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Modafinil-induced modulation of working memory and plasma corticosterone in chronically-stressed mice

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Abstract

The original aims of our study were to investigate the dose–effect relationship of modafinil administration on working memory performance, in parallel with the measurement of plasma corticosterone in chronically-stressed mice, as compared to control mice. Memory performance was evaluated by spontaneous alternation in a T-maze. Vehicle or modafinil (8, 16 or 32 mg/kg) were administered after or without chronic stress (immobilization and exposure to light) for 15 min/day over a period of consecutive 14 days. Immediately after behavioral testing, blood was sampled to measure plasma corticosterone levels.

Under non-stress conditions, corticosterone significantly increased with 16 and 32 mg/kg modafinil administration. Interestingly, optimal working memory performance was revealed at the 16 mg/kg dose. Moreover, no correlation was evidenced between working memory performance and plasma corticosterone level in modafinil-treated animals.

Under stress conditions, corticosterone level was lowered at 8 mg/kg and remained unchanged at 16 and 32 mg/kg modafinil. An optimal working memory performance was evidenced at 8 mg/kg, which indicated a decrease in the efficiency threshold of modafinil under stress. Furthermore, an inverse correlation emerged between working memory performance and corticosterone level. Our study evidenced for the first time the interaction between stress and memory, in the emotional modulation of working memory performance, as a function of the administered dose of modafinil.

Keywords: Modafinil; Spatial working memory; Alternation task; T-maze; Psychomotor performance; Glucocorticoids; Stress

1. Introduction

The issue of drug and stress interconnection with respect to performance, remains a boundless field for research. A wide variety of molecules operate as modulators of neurochemical pathways of vigilance, mainly depending on the interaction between noradrenergic and cholinergic neurons (Buccafusco, 2004).

Among them, modafinil or [(diphenylmethyl) sulfinyl]-2 acetamide has been reported as having stimulant and awakening properties without amphetamine-like side effects (Bastuji and Jouvet, 1988; Hermant et al., 1991; Lyons and French, 1991). This drug is successfully used in the treatment of narcolepsy and idiopathic hypersomnia without interfering, however, with nocturnal sleep (Bastuji and Jouvet, 1988). Indeed, according to our research, modafinil has been proven to enhance wakefulness by acting on both norepinephrine and dopaminergic systems, through ascending pathways that are likely to promote wakefulness by activating the cortex and other forebrain targets, possibly through interaction with the hypocretin/orexin system (Boutrel and Koob, 2004). We hypothesize that the mechanism of modafinil action may also involve a reduction of GABA release in the cerebral cortex (Tanganelli et al., 1992; Piérard et al., 1997) as well as an involvement of excitatory amino acids system (Piérard et al.,

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1995, 1997) and its receptors (Lagarde et al., 1996). Modafinil also enhances energy metabolism, by increasing the energetic pool of phosphocreatine in cortex, thus contributing to vigilance-enhancing properties of this drug (Piérard et al., 1995). As yet, however, the mechanism of modafinil action is not fully understood. As such, it is still largely surrounded by controversy (Saper and Scammel, 2004) and its brain targets remain a matter for debate (Gallopin et al., 2004).

When administered to healthy subjects, modafinil provides a military interest in cases of total or partial sleep deprivation resulting from either continuous or sustained operations (Lagarde et al., 1995; Lagarde and Batejat, 1995; Caldwell et al., 2004), despite its "overconfidence" and hyperthermia-inducing effects (Buguet et al., 2003). In addition, modafinil provides a major interest in survival conditions after aircraft ejection or ship wreck, in order to maintain sustained vigilance while waiting for rescue under major stress circumstances. Moreover, it has been alleged that the use of modafinil is likely to improve performance in the field of sports practice (Starr, 2004). Recent studies have demonstrated that modafinil is able to improve working memory both in animals (Béracochéa et al., 2001) as well as in humans (Muller et al., 2004); there is further evidence that modafinil enhances learning processes after chronic (Béracochéa et al., 2002) or acute (Béracochéa et al., 2003) systemic administration in mice. Moreover, such evidence of learning enhancement is due to the faster emergence of a cognitive win-stay strategy in modafinil-treated animals as compared to controls (Béracochéa et al., 2003). These cognitive-enhancing effects of modafinil (see also Turner et al., 2003), in addition to neuroprotective properties against hypoxia (Lagarde et al., 1993) and organophosphate intoxication (Lallement et al., 1997), could be partly mediated by the involvement of excitatory amino acids neurotransmission system (Piérard et al., 1997).

It is noteworthy, however, that studies intended to determine the effects of psychostimulant drugs including modafinil on hormone secretions, remain critically scarce (Brun et al., 1998), particularly with reference to stress conditions. Their action might induce an increase in glucocorticoids secretion (cortisol in humans or corticosterone in rodents) through adrenal cortex, that could improve psychomotor performance in healthy subjects. Indeed, owing to both fat and protein mobilization, the primary mechanism of glucocorticoid effect is likely to enhance the rate of glucose production in the liver (Shephard, 1987), and to increase plasma glucose, as well as glycerol and fatty acids concentrations, in the same manner as epinephrine does (Cerretelli, 2002). Moreover, glucocorticoids and their receptors are important mediators of stress response and learning/memory processes (Lupien et al., 1999; De Quervain et al., 1998; Roozendaal et al., 1996; De Kloet, 2004; Kitraki et al., 2004; Célérier et al., 2004). We wish to hypothesize therefore that glucocorticoids are likely to be involved in the mechanism of modafinil action on psychomotor and memory processes, and that this effect could be modulated under stress conditions.

Hence, our study was aimed at evidencing the dose–effect relation of modafinil administration on psychomotor and memory performance, in parallel with the determination of corticosterone level in chronically-stressed mice, as compared with control mice.

2. Materials and methods

2.1. Animals

The study was conducted using male mice of the C57BL/6J strain obtained at 8 weeks of age from Iffa-Credo, Lyon, France. On arrival, mice were housed collectively in colony cages (40 cm long × 25 cm high × 20 cm wide) matched for weight and placed in an animal room (ambient temperature 22 °C; automatic light cycle: 07:00 and 19:00 h) with free access to food and water. They remained in collective cages for 4 weeks. Five weeks before behavioral testing began, mice were transferred into individual cages, with free access to food and water.

This study was carried out according to the European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purposes, and under the agreement # 94001 delivered by the French Ministry of Defence, after the protocol was examined by the local ethical committee.

2.2. Memory testing

The behavioral task used to test working memory is based on spontaneous alternation behavior (SA); as such, it does not require use of food reinforcement to emerge. Indeed, SA is the innate tendency of rodents whereby over a series of trials run in a T-maze (except for the first trial), mice alternate at each successive trial, the choice of the goal arm. Repetitive testing constitutes a potent source of proactive interference. From trial to trial, accurate performance at a given trial (N) requires that subjects are able to discriminate the specific target trial N-1 from the interfering trial N-2. The target information required for successful performance varies from trial to trial; thus, the subject is not only required to keep temporarily in short-term memory a specific information, but also to reset it over consecutive runs. The resetting mechanisms and cognitive flexibility required to alternate over successive runs are major components of working memory processes. Working memory is a major component of the sequential alternation performance, since SA rates are dependent on the length of the intertrial delay interval, and/or on the place of the trial in the series. Thus, the sequential alternation procedure is of utmost relevance to assess delay-dependent working memory performance in mice (Béracochéa and Jaffard, 1990; Béracochéa et al., 1995).

The tests were carried out in a T-maze made of opaque grey Plexiglas. Stem and arms were 35 cm long, 10 cm wide, and 25 cm high. The start box (10 × 12 cm) was separated from the stem by a vertical sliding door. Vertical sliding doors were also placed at the entrance of each arm. A low-intensity diffuse illumination (10 lx) was provided above the apparatus. Between two trials, the apparatus was cleaned using 70% alcohol and water, in order to remove any olfactive cue.

2.3. Experimental protocol

2.3.1. Manipulation of mice, familiarization with the apparatus, and training sessions

Between 5 and 3 weeks before testing (from W-5 to W-3), animals were placed in their individual cages and were manipulated for 10 min each day, in order to reduce further interference with the experimenter. Familiarization with the apparatus began the day after the end of this period, i.e., 20 days before the day of the test, for 3 consecutive days (from D-20 to D-18). During this habituation period, all the animals were allowed 10-min free exploration of the apparatus in order for them to become familiar with the experimental conditions. Subsequently, between 17 and 15 days before testing (from D-17 to D-15), animals were submitted to 3 daily training sessions of SA, in order to foster the development of the alternation behavioral pattern and to familiarize mice with the opening and/or closing of the doors over successive runs (Fig. 1).

2.3.2. Chronic stress

A non-nociceptive chronic stress model was used in order that behavioral results were not impaired by neurochemical pathways of pain, as in the case of the electric footshocks stress model. Therefore, animals were submitted over a period of 14 consecutive days (up to the day before alternation task) to an immobilization phase under dense light exposure stress. For this purpose, mice were placed for 15 min/day in a narrow transparent Plexiglas tube, with 1300 lx exposure.

2.3.3. Modafinil administration

Modafinil was suspended in a 0.5% tragacanth gum solution (vehicle) and administered intraperitoneally (0.1 ml/10 g mouse) at the doses of 8 mg/kg (M8), 16 mg/kg (M16), and 32 mg/kg (M32). Control animals received the vehicle only. Behavioral testing started 30 min after modafinil or vehicle injections.

2.3.4. Alternation task and blood sampling

For all the mice groups involved in our study, behavioral testing as well as blood sampling were performed between 08:30 and 12:00 a.m. All the subjects were given 6 successive trials separated by a 60-s intertrial interval. To begin a trial, the mouse was placed in the start box for 60 s before the door to the stem was opened. When the subject entered one of the goal arms, the door to that arm was closed. The chosen arm and the time that elapsed between opening the door and the arrival to the end of the chosen arm (task achievement time) were registered. Following a 30-s confinement period in the chosen arm, the animal was removed and placed in the start box for a new trial. An alternation response was considered each time the subject entered the arm opposite to the one visited on the immediately previous trial. Alternation rate was calculated taking into account the 6 successive trials, and expressed in percentage relative to the maximal alternation rate of 100% (obtained when the subject never returned into the same arm over two consecutive trials).

Immediately after the alternation task (about 30 s), animals were sacrificed by decapitation, and blood sampled in order to measure plasma corticosterone level that is considered to be a relevant biochemical index of stress intensity in rodents.

2.3.5. Corticosterone measurement

Plasma corticosterone was quantified on plasma samples of 50 μl, using an original HPLC method with fluorometric detection (λ_{ex}=375 nm; λ_{em}=485 nm), preceded by 2 liquid–liquid extractions with ethyl acetate. This method was validated according to AFNOR guidelines XP T 90-210.

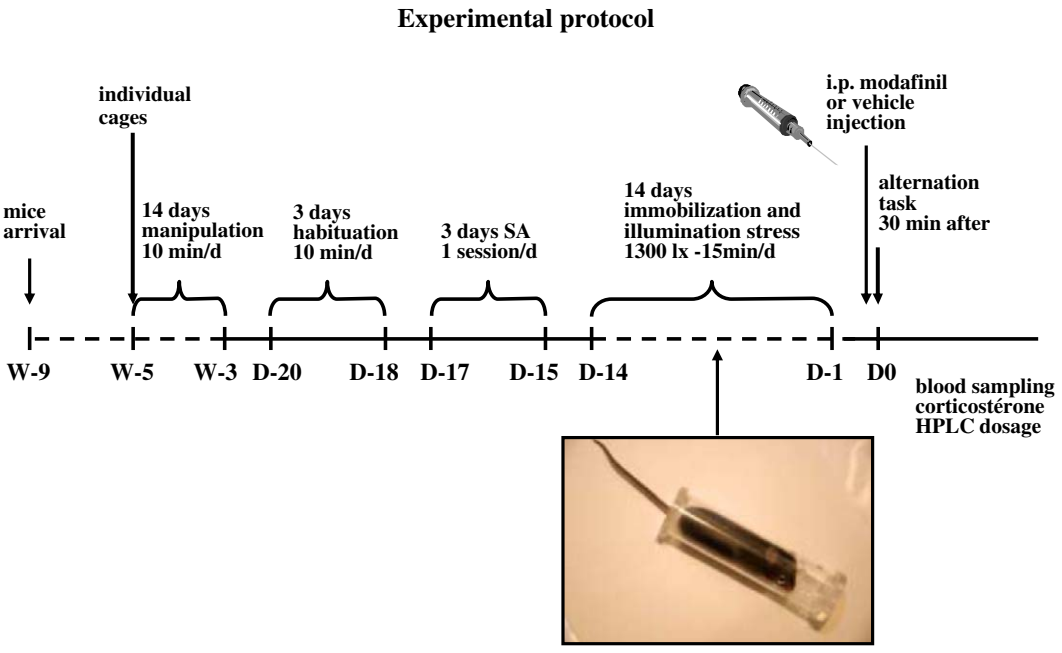

Fig. 1. Experimental protocol. W, D: week, day.

2.3.6. Statistical analyses

In our behavioral procedure, only mice having alternated at the second trial were selected for further behavioral analysis ($n=96$). This procedure is based on our previous papers showing that memory effects of various treatments (chronic alcohol consumption, brain lesions, etc) are observed following alternated trials *only* (for example right–left) but not after repeated choices (such as left–left for instance) (Béracochéa et al., 1987; Béracochéa and Jaffard, 1994). Insofar as mice were tested in a single alternation session in our procedure, we thus optimised observation conditions as regards the memory effects of modafinil by choosing mice that alternated immediately at the second trial of the series. Furthermore, this behavioral criterion ensured that motor abilities and motivation to alternate are not impaired by the previous chronic stress procedure, in a situation without interference (2nd trial of the series). Statistical analyses included: i) M8 groups, after ($n=14$) or without chronic stress ($n=9$), ii); M16 groups, after ($n=10$) or without chronic stress ($n=11$); iii) M32 groups, after ($n=13$) or without chronic stress ($n=9$); iv) control groups, after ($n=15$) or without chronic stress ($n=15$).

Statistical analyses were performed using Statview® v. 5.0 software. Two-way analysis of variance (ANOVA) was performed to assess the effects of treatments on the animals' performance (alternation rate and task achievement time) and corticosterone level. Further comparisons between individual groups were performed with the Scheffe post hoc test. Moreover, correlation analyses were performed between alternation rates, task achievement times and corticosterone levels, in both non-stress and stress conditions.

3. Results

3.1. Working memory performance

Working memory performance variations are summarized in Fig. 2. ANOVA on alternation rates reveals an overall significant difference between groups ($F(7,88)=5.14$; $p<0.0001$). Intragroup comparisons (i.e., between stress and non-stress conditions) show that stress significantly decreases alternation rates for control groups (50.0% vs. 76.0%; $p<0.05$), M16 groups (64.8% vs. 91.7%; $p<0.05$) and M32 groups (29.6% vs. 55.6%; $p<0.05$), whereas alternation rate remains unchanged for M8 groups. Intergroup comparisons (i.e., between vehicle and modafinil-treated animals) under non-stress conditions, show that modafinil administration significantly increases alternation rate for the M16 group only (91.7% vs. 76.0%; $p<0.05$). Intergroup comparisons under stress conditions show that modafinil administration induces a significant increase in alternation rate for the M8 group, and a decrease for the M32 group (respectively 72.2% vs. 50.0%; $p<0.05$ and 29.6% vs. 50.0%; $p<0.05$). Moreover, in stress conditions, the alternation rate for the M32 group is significantly lower than the alternation rate for the M8 group (29.6 vs. 72.2%; $p<0.001$), but not different from the M16 group.

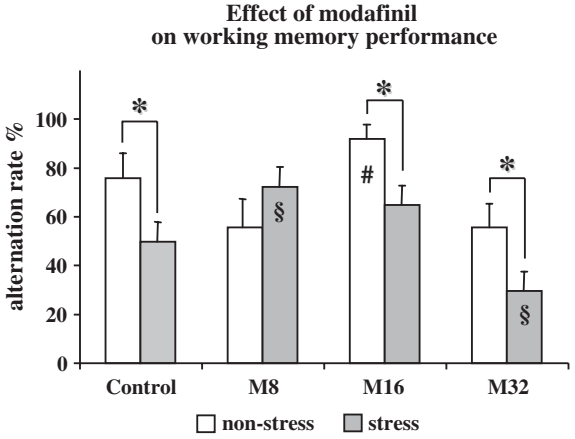

Fig. 2. Effect of modafinil on spatial working memory evaluated by spontaneous alternation rate (%) in T-maze, for 6 successive trials (60-s intertrial delay). Non-stress conditions (open bars); stress conditions (grey bars). Control: vehicle injected animals (0.5% tragacanth gum solution); M8, M16, M32: modafinil treated animals (respectively 8, 16 or 32 mg/kg i.p.). Values are mean ± SEM. *: intragroup comparisons; #: comparisons to non-stressed control group; §: comparisons to stressed control group. *, #, §: $p<0.05$.

3.2. Task achievement time

Task achievement time variations are summarized in Fig. 3. ANOVA on task achievement times shows a global significant difference between groups ($F(7,88)=2.81$; $p<0.05$). Intragroup comparisons (i.e., between stress and non-stress conditions) show that stress significantly increases task achievement time for the M32 group (91.5 vs. 25.6 s; $p<0.001$), whereas task achievement times remain unchanged for control, M8 and M16 groups. Intergroup comparisons (i.e., between vehicle and modafinil-treated animals) under non-stress conditions show that modafinil administration at any dose does not significantly impair task achievement times. Intergroup comparisons under stress conditions show that modafinil administration induces a significant increase in task achievement time for M32 group only (91.5 vs. 36.8 s; $p<0.001$). Moreover, in stress conditions, the task achievement time for the M32 group is significantly higher than the task achievement time for the M8 group (91.5 vs. 45.8 s; $p<0.05$), but not different from the M16 group.

3.3. Plasma corticosterone level

Plasma corticosterone variations are summarized in Fig. 4. ANOVA on corticosterone concentrations showed a global significant difference between groups ($F(7,86)=17.35$; $p<0.0001$). Intragroup comparisons (i.e., between stress and non-stress conditions) revealed that stress significantly increases corticosterone levels for controls only (0.23 vs. 0.14 μg/ml; $p<0.01$), whereas corticosterone levels remain unchanged for M8, M16 and M32 groups. In other words, there is no significant difference as regards plasma corticosterone level, between stressed or non-stressed conditions in all modafinil-treated groups. Intergroup comparisons (i.e., between vehicle and modafinil-treated animals) in non-stress conditions show that modafinil administration significantly

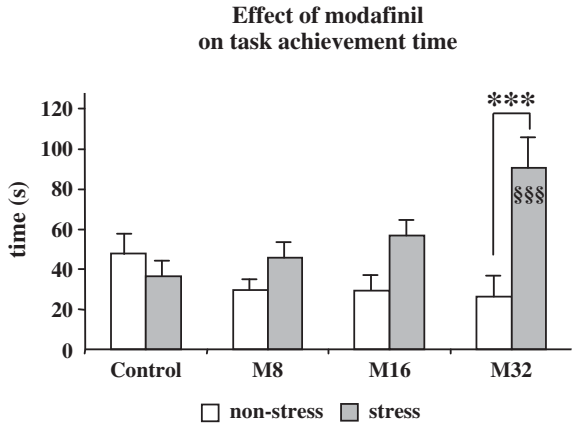

Fig. 3. Effect of modafinil on task achievement time (s) in T-maze (time elapsed between opening of the door and the arrival to the end of the chosen arm). Non-stress conditions (open bars); stress conditions (grey bars). Control: vehicle injected animals (0.5% tragacanth gum solution); M8, M16, M32: modafinil treated animals (respectively 8, 16 or 32 mg/kg i.p.). Values are mean ± SEM. *: intragroup comparisons; §: comparisons to stressed control group. **: $p<0.01$; §§§: $p<0.001$.

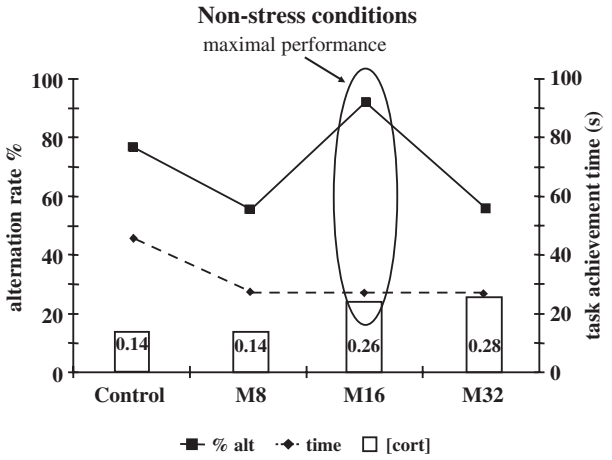

Fig. 5. Summary of behavioral and biochemical results obtained in non-stress conditions. Alternation rate (full lines); task achievement time (dotted lines); corticosterone level (clear bars). The maximal performance is obtained for 16 mg/kg modafinil. Control: vehicle injected animals (0.5% tragacanth gum solution); M8, M16, M32: modafinil treated animals (respectively 8, 16 or 32 mg/kg i.p.).

increases corticosterone levels for M16 and M32 groups (0.26 vs. 0.14 μg/ml, respectively; $p<0.001$ and 0.28 vs. 0.14 μg/ml; $p<0.001$), but not for the M8 group. Moreover, in non-stress conditions, the corticosterone levels for M16 and M32 groups are significantly higher than for the M8 group (0.26 vs. 0.14 μg/ml, respectively; $p<0.001$ and 0.28 vs. 0.14 μg/ml; $p<0.0001$), whereas corticosterone levels for M16 and M32 groups are not statistically different. Intergroup comparisons in stress conditions demonstrate that modafinil administration induces a significant decrease in corticosterone levels for the M8 group only (0.17 vs. 0.23 μg/ml; $p<0.01$), whereas corticosterone levels remain statistically unchanged for the M16 and M32 groups. Moreover, in stress conditions, the corticosterone levels for the M16 and M32 groups are significantly higher than the corticosterone level for the M8 group (respectively 0.23 vs. 0.17 μg/ml; $p<0.01$ and 0.28 vs. 0.17 μg/ml; $p<0.0001$), with corticosterone level for the M32 group being higher as compared to the M16 group (0.28 vs. 0.23; $p<0.05$).

3.4. Non-stress conditions

Fig. 5 summarizes the behavioral and biochemical results obtained in non-stress conditions. No statistical correlation has been evidenced between the two behavioral parameters on the one hand, and plasma corticosterone level on the other. Moreover, no correlation has been found between alternation rate and task achievement time in modafinil-treated animals, thus evidencing the independence, in non-stress condition,

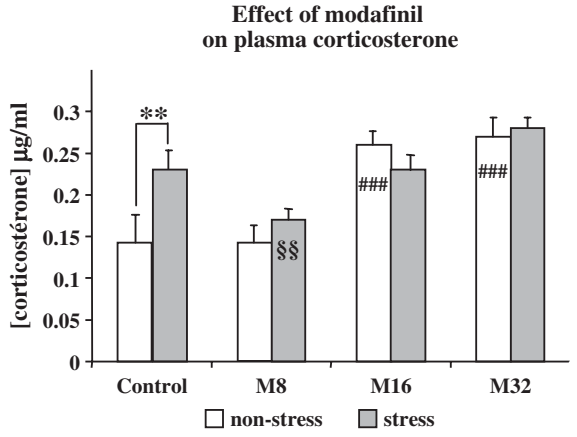

Fig. 4. Effect of modafinil on plasma corticosterone concentration (μg/ml). Blood sampled immediately after alternation task by decapitation. Non-stress conditions (open bars); stress conditions (grey bars). Control: vehicle injected animals (0.5% tragacanth gum solution); M8, M16, M32: modafinil treated animals (respectively 8, 16 or 32 mg/kg i.p.). Values are mean ± SEM. *: intragroup comparisons; #: comparisons to non-stressed control group; §: comparisons to stressed control group. **, §§: $p<0.01$; ###: $p<0.001$.

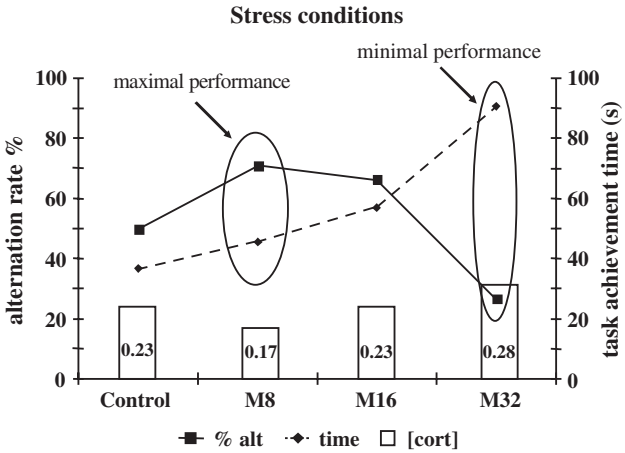

Fig. 6. Summary of behavioral and biochemical results obtained in stress conditions. Alternation rate (full lines); task achievement time (dotted lines); corticosterone level (clear bars). The maximal performance is obtained for 8 mg/kg modafinil. Control: vehicle injected animals (0.5% tragacanth gum solution); M8, M16, M32: modafinil treated animals (respectively 8, 16 or 32 mg/kg i.p.).

between the achievement of alternation task and the time needed for it.

3.5. Stress conditions

Fig. 6 summarizes the behavioral and biochemical results obtained in stress conditions. An inverse correlation was observed on the one hand between the alternation rate and the task achievement time ($r = -0.40$; $p < 0.05$), and, on the other hand, between the alternation task and the corticosterone level ($r = -0.38$; $p < 0.05$). There was no evidence, however, of any significant correlation between task achievement time and corticosterone level.

4. Discussion

First of all, in our experimental conditions, both behavioral testing and blood sampling were carried out between 08:30 and 12:00 a.m., i.e., several hours before the onset of the plasma corticosterone peak, during the period for which its level remained low and stable. Indeed, the peak of plasma corticosterone occurred 2 h before the beginning of the activity phase (Halberg et al., 1959), i.e., at about 05:00 p.m., because light was turned off at 07:00 p.m. in our animal room. Thus, we can assume that circadian influence on corticosterone level and behavior did not interfere with the interpretation of our results.

Our present study confirms a preliminary findings from the Laboratoire de Neurosciences Cognitives (Bordeaux 1 University). Indeed, the latter evidenced the lack of effect of 8 mg/kg modafinil on working memory, between non-stress and stress conditions. Furthermore, we found in the present study an effect at 16 and 32 mg/kg modafinil on working memory, between both conditions (Fig. 2).

In non-stress conditions, the enhancing effect of modafinil on spatial working memory is evidenced at the 16 mg/kg dose, whereas in an earlier study (Béracochéa et al., 2001), the enhancement of alternation rates was observed at a higher modafinil dose (64 mg/kg). This discrepancy could be explained by an important methodological difference between the two studies : indeed, the present experimental protocol (Fig. 1), as opposed to the previous one involves 3 successive days of spontaneous alternation (1 session per day, between D-17 and D-15), in order to foster the emergence of the alternation behavioral pattern prior to the test session, and to enable the animals to perform a better selection of the spatial cues on the day of testing (D0). Thus, it is likely that the 3 training sessions emphasize the emergence of the alternation behavior at lower modafinil doses.

Our study provides the demonstration for the first time that modafinil is able to modulate plasma corticosterone level in mice, in both control and chronic stress conditions. This effect could be either of peripheral origin, through a direct action of modafinil on adrenal glands, or of central origin, via the hypothalamo–hypophyso–adrenal axis. In humans, the enhancement of physical performance after modafinil administration (Starr, 2004), namely in prolonging exercise time to exhaustion by dampening the sensation of fatigue (Jacobs and Bell, 2004), could be bound up with the enhancement of glucocorticoid release. The alerting property of modafinil may not be related, however, to an alteration of cortisol profile in sleep-deprived healthy volunteers (Brun et al., 1998). Nevertheless, our data show that modafinil and stress effects are non-cumulative on the increase in plasma corticosterone level.

A relevant result is the inverse correlation evidenced between working memory performance and corticosterone level in stress conditions, but not in non-stress conditions, thus suggesting that behavioral and neuroendocrine responses to stressful stimuli may be distinct (Mueller et al., 2004). Indeed, mnemonic performance under stress conditions decreased as corticosterone level increased, thus confirming that chronic stress in rodents has mostly impairing effects on memory (Wolf, 2003). Moreover, 21 days of immobilization stress has been shown to affect spatial memory (Kitraki et al., 2004). Nevertheless, although the relationship between stress, glucocorticoids and memory is empirically supported, there are other factors, such as stress condition and gender, as well as individual differences within groups, that influence the cross-impact between these variables (Sauro et al., 2003).

From a behavioral point of view, we allege the following points, namely that:

- *Without modafinil*, the working memory performance of stressed animals is significantly lower as compared to the non-stress conditions, whereas stressed animals tend to perform quicker than non-stressed ones.
- *With modafinil*, the memory performance of stressed animals tends to be higher (M8) or is significantly decreased (M16, M32), as compared to non-stress conditions (Fig. 2). Moreover, modafinil-treated mice have a tendency (M8 and M16) or are significantly slower (M32) under stress, as compared to the non-stress conditions (Fig. 3). The latter result coincides with a recent study (Stone et al., 2002) to the effect that stress induced subsensitivity to modafinil, as regards locomotor activity. According the authors, this effect may be due to a selective desensitization or inhibition of motor-related brain α1-adrenoceptors and can be prevented by corticosterone treatment.

Therefore, we hypothesize that alternation rate and task achievement time are components of a global psychomotor index. In treated animals, the best performances are obtained in the M16 group in non-stressed conditions (Fig. 5), and in the M8 group in stress conditions (Fig. 6). Indeed, at such doses, alternation rates are the highest, and task achievement times are the lowest, as evidence of optimal psychomotor performance. In addition, at the same 8 mg/kg dose of modafinil, the global psychomotor performance of mice is higher in stress conditions, as compared to non-stress conditions. Thus, these results demonstrate that a decrease in the dose of modafinil induces an optimal psychomotor performance under stress conditions, as compared to non-stress conditions. Conversely, as regards higher doses of modafinil (i.e., at 32 mg/kg) we observe an impairment of psychomotor performance in both conditions. Indeed, in non-stressed animals (Fig. 5), only the decrease in

the alternation rate is responsible for this performance lowering, whereas, in stress conditions (Fig. 6), the reduction in the task achievement time also contributes to this deterioration. Such a finding is in agreement with a recent study from Ward et al. (2004), showing that rats given 100 mg/kg modafinil exhibited a drastic enhancement of locomotor activity even though this effect, however, did not induce more efficient goal-directed behavior. On the other hand, rats that received 32–128 mg/kg were unable to significantly enhance five-choice serial reaction time test performance under standard conditions, suggesting that attentional processes in normal awake rats remain unaltered, and that high doses of modafinil increase impulsivity (Waters et al., 2005).

Concerning the selection of mice according to our selection criterion (i.e., successful first alternation), we observe that, for *control* animals (after or without stress, $n=15$ for both groups), neither of the subjects was excluded, contrary to modafinil-treated animals. Indeed, for modafinil-treated animals in *non-stress conditions*, the number of excluded mice was comparable for M8, M16 and M32 groups (i.e., 6, 4 and 6, respectively; 16 mice as a whole), whereas *in stress conditions*, the number amounted to 1, 5 and 2 (8 mice as a whole) for M8, M16 and M32 groups, respectively. Thus, the effect of modafinil in non-stress conditions seems to be detrimental as regards our selection criterion (the success of the first alternation), whereas this effect appears to be attenuated in stress conditions. This point provides further evidence of increased modafinil efficiency on psychomotor performance in stress situations. Finally, the differences between the sizes of experimental groups, from a behavioral point of view, are due to modafinil/stress interaction effects.

From an operational point of view, a negative psychomotor effect is likely to occur if too much modafinil was administered to ejected pilots or wrecked sailors, in survival conditions exposed to major stress. We could also hypothesized that modafinil overdose could impair sports performance in stress-generating competition. Hence, the active dose of modafinil for optimal global psychomotor performance seems to be highly dependent on environmental stressors, such as sleep deprivation (Randall et al., 2004), but also on the emotional status of the subjects. Moreover, we previously found using elevated plus maze, that modafinil had anxiogenic action by itself at the dose of 64 mg/kg (unpublished data). Our present study, however, evidences the fact that the benefits of modafinil treatment are not clearly dose-related (Randall et al., 2005).

5. Conclusion

Our current study evidenced for the first time the involvement of glucocorticoids *and* stress in the modulation (enhancement or decrease) of psychomotor performance, as a function of the administered dose of modafinil.

Indeed, our work highlights the interaction between emotion and memory, involving many pathways other than glucocorticoids. Hence, in order to dissociate this interaction, future experiments will aim at measuring and modifying the emotional status of subjects, using anxiolytic drugs such as benzodiazepines or antidepressant drugs such as fluoxetine, prior to modafinil administration. We will also block the corticosterone secretion using metyrapone (inhibitor of 11-β hydroxylase involved in corticosterone biosynthesis), with a view to identifying the specific role of glucocorticoids in the modulation of performance by modafinil, with or without added stress. Further, we intend to investigate the effect of modafinil on different memory systems using the Contextual Serial Discrimination task (CSD) in the four-hole board, so as to evaluate the effects of stress on spatial and contextual information retrieval (Célérier et al., 2004).

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