

# Modafinil Prevents Inhibitory Avoidance Memory Deficit Induced by Sleep Deprivation in Rats

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**Study Objectives:** Evaluation of modafinil effects on the inhibitory avoidance task (IA).

**Design:** Rats were trained on a multiple trial IA task after receiving modafinil or vehicle injections. In experiment 1 they were trained with a weak protocol under baseline condition and in experiment 2, with a stronger protocol under sleep-deprivation condition.

**Results:** In experiment 1 modafinil improved rats' acquisition whereas the retention test remained unaffected. In Experiment 2 modafinil did not interfere with training performance, but the lower dose prevented the retention impairment in sleep-deprived animals.

**Conclusions:** Modafinil is able to improve acquisition in normal rats and reverse the long-term memory impairment induced by sleep-deprivation.

**Keywords:** Cognitive enhancer, emotional memory, acquisition, retrieval, learning, sleep

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MODAFINIL (DIPHENYL-METHYL-SULPHINIL-2-ACETAMIDE) IS A NONAMPHETAMINE WAKE-PROMOTING DRUG THAT HAS BEEN FOUND TO BE EFFECTIVE IN the treatment of excessive daytime sleepiness associated with narcolepsy, obstructive sleep apnea syndrome, and shift work sleep disorder.<sup>1</sup> Experimental studies have shown that, in addition to treating excessive daytime sleepiness, modafinil also has beneficial effects on cognitive tasks such as attention and working memory in experimentally sleep-deprived humans, primates, and rodents.<sup>1-3</sup> Moreover, recent studies without experimental sleep deprivation have demonstrated that modafinil is able to improve learning, working memory, and attention in animals and in humans,<sup>1,4-6</sup> although Waters et al.<sup>7</sup> did not observe a beneficial effect of modafinil on attention in normal non-sleep-deprived rats.<sup>7</sup> In non-sleep-deprived animals, Beracochea et al.<sup>6</sup> found that chronic treatment with modafinil before testing improved working memory of mice evaluated by spontaneous alternation rates in a T maze in a delayed-dependent manner. The researchers observed a beneficial effect with an intertrial interval of 60 and 180 seconds but not with 5 seconds.<sup>6</sup> In addition, the same group demonstrated that administration of modafinil also induced a significant facilitation in acquisition of a T-maze serial spatial discrimination task, which was dependent on the level of training.<sup>8</sup> In both cases, modafinil-treated mice required significantly fewer trials to reach the criterion level of performance compared with vehicle-treated control mice.

More recently, it has been shown that glucocorticoids and stress are involved in the modulation of modafinil-induced cog-

nitive effects.<sup>9,10</sup> Acute stress decreased the efficiency threshold of modafinil, as performance was enhanced at a lower dose (16 mg/kg) and impaired at a higher (32 mg/kg) dose, whereas, under nonstress conditions, this enhancement was obtained for the higher dose (32 mg/kg) only.<sup>9</sup> In addition, Shuman et al.<sup>9</sup> found dissociated effects of modafinil with differently motivated learning tasks. The same dose that benefitted performance of mice in the Morris water maze (75 mg/kg) impaired performance on contextual fear conditioning.<sup>11</sup>

As postulated by Pierard et al.,<sup>10</sup> given that psychostimulants are generally used under stressful conditions such as prolonged sleep deprivation or cognitive sustained tasks, it is of interest to consider the interaction of stress and modafinil under these conditions. Therefore, the aim of the present study was to evaluate modafinil effects on emotional memory acquisition in non-sleep-deprived and sleep-deprived rats using a multiple-trial inhibitory avoidance paradigm (MTIA), an aversive memory task in which it is possible to evaluate acquisition and retention in the same task.

## METHODS

Male Wistar rats, weighing between 250 and 350 gm, from the animal facility of the Department of Psychobiology and CEDEME (Centro de Desenvolvimento de Modelos Experimentais) from the Universidade Federal de São Paulo were used. All of the procedures in this report were approved by the Institution's Ethics Committee (CEP-UNIFESP, #1391/06) and followed international guidelines for animal use and care. Animals were maintained under controlled temperature (23°C ± 2°C) and 12-hour light:12-hour dark cycle (lights on at 07:00) conditions. All behavioral procedures were carried out from 12:00 to 16:00. Rat chow and tap water were provided ad libitum. Twenty-eight, 62, and 23 animals were used in Experiment 1, Experiment 2, and the open field test, respectively. The inhibitory avoidance chamber consisted of 2 acrylic boxes, each measuring 30 × 30 × 60 cm connected by a sliding door. The safe compartment is made of white acrylic, whereas the aver-

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sive compartment is made of black acrylic. The floor is made of parallel metallic rods, each 0.4 cm in diameter, 1.2 cm apart from each other, and connected to an electric shock generator (AVS Projetos Especiais, Brazil). Training of the MTIA consisted of placing the animal in the safe compartment of the inhibitory avoidance chamber. Each time the animal crossed to the dark compartment of the apparatus, it received a 0.4- or 0.7-mA per second footshock (Experiment 1 and Experiment 2, respectively), and the first training latency was recorded. The stronger footshock protocol in Experiment 2 was used to permit sleep-deprived animals to perform the task, otherwise they would have been unable to reach the criterion and the task would not have been completed (parameter obtained in a pilot experiment; data not shown). Immediately after each footshock, the animal was again placed in the safe compartment. The criterion for ending the task was the animal remaining in the light compartment for 120 seconds. The number of crosses to the dark compartment was the measure of acquisition. After reaching the criterion, each rat was immediately replaced in its home cage. Twenty-four hours later, the test session was carried out, in which the latency to cross to the dark compartment was recorded as a measure of retention. If the animal did not cross within 300 seconds, it was removed from the apparatus and a 300-second latency was recorded. No footshock was delivered. In Experiment 2, sleep deprivation was carried out by the modified multiple platform method<sup>12</sup> during 96 hours before training. Modafinil administration (L. Lafon, Maisons-Alfort, France; 100 and 200 mg/kg, by gavage, suspended in distilled water) was carried out 60 minutes before training on the MTIA. Another group of non-sleep-deprived animals was used to evaluate whether administration of modafinil at the same doses affected locomotor activity using the open-field arena for 5 minutes. To do so, we used an open-field apparatus consisting of a circular arena (80 × 36 cm) with the floor divided into 3 concentric circles, subdivided in 18 equal sectors.

## RESULTS

Figure 1A shows the mean number of trials required by each group to reach the criterion in Experiment 1. One-way analysis of variance (ANOVA) revealed a significant treatment effect on the task acquisition ( $F_{2,25} = 7.0594$ ;  $P < 0.004$ ). Duncan posthoc analysis indicated that both groups of modafinil-treated animals reached the criterion within fewer trials than did the vehicle-treated animals ( $P < 0.006$ ). For retention latencies, a 2-way repeated-measures ANOVA with Group and Session (First Training Latency × Test Latency) as factors showed significant effect of session ( $F_{1,25} = 15.36$ ;  $P < 0.0006$ ). The latencies of all groups were higher at the test session compared with the first training latency (Figure 1B). No Group effect ( $F_{2,25} = 0.40$ ;  $P = 0.68$ ) or interaction between Session and Group effects were observed ( $F_{2,25} = 0.18$ ;  $P = 0.84$ ). Figure 1C shows the mean number of trials to reach the criterion by each group in Experiment 2. One-way ANOVA revealed no significant Group effect on task acquisition ( $F_{3,56} = 1.54$ ;  $P = 0.19$ ). For retention latencies, a 2-way repeated-measures ANOVA with Group and Session (First Training Latency × Test Latency) as factors showed significant effect of Session ( $F_{1,56} = 98.13$ ;  $P < 0.001$ ) and an interaction between Session and Group effects ( $F_{5,56} = 6.13$ ;  $P < 0.001$ ). Posthoc analysis revealed that the retention latencies

of all groups were higher at the test session compared with the first training latency and that the sleep-deprived vehicle group and the sleep-deprived group that received modafinil at the dose of 200 mg/kg had worse performance than did the other groups at test ( $P < 0.03$ ) (Figure 1D).

A 1-way ANOVA for the open-field test did not show significant differences between groups ( $F_{2,20} = 3.41$ ;  $P = 0.053$ ). The mean ( $\pm$  SEM) number of crosses in the open-field arena ( $77.14 \pm 12.19$ ,  $100.0 \pm 10.88$ , and  $121.71 \pm 11.60$ ) for vehicle, modafinil 100mg/kg, and modafinil 200mg/kg, respectively.

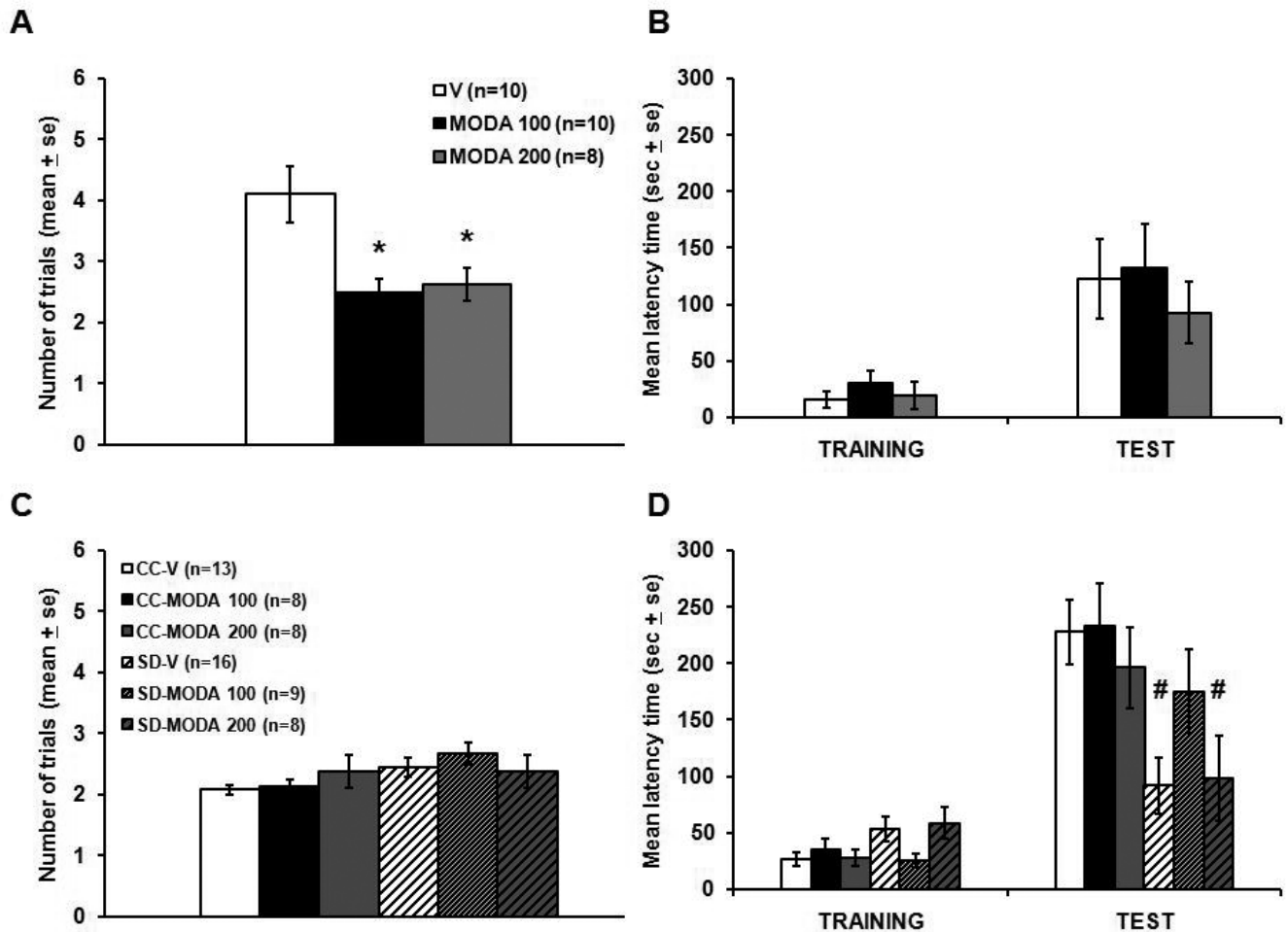
## DISCUSSION

The main findings of the present study were that modafinil facilitates acquisition of the inhibitory avoidance response with a weak footshock protocol and did not affect retention of the task in non-sleep-deprived rats (Experiment 1). In Experiment 2, using a stronger footshock protocol, modafinil failed to produce such an acquisition improvement but prevented the retention impairment induced by sleep deprivation in vehicle-treated animals only with the lower dose was used.

A slight increase in activity level was observed with the higher dose of 200 mg/kg of modafinil in the open field, but this effect does not seem to be related to the animals' performance because both doses of modafinil enhanced task acquisition in Experiment 1. Moreover, an increase in locomotor activity would be expected to impair acquisition through an increase in the number of crosses to the dark compartment, which was not the case.

The cognitive-enhancing effects of modafinil on the MTIA acquisition (Experiment 1) is in agreement with previous work showing the specificity of positive effects in short-term memory tasks in humans and animals.<sup>1,4,6,8</sup> Beracochea et al.<sup>6</sup> attributed the action of modafinil to an enhancement of working memory, whereas long-term memory was found to not be affected when evaluated by a serial spatial discrimination task.<sup>6</sup> Although our results are in accordance with others, suggesting that the effects of modafinil occur during the acquisition phase, we cannot rule out the possibility of consolidation effect, since the drug was also present during the early stages of memory consolidation. However, this is unlikely because a study that evaluated modafinil effects on acquisition and consolidation in contextual fear conditioning, which is also an emotional memory task, showed that the drug had no effect when administered after training.<sup>11</sup>

In Experiment 2, with a stronger footshock, the same doses of modafinil that improved acquisition in Experiment 1 (100 and 200 mg/kg) produced no effect on task acquisition, possibly because the stronger footshock per se masked the modafinil-induced effects during the acquisition phase due to a ceiling effect observed in the vehicle-treated rats (2 trials to reach the criterion). As seen in Experiment 1, in Experiment 2, modafinil had no effect on memory retention in control animals but prevented performance impairment during the test session in sleep-deprived animals. However, this effect occurred only with the lower dose of modafinil, suggesting a possible interactive effect between sleep deprivation and modafinil in a way that a more stressful situation counteracts the effects of modafinil, decreasing its efficiency threshold. This interaction with stress was first demonstrated by Pierard et al.<sup>10</sup> who showed that, in



**Figure 1**—Effects of modafinil at acquisition (A; trials to criterion) and retention (B; TRAINING bars represent the first training latency, and TEST bars represent the test latency) on the inhibitory avoidance response in non-sleep-deprived animals (Experiment 1). V refers to the group of rats that received vehicle; MODA 100, rats that received 100 mg/kg of modafinil; MODA 200 rats that received 200 mg/kg of modafinil (\* $P < 0.05$  compared with V). Effects of modafinil on the inhibitory-avoidance acquisition (C; trials to criterion) and retention (D TRAINING BARS represent the first training latency, and TEST bars represent the test latency) after sleep deprivation (Experiment 2). CC-V refers to cage control animals that received vehicle; CC-MODA 100, cage control animals that received 100 mg/kg of modafinil; CC-MODA 200, that received 200 mg/kg of modafinil; SD-V, sleep-deprived animals that received vehicle; SD-MODA 100, sleep-deprived animals that received 100 mg/kg of modafinil; SD-MODA 200, sleep-deprived animals that received 200 mg/kg of modafinil. The number of animals is shown in parentheses; # $P < 0.05$  comparing control animals and SD-MODA 100 groups during the same test session.

a stress condition, only a lower dose of modafinil was able to produce the effect of improvement compared with a nonstress condition. Although a stress-modafinil interaction is likely to occur, this interaction seems to not be related to corticosterone levels, as has been shown by Pierard et al.<sup>10</sup> In the present study, we observed that modafinil only at the dose of 100 mg/kg prevented memory impairment in sleep-deprived rats; the lack of effect with the dose of 200 mg/kg could be related to a stress-modafinil interaction. Sleep deprivation is known to increase corticosterone levels,<sup>12</sup> but a previous study showed that corticosterone inhibition by metyrapone administration did not prevent memory impairment on contextual fear conditioning in sleep-deprived rats.<sup>13</sup> The findings suggest that the interaction between stress and modafinil would not be excluded; however, other aspects of a stress response, other than the corticosterone itself, could be involved.

Our results confirm and extend the previous findings showing the beneficial effects of modafinil on learning<sup>1,4,6,8,11</sup> and the reversal of deleterious sleep-deprivation effects.<sup>2,3</sup> In addition,

the effects of modafinil seem to be influenced by stress, showing different profiles of effect depending of the protocol used—i.e., in Experiment 1, we observed an effect at acquisition, and, in Experiment 2, we observed an improvement in retention. Moreover, the present work suggests that modafinil is able to reverse the long-term memory impairment induced by sleep deprivation in aversively motivated emotional memory tasks.

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#### DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

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