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Effect of Midazolam on Propofol-induced Positive Affective State Assessed by Place Conditioning in Rats

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Background: **The effect of either midazolam or the combination of midazolam and propofol on the affective state was assessed in rats at subanesthetic doses and at recovery from anesthesia.**

Methods: **The putative drug(s)-induced affective states were repeatedly paired with one of two distinguishable compartments of an experimental cage, whereas the vehicle(s)-induced effect was repeatedly paired with the other compartment. During a subsequent choice test for one compartment over the other, the rats' preference for the drug(s)-paired compartment over the vehicle(s)-paired compartment is indicative of a pleasant state induced by the drug(s). In experiment 1, rats were conditioned with different doses of midazolam either at subanesthetic states or at recovery from anesthesia. In experiment 2, groups of rats were conditioned with different combinations of midazolam and propofol either at subanesthetic states or at recovery from anesthesia induced jointly by midazolam (10 mg/kg) and propofol (60 mg/kg). Experiment 3 was conducted in the same way as experiment 2, except that midazolam was paired with both compartments. In addition, these groups were tested not only in an undrugged state but also in a drugged (with midazolam) state.**

Results: **In experiment 1, rats exhibited a place preference for the environment previously associated with midazolam, at subanesthetic and anesthetic doses. Experiment 2 showed that a propofol-induced place preference was found to be dose-dependently suppressed by midazolam. Experiment 3 replicated the findings of experiment 2 and extended them to the mechanism by which midazolam blocked a propofol-induced place preference.**

Conclusions: **Midazolam administered before propofol blocked the expression of a propofol-induced pleasant state. (Key words: Anesthetics, hypnotics: propofol; midazolam. Side effects. Behavior: affective states; pleasant effect; state dependency; memory; place conditioning. Animal: rat.)**

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PROPOFOL is often used as part of a combination of hypnotic drugs. A typical combination is a regimen in which the short-acting benzodiazepine midazolam is injected before propofol.[1] The administration of low doses of midazolam before propofol has been shown to potentiate propofol-induced hypnosis.[2,3] However, whether the synergistic interact[4] between the two drugs also applies to the nonhypnotic effects of the drugs still remains to be determined.

Some (but not all) clinical studies have suggested that propofol induces a subjective feeling of well-being at subanesthetic doses and at recovery from anesthesia.[5–7] In a previous study,[8] using a place conditioning paradigm,[9] we demonstrated in rats that propofol had a pleasant effect at subanesthetic doses and at recovery from anesthesia. Briefly, our paradigm is based on two distinctive environments: During a conditioning phase, a rat learns to associate the effect of the drug with one environment and the effect of the drug vehicle with the other environment. As a result of this association, a drug displaying a pleasant or an unpleasant effect will motivate the animal to spend more or less time in the environment that has been positively or negatively reinforced. This is called a "conditioned place preference" or a "conditioned place aversion." The locomotor activity of rats is systematically recorded to identify any im-

portant decrease in exploratory behavior that could account for a defect of conditioning.

The initial purpose of the present study was to extend our initial finding on propofol to midazolam and to determine the affective state induced by the widely used combination of midazolam followed by propofol. This was performed at a subanesthetic level and during recovery from anesthesia. Our initial results suggested that the combination of midazolam and propofol could be devoid of any pleasant effect. Therefore, an additional experiment was designed to further explore the mechanisms of interaction between midazolam and propofol. Indeed, during place conditioning, the association of the effects of the drug and the environment involves learning mechanisms, such as acquisition and retrieval of the association realized between the effects of the drug and the environment. Propofol[10-12] and midazolam[13-18] have been shown to impair memory mechanisms. We sought to determine whether the combination of midazolam and propofol was truly devoid of any pleasant effect or if this could result from any mnemonic processes.

Materials and Methods

Animals

Our subjects were 138 naive male Long-Evans rats (Janvier, Le Genest-St-Isle, France) that weighed 300–350 g. They were housed two per cage in a colony room maintained on a 12-h light:dark cycle (light on at 8:00 A.M.) with food and water provided *ad libitum*.

Drugs

Propofol (10 mg/ml Diprivan; Zeneca, London, U.K.) was dissolved in 10% "intralipid." Midazolam (5 mg/ml Hypnovel; Roche, Basel, Switzerland) was dissolved in 0.9% sodium chloride. All drugs were injected intraperitoneally.

During preliminary studies on these rats, the hypnotic doses of midazolam and propofol, as assessed by the loss in 50% of the rats of the righting reflex within 30 s and lasting at least 5 min, were 10 mg/kg and 80 mg/kg, respectively. The anesthetic doses of midazolam and propofol, as assessed by the loss of righting reflex within 30 s and the absence of reaction to a tail pinch for at least 5 min, were 20 mg/kg and 100 mg/kg, respectively.

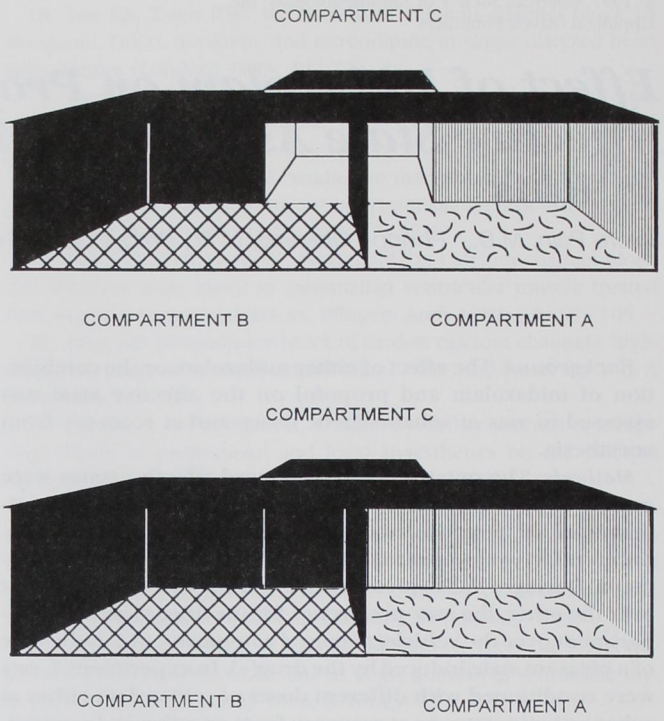

Fig. 1. The place conditioning apparatus (front view) consisted of three compartments: two large compartments A and B (45 × 45 × 30 cm) with a distinct roof, walls, and floor (A: White roof, black and white vertical stripped walls, methacrylate polymer floor, wooden chips. B: Black roof, black walls, and wire grid floor) and a side-painted grey compartment C (36 × 18 × 36 cm). Compartment C is adjacent to the rear of compartments A and B and had removable wooden partitions between compartments A and B. (*A*) When the partitions are removed, the animal can move freely between the two large compartments *via* compartment C. (*B*) When the partitions are in place, the animal is confined in one of the large compartments.

Apparatus

Four identical copies of a place conditioning apparatus were used. The apparatus has been described in previous studies.[8,19] Briefly, the apparatus consisted of three compartments (fig. 1). Two large compartments, A and B, had a distinctive roof, walls, and floor and were separated by a wooden partition. The third compartment, C, was a side compartment adjacent to the rear of compartments A and B and had removable wooden partitions separating it from 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 infrared radia-

tion emitted by the animal. Recording the number of infrared beams disrupted by the rat permitted us to locate it and measure its locomotor activity within the compartments. The signal was fed into a programmable controller (Sysmac C20; Omron, Paris, France) that summed up and recorded the time spent and the locomotor activity of the rat in each compartment.

Place Conditioning Procedure

Behavioral testing and conditioning started after three daily handling sessions. The procedure was divided into three consecutive phases.

Preconditioning Test. On the first day of the experiment, the partitions were removed. Each rat was placed in side compartment C and allowed to move freely throughout the apparatus for 15 min. The time spent by the rats in each compartment was recorded electronically. The preconditioning test was systematically performed in all experiments because it allowed us to verify that rats do not exhibit any spontaneous preference for a given compartment.[8,19]

Eight-day Conditioning Phase. During this phase, the partitions between the compartments were in place. The conditioning phase lasted 8 days and consisted of four pairings of the drug with one compartment intermingled with four pairings of the vehicle with the other compartment. Each rat was injected with the drug on one day and confined for 30 min in compartment A or B. On the alternate day, the rat was injected with the vehicle of the drug and confined for 30 min in the other compartment B or A. 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. During this phase, the locomotor activity of the rat in the compartment was recorded during the initial 15 min.

Postconditioning Test. After the 8-day conditioning phase, each rat was placed into the side compartment C with the partitions removed and was allowed to move freely throughout the apparatus for 15 min. The time spent by the rats in each compartment was recorded. This postconditioning test could be done with no drug being administered to the rat (undrugged state) or with a drug being administered (drugged state).

Experiment 1.

Experiment 1a: Subanesthetic Doses of Midazolam. During the 8-day conditioning phase, rats were injected with midazolam on one day and with vehicle on the alternate day. Ten minutes after the injection of either midazolam or midazolam vehicle, rats were confined for 30 min in one of the large compartments (A or B), as described in the place conditioning procedure section. Forty-eight rats were randomly assigned to one of four groups (n = 12) according to the dose of midazolam they received: 0, 1.25, 2.5, or 5 mg/kg. The postconditioning test was performed with rats in an undrugged state.

Experiment 1b: Recovery from Anesthesia Induced by Midazolam. To assess the affective properties associated with recovery from short-term anesthesia, a modified conditioning procedure previously described[8] was performed on 12 rats. Briefly, during the conditioning phase, anesthesia was achieved by an intraperitoneal injection of midazolam (20 mg/kg) on one day. At the end of the hypnotic period, as evident by recovery of righting reflex, rats were confined for 30 min in one of compartments A or B. On the alternate day, the rats were injected with the vehicle and then confined for 30 min in compartment B or A after a postinjection delay equal to that observed after midazolam injection. The postconditioning test was performed in an undrugged state as described earlier.

Experiment 2

Experiment 2a: Subanesthetic Combination of Midazolam and Propofol. During the 8-day conditioning phase, rats were injected on one day with midazolam followed 5 min later by propofol (60 mg/kg). On the alternate day, rats were injected with the vehicle of midazolam followed 5 min later by the vehicle of propofol. Ten minutes after the injection of either propofol or propofol vehicle, rats were confined for 30 min in one of the two large compartments, as described in the place conditioning procedure. Thirty rats were randomly assigned to one of three groups (n = 10) according to the dose of midazolam they received: 0, 1.25, or 2.5 mg/kg. The postconditioning test was realized in an undrugged state; that is, with no drug (midazolam or propofol) being administered.

Experiment 2b: Recovery from Anesthesia Induced by a Combination of Midazolam and Propofol. During the conditioning phase, anesthesia was achieved in 12 rats by an intraperitoneal injection of midazolam (10 mg/kg) followed 5 min later by an injection of propofol (60 mg/kg) on 1 day. At the end of the hypnotic period, as evident by recovery of righting reflex, rats were confined for 30 min in one of compartments A or B. On the alternate day, the rats were injected with the vehicle of midazolam followed 5 min

later by the vehicle of propofol. The rats were confined for 30 min in compartment B or A after a postinjection delay equal to that observed after propofol injection. The postconditioning test was performed in an undrugged state, as described previously.

Experiment 3

Effect of Midazolam on Propofol-induced Pleasant State. During the 8-day conditioning phase, rats were injected on one day with propofol (60 mg/kg) and with propofol vehicle on the alternative day. Ten minutes after the injection of either propofol or propofol vehicle, rats were confined for 30 min in one of the two large compartments, as described in the place-conditioning procedure. However, on each of the 8 days of conditioning, rats also received midazolam 5 min before the injection of either propofol or propofol vehicle. Thirty-six rats were randomly assigned to one of three groups (n = 12) according to the dose of midazolam they received: 0, 1.25, or 2.5 mg/kg. Two postconditioning tests were realized. The first one was performed in an undrugged state; *i.e.*, in the absence of any drug. The second one was performed in a midazolam state (drugged state); that is, rats were injected with the same dose of midazolam as during conditioning and were placed in the apparatus for testing after a 15-min postinjection delay.

Statistical Analyses

Place Preference. During the preconditioning and the postconditioning tests, the difference between the time spent in the drug-paired compartment and in the vehicle-paired compartment (drug minus vehicle) was the dependent variable used to assess the preference for one compartment.[20] This variable was called "place preference." Thus it should take positive values in the case of a positive affective (pleasant) state induced by the drug and negative values in the case of a negative affective (unpleasant) state induced by the drug.

Statistical analysis of the place preference exhibited during the preconditioning test was performed using a one-way analysis of variance. Statistical analysis of the place preference exhibited during the postconditioning test depended on the procedure used in each experiment. In experiment 1a, statistical analysis of the place preference exhibited by the different doses of midazolam was performed using a one-way analysis of variance (factor: dose of midazolam) followed by multiple comparisons using Dunett's test. In experiment 2a, statistical analysis of the place preference exhibited for the different doses of midazolam combined with propofol (60 mg/kg) was performed using a one-way analysis of variance (factor: dose of midazolam) followed by multiple comparisons using Dunett's test. In experiments 1b and 2b, statistical analysis of the place preference expressed after conditioning during recovery from anesthesia was realized by comparing results from the preconditioning test and the postconditioning test, using a paired *t* test. In experiment 3, the difference of place preference exhibited by rats during the two postconditioning tests (undrugged and drugged tests) was assessed by an analysis of variance of place preference with repeated measures and covariates (factor, test; covariate, dose of midazolam).

Locomotor Activity. 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 was used as the dependent variable. In experiments 1a, 2a, and 3, statistical analyses of the locomotor activity were performed using a two-way analysis of variance with repeated measures followed, when necessary, by multiple comparisons using the Tukey studentized range method test. In experiments 1b and 2b, statistical analysis of the locomotor activity was performed using a paired *t* test.

Results

Preconditioning Tests

No initial preference for a given compartment (drug *vs.* vehicle) was observed in any group. In experiment 1a, the mean time difference between the midazolam-paired compartment and the vehicle-paired compartment was similar among the four groups: -9, 24, 24, and 20 s for, respectively, 0, 1.25, 2.5, and 5 mg/kg midazolam (not significant). In experiment 2a, the mean time difference between the midazolam-propofol-paired compartment and the vehicle-paired compartment was similar across the three groups: 20, -19, and 17 s for, respectively, 0, 1.25, and 2.5 midazolam (not significant). In experiment 3, the mean time difference between the propofol-paired compartment and the vehicle-paired compartment was similar across the three groups: -22, -16, and -18 s for, respectively, 0, 1.25, and 2.5 mg/kg midazolam (not significant).

Experiment 1a: Subanesthetic Doses of Midazolam

Place Preference. Figure 2A depicts the place preference elicited during the postconditioning test by condi-

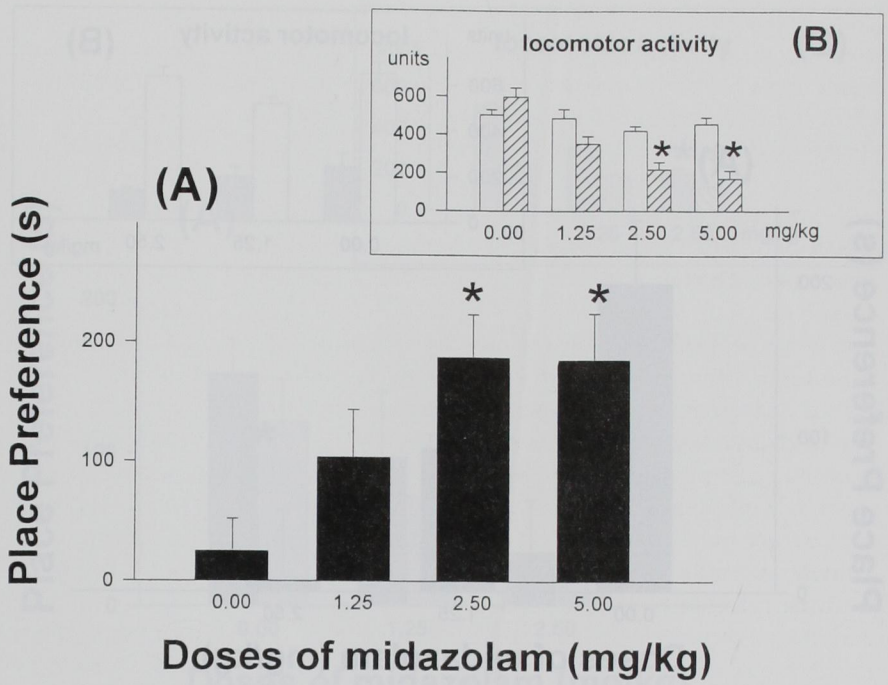

Fig. 2. The effect of subanesthetic doses of midazolam. (*A*) During the postconditioning test, place preference (defined as the difference between time spent in the drug-paired compartment and the vehicle-paired compartment) for the different doses of midazolam. Error bars represent SEM. *$P < 0.05$ when compared with the 0-mg/kg dose. (*B*) Locomotor activity (defined as the mean activity counts/15 min for the four conditioning sessions) in the vehicle-paired compartment (*white boxes*) and in the midazolam-paired compartment (*batched boxes*) for the different doses of midazolam. Error bars represent SEM. *$P < 0.05$ when compared with the 0-mg/kg dose.

tioning with midazolam 0, 1.25, 2.5, and 5 mg/kg. The place preference increased with the dose, up to 2.5 mg/kg midazolam. Analysis of variance showed a significant effect of the dose of midazolam on place preference ($P < 0.01$). The multiple comparisons test showed that the 2.5- and 5-mg/kg doses differ significantly from the 0 mg/kg dose; *i.e.*, a conditioned place preference was observed for 2.5-mg/kg and 5-mg/kg doses of midazolam.

Locomotor Activity. Figure 2B depicts the mean activity in the midazolam-paired compartment and in the vehicle-paired compartment during conditioning. Analysis of variance showed significant effects of the compartment (midazolam or vehicle-paired compartment) and the dose of midazolam as well as an interaction between compartments and doses on the mean activity (compartment, $P < 0.0001$; dose, $P < 0.0001$; compartment x dose, $P < 0.0001$). The multiple comparisons test showed that the mean activity in the midazolam-paired compartment was significantly decreased at 2.5-mg/kg and 5-mg/kg doses compared with the 0-mg/kg dose.

Experiment 1b: Recovery from Short-term Anesthesia Induced by Midazolam

Place Preference. When conditioning occurred during recovery from anesthesia induced by 20 mg/kg midazolam, a significant place preference was observed during the postconditioning test, compared with the preconditioning test (postconditioning, 264 s [SEM = 26]; preconditioning, 16 s [SEM = 42]; $P < 0.0001$).

Locomotor Activity. During conditioning, the mean activity was significantly reduced in the compartment paired with recovery from anesthesia compared with the vehicle compartment (recovery, 166 units [SEM = 54]; vehicle, 599 units [SEM = 15]; $P < 0.0001$).

Experiment 2a: Subanesthetic Combination of Midazolam and Propofol

Place Preference. Figure 3A shows the place preference induced by 60 mg/kg propofol was reduced by 0, 1.25, and 2.5 mg/kg midazolam administered before propofol. Increasing the dose of midazolam led to a decrease in the place preference induced by propofol ($P < 0.01$). The multiple comparisons test showed that the 2.5-mg/kg dose differed significantly from the 0-mg/kg dose; *i.e.*, the place preference induced by propofol was significantly reduced by the 2.5-mg/kg dose of midazolam.

Locomotor Activity. Figure 3B depicts mean activity in the midazolam–propofol-paired compartment and in the vehicle-paired compartment during conditioning. Analysis of variance revealed a significant effect of the compartment (propofol or vehicle-paired compart-

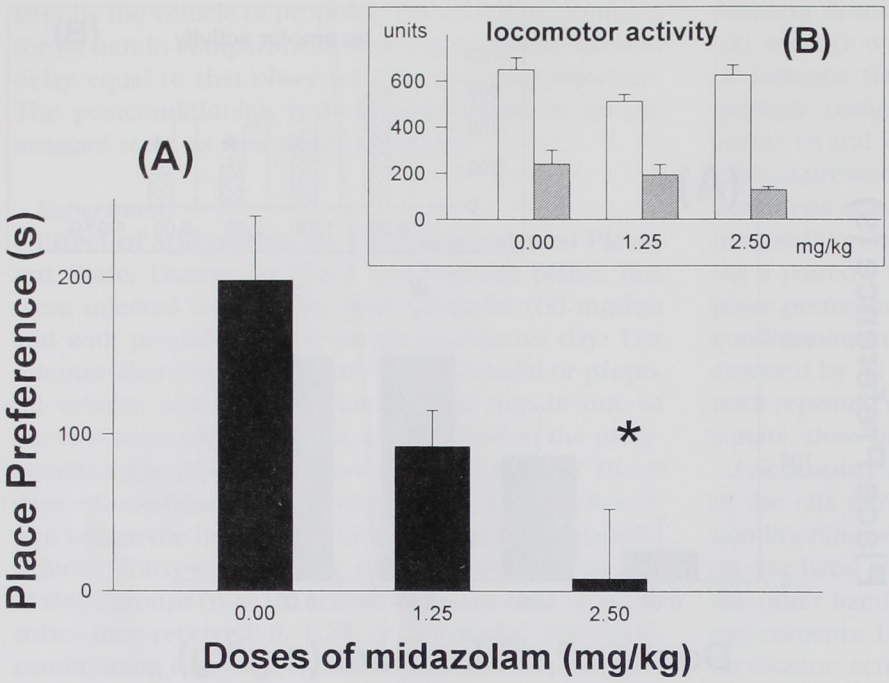

Fig. 3. Effect of midazolam administered before propofol on propofol-induced conditioned place preference. (*A*) During the postconditioning test, place preference (defined as the difference between time spent in the midazolam–propofol-paired compartment and the vehicle-paired compartment) elicited by 60 mg/kg propofol preceded by midazolam for the different doses of midazolam. Error bars represent SEM. *$P < 0.05$ when comparing the 0-mg/kg dose with the other doses of midazolam. (*B*) Locomotor activity (as defined in fig. 2B) in the vehicle-paired compartment (*white boxes*) and in the midazolam–propofol-paired compartment (*hatched boxes*) for the different doses of midazolam. Error bars represent SEM. No significant effect of the dose of midazolam.

ment) and no significant effect of the dose of midazolam but a significant interaction between the compartment (propofol or vehicle-paired compartment) and the dose of midazolam on the mean activity (compartment, $P < 0.0001$; dose, not significant; compartment x dose, $P < 0.01$).

Experiment 2B: Recovery from Anesthesia Induced by a Combination of Midazolam and Propofol

Place Preference. When conditioning occurred during recovery from anesthesia induced jointly by 10 mg/kg midazolam and 60 mg/kg propofol, no place preference was observed during the postconditioning test compared with the preconditioning test (postconditioning, -17 s (SEM = 60); preconditioning, -26 s (SEM = 46); not significant).

Locomotor Activity. During the conditioning, the mean activity was significantly reduced in the compartment paired with recovery from anesthesia compared with the vehicle compartment (recovery, 176 units [SEM = 52]; vehicle, 514 units [SEM = 40]; $P < 0.01$).

Experiment 3: Effect of Midazolam on the Propofol-induced Pleasant State

Place Preference. Figure 4A depicts the place preference elicited by 60 mg/kg propofol during the postconditioning undrugged test and the subsequent postconditioning drugged test, for the three doses of midazolam (0, 1.25, and 2.5 mg/kg) administered during the conditioning phase. Increasing the dose of midazolam led to a decrease in the place preference induced by propofol during the undrugged test and the drugged test. Analysis of variance showed no significant effect of the test (undrugged or drugged test: not significant), but still a significant effect of dose ($P < 0.001$).

Locomotor Activity. Figure 4B depicts the mean activity in the propofol-paired compartment and in the propofol vehicle-paired compartment during conditioning for the three doses of midazolam. Analysis of variance showed significant effects of the compartment (propofol or vehicle-paired compartment) and the dose of midazolam and a significant interaction between the compartment (propofol or vehicle-paired compartment) and the dose of midazolam on the mean activity (compartment, $P < 0.0001$; dose, $P < 0.0001$; compartment x dose, $P < 0.0001$). The multiple comparisons test showed that mean activity was significantly reduced for the 2.5 mg/kg dose compared with the 0 mg/kg dose of midazolam in the propofol-paired compartment; mean activity was also significantly reduced for the 2.5-mg/kg dose compared with the 0 mg/kg dose of midazolam in the propofol vehicle-paired compartment.

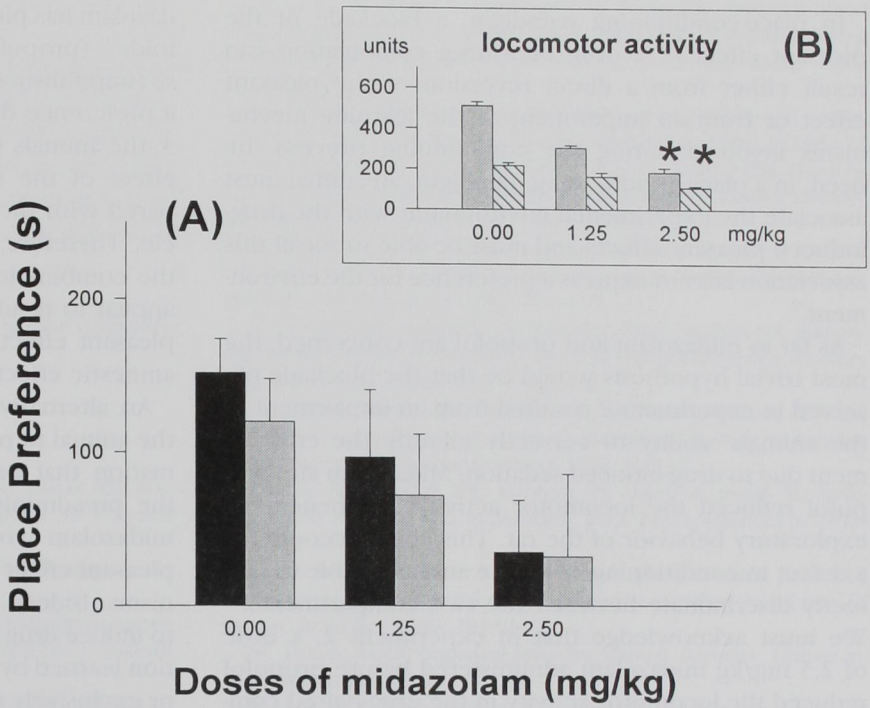

Fig. 4. Effect of midazolam treatment on propofol-induced conditioned place preference. During the conditioning for propofol (60 mg/kg), each rat received midazolam before propofol and before "intralipid." (*A*) Place preference (as defined in fig. 3A) elicited during the postconditioning undrugged test (*black boxes*) and during the postconditioning drugged test (*grey boxes*). See text for more explanation. Error bars represent SEM. No significant effect of the test (undrugged *vs.* drugged test). (*B*) Locomotor activity (as defined in fig. 2B) in the propofol vehicle-paired compartment (*grey boxes*) and the activity in the propofol-paired compartment (*batched boxes*) for the different doses of midazolam. Error bars represent SEM. *$P < 0.05$ when comparing the 0-mg/kg dose with the other doses of midazolam.

Discussion

In experiment 1, we found that rats exhibited a preference for the environment previously associated with midazolam; *i.e.*, midazolam motivated the animal to spent more time in a compartment that had been positively reinforced by the midazolam effect. This conditioned place preference was observed at subanesthetic and at anesthetic doses and was dose-dependent, with a significant preference observed at doses of 2.5 mg/kg and higher. This result demonstrates the pleasant effect of subanesthetic doses of midazolam in a place-conditioning paradigm, thereby adding midazolam to the list of benzodiazepines that are already known to induce conditioned place preference.[9,21,22] Further, using our procedure, we showed that recovery from midazolam-induced anesthesia also produced a pleasant effect. It is noteworthy that the magnitude of the place preference obtained here with 20 mg/kg midazolam was similar to that produced by the anesthetic dose of 100 mg/kg propofol[8] or by other drugs that induce a pleasant effect (*e.g.*, amphetamine[23]) in our experimental conditions. This suggests that the pleasant effect induced by midazolam during recovery is strong. Of the three short-term anesthetic agents that we have studied so far (propofol, methohexital, and midazolam), propofol and midazolam, but not methohexital, induced a pleasant effect at subanesthetic and anesthetic doses. The fact that we did not observe a methohexital-induced pleasant effect[8] suggests that pleasant effect is not an inevitable effect of short-term anesthetic agents.

In experiment 2, we found that pretreatment with midazolam resulted in a combination of midazolam and propofol that did not induce conditioned place preference. Such a blockade could be evidenced for a subanesthetic dose of midazolam and at recovery from an anesthesia induced by a combination of midazolam and propofol. Furthermore, this blockade was significant for a dose of midazolam for which we observed a significant midazolam-induced conditioned place preference. Midazolam and propofol have a synergistic effect on hypnosis.[3,4]

Therefore, the finding that the combination of midazolam and propofol did not induce conditioned place preference is surprising and invites closer examination. To our knowledge, there are two previous reports of the blockade of a drug-induced conditioned place preference by a benzodiazepine: diazepam has been shown to block morphine-induced conditioned place preference[24] and triazolam has been shown to block amphetamine-induced conditioned place preference.[25]

In place-conditioning paradigm, a blockade of the pleasant effect of a drug or a drug combination can result either from a direct reversion of the pleasant effect or from an impairment of the learning mechanisms involved during the conditioning process. Indeed, in a place-conditioning paradigm, an animal must associate the experimental environment with the drug-induced pleasant effects and must be able to recall this association later to express a preference for the environment.[9]

As far as midazolam and propofol are concerned, the most trivial hypothesis would be that the blockade observed in experiment 2 resulted from an impairment of the animals' ability to correctly identify the environment due to drug-induced sedation. Midazolam and propofol reduced the locomotor activity and hence the exploratory behavior of the rat. This could account for a defect in conditioning, with the animal unable to correctly discriminate between the two compartments.[26] We must acknowledge that in experiment 2, a dose of 2.5 mg/kg midazolam administered before propofol reduced the locomotor activity in the drug-paired compartment by one sixth compared with the activity observed in the vehicle-paired compartment. Therefore, our results do not allow us to rule out this hypothesis. Such an explanation is, however, unlikely because in our preparation, 2.5 and 5 mg/kg midazolam induced a similar drastic reduction of the locomotor activity but still yielded a significant conditioned place preference, as attested by experiment 1.

A viable hypothesis would be that the blockade observed in experiment 2 has resulted either from the combination of midazolam and propofol lacking of pleasant effect or from an inability for the animal to acquire the information that the combination had a pleasant effect, due to a powerful synergistic amnestic effect of the combination. Rather the results from the undrugged test in experiment 3 suggest that the animals were able to acquire the pleasant effect of the combination of midazolam and propofol. The animals were experiencing the combination of midazolam and propofol in one large compartment and the combination of midazolam and propofol vehicle in the other large compartment during conditioning. Because place conditioning relies on a discrimination between two differentially affectively loaded compartments, the suppression of the pleasant effects of midazolam and propofol when combined would have led the animals to exhibit a preference for the compartment paired with midazolam and propofol vehicle during the undrugged test because midazolam has pleasant effects (experiment 1) and "intralipids" (propofol vehicle) has no pleasurable effect *per se* (unpublished data). Because we did not observe such a preference during the undrugged test in experiment 3, the animals were able to acquire a preference for the effect of the midazolam propofol combination compared with the effect of midazolam and propofol vehicle. Therefore, the blockade of the pleasant effect of the combination of midazolam and propofol does not appear to result from the combination being devoid of pleasant effects, nor does it appear to result from an amnestic effect (acquisition deficit).

An alternative hypothesis would be an inability for the animal to retrieve (rather than to acquire) the information that propofol has pleasant effects because of the preadministration of midazolam. In other words, midazolam would have blocked the propofol-induced pleasant effect because of its effects on retrieval mechanisms. Indeed, midazolam has been shown frequently to induce drug state dependency[17,18]; that is, an information learned by a subject under midazolam can be better or exclusively recalled when the subject is tested under midazolam (drugged state) rather than in an undrugged state. Because midazolam was administered 5 min before propofol, it is conceivable that a propofol-induced pleasant effect has been locked into the midazolam state.[27] In experiment 3, we found that reinstating the midazolam condition during the drugged test did not alleviate the blockade by midazolam of propofol-induced pleasant effect. This later result suggests that midazolam-induced blockade is not a state-dependent retrieval deficit induced by midazolam.

From experiments 2 and 3, when administrated before propofol, midazolam blocked the expression of the pleasant effect of the former drug. This blockade does not appear to result from the combination of midazolam and propofol being devoid of any pleasant effect, nor does it appear to result from an acquisition deficit or a state-dependent retrieval deficit induced by midazolam. This suggests that midazolam blocked the pleasant effect induced by propofol by an alternative mechanism. In this respect, it is noteworthy that Mariatasan and Stolerman[28] recently found in rats that midazolam masked properties of another drug, nicotine. Midazolam-induced masking (overshadowing) resulted from a retrieval deficit and not from an acquisition deficit.[29] On these grounds, we propose that in the current study, midazolam masked propofol-induced pleasant effects by a similar mechanism, namely overshadowing. This deserves mention because it emphasizes the putative

powerful retrieval effect of midazolam when administered before anesthesia.

Our results with rats raise interesting issues for future research and suggest that interactions between drugs such as propofol and midazolam are complex. Indeed, the combination of midazolam followed by propofol expresses a neutral affective state that could not have been predicted from the individual pleasant effects of each drug.

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