

# Rewarding Effects of Ethanol Combined with Low Doses of Morphine through Dopamine D1 Receptors

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## Abstract

This study investigated whether ethanol combined with low doses of morphine produces rewarding effects in rats. Ethanol (0.075–1.2 g/kg, intraperitoneal [i.p.]) alone did not induce place preference. A moderate dose (1 mg/kg, s.c.), but not a low dose (0.1 mg/kg), of morphine induced a significant place preference. The combination of ethanol (0.075–0.6 g/kg, i.p.) and 0.1 mg/kg of morphine, as well as low doses of morphine (0.03–0.1 mg/kg, subcutaneous [s.c.]) combined with ethanol (0.3 g/kg, i.p.), induced a significant place preference. The combined effect of ethanol and morphine was significantly attenuated by naloxone (0.3 mg/kg, s.c.), naltrindole (1.0 mg/kg, s.c.), or long-term administration of the dopamine D1 receptor antagonist SCH23390 (1.0 mg/kg/day, s.c.). These results suggest that the rewarding effect induced by ethanol and a low dose of morphine is mediated by activation of the central opioidergic and dopaminergic systems through dopamine D1 receptors.

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**Key words:** ethanol, morphine, conditioned place preference, rats

## Introduction

The reinforcing/rewarding effects of abused drugs have been assessed with self-administration and with conditioned place preference procedures. Ethanol has been self-administered through both the oral and intravenous routes in rats<sup>1,2</sup> and in monkeys<sup>3</sup>. On the other hand, identifying the ethanol-induced rewarding effect has been difficult with the regular conditioned place preference procedure<sup>4–11</sup>. Stewart and Grupp<sup>12</sup> first showed the

rewarding effect using the conditioned place preference procedure with a moderate dose of ethanol (0.5 g/kg) and food motivation. Others have used alternative techniques to establish the ethanol-induced place preference including exposing subjects to ethanol for a long period of time before the study<sup>13</sup>; increasing the conditioning trials<sup>14</sup>; and combining ethanol with pyrazole, an alcohol dehydrogenase inhibitor<sup>15</sup>. Thus, special conditioning or long-term conditioning may be required to establish the ethanol-induced place preference in rodents<sup>16</sup>.

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Several lines of evidence in animals suggest that an endogenous opioidergic system plays a critical role in the rewarding effects of ethanol<sup>17-19</sup>, because ethanol increases the activity of the endogenous opioid,  $\beta$ -endorphin<sup>17,20,21</sup>. Furthermore, low doses of morphine can stimulate ethanol consumption<sup>22-24</sup>. Conversely, treatment with opioid receptor antagonists decreases alcohol consumption<sup>18,22,25-30</sup> and ethanol-induced place preference<sup>31</sup>.

The rewarding effects of ethanol may involve stimulation of the central dopaminergic system through activation of the endogenous opioidergic system. *In vitro* experiments with slice preparations from rat striatum showed that ethanol dose-dependently increases basal dopamine release and that this effect is blocked by opioid receptor antagonists<sup>32</sup>. Furthermore, *in vivo* microdialysis studies have shown that the ethanol-induced increase in dopamine release from the nucleus accumbens is attenuated by pretreatment with opioid receptor antagonists<sup>33-35</sup>. These observations suggest that dopamine-opioid interactions are involved in the rewarding effects of ethanol. The signaling mechanisms that result from the interaction between opioids and ethanol to produce rewarding effects have not been defined. The present study investigated the interaction between ethanol and morphine to produce place preference and the possible involvement of dopaminergic system activation in opioid-ethanol interaction.

### Materials and Methods

Male Sprague-Dawley rats (Tokyo Experimental Animals, Ltd., Tokyo, Japan), weighing 190 to 230 g were housed in groups of 4 in a temperature-controlled (25°C  $\pm$  1°C), specific pathogen-free room. The animals were maintained on a 12-hour light/dark cycle (lights on 8:00 a.m. to 8:00 p.m.) with laboratory rat chow and tap water available *ad libitum*. This study was performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University School of Pharmacy and Pharmaceutical Sciences, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of

Education, Science, Sports and Culture of Japan.

Place conditioning was performed as described previously<sup>36</sup>. The apparatus consisted of a shuttle box (30 cm wide  $\times$  60 cm long  $\times$  30 cm high) made of acrylic resin sheet and divided into 2 compartments of equal size. One compartment was white with a textured floor, and the other was black with a smooth floor to create equally preferable compartments. The experimental sessions on days 1 and 2 were performed by raising the partition that separated the two compartments to 12 cm above the floor and inserting a neutral platform in the seam separating the compartments. Then the rats were allowed to move freely in the shuttle box for 900 seconds. On day 3, conditioning pre-tests were performed. Rats that had not been treated with either drugs or saline were placed on the platform, and the time spent in each compartment during a 900-second session was measured automatically in a blinded fashion with an infrared beam sensor (KN-80, Natsume Seisakusho, Tokyo, Japan). The position of the rat was defined by the position of its body. All sessions were conducted under conditions of dim illumination (40 lux) and masking white noise.

Conditioning sessions were performed on days 4 to 9. The rats were given subcutaneous (s.c.) injections on day 4 with a volume of saline equal to that of ethanol used for the conditioning session. The rats were then immediately confined to the preferred compartment of the place conditioning apparatus for 50 minutes. On day 5, the rats were treated with ethanol (0.075–1.2 g/kg, i.p.) or morphine (0.1–1 mg/kg, s.c.) and were confined to the nonpreferred compartment for 50 minutes. Three conditioning sessions were performed with a total of 3 injections of drug and 3 injections of saline. The control rats were given injections of saline instead of ethanol in the conditioning session. On day 10, both pre-testing and post-testing conditioning were performed. The time spent in each compartment during the 900-second session was again measured. The degree of place preference was estimated by subtracting the time spent on the nonpreferred side (ethanol-injected side) during the preconditioning session from the time spent there during the postconditioning session. In the combination tests,

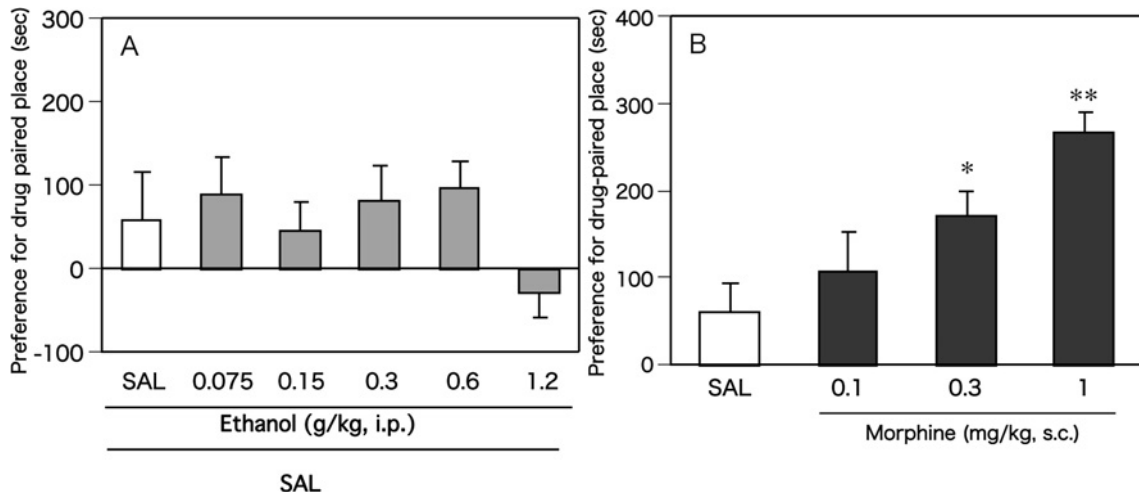


Fig. 1 A: Motivational effect of ethanol combined with saline (SAL) in rats. Rats were given injections of saline (1 mL/kg, s.c.) 30 minutes before treatment with ethanol (0.075–1.2 g/kg, intraperitoneal [i.p.]). Each column represents the mean conditioning score and S.E.M. of 8 to 16 rats. B: Place preference conditioning produced by morphine in rats. Rats were given injections of morphine (0.1–1 mg/kg, s.c.). Each column represents the mean conditioning score  $\pm$  S.E.M. of 8 to 16 rats. \* $P < 0.05$ , \*\* $P < 0.01$  vs. saline-treated group.

morphine (0.03–0.1 mg/kg, s.c.) was injected 30 minutes before ethanol injection. In the antagonism tests, either naloxone (0.03–0.3 mg/kg, s.c.) or naltrindole (1.0 mg/kg, s.c.) was injected 30 minutes before ethanol injection. Osmotic minipumps (Alzet model 2001, Durect Corp., Cupertino, CA, USA) with a flow rate of 1.0  $\mu$ L/h were used for long-term s.c. infusion of SCH23390 at a constant rate (1.0 mg/kg/day), according to our previous report<sup>36–38</sup>. Rats were anesthetized with diethylether to permit s.c. implantation of a minipump. Conditioning sessions were performed in the presence of pumps on the day after implantation.

The drugs used were ethanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan), morphine hydrochloride (Sankyo Co., Tokyo, Japan), naloxone hydrochloride (Research Biochemicals Inc., Wayland, MA, U.S.A.), naltrindole methanesulfonate, (Toray Industries, Tokyo, Japan), and SCH23390 [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1 H-3-benzazepine-OH] hydrochloride (Research Biochemicals, Inc.). Ethanol was diluted in saline to 20% (v/v); morphine, naloxone, and naltrindole were dissolved in saline; and SCH23390 was dissolved in 30% dimethylsulfoxide in water. All drugs refer to the salt forms in aqueous solution.

Conditioning scores represent the time spent in

the post-conditioning score of the drug-injected place minus the time spent in the pre-conditioning score of there and are expressed as the mean(s)  $\pm$  S.E.M. Behavioral data were statistically evaluated with one-way analysis of variance (ANOVA) followed by a Newman-Keuls *post hoc* test or two-way ANOVA followed by Bonferroni's *post hoc* test. A  $P$  value of  $< 0.05$  was considered to reflect significance.

## Results

Rats treated with saline did not exhibit any place preference (Fig. 1A and B). Ethanol (0.075–1.2 g/kg) did not produce either significant place preference or place aversion ( $F_{5,42}=1.03$ ,  $P > 0.05$ , Fig. 1A). On the other hand, morphine (0.1–1.0 mg/kg) produced a dose-dependent place preference ( $F_{3,34}=10.12$ ,  $P < 0.01$ ), with significant place preference produced with 0.3 mg/kg ( $P < 0.05$ ) or 1.0 mg/kg ( $P < 0.01$ ) of morphine (Fig. 1B).

In combination tests, ethanol (0.075–0.6 g/kg) and morphine (0.1 mg/kg) induced a significant place preference ( $F_{1,64}=4.68$ ,  $P < 0.05$ ) compared with ethanol alone. There was no significant effect of dose ( $F_{3,64}=0.95$ ,  $P > 0.05$ ) or of treatment  $\times$  dose interaction ( $F_{3,64}=0.61$ ,  $P > 0.05$ ). Furthermore, 0.3 g/kg of ethanol and 0.1 mg/kg of morphine induced a

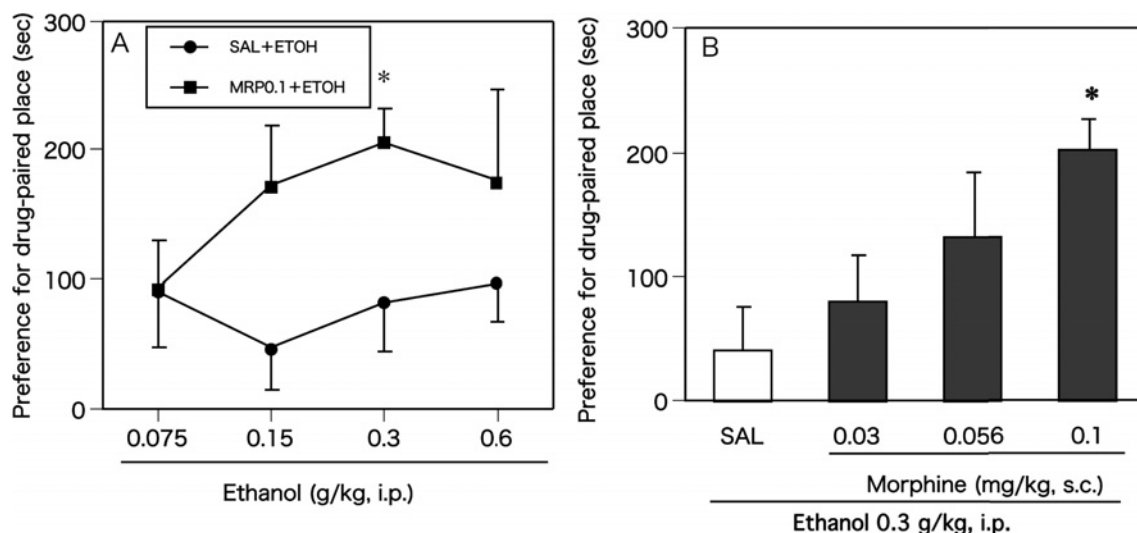


Fig. 2 **A:** Effect of morphine (MRP) on ethanol (ETOH)-induced place preference in rats. Rats were given injections of saline (1 mL/kg, s.c.) or morphine (0.1 mg/kg, s.c.) 30 minutes before treatment with ethanol (0.075–0.6 g/kg, i.p.). Each point represents the mean conditioning score and S.E.M. of 8 to 16 rats. \* $P < 0.05$  vs. group treated with ethanol (0.3 g/kg) and saline.

**B:** Effect of morphine on ethanol-induced place preference in rats. Rats were given injections of saline (1 mL/kg, s.c.) or morphine (0.03–0.1 mg/kg, s.c.) 30 minutes before treatment with ethanol (0.3 g/kg, i.p.). Each column represents the mean conditioning score and S.E.M. of 8 to 10 rats. \* $P < 0.05$  vs. ethanol (0.3 g/kg) combined with saline-treated group.

significant place preference compared with saline and 0.3 g/kg of ethanol ( $P < 0.05$ ) (**Fig. 2A**). Although a low dose of morphine (0.1 mg/kg) did not induce any place preference, the combination of ethanol (0.3 g/kg) and morphine (0.03–0.1 mg/kg) induced dose-dependent place preference ( $F_{3,30} = 3.67$ ,  $P < 0.05$ ). Significant place preference was observed with treatment with ethanol (0.3 g/kg) and 0.1 mg/kg of morphine ( $P < 0.05$ , **Fig. 2B**).

In the antagonism tests, place preference induced by ethanol (0.3 g/kg) and morphine (0.1 mg/kg) was dose-dependently attenuated ( $F_{3,28} = 3.47$ ,  $P < 0.05$ ) by naloxone (0.03–0.3 mg/kg,  $P < 0.05$ ). Significant inhibition of place preference was observed with treatment with naloxone at 0.3 mg/kg ( $P < 0.05$ , **Fig. 3A**). Furthermore, pretreatment with naltrindole attenuated the place preference induced by ethanol (0.3 g/kg) and morphine (0.1 mg/kg) ( $P < 0.05$ , **Fig. 3B**). The place preference induced by ethanol (0.3 g/kg) and morphine (0.1 mg/kg) was completely abolished by long-term treatment with 1 mg/kg/day of SCH23390 ( $P < 0.01$ , **Fig. 4**).

## Discussion

In the present study, we investigated the effect of ethanol, administered with a low dose of morphine, on the behavior of rats, and observed that ethanol in combination with low doses of morphine induced a place preference. Marglin *et al.*<sup>39</sup> have also reported that treatment with ethanol in the presence of morphine establishes a significant conditioned place preference in rats. In their study, however, only a single dose of morphine (2 mg/kg) was used. In addition, dose (2 mg/kg) of morphine, which induced a significant place preference compared to control, was used. Therefore, the data presented by Marglin *et al* did not indicate that ethanol in combination with morphine induces a conditioned place preference. In the present study, a range of low doses (0.03–0.1 mg/kg) of morphine did not induce a significant place preference, but low doses of morphine developed ethanol-induced place preference in a dose-dependent manner. These data confirm that ethanol plus small doses of morphine (0.1 mg/kg) induces a conditioned place preference.

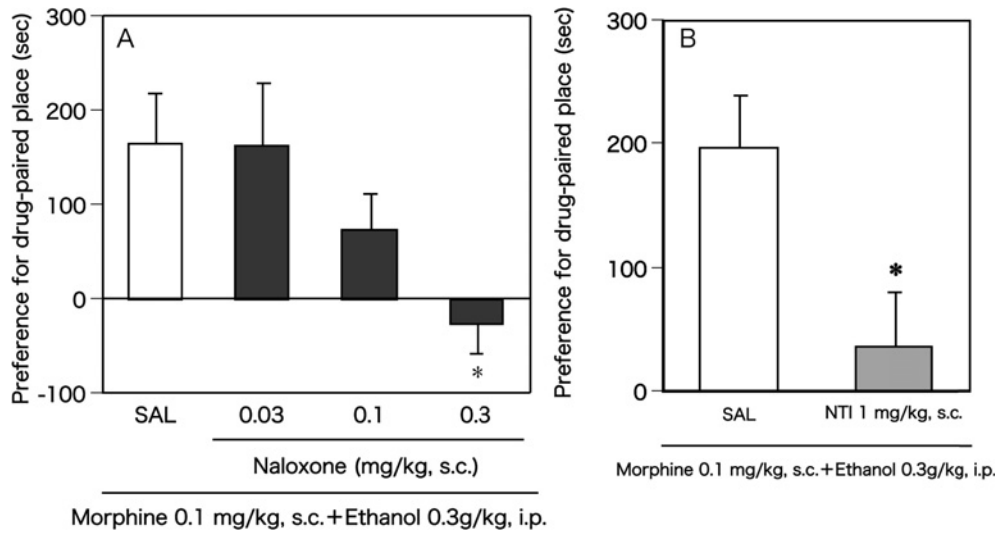


Fig. 3 **A:** Effect of naloxone on place preference induced by ethanol and morphine in rats. Rats received injections of saline (1 mL/kg, s.c.) or naloxone (0.03–0.3 mg/kg, s.c.) just before treatment with ethanol (0.3 g/kg, i.p.). Each column represents the mean conditioning score and S.E.M. of 8 rats. \*P<0.05 vs. group treated with ethanol (0.3 g/kg) and morphine (0.1 mg/kg). **B:** Effect of naltrindole (NTI) on place preference induced by ethanol and morphine in rats. Rats received injections of saline (SAL, 1 mL/kg, s.c.) or NTI (1 mg/kg, s.c.) 30 minutes before treatment with ethanol (0.3 g/kg, i.p.). Each column represents the mean conditioning score and S.E.M. of 8 to 16 rats. \*P<0.05 vs. group treated with ethanol (0.3 g/kg) and morphine (0.1 mg/kg).

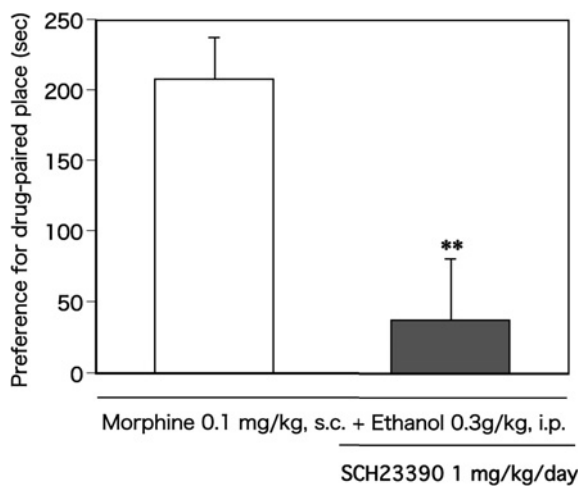


Fig. 4 Effect of long-term treatment with SCH23390 on place preference induced by ethanol and morphine in rats. Rats received long-term treatment with SCH23390 (1 mg/kg/day, s.c.) by means of an osmotic minipump before the start of conditioning. Each column represents the mean conditioning score and S.E.M. of 8 to 12 rats. \*\*P<0.01 vs. group treated with ethanol (0.3 g/kg) and morphine (0.1 mg/kg).

rewarding effects of morphine by up-regulating functional changes in  $\mu$ -opioid receptors, mediated by G protein-coupled receptor-specific serine/threonine kinase 2 (GRK2) in the ventral tegmental area<sup>40</sup>. The most significant place preference observed in the present study was produced by intraperitoneal (i.p.) administration of ethanol at a dose of 0.3 g/kg when combined with morphine. We have also observed that ethanol (0.3 g/kg) combined with pylazole or stress (conditioned fear and foot shock) induced a significant place preference<sup>15,41,42</sup>. A similar dose of ethanol (0.5 g/kg) combined with morphine was observed by Marglin *et al.*<sup>39</sup> to induce a significant place preference. Thus, low doses of ethanol induce a place preference when combined with other treatments in rats. The report of Marglin *et al.* supports our findings that ethanol combined with low doses of morphine induces a conditioned place preference. A high dose of ethanol tended to induce place aversion. Therefore, ethanol combined with morphine did not produce place preference in a dose-dependent manner.

Numerous studies have shown that the endogenous opioidergic system plays an important

Our present laboratory data strongly indicate that prior treatment with ethanol enhances the

role in the rewarding effects of ethanol<sup>17-19,29-31</sup>. These studies suggest that the reinforcing value of ethanol is enhanced when the opioidergic system is activated by morphine. The combined effects of morphine and ethanol are attenuated by pretreatment with naloxone (0.3 mg) and naltrindole (1.0 mg), which produced neither place preference nor aversion in our previous study<sup>42</sup>. Therefore, the endogenous opioidergic system may be responsible for expression of the ethanol-induced rewarding effect.

Several lines of evidence indicate that activation of the central dopaminergic system is critical for the rewarding effects of abused drugs, including ethanol<sup>35,43</sup>. For example, dopamine release in the nucleus accumbens correlates with the operant response to orally self-administered ethanol<sup>44</sup>. Ethanol administered into the nucleus accumbens in rats enhances extracellular dopamine in this structure<sup>45,46</sup>, and this effect can be reversed by systemic administration of naltrexone<sup>34</sup>. Furthermore, the ethanol-induced increase in dopamine release in the nucleus accumbens can be attenuated by pretreatment with opioid-receptor antagonists, such as naloxanazine (an irreversible  $\mu$ -opioid receptor antagonist), naltrindole, and ICI174,864<sup>33,47</sup> (selective  $\delta$ -opioid receptor antagonists). Infusion of the opioid-receptor antagonist methylnaloxonium into the ventral tegmental area inhibits ethanol-induced place preference in mice<sup>48</sup>. Therefore, in the present study, the combined effects of ethanol and morphine were attenuated by pretreatment with naloxone (a nonselective opioid receptor antagonist) or naltrindole (selective  $\delta$ -opioid receptor antagonists). These results suggest that activation of the opioidergic system, including the  $\delta$ -opioidergic system, is related to the rewarding effects of ethanol.

The activation of the central dopaminergic system, especially D1 receptors, may be required for expression of the abused drug-induced place preference<sup>36-38,49</sup>. Therefore, we investigated the involvement of D1 receptors in the effects of ethanol on place preference induced by small doses of morphine. Long-term treatment with SCH23390, a selective D1 receptor antagonist, completely blocked

the place preference induced by ethanol combined with morphine. These data clearly indicate that place preference induced by ethanol combined with morphine is mediated by the mesolimbic dopaminergic system and, in particular, involves D1 receptors.

In conclusion, we have provided additional evidence that ethanol induces a conditioned place preference by activating the central opioidergic system through morphine. Furthermore, place preference induced by combining ethanol with low doses of morphine may be produced by activation of the central dopaminergic system, which involves dopamine D1 receptors via the  $\delta$ -opioidergic system. Opioid-receptor antagonists may be useful for reducing the actions of ethanol in humans through the naltrexone-induced reduction of drinking behavior and alcohol craving and the prevention of relapse in alcohol-dependent persons<sup>30</sup>.

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