Contribution of the α5 $GABA_A$ receptor to the discriminative stimulus effects of propofol in rat

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Propofol as an agonist of $GABA_A$ receptor has a rewarding and discriminative stimulus effect. However, which subtype of the $GABA_A$ receptor is involved in the discriminative stimulus effects of propofol is still not clear. We observed the effects of an agonist or an antagonist of the subtype-selective $GABA_A$ receptor on discriminative stimulus effects of propofol. Male Sprague-Dawley rats were trained to discriminate 10 mg/kg (intraperitoneal) propofol from intralipid under a fixed-ratio 10 schedule of food reinforcement. We found that propofol produced dose-dependent substitution for propofol at 10 mg/kg, with response rate reduction only at a dose above those producing the complete substitution. CL218,872 (1–3 mg/kg, intraperitoneal), an α1 subunit-selective $GABA_A$ receptor agonist, and SL651,498 (0.3–3 mg/kg, intraperitoneal), an α2/3 $GABA_A$ receptor selective agonist, could partially substitute for the discriminative stimulus effects of propofol (40–80% propofol-appropriate responding). Meanwhile, L838,417 (0.2–0.6 mg/kg, intravenous), a α2/3/5 $GABA_A$ receptor selective agonist, could produce near 100% propofol-appropriate responding and completely substitute for propofol effects. Moreover, the administration of L655,708, the α5 $GABA_A$ receptor inverse agonist, could dose dependently attenuate the discriminative stimulus of propofol. In contrast, the α1 $GABA_A$ receptor antagonist β-CCt (1–3 mg/kg) combined with propofol (10 mg/kg) failed to block the propofol effect. The data showed that propofol produces discriminative stimulus effects in a dose-dependent manner and acts mainly on the α5 $GABA_A$ to produce the discriminative stimulus effect. *NeuroReport* 29:347–352 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Propofol has been used widely for clinic anesthesia and sedation because of short-acting and quick effectiveness. Clinical data indicate that propofol may make individuals feel good, relaxed, and euphoric [1]. Also, the rewarding characteristics of propofol have been reported in studies using conditioned place preference [2,3] and self-administration [4,5]. These results of studies indicate that propofol has psychic dependence and abuse potential.

In a drug-discriminative paradigm, the subjects recognize the effects of a drug by behavioral responses emitted to obtain reward. Propofol is an agonist of $GABA_A$ receptor, and also exerts discriminative stimulus (DS) effects [6]. We had found that $GABA_A$ receptors may be involved in propofol self-administration [7]. The $GABA_A$ receptors are assembled from a repertoire of at least 19 subunits, and the majority of $GABA_A$ receptors contain at least one α, β, and γ subunit. $GABA_A$ receptor subtypes have different functions that are determined mainly by a subunit. Several studies have shown that α1 $GABA_A$ receptor exerts a sedative effect, the α2/3 $GABA_A$ receptor is involved in anxiolytic effects, and the α5 $GABA_A$ receptor is mainly involved in the memory processes [8,9] and chronic pain [10]. The action of α5 $GABA_A$ receptors is greater than that of α1 $GABA_A$ receptors in a rhesus alcohol discrimination model [11], whereas the α1 $GABA_A$ receptor plays a major role in the muscle relaxant carisoprodol discriminative effect [12]. However, the contribution of the $GABA_A$ receptor subtypes toward DS effects of propofol is still unclear. The aim of this study was to investigate the characteristics of different α subunits of the $GABA_A$ receptor in the DS effects of propofol.

Materials and methods

Animals

One hundred and fifty-six male Sprague-Dawley rats (14 weeks of age) were obtained from the Experimental

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Animal Center of Zhejiang Province (Hangzhou, China). All rats were housed individually in home cages and maintained on a 12/12-h dark/light cycle with light on from 8 : 00 a.m. to 8 : 00 p.m. The animal weights were maintained at 250–300 g and water was provided freely. We followed the Principle of Laboratory Animal Care (NIH publication no.86-23, 1996). All housing and procedures were approved by the Ningbo Addiction Research and Treatment Center (Ningbo, China).

Drugs

Propofol (10 mg/ml, Diprivan; AstraZeneca, Basiglio, Italy) was prepared immediately in 20% intralipid before use and injected intraperitoneally at 1 ml volume. According to the earlier study [6], the dose of propofol (10 mg/kg, intraperitoneal) was chosen for the discrimination testing. β-CCt (Cat. No. SML0249), L655,708 (L9787), and L838,417 (L9169) were obtained from Sigma-Aldrich Inc. (St Louis, Missouri, USA). CL218,872 (1709) or SL651,498 was purchased from Tocris Inc. (Minneapolis, Minnesota, USA) or Sanofi-Aventis Inc. (Paris, France), respectively. β-CCt and L838,417 were dissolved in 50% propylene glycol in a 50% saline solution; CL218,872, L655,708, and SL651,498 were diluted to an 80% propylene glycol and 20% saline solution.

Nose-poke discrimination test

The procedure for training and experimental sessions was performed in ventilated and sound-attenuating Plexiglas chambers. Each chamber was equipped with two nose-poke holes, both of which contained a photocell and a yellow cue light. Between the holes was a tray for food delivery. A computer controlled the scheduling of reinforcement contingencies, reinforcement delivery, and data recording. Before discrimination training, each rat was exposed to a fixed-ratio (FR) schedule of food reinforcement to get them used to the sound and place of food pellet delivery. Initially, a white house light was turned on and rats were trained under an FR-1 schedule, and then the FR value was increased to the final FR-10 gradually. The completion of the corresponding response schedule on the active hole resulted in food delivery and initiated a 3 s timeout during which nose-poke could not produce food delivery. Responses on the inactive poke produced no food, but they were recorded. After each timeout, the white light was turned on again and the next trial was begun. At the end of the preliminary training, the rats could earn one food pellet by active nose-poke 10 times consecutively. Every training session ended after 30 min or 100 food pellet rewards irrespective of which occurred first. Once rats established stable response rates (complete 100-time food reinforcement in 30-min training sessions on 2 consecutive days), the drug-discrimination training was started.

In discrimination training sessions, propofol or 20% intralipid as a vehicle solution was injected randomly to ensure that no olfactory cues related to two pokes would bias the discrimination.

Propofol (10 mg/kg) or vehicle (20% intralipid) was administered 20 min before the training sessions and then placed in the experimental chamber. All rats were exposed to training sessions in the double alternation, which consisted of 2-day propofol, followed by 2-day vehicle. One nose-poke was set to produce food reinforcement after an injection of propofol, and conversely, another poke to produce a food pellet after injection of vehicle. Training was started under an FR-1 schedule and the FR value increased gradually to FR-10 until they fulfilled the training criteria (the errorless propofol-appropriate poking responding > 80%). Every session ended after delivering 100 food pellets or ending after 30 min.

Substitute testing

Once the discrimination procedure was performed, rats were surgically implanted with catheters in the external jugular vein to administer the testing drugs intravenously using a previously described method [13]. After recovery from surgery, all rats restarted the discrimination drug test till they fulfilled the criterion of 80% accuracy under the FR-10 schedule [11,14]. Once the rats reliably discriminated propofol from intralipid for two consecutive training sessions, dose–response effects of propofol were tested. The rats were divided into four groups ($n = 8$) randomly to test with intralipid or 5.0, 7.5, 10, and 15 mg/kg propofol by an intraperitoneal injection. The test session was the same as the training sessions, except that 10 successive poking on either hole led to a food pellet delivery. Other rats ($n = 6$, each group) were tested with the $GABA_A$ receptor agonists that were administered 30 min before the test: CL218,872 (1–3 mg/kg, intraperitoneal), L838,417 (0.2–0.6 mg/kg, intravenous), and SL651,498 (0.3–3 mg/kg, intraperitoneal).

Antagonism testing

Antagonism testing ($n = 6$ per group) was performed as follows: propofol (10 mg/kg, intraperitoneal) was injected at 20 min before testing and β-CCt (1.0–3 mg/kg, intravenous) or L655,708 (0.5–2 mg/kg, intravenous) was administered 5 min before the start of testing, respectively.

Statistical analysis

Drug-discrimination data are expressed as the mean percentage of responses on the propofol-appropriate poke in each test period. Response rate was expressed as a function of the number of responses made divided by the total session time. To assess the degree of similarity in DS effects, complete substitution was defined as at least 80% propofol-appropriate responding and not statistically different from training propofol, and partial substitution as at least 40% and less than 80% propofol-appropriate responding [15]. Data were analyzed using a one-way analysis of variance and the significance level in all analyses was set at P value less than 0.05.

Results

The discriminative stimulus effects of propofol

Thirty-two subjects discriminated reliably propofol (10 mg/kg, intraperitoneal) from vehicle (20% intralipid) in 42.7 ± 1.3 session. In the last session of training, the percentage responding on the propofol-associated pokes was 99.7 ± 0.3% and the response rate was 0.68 ± 0.02. Meanwhile, the percentage responding on the propofol-associated pokes of vehicle training was only 2.5 ± 0.6% and the response rate was 0.83 ± 0.05. Under test conditions, cumulative doses of propofol from 5 to 15 mg/kg increased in the percentage responding on the propofol-associated pokes in a dose-dependent manner (Fig. 1a). The percentage responding on the propofol-associated pokes of propofol at doses of 5, 7.5, 10, or 15 mg/kg was 50.79 ± 12.07, 67.46 ± 13.08, 99.74 ± 0.09, and 99.40 ± 0.15%, respectively. As shown in Fig. 1b, the statistics indicated a significant difference in the response rate among the groups [$F(3,31) = 10.09$, $P < 0.05$] and multiple comparison showed a significant reduction in the response rate of propofol at 15 mg/kg. Therefore, propofol at 10 mg/kg was selected for use in the subsequent substitution tests.

Substitutive effects of $GABA_A$ receptor subtype agonists for propofol

As shown in Fig. 2a, pretreatment with CL218,87, a selective agonist of the α1 $GABA_A$ receptor at doses of 1.0–3.0 mg/kg, could be substituted partially for the DS effects of propofol (10 mg/kg). The statistical analysis indicated a significant difference in propofol-appropriate responding after treatment with CL218,872 [$F(3,23) = 9.30$, $P < 0.01$]. Pretreatment with L838,417, a selective agonist of the α2/3/5 $GABA_A$ receptor at doses of 0.4 and 0.6 mg/kg, could produce near 100% propofol-appropriate responding and fully substitute for the DS effects of propofol, but L838,417 at a dose of 0.2 mg/kg, reached near 80% of propofol-appropriate responding ($P < 0.05$) as shown in Fig. 2b. Pretreatment with SL651,498, an agonist of the α2/3 $GABA_A$ receptor (0.3–3 mg/kg), could partially substitute for the DS effects of propofol [$F(3,23) = 96.08$, $P < 0.01$], but the substitutive effect less than 80% of propofol-appropriate responding in Fig. 2c.

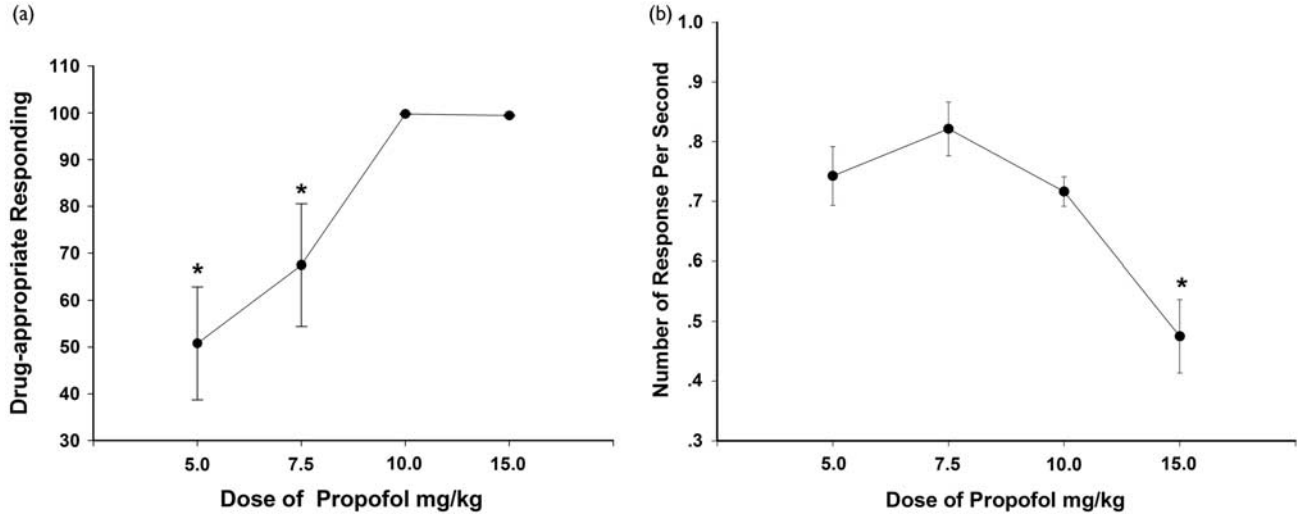
Antagonism effects of antagonist or inverse agonist on the α1 or the α5 $GABA_A$ receptor

Combined with propofol (10 mg/kg), β-CCt, an antagonist of the α1 $GABA_A$ receptor, at doses from 1 to 3 mg/kg partially the DS effects of propofol decreased, but there was no significant difference among the groups [$F(3,23) = 2.99$, $P = 0.055$] as shown in Fig. 3a. In contrast, L655,708, an inverse agonist of the α5 $GABA_A$ receptor, inhibited markedly the propofol responding in a dose-dependent manner [$F(3,23) = 106.027$, $P < 0.001$] as shown in Fig. 3b.

Discussion

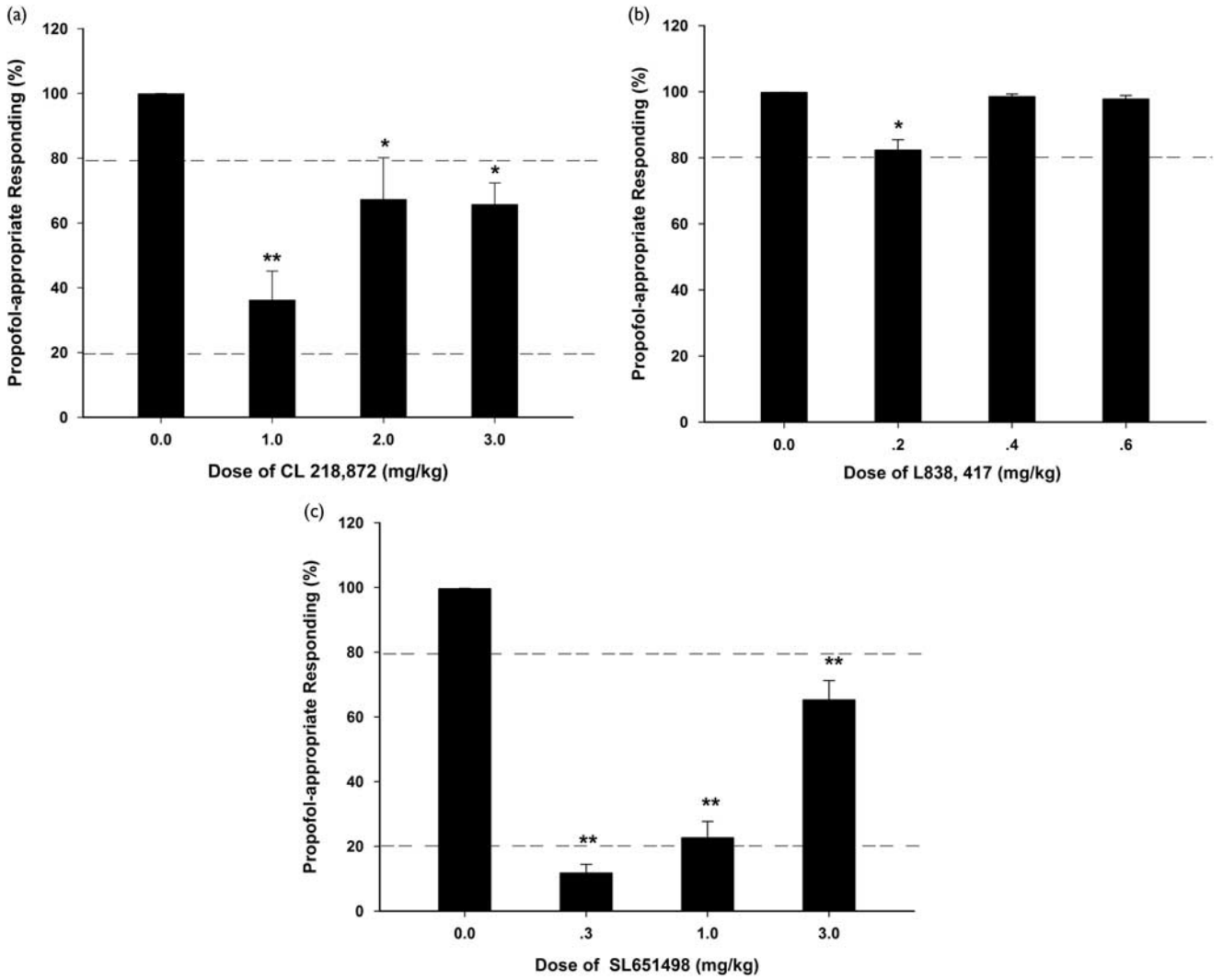
Drug discrimination has remained an important technique in behavioral pharmacology for testing drugs' abuse liability. Here, the DS effects of propofol were investigated in rats trained to discriminate 10 mg/kg propofol from intralipid under a two-poke FR-10 schedule of food reinforcement. Propofol produced dose-dependent substitution for the training dose of 10 mg/kg propofol with response rate reductions only at doses above those

Fig. 1



Discriminative stimulus effects of propofol. Percentage responding decreased at the low dose of propofol shown in (a). The rate of responding reduced at a dose of 15 mg/kg propofol (b). Data are shown as mean ± SEM, $n = 8$ for each group. Compared with the 10 mg/kg propofol group, $^*P < 0.05$.

Fig. 2

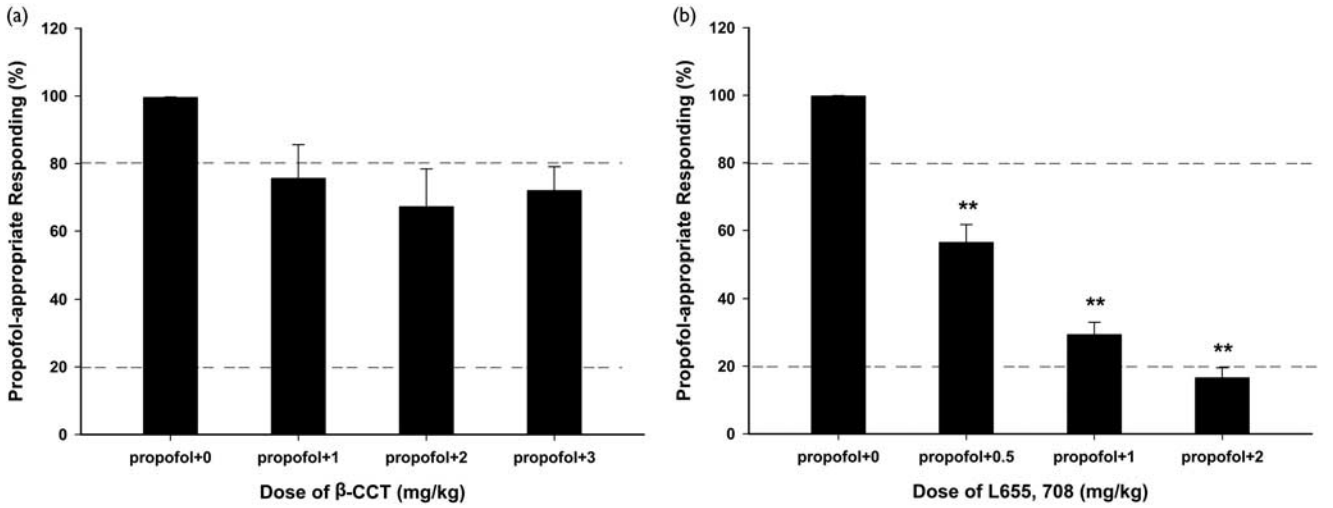


Substitutive effects of $GABA_A$ receptor subtype agonists for propofol. Pretreatment with CL218,872 (a) or SL651,498 (c) only partially substituted propofol DS effects. In contrast, L838,417 (b) treatment could fully substitute the propofol effect. The data are shown as mean ± SEM, $n = 6$ for each group. Compared with the propofol, $^*P < 0.05$, $^{**}P < 0.01$.

producing complete substitution. The result is in agreement with other studies showing the DS effect of propofol [6], and is similar to $GABA_A$ receptor agonist muscimol [16] and carisoprodol [12]. Previous studies showed that subanesthetic doses of propofol can acquire self-administration behavior [7]; thus, both reinforcement and discriminative effects identified could contribute toward the abuse potential of propofol.

The distribution of heterogeneously constituted $GABA_A$ receptor complexes may exert different pharmacological properties upon stimulation by GABA or its agonists. The α1 $GABA_A$ receptor is the major subtype, contributing toward about 60% of all $GABA_A$ receptors in the brain. The evidence confirms the essential roles of α1 $GABA_A$ in sedation, anxiety, and sleep [17]. In the present study, the α1 $GABA_A$ receptor agonist CL218,872 only partly replaced (40–80%) the DS effects of propofol. Similarly, CL218,872 has been shown to partially reproduce the DS effects of ethanol [11], and the α1 $GABA_A$ agonist zolpidem partially reproduced the DS effects of ethanol [18, 19]. Zolpidem also significantly abolishes methamphetamine conditioned place preference formation, indicating that α1 $GABA_A$ receptors may be strongly implicated in drug-associated rewarding memories [20]. The present study showed that the α1 receptor antagonist β-CCt failed to antagonize the DS effects of propofol, which is consistent with the previous studies [11,14,21]. Meanwhile, α2/3 receptor agonists only partly replaced the propofol DS effects. $GABA_A$ receptors containing the α2 or the α3 subunit account for the anxiolytic/

Fig. 3



Antagonism effect of the α1 GABA$_A$ receptor antagonist or the inverse agonist on the α5 GABA$_A$ receptor. β-CCt failed to block the effects of propofol in (a), whereas L655,708 antagonized propofol discrimination (b). The data are shown as mean ± SEM, $n = 6$ for each group. Compared with the vehicle group, $^{**}P < 0.01$.

anticonvulsant effects, and the GABA$_A$ α2 agonist in the ventral hippocampus inhibits anxiety [22]. Thus, the present data suggested that the DS effects of propofol may not be dependent mainly on the activation of α1, α2, or α3 subunit GABA$_A$ receptor.

The sustained increase in α5 GABA$_A$ activity impairs memory performance, and inhibition of the α5 GABA$_A$ receptor completely reverses the memory deficits after anesthesia [23]. The α5 receptor inverse agonist selectively attenuates the effects of GABA at α5 GABA$_A$ receptors and enhances performance in learning and memory [24]. The α5 receptor inverse agonist L655,708 reduces the potentiation of GABA-evoked current by inhaled anesthetics [25]. The present data showed that the α2/3/5 receptor agonist could completely replace the DS effects of propofol and α5 receptor inverse agonist blocked the DS effects of propofol. These results are in agreement with findings that L655,708 completely reverses the DS effects of ethanol [11]. Similarly, other α5 GABA$_A$ agonists also substitute fully for the DS effects of ethanol [11]. Together, these results suggested that α5 subunits of GABA$_A$ play a more important role than α1 and α2/3 subunits in the DS effects of propofol.

Conclusion

These results indicate the pharmacological specificity of propofol discrimination by showing that a direct agonist or an inverse agonist for the α5 GABA$_A$ receptor produces complete substitution or deletes the DS effects of propofol, suggesting that activation of the α5 GABA$_A$ receptor by propofol contributes toward the discriminative effects.

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Conflicts of interest

There are no conflicts of interest.

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