Effect of Propofol on Affective State as Assessed by Place Conditioning Paradigm in Rats

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Background: Whether propofol produces a pleasant affective state remains unclear from clinical studies. In the current study, the effect on affective state of subanesthetic and anesthetic doses of propofol was assessed at a preclinical level with rats in a place conditioning paradigm. Propofol was compared with methohexitol.

Methods: In the place conditioning paradigm, propofol-induced effect was repeatedly paired with one of two distinguishable compartments of the apparatus, whereas the vehicle-induced effect was repeatedly paired with the other compartment. During a subsequent free-choice test, a preference for the drug-paired compartment over the vehicle-paired compartment would be indicative of pleasant state induced by the drug. For all experiments, the conditioning session lasted 8 days and consisted of four pairings of the drug with one compartment and four pairings of the equivalent volume of vehicle with the other compartment. In experiment 1A, four groups of rats were designated according to the dose of propofol that they received intraperitoneally: 0, 30, 60, or 90 mg/kg. In experiment 1B, the same procedure was used with subanesthetic doses of intraperitoneal methohexitol: 0, 10, 20, or 30 mg/kg. In experiment 2, the rats were conditioned during the recovery period from short-term anesthesia. For one group, anesthesia was induced by propofol (100 mg/kg) whereas for the other group, anesthesia was induced by an equivalent anesthetic dose of methohexitol (40 mg/kg).

Results: In experiment 1A, the 30-mg/kg, 60-mg/kg, and 90-mg/kg groups showed a place preference for the drug-paired compartment, but only the group conditioned with 60 mg/kg propofol significantly differed from the 0-mg/kg group. In experiment 1B, the groups conditioned with methohexitol showed no place preference for the drug-paired compartment. In experiment 2, the rats showed a place preference for the compartment in which they recovered from propofol-induced anesthesia but no place preference for the compartment in which they recovered from methohexitol-induced anesthesia.

Conclusions: Propofol, but not methohexitol, induced a pleasant affective state in rats at subanesthetic doses as well as during recovery from an anesthetic dose. (Key words: Anesthesia: affective state; behavior; place conditioning; side effects. Anesthetics, hypnotic: methohexitol; propofol. Animals: rat.)

PROPOFOL (2,6-dissopropylphenol) is a short-acting intravenous anesthetic† often administered for short-term anesthesia as well as in anesthetic procedures such as colonoscopy‡ or regional anesthesia§ for which conscious sedation is desired. Moreover, in intensive care units, propofol often is given as a sedative agent because it allows rapid recovery after long-term infusion.‡ Propofol is chemically unrelated to other anesthetic agents and the molecular mechanism underlying the hypnotic effects of propofol remains partly unknown. Yet propofol is believed to potentiate gamma-aminobutyric acid-mediated synaptic inhibition by acting at the level of the GABA_A receptor complex.§ However, the precise binding site of propofol remains undetermined, although it is known to differ from that of barbiturates, benzodiazepines, steroids, and etomidate.‡ Recently, it has been proposed that propofol could act by binding directly to the GABA_A chloride channel or to the channel regulatory proteins.

In addition to the anesthetic properties of propofol, various psychological side effects have been occasionally reported during recovery, such as hallucinations, sexual disinhibition, or euphoria.§ However, the most frequent side effect reported by patients during the recovery from propofol is a feeling of well-being.†† In addition, at subanesthetic doses, propofol has been...
found to induce unexpected effects such as antinomically, anxiolytic, and pleasant mood-change effects in patients. Few studies have examined the putative effects of propofol on affective states. To date, in clinical studies, the question of whether propofol has a pleasant effect is still a matter of debate. From our point of view, when dealing with drug-induced affective states, preclinical studies are well-suited to inform the debate because feelings and motivations are submitted to larger interindividual variability in human than in infrahuman species.

The current study was therefore designed to examine the putative pleasant affective state induced by propofol in rats. For this purpose, a place conditioning paradigm, which is a widely used situation in psychophysiology, was employed. Place conditioning is the only paradigm that allows assessment of the affective properties of a drug without the drug being administered to the animal during testing. Thus, it eliminates or reduces the possible sensory or locomotor side effects induced by the drug. Briefly, the place conditioning paradigm uses an apparatus with two distinctive environments. The procedure consists of pairing one of these environments with the effect of a drug and the other one with the effect of the drug vehicle, both over a number of consecutive conditioning sessions. During a subsequent postconditioning test, the animal will exhibit a preference for the drug-paired compartment. i.e., more time will be spent in the drug-paired compartment than in the vehicle-paired compartment, for drugs that induce a pleasant affective state. If a drug elicits an unpleasant affective state, the animal will express this during a postconditioning test by avoiding the drug-paired compartment. In experiment 1A, the putative pleasant properties of propofol in rats were studied at different subanesthetic doses. Experiment 1B was designed to control for any nonspecific effect induced by a short-acting hypnotic agent. For this purpose, experiment 1B was run in a way similar to experiment 1A, but using an alternative hypnotic agent, namely methohexital. In experiment 2, the putative pleasant properties of propofol and methohexital during recovery from an anesthetic dose were compared.

Materials and Methods

Animals

Subjects were 123 male Long-Evans rats (Janvier, France), weighing 300–450 g. They were housed two per cage in a colony room maintained on a 12 h:12 h light-dark cycle (light on at 8:00 AM) with food and water provided ad libitum. For experiments 1A and 1B, rats had been previously used in a taste experiment for which they had received a single injection of lithium chloride and a maximum of two injections of a subanesthetic dose of ketamine and were tested for response to sweetened water in a different apparatus. A delay of 6 weeks was allowed between that experiment and the current one. Previous taste experiments do not affect a place conditioning paradigm. For experiment 2, the rats were previously untested.

Drug Conditions

Preliminary studies have been conducted to determine the hypnotic and anesthetic doses of propofol and methohexital injected intraperitoneally on different Long-Evans male rats weighing 300–450 g. Hypnosis was assessed by the loss of righting reflex within 30 s, lasting at least 5 min. The hypnotic dose causing loss of righting reflex in 50% of rats for propofol and methohexital were 80 and 30 mg/kg, respectively. The anesthetic dose was assessed by the loss of righting reflex within 30 s and the absence of reaction to a tail pinch for at least 5 min. The anesthetic dose was 100 mg/kg propofol or 40 mg/kg methohexital.

In experiment 1A, 10 mg/ml propofol (Diprivan, Zeneca, London, UK) dissolved, in 10% Intralipid, was injected intraperitoneally at four different doses: 0, 30, 60, and 90 mg/kg. In experiment 1B, methohexital (10 mg/ml; Brietal, Lilly, Paris, France), dissolved in 0.9% sodium chloride, was injected intraperitoneally at four different doses: 0, 10, 20, and 30 mg/kg. In experiment 2, an anesthetic dose of 100 mg/kg propofol or 40 mg/kg methohexital was used.

Apparatus

The place conditioning apparatus is one in which rats normally exhibit no spontaneous compartment preference. Each rat was tested and conditioned in one of four wooden place conditioning apparatuses. The apparatus consisted of three compartments (Fig. 1). Two large compartments (A and B, 45 × 45 × 30 cm) were separated by a wooden partition. Each of these compartments had a methyl methacrylate polymer front and distinct roof, walls, and floor. The methyl methacrylate polymer front allowed observation of the animals' behavior during conditioning. One compartment had a white roof, black and white vertical striped walls, and a methyl methacrylate polymer floor covered
Affective State Induced by Propofol

Fig. 1. The place conditioning apparatus consisted of three distinctive compartments: A (white roof, black and white vertical striped walls, methyl methacrylate polymer floor, wooden chips), B (black roof, black walls, wire grid floor), and C (grey roof, walls, and floor). C is adjacent to A and B. Removable partitions could be placed between C and A, and C and B. See text for details.

by wood chips; the other had a black roof, black walls, and a wire grid floor. The third compartment (C: 36 x 18 x 30 cm) was a side painted grey compartment. It was adjacent to the rear of both compartments A and B and had removable wooden partitions between compartments A and B. When the partitions were in place, the rat was confined in one of the large compartments.

When the partition was removed, the animal could move freely between the two large compartments (via compartment C). A detector (IRP124, Talco, Paris, France) at the roof of each compartment was used to locate the infrared radiations emitted by the animal. Recording the number of infrared beams disrupted by the rat permitted a gross evaluation of its locomotor activity within the compartments. The signal was fed into a programmable controller (Sysmac C20, Omron, Paris, France) that added up and recorded the time spent and the locomotor activity of the rat in each compartment.

Procedure

Subanesthetic Doses of Propofol or Methohexital. In experiment 1A, 48 rats were randomly assigned to one of four groups (n = 12) according to the dose of propofol that they received (0, 30, 60, and 90 mg/kg, respectively). The first group (0 mg/kg) was divided into three subgroups (n = 4), each of them receiving a volume of vehicle equivalent to that received by each of the other groups. In experiment 1B methohexital was injected in an identical manner to that used for propofol. Forty-eight rats were randomly assigned to one of four groups (n = 12) according to the dose of methohexital received (0, 10, 20, and 30 mg/kg). Behavioral testing and conditioning started after three daily handling sessions and was always conducted between 2:00 and 7:00 PM. The procedure was divided into three successive phases.

Preconditioning Test. On the first day of the experiment, the partitions were removed. Each rat was placed into the side compartment C and allowed to move freely throughout the apparatus for 15 min. The time spent by the rats and their locomotor activity in each compartment were recorded.

Conditioning. The conditioning phase lasted 8 days and consisted of four pairings of the drug with one compartment (A or B) and four pairings of the vehicle with the other one. During this phase, the partitions between the compartments were in place. Each rat was injected with the drug on one day and with the vehicle on the alternate day. After a 10-min postinjection delay in the home cage, the rats were confined for 30 min in compartment A or B. The order of injection (drug or vehicle) and the number of animals experiencing the drug in a given compartment (A or B) were counterbalanced in each group. For the 0 mg/kg group, the vehicle was paired with both compartments; the compartment considered as the "drug-paired" compartment was randomly assigned before the experimentation to each animal. During this phase, the locomotor activity was recorded for each rat during the initial 15 min.

Postconditioning Test. On day 10, each rat was placed into the side compartment with the partitions opened and was allowed to move freely throughout the apparatus for 15 min. The time spent by the rats and their locomotor activity in each compartment were recorded.

Recovery from Short-term Anesthesia. Twenty-seven rats were assigned to one of two groups according to the drug administered: 100 mg/kg propofol or 40 mg/kg methohexital. The preconditioning test, the conditioning phase, and the postconditioning test were performed as described for the experiments earlier. Anesthesia was achieved by an intraperitoneal injection of propofol or methohexital. During anesthesia, the rats were kept in a laboratory room with the temperature maintained at 24°C. Rats were placed in plastic cages (36 x 40 x 15 cm) in which the floor had been covered with cotton wool. During this period, they were carefully monitored for hypothermia and cardiac or respiratory depressant effects of the drug. Each rat

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was tested every 2 min for recovery of the righting reflex, indicating the end of hypnotic period. Each rat
was then confined for 30 min in compartment A or B. On the alternate day, the rats were injected with vehicle
and later confined for 30 min in compartment A or B, but after a postinjection delay equal to that observed
after drug injection.

**Statistical Analyses**
During the preconditioning and the postconditioning
tests, the dependent variable used to assess the
preference for one compartment was the difference
between the time spent in the drug-paired compart-
ment and the vehicle-paired compartment (drug mi-
minus vehicle). This variable was named “place pre-
ference.” Thus, it takes positive values in the case of
a pleasant affective state induced by the drug and
negative values in the case of an unpleasant affective
state induced by the drug. During the conditioning,
the dependent variable was the pooled locomotor
activity of the rats during the first 15 min of each of
the four conditioning sessions in the drug-paired
compartment on one hand and in the vehicle-paired
compartment on the other hand. In experiments 1A
and 1B, statistical analyses of the place preference
were performed using one-way analysis of variance
followed by a Student-Newman-Keuls multiple range
test. Locomotor activity was tested using two-way
one-way analysis of variance. In experiment 2, analy-
ses of place preference between the two groups
were performed using a one-way analysis of variance.
In all experiments, statistical analyses of the loco-
motor activity were performed using one-way analysis
of variance with repeated measures followed by a
Tukey Studentized range method test.20

**Results**

**Subanesthetic Doses of Propofol**

One rat in the 90-mg/kg group was discarded during
the experiment because of illness. During conditioning,
the mean locomotor activity in the compartment con-
sidered as the drug-paired compartment and the mean
locomotor activity in the compartment considered as
the vehicle-paired compartment did not differ across
the three vehicle subgroups. During the precondition-
ing and postconditioning tests, the place preference
did not differ across the three vehicle subgroups.
Therefore, these three subgroups were pooled for the
further analyses, and therefore were collectively iden-
tified as the 0-mg/kg group.

**Preconditioning Test**

No initial preference for a given compartment was observed, that is, the rats spent roughly the same
in the compartments A, B, and C (302, 299, and 299 s,
respectively). The mean time difference between
the drug-paired compartment and the vehicle-paired
compartment was similar across the four groups: +11,
−28, +3, and −17 s for the 0-, 30-, 60-, and 90-mg/kg
groups, respectively (F(3,43) < 1).

**Locomotor Activity.** Figure 2 depicts the mean act-
ivity in the vehicle-paired compartment and in the
drug-paired compartment during conditioning for the
four doses. For the largest dose, a transient hypnotic
phase of approximately 10 min occurred in 7 of the
11 animals during conditioning during at least one
of the sessions. Analysis of variance for repeated meas-
ures showed a significant effect of dose and compart-
ment on the mean activity (dose: F(3,45) = 27.23, com-
partment: F(1,45) = 138.64, interaction: F(3,45) =
41.49, P < 0.0001 for all). No difference appeared in
the mean activity in the vehicle-paired compartment
between the four groups. Conversely, a dose-dependent
decrease of the mean activity was observed in the drug-
paired compartment. The multiple comparison test
showed that the mean activity in the drug-paired comp-
artment was significantly decreased at the 60-mg/kg
and 90-mg/kg doses compared to the 0-mg/kg dose.

![Chart showing locomotor activity at different doses of propofol](chart.png)

**Doses of Propofol (mg/kg)**

- 0
- 30
- 60
- 90

**Place Preference.** Figure 3 shows the dose-depen-
dent decrease of place preference in the drug-paired
dose, up to 60 mg/kg, with a further increase of 90 mg/kg.
The rat preference for the drug-paired compart-
magnitude less than for 60 mg/kg, which showed a signifi-
cant effect of the drug-paired compartment (F(3,45) = 3.69,
comparison showed that the conditioned place prefer-
ence differed significantly from the 0-mg/kg group.

**Subanesthetic Doses of Propofol**

**Preconditioning Test**

No initial preference for a given compartment was observed, that is, the rats spent roughly the same
in the compartments A, B, and C (304, 301, and 295 s,
respectively). The mean time difference between the
compartment and the vehicle-paired compartment was
similar across the four groups: +7, −2, +3, and −3 s,
and 30- and 90-mg/kg groups, respectively.

**Locomotor Activity.** Figure 4 shows the mean locomotor activity in the vehicle-paired compara-
tment and in the drug-paired compartment for the
different doses of propofol. Error bars mean SEM. *P <
0.05 when compared to the 0-mg/kg group.

![Chart showing locomotor activity at different doses of propofol](chart.png)

**Doses of Propofol (mg/kg)**

- 0
- 30
- 60
- 90

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**AFFECTIVE STATE INDUCED BY PROPOFOL**

![Graph 1](image1)

**Place Preference.** Figure 3 depicts the results observed for the different groups during the postconditioning test. The place preference increased with the dose, up to 60 mg/kg propofol. For the largest dose tested (90 mg/kg), the rats still exhibited a place preference for the drug-paired compartment, but with a magnitude less than for 60 mg/kg. Analysis of variance showed a significant effect of the dose of propofol on place preference (F(3,43) = 2.86, P < 0.05). Planned comparison showed that only the 60-mg/kg group differed significantly from the 0-mg/kg group; that is, a conditioned place preference was observed for the 60-mg/kg group.

**Subanesthetic Doses of Methohexital**

**Preconditioning Test.** No initial preference for a given compartment was observed; that is, the rats spent roughly the same time in the compartments A, B, and C (301, 301, and 295 s, respectively). The mean time difference between the drug-paired compartment and the vehicle-paired compartment was similar across the four groups: +7, −2, −3, and +5 s for the 0-, 10-, 20-, and 30-mg/kg groups, respectively (F(3,43) P < 1).

**Locomotor Activity.** Figure 4 depicts the mean activity of the four groups in the vehicle-paired compartment and in the drug-paired compartment. The analysis of variance for repeated measures showed a significant effect of the dose and the compartment on the mean activity (dose: F(3,43) = 31.40, compartment F(1,43) = 87.35, interaction F(3,43) = 27.33, P < 0.0001 for all). No difference of the mean activity in the vehicle-paired compartment appeared between the four doses. Conversely, a dose-dependent decrease of the mean activity was observed in the drug-paired compartment. The multiple comparison test showed that the mean activity in the drug-paired compartment was significantly decreased at the 20-mg/kg and 30-mg/kg doses compared to the 0-mg/kg dose.

![Graph 2](image2)

![Graph 3](image3)

![Graph 4](image4)

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Place Preference. Figure 5 depicts the results observed for the different groups during the postconditioning test. Analysis of variance showed no effect of methohexital on place preference.

Recovery from Short-term Anesthesia

Preconditioning. No initial preference for a compartment was observed; that is, the rats spent roughly the same time in the compartments A, B, and C (311, 308, and 281 s, respectively). The mean time difference between the drug-paired and the vehicle-paired compartments was roughly similar across the two groups: +28 and +12 s for the groups methohexital and propofol, respectively ($F(1,25) P < 1$).

Locomotor Activity. Figure 6 depicts the mean activity for the two groups in the vehicle-paired and the drug-paired compartments. Analysis of variance for repeated measures showed a significant effect of group and compartment on the mean activity (group: $F(1,25) = 7.40, P < 0.05$, compartment: $F(1,25) = 54.66, P < 0.0001$, no interaction: $F(1,25) = 1.15$). The multiple comparison test showed that for each group the mean activity in the drug-paired compartment significantly differed from the mean activity in the vehicle-paired compartment. Mean activity in the drug-paired compartment in the propofol and methohexital groups was not significantly different.

![Graph showing place preference](image)

Fig. 7. Place preference (as defined in fig. 2) exhibited at recovery period from anesthesia induced by 40 mg/kg methohexital or 100 mg/kg propofol. Error bars mean SEM. *$P < 0.05$ when comparing the propofol and methohexital groups.

![Graph showing locomotor activity](image)

Fig. 6. Mean activity counts per 15 min pooled over the four conditioning sessions in the vehicle-paired compartment (grey boxes) and in the drug-paired compartment (black boxes) at recovery period from anesthesia induced by 40 mg/kg methohexital or 100 mg/kg propofol. Error bars mean SEM. *$P < 0.05$ when comparing vehicle-paired and drug-paired compartments for each group.

Place Preference. Figure 7 depicts the results observed for the two groups during the postconditioning test. The group conditioned at the recovery period from propofol-induced anesthesia exhibited a significant place preference when compared to the control group conditioned at the recovery period from methohexital-induced anesthesia ($F(1,25) = 34.26, P < 0.0001$).

Discussion

Our experiments show that subanesthetic doses of propofol and recovery from short-term anesthesia by propofol induced a conditioned place preference, suggesting that propofol induces a pleasant affective state.

Subanesthetic doses of propofol, i.e., doses less than that causing loss of righting reflex in 50% of rats, induced a dose-dependent conditioned place preference (experiment 1A). Conversely, subanesthetic doses of methohexital induced no conditioned place preference (experiment 1B). This is not really surprising, because long-term barbiturate agents such as pentobarbital or phenobarbital have been shown to exhibit even aver- age properties when tested in a place conditioning. Therefore, results from both experiments 1A and 1B suggest that conditioned place preference for subanesthetic doses was not owing to any nonspecific general property of short-term anesthetics. It must be empha-
sized that the magnitude of the place preference obtained at 60 mg/kg propofol was similar to that produced by other drug treatment causing a pleasant affective state (e.g., 1.5 mg/kg amphetamine).23 In our experimental conditions. This suggests that the pleasant properties of propofol are strong rather than weak.

For one of the subanesthetic doses of propofol tested above that causing loss of righting reflex in 50% of rats (90 mg/kg), a significant conditioned place preference cannot be demonstrated. This could suggest that, when close to the anesthetic doses, propofol loses its rewarding effects. However, the decline of the magnitude of place preference observed for 90 mg/kg could be caused by a side effect (such as sedation) interfering with the normal conditioning process, for example by shortening the time during which the animals were awake at the same time and exposed to the drug-paired compartment. In favor of this hypothesis, activity counting during the conditioning phase showed that propofol induced a dose-dependent decrease in locomotor activity, with drastic effects at 90 mg/kg. Moreover, visual examination of the rats during this phase also indicated that at this dose, a transient hypnogenic phase of about 10 min occurred in 7 of 12 rats. However, the results for this group did not allow us to rule out the possibility that a hypnotic dose of propofol loses its pleasant properties. Experiment 2 was designed to explore this possibility.

In experiment 2, an original procedure was used to assess the putative pleasant properties of anesthetic doses of propofol. To control for the duration of the conditioning phase, animals were conditioned during the recovery period from short-term anesthesia, as attested by the recovery of righting reflex. To our knowledge, it is the first time that such a conditioning method was used in the place conditioning paradigm. With this method, rats treated with propofol exhibited a conditioned place preference with an anesthetic dose of 100 mg/kg. Here too, place preference induced by an anesthetic dose was not caused by any nonspecific property from drug-induced anesthesia in rats, because control rats anesthetized by methohexitol did not display any place preference.

Taken together, our results demonstrate that propofol has pleasant properties at subanesthetic as well as at anesthetic doses and agree with what has been suggested or observed in humans. At subanesthetic doses, our data confirm and extend the observations by Zaccari et al.13,14 on healthy volunteers, that propofol induces a pleasant effect. At an anesthetic dose, our animal model demonstrated that recovery from propofol anesthesia also has pleasant properties.

The pleasant affective state induced by propofol could appear as beneficial side effects of an anesthetic in light of clinical practice. However, human studies using discrete trials choice procedure14,24 have suggested that the pleasant affective state of propofol could be indicative of potential abuse. Indeed, one case of an anesthesiologist who abused subanesthetic doses of propofol because of its pleasant effects has been reported.25 A variety of abused drugs21,26,27 within the classes of opioids and psychostimulants, exhibit pleasant properties in the place conditioning paradigm. However, place conditioning paradigm cannot itself be a tool to predict addictive properties of a drug, because drug dependence depends on more than pleasant properties.28

In conclusion, our study clearly establishes in rats that propofol induces a pleasant affective state at subanesthetic as well as anesthetic doses.

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