Drug dependence is defined as the loss of control over drug use, or the compulsive seeking and taking of drugs despite adverse consequences (Koob, 1999). It is caused by drug activity in the brain, but relates to physiologic and social factors. Drug dependence can show a life-long effect. Animal experiments can measure two types of drug dependence: physical dependence and psychological dependence. Physical dependence refers to the state resulting from chronic use of a drug, to the point of tolerance, in which negative physical symptoms or withdrawal result from abrupt drug discontinuation or dosage reduction. The jumping behavior test is used to determine a drug’s potential to lead to physical dependence. Psychological dependence indicates non-self restraint of drug use, and involves reinforcement and reward. Reinforcement is an event that increases the probability of a response. Reward has a similar meaning but it is usually related to positive sensations such as pleasure (Koob, 1992). The conditioned place preference test and self-administration test are valid models for investigating the reward effect and reinforcing effect, respectively, of drugs (Mucha et al., 1982; Gorelick et al., 2004). The climbing behavior has been used in many studies as pre-evaluation test to evaluate a drug’s dopaminergic effect.

Propofol is a common anesthetic for conscious sedation or to induce and maintain general anesthesia (Pain et al., 1996; LeSage et al., 2000). Its pharmacological action sites are gamma-aminobutyric acid (GABA) receptors, N-methyl-D-aspartate (NMDA) receptors, and glycine receptors (Iwersen-Berqmann et al., 2001; Nguyen et al., 2009). Propofol shows rapid anesthesia induction and rapid recovery after medical processes (Roussin et al., 2007). However, there are several reports on its dependence potential (LeSage et al., 2000; Pain et al., 2002), and further studies are needed to evaluate the dependence potential and abuse liability of propofol. We therefore performed several animal behavioral tests including climbing behavior, jumping behavior, conditioned place preference test, and self-administration test using experimental mice or rats to assess the dependency of propofol.

**MATERIALS AND METHODS**

**Animals and drugs**

Male Sprague-Dawley rats (180-220 g) and ICR mice (15-20 g) were obtained from Korea Food and Drug Administration.
(AAALAC member, Seoul, Korea) and they were housed in groups, or adequate size, in a temperature-controlled 23 ± 2°C room with a 12 hour light/dark cycle (lights on 08:00 to 20:00). The animals received a solid diet and tap water ad libitum, and their treatment conformed to the Guide for the Care and Use of Laboratory Animals (NRC 1996). We performed all experiments between 09:00 and 18:00. Methamphetamine HCl and propofol were obtained from Sigma (St. Louis, MO, USA).

**Apparatus**

The climbing behavior test apparatus was a stainless steel cylinder with many vertical bars, which an experimental mouse could climb. Its floor diameter was 12 cm, and each vertical bar’s length was 24 cm. To evaluate jumping behavior test, a transparent box sans ceiling, measuring 30×30×40 cm was used.

The conditioned place preference test chamber had three distinct compartments (white, black, and grey) separated by automatic guillotine doors. To automate data collection, 15 infrared photo-beam detectors were added. The overall inside dimensions were 21×21×68 cm, and the unit’s base measured 86.4×25.4 cm. The manufacturer provided the mounting holes for the ENV-013 IR Infrared Sensor Package (Med Inc., USA), which places six photo-beams across the white and black zones, 1.25 cm from each end wall, with 5 cm intervals between the beams. The choice compartments were 28 cm long. One choice compartment was all black, with a stainless steel grid rod floor consisting of 4.8 mm rods on 16 mm centers. The other compartment was all white, with a 1.25×1.25 cm stainless steel mesh floor.

The self-administration test chamber was from Med Inc. (USA) and measured 29×21×24 cm. The chambers contained two levers, an active lever to deliver a drug dose, via the jugular vein, through a connected catheter and an inactive lever, not connected to the experimental animal. Infusion pumps were placed outside the chamber and connected to a 10 ml syringe. We connected the chamber to a computer, to record test data and control the experimental processes.

**Methods**

**Climbing behavior test:** One group of mice was administered with the negative control (saline, 1 mg/kg, i.p.) or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) for 40 min. Then for 1 min, their climbing duration was checked, using a stopwatch. The other group of mice was pre-treated with the negative control (saline, 1 mg/kg, i.p.) or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) for 40 min before the test. Then just before testing, apomorphine (2 mg/kg, i.p.) was administered to each subject and timed their climbing duration as above. The tests were repeated three times, with a time-out period of 10 min.

**Jumping test:** One group of mice was administered the negative control (saline, 1 mg/kg, i.p.), or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) for 40 min and followed by naloxone (10 mg/kg, i.p.). Then for 15 min, the jumping numbers of the animals were counted. The other group of mice was pre-treated with the negative control (saline, 1 mg/kg, i.p.) or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) for 40 min before the test. Next, morphine (150 mg/kg, s.c.) was administered and followed by naloxone administration (10 mg/kg, i.p.) 4 hrs after the morphine treatment. The jumping number was counted for 15 min. The experiment was repeated three times.

**Conditioned place preference test:** Before starting the experiment, the rats were acclimated to the experimental apparatus and handled for 6 days. The procedure was similar to that described previously (Bardo et al., 1995; Narita et al., 2004).

Each experiment consisted of three phases, as follows.

Pre-conditioning: For 2 days (days 1 and 2) the rats were allowed free access to both compartments of the apparatus for 15 min (900 s) each day. One day 2, the time spent by the rats in each compartment was recorded and served as a baseline. The rats showed preference for the black compartment was selected for further experiments and divided into two groups.

Conditioning: Conditioning was conducted for 8 days (days 3 to 10), for one session per day. On day 3, one group of the selected rats was treated with drugs (methamphetamine, 1 mg/kg, i.p., one of the three doses of propofol, 30, 60, and 90 mg/kg, i.p.), and placed in the non-preferred compartment (white) for 30 min. The other group of rats was treated with saline, and placed in the preferred compartment (black) for 30 min. The groups were switched everyday and the same procedure was conducted.

Post-conditioning: On day 11, the rats were allowed to access freely both compartments of the apparatus for 15 min (900 s). The time spent by the rats in each compartment was recorded, with these values serving as a test line.

**Self-administration test:** Surgical procedures were as follows. The rats were anesthetized with pentobarbital sodium (Entobar®). The surgical procedures adhered to aseptic conditions described previously (Weeks, 1972; Mucha et al., 1982). Briefly, a catheter was inserted into each rat's right jugular vein. The catheter exited on the rat’s shoulder. The rats received heparin everyday of the experimental periods. After surgery, each rat recovered for at least 14 days in a controlled cage, receiving a solid diet and tap water ad libitum.

The testing procedures were as follows. The rats self-administered 2 mg/kg of propofol for 3 days to stabilize the response (Picetti et al., 2011). Then the experiment was continued for more than 30 days at 1 mg/kg of propofol in the rats that showed stabilized response. The self-administration test was performed for 6 s followed by 20 s of time-out, during daily 1 h session on a fixed-ratio 1 (FR1) reinforcement schedule. With this schedule, when a rat presses the active lever, it receives a certain drug dose injected into the jugular vein through the catheter. The self-administration chamber contains two levers linked to a computer program which records the experimental data. The vehicle substance (intrlalipid) was used as a negative control.

**Statistics:** The data are expressed as the mean ± S.E. The climbing and jumping data were analyzed via paired t-tests. Likewise, paired t-tests were used to compare time spent in the drug- and saline-paired compartments in the CPP test. To analyze the self-administration test data, a two-way ANOVA was employed (p<0.05).

**RESULTS**

**Climbing behavior test**

We measured climbing behavior in experimental mice with