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Inhibitory effects of modafinil on emotional memory in mice

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ABSTRACT

Modafinil (MOD), a psychostimulant used to treat narcolepsy, excessive daytime sleepiness, and sleepiness due to obstructive sleep apnea, appears to promote a possible facilitatory effect on cognitive function. In the present study, we investigated the effects of the acute administration of MOD on the different steps of emotional memory formation and usage (acquisition, consolidation and retrieval) as well as the possible participation of the state-dependency phenomenon on the cognitive effects of this compound. Mice were acutely treated with 32, 64 or 128 mg/kg MOD before training or testing or immediately after training and were subjected to the plus-maze discriminative avoidance task. The results showed that although pre-training MOD administration did not exert any effects on learning, the doses of 32 or 64 mg/kg induced emotional memory deficits during testing. Still, the post-training acute administration of the higher doses of MOD (64 and 128 mg/kg) impaired associative memory consolidation. When the drug was administered pre-test, only the 32 mg/kg dose impaired the task retrieval. Importantly, the cognitive impairing effects induced by 32 mg/kg MOD were not related to the phenomenon of state-dependency. In all, our findings provide pre-clinical evidence of potential emotional memory amnesia induced by MOD.

This article is part of a Special Issue entitled 'Cognitive Enhancers'.

1. Introduction

Modafinil (MOD) is a psychostimulant that acts as a wake-promoting drug and has been approved for the treatment of excessive daytime sleepiness in narcolepsy, obstructive sleep apnea, and shift work syndrome. Clinical studies have pointed MOD positive effects when used to treat Parkinson's disease (Ferraro et al., 1998; Nieves and Lang, 2002), multiple sclerosis (Kraft and Bowen, 2005), schizophrenia (Turner et al., 2004) and attention deficit hyperactivity disorder (Taylor and Russo, 2000). Additionally, MOD seems to be widely prescribed off-label to enhance alertness, attention, memory for dementia and depression (Joos et al., 2010). Of note, an illicit market exists for academic doping as well (Cakic, 2009).

There are several hypotheses that attempt to explain the mechanisms of action of MOD. Studies have suggested that it may increase the release of catecholamines (dopamine and norepinephrine), serotonin and glutamate, thereby reducing the release of GABA in various brain regions, and activating hypothalamic neurons containing hypocretin/orexin neurons of the tuberomammillary nucleus (Minzemberg and Carter, 2008).

It has been demonstrated that MOD modifies the activity of brain areas involved with memory, such as the hippocampus and prefrontal cortex (Béracochéa et al., 2003). Within this context, studies indicate that MOD has cognitive-enhancing abilities in rodents performing a variety of learning/memory exercises in the T-Maze based on spontaneous alternation behavior (Béracochéa et al., 2001), and also enhanced learning (Béracochéa et al., 2003) and memory retrieval (Béracochéa et al., 2008). Still, it was reported that this drug was able to restore the memory impairments observed after a 10 h total sleep deprivation period in a contextual memory task (Piérard et al., 2007) and also prevented the 96 h of paradoxical sleep deprivation-induced memory deficits in the multiple trial inhibitory avoidance paradigm (Moreira et al., 2010).

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Concerning its chronic administration, MOD was reported to improve learning (Béracochéa et al., 2002).

However many studies have reported cognitive enhancing abilities of MOD (Shuman et al., 2009), the facilitative effects of MOD on memory have not always been verified when memory consolidation was specifically investigated. In fact, a study conducted by Shuman et al. (2009) reported that the administration of MOD immediately after training had no effects on either cued or contextual fear paradigms.

Although experimental evidence suggests a possible positive effect of MOD on memory, the facilitatory effects of this drug on memory formation still require systematic experimentation with distinct models of learning and memory. Indeed, most of the facilitatory effects of MOD on learning/memory have been obtained in tasks which were devoid of any emotional component (Béracochéa et al., 2001; Piérard et al., 2007, 2011) or in tasks that involve positive reinforces (Béracochéa et al., 2002, 2003). In this scenario, the investigation of the effects of MOD on the plus-maze discriminative avoidance task (PM-DAT) can be interesting because this animal model can evaluate learning and the retention of an emotional discriminative avoidance task (Gulick and Gould, 2011; Patti et al., 2006, 2010; Sanday et al., 2012; Silva et al., 1997; Silva and Frussa-Filho, 2000; Zanin et al., in press). Thus, the objective of the present study was to investigate the effects of MOD on the different steps of emotional memory formation in the PM-DAT. The present findings provide evidence of potential emotional amnestic proprieties of acutely administered MOD.

2. Material and methods

2.1. Subjects

Three-month-old Swiss male mice (raised and maintained in the Centre for Development of Experimental Models in Medicine and Biology of Universidade Federal de São Paulo) were used in the experiments. Animals weighing 30–35 g were housed under controlled temperature (22–23 °C) and lighting (12 h light, 12 h dark; lights on at 6:45 a.m.) conditions. Food and water were available *ad libitum* throughout the experiments.

All efforts were made to minimize animal suffering and to reduce the number of animals used. The animals used in this study were maintained in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications N° 8023, revised 2011) and in accordance with the Brazilian Law for Procedures for Animal Scientific Use (#11794/2008). All experimental procedures were approved by the Ethics Committee under protocol #1162/08.

2.2. Drug

MOD (Cephalon®) was dissolved in 0.5% Arabic gum and intraperitoneally (i.p.) administered in a volume of 10 ml/kg body weight at doses of 32 (MOD32), 64 (MOD64), or 128 mg/kg (MOD128). MOD vehicle was used as the control solution and administered i.p. This dose range was selected based on previous work of our group (Wuo-Silva et al., 2011).

2.3. Behavioral test: PM-DAT

The apparatus employed in the PM-DAT is a modified elevated plus-maze made of wood. The apparatus has two enclosed arms with sidewalls and no top (28.5 × 7 × 18.5 cm). The enclosed arms are opposite to two open arms (28.5 × 7 cm). A non-illuminated, 100-W lamp and a hair dryer were placed over the center of one of the enclosed arms (aversive enclosed arm). In the training session, each mouse was placed at the center of the apparatus, and during a 10-min period, the aversive stimuli were administered every time the animal entered the enclosed arm containing the lamp and the hair dryer and was continued until the animal left the arm. The aversive stimuli consisted of both the illumination of the 100-W light and cold air blow produced by the hair dryer. In the test session, which was performed in the same room 10 days after the training, mice were again placed in the center of the apparatus and were observed for 3 min; however, the mice did not receive the aversive stimuli when they entered the aversive enclosed arm even though the non-illuminated lamp and the hair dryer were still placed on the middle of this arm to help distinguish between the aversive and non-aversive arms. In all experiments, the animals were observed in a blind manner, and the apparatus was cleaned with a 5% alcohol solution after each behavioral session. The percent time spent in the aversive enclosed arm (time spent in aversive enclosed arm/time spent in both enclosed arms × 100) was calculated. Learning and memory were evaluated by the percent time spent in the aversive enclosed arm during training and testing, respectively. All the measures taken during the PM-DAT were obtained manually.

2.4. Statistical analysis

The comparisons were made using the 1-way analysis of variance (ANOVA) followed by Duncan's test when necessary. Significance was accepted at p-values less than 0.05.

2.5. Experimental design

2.5.1. Experiment 1: effect of the acute pre-training administration of MOD in mice subjected to the PM-DAT

Forty-eight animals were randomly assigned to one of the following groups ($n = 12$): vehicle, 32 (MOD32), 64 (MOD64) or 128 mg/kg MOD (MOD128). Mice received an acute i.p. administration of vehicle or MOD. Thirty min after the injection, animals were trained in the PM-DAT. Ten days later, they were tested. This experiment was designed to evaluate the effects of acute MOD administration on acquisition and encoding of the emotional discriminative avoidance memory.

2.5.2. Experiment 2: effects of the acute post-training administration of MOD in mice subjected to the PM-DAT

Forty-eight animals were randomly assigned to one of the following groups ($n = 12$): vehicle, 32 (MOD32), 64 (MOD64) or 128 mg/kg MOD (MOD128). Mice were trained in the PM-DAT and, immediately afterwards, received an i.p. administration of vehicle or MOD at different doses. Ten days later, animals were tested. In this experiment, we aimed to investigate the effects of acute MOD administration exclusively on the encoding phase of memory formation.

2.5.3. Experiment 3: effects of the acute pre-test administration of MOD in mice subjected to the PM-DAT

Forty-eight animals were randomly assigned to one of the following groups ($n = 12$): vehicle, 32 (MOD32), 64 (MOD64) or 128 mg/kg MOD (MOD128). Mice were trained in the PM-DAT. Ten days later, the animals received an acute i.p. administration of vehicle or different doses of MOD. Thirty min after the injection, the animals were tested. With pre-test administration, we investigated the effects of acute MOD administration in the retrieval of the emotional memory task.

2.5.4. Experiment 4: role of the state-dependency phenomenon on the cognitive effects of 32 mg/kg MOD in mice subjected to the PM-DAT

Forty-eight mice were randomly assigned to one of the following groups ($n = 12$): pre-training/pre-test administration of vehicle (VEH–VEH), pre-training administration of MOD (MOD–VEH), pre-test administration of MOD (VEH–MOD), or pre-training/pre-test administration of MOD (MOD–MOD). Groups of 24 mice received vehicle or MOD. Thirty min after the injection, all animals were trained in the PM-DAT. Ten days after the training, 12 animals from the pre-training vehicle group received another vehicle injection, whereas the other 12 mice from the same group received an injection of MOD. Similarly, 12 animals from the pre-training MOD group received a vehicle injection, whereas the other 12 mice received another 32 mg/kg MOD injection. The test session was performed 30 min after the 2nd injection.

3. Results

3.1. Experiment 1: effect of the acute pre-training administration of MOD in mice subjected to the PM-DAT

In the training session, the ANOVA revealed that there were no significant differences among groups in the percent time spent in the aversive enclosed arm [$F(3,44) = 0.17$; $p > 0.05$] (Fig. 1A).

In the test session, performed 10 days after training, the ANOVA followed by Duncan's test showed that the MOD32 and MOD64 groups spent a significantly longer percent time in the aversive enclosed arm compared to the other groups (the vehicle and MOD128 groups) [$F(3,44) = 6.91$; $p < 0.05$] (Fig. 1B).

3.2. Experiment 2: effects of the acute post-training administration of MOD in mice subjected to the PM-DAT

In the training session, as expected, the ANOVA revealed that there were no significant basal differences among the groups for the percent time spent in the aversive enclosed arm [$F(3,44) = 1.88$; $p > 0.05$] (Fig. 1C).