA neurons in this region through purinergic mechanisms and this could reflect one way in which alcohol intake enhances DA levels in NAc. That is, alcohol would cause a decrease in $P2X_4$ receptor activity that would lead to a disinhibition of DA neurons through a reduction in GABA interneuron inhibition. However, ivermectin, a positive allosteric modulator of $P2X_4$ receptors, enhances the excitatory $P2X_4$ system and this would cause a reduction in DA neural activity due to an increase in GABA inhibition. Presumably, an indirect alcohol-induced increase in NAc DA levels could be counteracted by ivermectin via the $P2X_4$ mechanism although effects mediated through other systems likely contribute as well.

Several lines of evidence suggest that ivermectin crosses the blood–brain barrier to affect CNS processes. Systemic ivermectin administration causes gross motoric problems including ataxia, staggering, and loss of righting in rats among other signs suggesting involvement of the CNS (Dadarkar et al., 2007). Further support for the ability of ivermectin to cross the blood–brain barrier and affect the CNS is shown by its ability to protect against the convulsant effects of pentylenetetrazole, lidocaine, or strychnine, reverse picrotoxin-induced anxiety in elevated plus maze, and decrease spontaneous locomotor activity when administered systemically as seen in the present study and in previous reports (Dawson et al., 2000; Spinosa et al., 2002; Trailovic and Varagic, 2007).

There are limitations in the present study. The ability of alcohol exposure to reinstate extinguishing responding on the lever previously associated with alcohol delivery is thought to be a model of relapse to drinking after abstinence in alcoholics due to its face validity. While this drug-induced reinstatement procedure in animals has been a popular tool for researchers in addiction (Le et al., 2002; Shaham et al., 2003), its construct and predictive validities as an animal model of relapse have not been confirmed (Katz and Higgins, 2003; Koob et al., 2009). Further, the reinstatement procedure in animals necessarily utilizes active extinction (i.e., the contingency between lever pressing and solution delivery is eliminated) whereas for humans, abstinence is not typically an active process of extinguishing a behavior. The specificity of the effect of ivermectin on behavior maintained by alcohol vs. another reinforcer is perhaps limited. P2 receptors have been implicated in motivated behaviors such as feeding although such effects are most likely due to P2Y receptor mediation and not P2X receptors (Kittner et al., 2001; Krugel et al., 2004). Although interest in role of the purinergic system in the CNS is growing, the literature about this system and behavior is limited and much of it relates to pain (Abbracchio et al., 2009). Another limitation is the lack of dose response function for ivermectin. Although in many cases, the ivermectin dose effects were significant, examination of the data in the figures and tables suggests that this likely reflect that assessments made under the vehicle condition differed from those made under any of the three doses of ivermectin tested (1–10 mg/kg). These doses of ivermectin are below the LD_{50} of 18.2 mg/kg and outside its lower 95% confidence interval which is 15.5 mg/kg (Trailovic et al., 2007). Thus, the maximum dose used in the present study is about as high as can be used to avoid lethality or gross toxic effects.

Ivermectin is used in humans to treat lymphatic filariasis and onchocerciasis, two parasite-induced diseases found mostly in Africa (WHO, 1997). It is generally considered safe with few side effects and is acceptable to patients (Pacqui et al., 1991). Thus, ivermectin could be

considered as a potential pharmacological treatment for alcohol dependence. Interestingly, an interaction between alcohol and ivermectin in humans has been described. Based on clinical reports of increased cases of ataxia and postural hypotension among individuals who ingested alcohol along with ivermectin, a study of plasma ivermectin levels was conducted (Shu et al., 2000). The group administered alcohol along with ivermectin showed significantly higher plasma ivermectin concentrations up to 4 h after administration compared to the group given ivermectin with water. Thus, if ivermectin is tested in alcohol dependent individuals, caution is needed in dose considerations. Another caution about the potential use of ivermectin to treat alcoholism is its limited specificity to alcohol.

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