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# Prior Repeated Exposure to a 5-$HT_3$ Receptor Agonist Does Not Alter the Ethanol-Induced Conditioned Taste Aversion in Rats

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BIENKOWSKI, P., K. IWINSKA, E. KOROS, I. PANOCKA, J. PIASECKI AND W. KOSTOWSKI. *Prior repeated exposure to a 5-$HT_3$ receptor agonist does not alter the ethanol-induced conditioned taste aversion.* PHARMACOL BIOCHEM BEHAV **59**(4) 975–980, 1998.—Several reports have indicated that the brain serotonergic 5-$HT_3$ receptors are involved in at least some central effects of ethanol in rats. However, using an operant drug discrimination procedure, we have shown that these receptors are not primarily involved in the discriminative stimulus effects of ethanol. The aim of the present study was to further elucidate the role of 5-$HT_3$ receptors in the formation of the ethanol-cueing effects in rats. To this purpose, a crossfamiliarization conditioned taste aversion (CF-CTA) procedure was used. Four daily injections of 1.5 g/kg ethanol (10% v/v) resulted in a significant attenuation of the subsequent ethanol-induced CTA. In contrast, four daily injections of the 5-$HT_3$ receptor agonist, 1-(*m*-chlorophenyl)-biguanide (mCPBG; 50 μg per rat, ICV) did not alter the subsequent ethanol-induced CTA. The 50 μg dose of mCPBG produced a marked CTA in a control experiment. These results taken together with some previous findings from our laboratory suggest that the brain 5-$HT_3$ receptors do not play any crucial role in the mediation of the discriminative stimulus effects of ethanol. © 1998 Elsevier Science Inc.

Ethanol 5-HT3 receptor 1-(m-Chlorophenyl)-biguanide Crossfamiliarization Conditioned taste aversion Rat

BRAIN serotonin (5-HT) is believed to regulate several behavioral effects of ethanol including development of tolerance and dependence (10,12,23). Specific 5-HT receptor subtypes that might be involved in these effects of ethanol have not been precisely identified yet. Some findings, however, are indicative of at least certain binding sites—particularly the 5-$HT_3$ receptor subtype (7,12,21,23). The 5-$HT_3$ receptor belongs to the "superfamily" of ionotropic receptors and is linked to the cation channel that conducts primarily $Na^+$ and $K^+$ ions (24,26). In a series of in vitro experiments ethanol has been reported to increase the 5-$HT_3$ receptor conductance by an enhancement of 5-HT potency (12,23,24,26). Moreover, a number of behavioral studies have shown that the 5-$HT_3$ receptor antagonists (e.g., ondansetron, tropisetron, bemesetron, and zacopride) may reduce ethanol drinking and preference in various animal species (12,21,23).

Both theory and empirical data support the belief that a drug discrimination procedure is a useful test for identifying receptor mechanisms of drug actions (1,3,8,11,14). Original experiments that made use of this test gave a limited support for the involvement of 5-$HT_3$ receptors in the mediation of the discriminative stimulus effects (the interoceptive cue) of

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ethanol. Bemesetron (MDL 72222) decreased the cueing properties of ethanol in pigeons while tropisetron and zacopride did not (13). Another study with rats showed that pharmacokinetic factors rather than any direct interaction at the receptor level were responsible for the attenuation of the ethanol stimulus by bemesetron (12,15). In line with the latter finding, other studies (22,32) did not reveal any specific role of 5-$HT_3$ receptors in the ethanol cueing effects in rats. 1-(*m*-Chlorophenyl)-biguanide, a potent 5-$HT_3$ receptor agonist [mCPBG; (20,25)], did not substitute for ethanol (32) and the 5-$HT_3$ receptor antagonists (i.e., tropisetron, bemesetron, and ondansetron) did not attenuate the ethanol cueing effects (22,32).

A crossfamiliarization conditioned taste aversion (CF-CTA) procedure has been proposed as an alternative method for assessing stimulus similarities between drugs (2,9,10). In the CF-CTA procedure the taste aversion conditioning induced by a given substance is weakened by preexposure to another substance with similar stimulus effects (2,9,10,17). Importantly, several other explanations of the crossfamiliarization phenomenon like drug tolerance or associative blocking, have also been considered (5,10,17). For instance, according to the associative blocking explanations prior paring of the environmental cues with drug action serves to attenuate subsequent attempts to condition a taste–drug association in the presence of the same environment (17).

It has recently been suggested that some aspects of the ethanol cue identified with the CF-CTA test may be overlooked by the operant drug discrimination procedure (9). Thus, to extend our previous findings from the operant drug discrimination test (32) we decided to study the role of central 5-$HT_3$ receptors in the ethanol cueing effects using the CF-CTA paradigm. To this end, rats received four daily injections of the 5-$HT_3$ receptor agonist, mCPBG before a subsequent taste aversion conditioning with ethanol (CF-CTA experiment). Because mCPBG has been reported to poorly penetrate the blood–brain barrier (19,25) this compound was administered ICV in the present study. Importantly, it has been repeatedly shown that central effects of mCPBG might be completely prevented by selective 5-$HT_3$ receptor antagonists (28,29). The 50 μg dose of mCPBG used in the CF-CTA experiment was chosen on the basis of our previous behavioral studies (18,32) and a separate CTA experiment. This dose of mCPBG significantly decreased locomotor activity in the "open field" test and reduced water drinking in the Vogel conflict test (18). Lower dose of mCPBG (35 μg) has been shown to suppress the rate of responding in the drug discrimination procedure (32). In the present study, 50 μg mCPBG have been shown to possess marked aversive stimulus effects in the CTA experiment (see below).

In addition, in a control experiment an ability of ethanol preexposure to attenuate the ethanol-induced CTA was tested.

## METHOD

### *Subjects*

Male Wistar rats (280–330 g at the beginning of each experiment) were used. The animals were supplied by a licensed breeder (HZL, Warsaw, Poland) at least 2 weeks prior to the start of the investigation. During the procedure, the animals were housed individually in wire cages (20 × 25 × 28 cm) with two removable drinking tubes (of 100 ml content each) mounted at the front. (The liquids available in the tubes will be described below.) Standard lab chow (Bacutil, Poland) was available ad lib. The subjects were kept in constant laboratory conditions at 22 ± 1°C, 60% humidity, and a 12 L:12 D cycle (lights on at 0700 h). All experiments were conducted between 1000–1400 h. Separate groups of rats were used in every experiment. All procedures used in the study were approved by our institutional ethical committee.

### *Surgery*

The rats were anesthetized with ketamine (75 mg/kg, IP; Gedeon Richter Ltd., Budapest, Hungary) and placed in a Stoelting stereotaxic apparatus with the incisor bar set 3.3 mm below the horizontal plane. Stainless steel guide cannulae (22 gauge) were implanted to terminate 2.0 mm above the final place of injection. The cannulae were attached to the skull with dental cement and stainless steel cranial screws and were sealed with removable stylets. The stereotaxic coordinates used for the lateral ventricle were: P = −0.8 mm from bregma, L = 1.5 mm lateral to the midline, and V = 1.5 mm from the skull surface (27). A recovery period of at least 7 days was used between the surgery and the experimentation. During this period the animals were regularly handled. Upon the completion of the experiment the rats were deeply anesthetized with ketamine (>100 mg/kg, IP) and injected ICV with a blue dye solution. The brains were removed and sectioned on a freezing microtome. The placement of the cannulae tip and the location of the dye were assessed by means of a magnifying glass. Only those animals with the correct cannulae placements and distribution of the dye in the lateral ventricle were included into the statistical analysis.

For ICV injections of mCPBG the 28 gauge internal cannula (C313I, Plastics One, Inc., Roanoke, VA) was connected with a tubing to a 10 μl microsyringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) mounted on an infusion pump (CMA/100, Microdialysis AB, Stockholm, Sweden). The drug or its vehicle was injected in the volume of 5 μl (2.5 μl/min). Additional 60 s were allowed for drug diffusion away from the cannula.

### *Procedure*

*mCPBG-induced conditioned taste aversion (CTA).* The animals were randomly assigned to three experimental groups ($n$ = 9–11 rats) differing in treatment received immediately after the conditioning session. The CTA procedure was similar to that described by others (10,16), and was essentially the same as the procedure used in our previous study (4). During the training period (6 days) the animals received tap water for 20 min/24 h. After six daily training sessions the water intake stabilized and the conditioning sessions (CSs; one-bottle tests) started. The CSs consisted of a 20-min access to 0.1% w/v saccharin solution (saccharin sodium salt; Aldrich, Dorset, UK) in place of water. The volume (ml) of saccharin consumed was measured for each rat. Depending on the group, the rats were injected ICV with mCPBG (10 or 50 μg per rat) or saline immediately after the CS 1 and 2. No drugs were given after the CS 3. The CSs were repeated every 48 h. On the intervening days the animals had access to water for 20 min and no drugs were administered. Twenty-four hours after the CS 3, a two-bottle test (TBT) was performed. During the TBT the subjects had unlimited access to both water and saccharin solution for 20 min. Preference (%) of saccharin was calculated for each rat as a ratio of saccharin consumption to total fluid intake (saccharin + water). Other experiments were conducted in accordance with this general procedure.

*Ethanol-induced CTA after ethanol preexposure.* Before the start of the experiment, the subjects were randomly assigned to four experimental groups ($n$ = 8–9 rats) differing in treatment received during the training period and the CSs: the

SAL-SAL, the EtOH-SAL, the SAL-EtOH, and the EtOH-EtOH group (SAL = saline, EtOH = ethanol). During the training period, 1.5 g/kg ethanol (10% v/v, 19.5 ml/kg) or saline were injected IP, at least 1 h after the 20-min water drinking session. There were four ethanol or saline injections that commenced every 24 h from the third to the sixth day of the training period. During the conditioning sessions (CS 1–2) the animals received 1.5 g/kg ethanol or saline immediately after the 20-min saccharin drinking period.

*Ethanol-induced CTA after mCPBG preexposure (CF-CTA experiment).* Before the start of the experiment, the subjects were randomly assigned to four experimental groups ($n$ = 8–10 rats) differing in the treatment received during the training period and the CSs: the SAL-SAL, the mCPBG-SAL, the SAL-EtOH, and the mCPBG-EtOH group. During the training period, mCPBG (50 μg per rat) or saline were injected ICV at least 1 h after the 20-min water drinking session. There were four mCPBG or saline injections that commenced every 24 h from the third to the sixth day of the training period. During the conditioning sessions (CS 1–2) the animals received IP 1.5 g/kg ethanol or saline immediately after the 20-min saccharin drinking period.

*Drugs*

The ethanol solution was prepared from 95% stock solution and saline (0.9% NaCl). 1-(*m*-Chlorophenyl)-biguanide hydrochloride (RBI, Natick, MA) was dissolved in saline. All solutions were prepared immediately prior to use and the doses of mCPBG refer to the salt form.

*Statistics*

In the CTA experiment with mCPBG, the data from the CSs (saccharin consumption in millilitres) were compared by a two-way ANOVA (treatment × session) with repeated measures on CSs (within-subject factor). The data from the TBT (percentage of saccharin preference) were analyzed with a one-way ANOVA. In other experiments, a three-way (pretreatment × treatment × session) or a two-way ANOVA (pretreatment × treatment) was used for the data from the CSs and the TBTs, respectively. (Both treatment and pretreatment were treated as between subject factors.) Newman–Keuls test was used for individual post hoc comparison.

## RESULTS

*mCPBG-Induced CTA*

As revealed by the one-way ANOVA, mean water intakes and body weights did not differ ($F < 1$) between the three experimental groups on the sixth day of the training period (data not shown).

The two-way ANOVA on the data from the CSs (Fig. 1A) revealed a significant effect of session, $F(2, 50) = 13.31$, $p < 0.001$, and a significant treatment × session interaction, $F(4, 50) = 4.28$, $p < 0.01$. The effect of treatment missed significance, $F(2, 25) = 3.06$, $p = 0.064$. The group treated with 50 μg mCPBG showed a significant reduction in its saccharin intake during the CS 3 ($p < 0.01$ vs. the saline-treated control group). The one-way ANOVA on the data from the TBT indicated a significant effect of treatment, $F(2, 25) = 4.31$, $p < 0.05$ (Fig. 1B). The group treated with 50 μg mCPBG revealed a significant reduction in its saccharin preference ($p < 0.05$ vs. the saline-treated control group). During the CS 3, 50 μg mCPBG produced CTA response comparable with that induced by 1.5 g/kg ethanol [(4); present results]. However, 50 μg mCPBG did not induce significant CTA during the CS 2, whereas ethanol consistently did (see below).

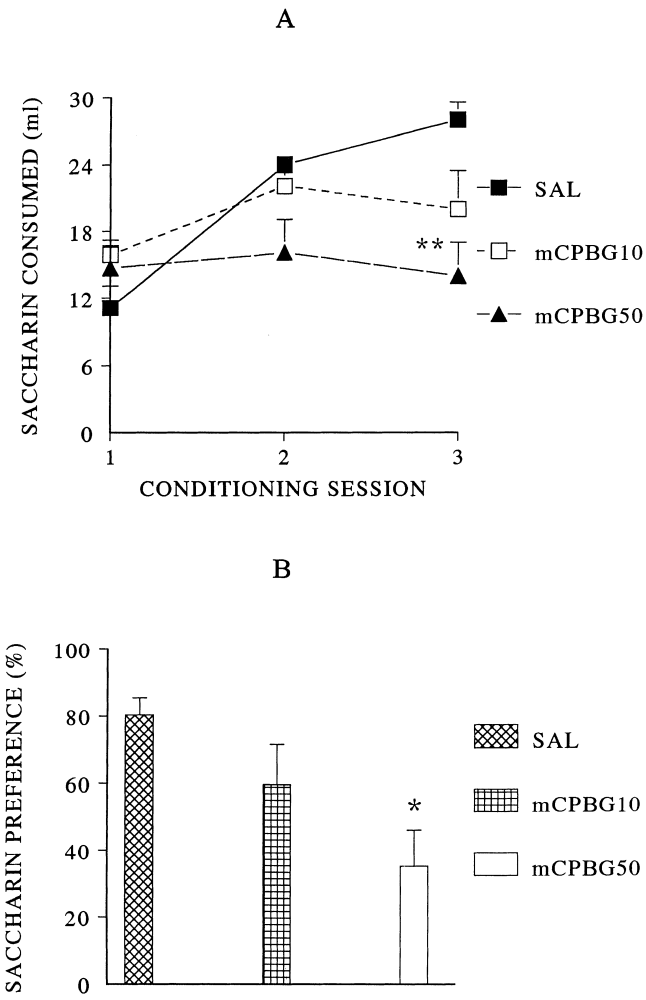


FIG. 1. Results of the conditioned taste aversion experiment with the 5-HT$_3$ receptor agonist, 1-(*m*-chlorophenyl)-biguanide (10 or 50 μg; ICV). Results from conditioning sessions (CS 1–3) are expressed as mean (±SEM) saccharin consumption (A). Results from the two-bottle test (TBT) are expressed as mean (±SEM) saccharin preference (B). *$p < 0.05$, *$p < 0.01$ vs. the saline-treated control group; $n$ = 9–11 rats. mCPBG = 1-(*m*-chlorophenyl)-biguanide.

*Ethanol-Induced CTA After Ethanol Preexposure*

As revealed by the one-way ANOVA mean water intakes and body weights did not differ between the four experimental groups on the sixth day of the training period ($F < 1$).

The three-way ANOVA on the data from the CSs indicated a significant effects of pretreatment, $F(1, 23) = 4.70$, $p < 0.05$; treatment, $F(1, 23) = 21.83$, $p < 0.001$, and session, $F(2, 46) = 11.59$, $p < 0.001$. Also, some interactions were significant, i.e., pretreatment × treatment, $F(1, 23) = 9.52$, $p < 0.01$; pretreatment × session, $F(2, 46) = 15.81$, $p < 0.001$, and treatment × session, $F(2, 46) = 15.79$, $p < 0.001$. The SAL-EtOH group decreased its saccharin intake during the CS 2 and 3 ($p < 0.01$). In contrast, the EtOH-EtOH group did not differ from the EtOH-SAL and the SAL-SAL group, al-

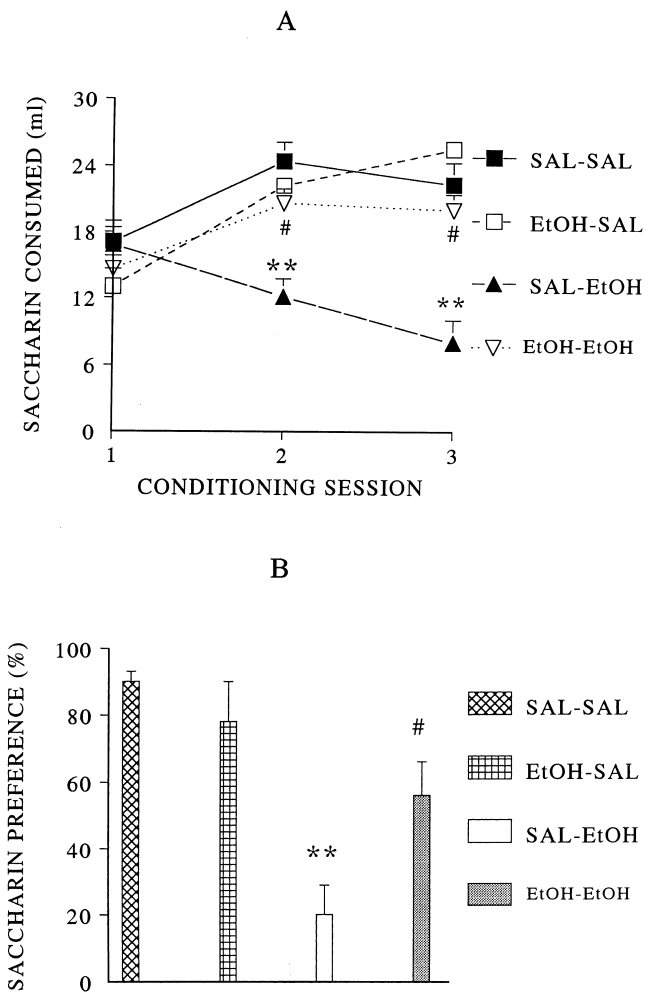


FIG. 2. Ethanol-induced conditioned taste aversion after ethanol preexposure. Results from conditioning sessions (CS 1–3) are expressed as mean (±SEM) saccharin consumption (A). Results from the two-bottle test (TBT) are expressed as mean (±SEM) saccharin preference (B). *$p < 0.05$, **$p < 0.01$ vs. the respective control group; #$p < 0.01$ vs. the SAL-EtOH group; $n$ = 8–9 rats.

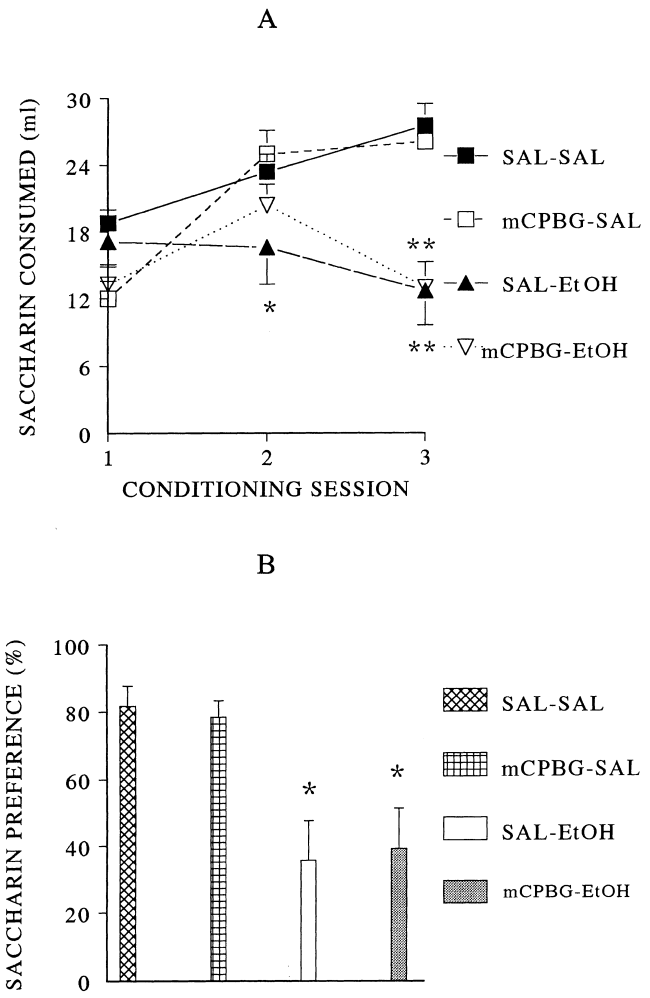


FIG. 3. Ethanol-induced conditioned taste aversion after 1-(*m*-chlorophenyl)-biguanide preexposure (crossfamiliarization experiment). Results from conditioning sessions (CS 1–3) are expressed as mean (±SEM) saccharin consumption (A). Results from the two-bottle test (TBT) are expressed as mean (±SEM) saccharin preference (B). *$p < 0.05$, **$p < 0.01$ vs. the respective control group; $n$ = 8–10 rats. mCPBG = 1-(*m*-chlorophenyl)-biguanide.

though it differed significantly from the SAL-EtOH group ($p < 0.01$) (Fig. 2A). The two-way ANOVA on the data from the TBT showed a significant effect of treatment, $F(1, 23) = 24.99$, $p < 0.01$, and a significant pretreatment × treatment interaction, $F(1, 23) = 6.94$, $p < 0.05$. The pretreatment effect was not significant ($p > 0.2$). the saccharin preference of the SAL-EtOH group was significantly reduced. In contrast, the EtOH-EtOH group did not differ from the saline-treated control groups, although it was significantly different from the SAL-EtOH group ($p < 0.01$) (Fig. 2B). Thus, four daily 1.5 g/kg ethanol injections were capable of reducing the subsequent CTA induced by the same dose of ethanol.

*Ethanol-Induced CTA after mCPBG Preexposure (CF-CTA Experiment)*

The three-way ANOVA showed a significant effect of treatment, $F(1, 25) = 16.41$, $p < 0.001$, and session, $F(2, 52) = 17.52$, $p < 0.001$. Neither the pretreatment effect nor its interactions with the treatment effect were significant (pretreatment × treatment, $F < 0.7$, $p = 0.4$; Pretreatment × Treatment × Session, $F < 0.1$, $p = 0.9$) (Fig. 3A). Both groups treated with ethanol after the CSs (SAL-EtOH, mCPBG-EtOH) reduced their saccharin intakes when compared with the saline-treated control groups (SAL-SAL, mCPBG-SAL). There were no differences between the SAL-EtOH and the mCPBG-EtOH group.

The two-way ANOVA on the TBT data revealed a significant effect of Treatment, $F(1, 25) = 18.24$, $p < 0.001$. Neither the effect of Pretreatment nor the interaction was significant ($F < 0.3$, $p > 0.6$) (Fig. 3B). Both groups treated with ethanol after the CSs showed a significantly lower saccharin preference when compared with the saline-treated control groups.

## DISCUSSION

The 50 μg dose of mCPBG induced significant CTA in the present study. A lower dose of mCPBG (10 μg per rat) did

not produce any taste aversion conditioning. The magnitude of the 50 μg mCPBG-induced CTA, as measured by saccharin drinking during the CS 3, was comparable (considering the percentage of the control group drinking) with the CTA induced by 1.5 g/kg ethanol in the present and previous experiments from this laboratory (4). Some rats treated with the lower dose of mCPBG (10 μg) remained motionless for at least 20–30 min after the microinjection. A clear-cut reduction of locomotor activity was observed after the higher dose of the drug. Our observations are in agreement with a previous report (16) showing that some rats remained virtually motionless after the ICV administration of 10 μg mCPBG. In this latter study, mCPBG and the structurally related 5-HT$_3$ receptor agonist, 1-phenylbiguanide have been shown to produce taste and place aversion conditioning after peripheral administration (16). More importantly, Higgins et al. (16) have shown that several patterns of behavior induced by centrally administered 1-phenylbiguanide were not attenuated by tropisetron and ondansetron, the selective 5-HT$_3$ receptor antagonists. Notably, both mCPBG and 1-phenylbiguanide have been reported to directly interact with the dopamine transporter (30). In line with the above, the 5-HT$_3$ receptor antagonist, zacopride, only partially attenuated the mCPBG cue in the drug discrimination procedure (11). Thus, although our data indicate that centrally administered mCPBG may produce clear-cut aversive stimulus effects, the receptor mechanisms involved in this phenomenon remain to be established.

In accordance with previous reports (5,9,10), our study demonstrated that the prior history of ethanol treatment attenuated the subsequent ethanol-induced CTA. Thus, four daily injections of 1.5 g/kg ethanol were sufficient for the complete elimination of the taste aversion conditioning evoked by the same dose of ethanol. This effect, named "familiarization" (2,9,10), has been proposed to model stimulus similarities between drugs (see the introductory paragraphs).

Pretreating rats with 50 μg mCPBG did not affect the ethanol-induced CTA in the present study. These results are in line with our previous findings from the standard two-lever drug discrimination procedure (32). Thus, the ICV administered 5-HT$_3$ receptor agonist, mCPBG, failed to mimic the ethanol stimulus either in the operant drug discrimination or in the CF-CTA procedure. In line with the above, none of the 5-HT$_3$ receptor antagonists tested [(22,32); but see also (15)] blocked the cueing effects of ethanol in the rat. Taken together, these results lead to the conclusion that the central 5-HT$_3$ receptors are not of primary importance for the formation of the ethanol interoceptive cue. Further studies are, however, needed to elucidate the role of 5-HT$_3$ in the mediation of the ethanol cue because only one 5-HT$_3$ receptor agonist with questionable selectivity (30) was used in this and the previous study (32) from our laboratory. Besides, according to the mixed, or compound, nature of the ethanol interoceptive cue (3,6,14,31), one could speculate that the elimination of only one component is not sufficient to block the ethanol discrimination by rats (14,33). This hypothesis, if true, might explain unsuccessful attempts to antagonize the ethanol cue with 5-HT$_3$ receptor antagonists (3,14,22,32,33).

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