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Differential effects of total sleep deprivation on contextual and spatial memory: Modulatory effects of modafinil

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ABSTRACT

The aim of the present work was to investigate in mice the effects of a total 10-hr sleep deprivation on contextual (episodic-like) and spatial (reference) memory tasks. For that purpose, mice learned two consecutive discriminations (D1 and D2) in a 4-hole board involving either identical (Serial Spatial Discrimination, SSD) or distinct (Contextual Serial Discrimination, CSD) internal contextual cues. In a second step, we intended to assess the corrective effect of modafinil on memory impairments generated by sleep deprivation. Sleep deprivation was triggered through an alternative platform apparatus (water box), previously validated using EEG recording and spectral analysis.

We showed that a 10-hr total sleep deprivation impaired the CSD task but not the SSD one. Moreover, the impairment of contextual memory in sleep-deprived animals was dose-dependently corrected by modafinil. Indeed, modafinil administered after the sleep deprivation period and 30 min before the test session restored a memory retrieval pattern identical to non sleep-deprived animals at the doses of 32 and 64 mg/kg, however not at 16 mg/kg.

Results hereby evidence that the vigilance-enhancing drug modafinil is able to restore the contextual memory performance at a low dose as compared to other memory tasks, possibly by an enhancement of hippocampal activity known to be both involved in the processing of contextual information and impaired following our sleep deprivation procedure.

1. Introduction

Sleep deprivation has metabolic and endocrine effects and causes marked impairments in neurotransmitter receptors function in brain areas involved in learning and memory, such as the hippocampus (Copinschi, 2005; Longordo et al., 2009). Thus, from a functional point of view, sleep deprivation has a deleterious impact on cognitive performance, mainly on attention and working memory, but further affects other functions such as long-term memory and decision-making (Durmer and Dinges, 2005; Versace et al., 2006; Alhola and Polo-Kantola, 2007). Moreover, sleep and sleep deprivation also affect definite phases of memory processes, namely: consolidation and reconsolidation (Stickgold and Walker, 2005).

Modafinil has stimulant and awakening properties without amphetamine-like side effects (Bastuji and Jouvet, 1988; Lagarde and Milhaud, 1990; Hermant et al., 1991; Lagarde and Batejat, 1995). Thus, modafinil is prescribed for the treatment of sleep pathologies without interfering with nocturnal sleep (Bastuji and Jouvet, 1988). Modafinil has been found to act via several neurotransmitters systems (Tanganelli et al., 1992; Pierard et al., 1995, 1997; Lagarde et al., 1996; Boutrel and Koob, 2004). In addition we recently evidenced an interaction between modafinil and glucocorticoids system in stress conditions (Pierard et al., 2006).

In non sleep-deprived (NSD) mice, we previously evidenced that modafinil has an enhancing effect in working memory tasks involving a flexible form of memory processes (Beracochea et al., 2001) and learning processes as well (Beracochea et al., 2002, 2003). In sleep-deprived (SD) animals, we evidenced that a 10-hr sleep deprivation-induced working-memory performance impairments, correlated with a distinct neuronal activity decrease in several brain areas, however mainly within the hippocampus. Both these memory and neurobiological impairments were reversed by modafinil, administered after the sleep deprivation period (Pierard et al., 2007).

Thus, the aim of the present work was to evaluate in mice the effects of a total 10-hr sleep deprivation on other memory task, *i.e.* contextual memory processes, and the corrective effect of modafinil. Moreover, plasma corticosterone level was measured in order to better assess the impact of stress on the memory impairments resulting from SD. To such an end, we developed two original memory paradigms in a 4-hole board,

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involving the acquisition of two consecutive discriminations allowing the evaluation of either context-independent (Serial Spatial Discrimination, SSD task) or context-dependent memory processes (Contextual Serial Discrimination, CSD task) (Celerier et al., 2004; Chauveau et al., 2009, 2010; Pierard et al., 2009; Tronche et al., 2010a). For that purpose, in the SSD task, the floor context (colour and texture) of both discriminations is the same, whereas in the CSD task the floor contexts of both discriminations are different. In contrast, the allocentric spatial environment of the hole-board was kept constant in both the CSD and SSD tasks. Given the change of the floor context in the acquisition phase, the CSD task involves more flexible form of memory processes as compared to the SSD one (Celerier et al., 2004; Tronche et al., 2010b). In a second step, given the results obtained in the first step, we intended to evaluate the corrective effect of modafinil on the contextual memory impairments induced by sleep deprivation in the CSD task.

2. Materials and methods

2.1. Animals

The study was conducted using male mice of the C57 BL/6 Jico strain (Iffa-Credo, Lyon, France). Upon 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 (22 °C ambient temperature; automatic light cycle 07:00 a.m. and 07:00 p.m.) with free access to food and water. Fifteen days before testing, mice were placed in individual cages and manipulated 10 min per day, in order to reduce interference with the experimenter. Animals were 5 month-old on the day of the experiment. Four days before behavioral testing, mice were submitted to a food deprivation schedule intended to reduce body weight as follows: at the time of training, mice weighed 86–88% of their initial free-feeding weights ranged between 25 and 30 g. Food ration was adjusted individually in order to maintain the same level of deprivation throughout the ensuing experimental period (acquisition and test sessions).

The present study was carried out in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, under agreement 2010/11 delivered by the French Ministry of Defence after the protocol was examined by the local ethical committee. Guidelines for proper laboratory animal care were fully implemented.

2.2. Sleep deprivation

An automatic total sleep deprivation apparatus has been developed (Pierard et al., 2007). This apparatus involves a thermostated (31 °C) water tank (42 × 32 × 22 cm high) including two small square platforms (10 × 10 cm) closely adjusted side by side and slightly emerging from the water level. Each platform alternatively moves every 10 s below the water surface, thus compelling the mouse to a permanent motion from one platform to the other in order to avoid water contact. Thus, at any moment, the animal has an emerging security platform readily available, allowing it to stay out of the water, thus reducing the stress level. Moreover, to that aim, mice were allowed to become familiar with the water box environment the days prior to the behavioral task. Finally, in order to obtain an index of stress intensity in sleep-deprived mice, we measured plasma corticosterone level at the end of the sleep deprivation period, as compared to non sleep-deprived control animals submitted or not to the CSD task. Animals were placed in the apparatus at 08:00 a.m. and removed at 06:00 p.m. Thus, the sleep deprivation period occurred during the diurnal phase corresponding to the resting phase. In a previous EEG study we showed that the efficacy of this sleep deprivation procedure reached 99.79% as regards the duration of micro-sleeps (Pierard et al., 2007).

2.3. Memory tests

Memory tests were performed in a 4-hole board apparatus (45 × 45 × 30 cm high) enclosed by a grey Plexiglas. The floor of the board was interchangeable as regards colour and texture. On the floor, four holes opening onto a food cup (3 cm diameter × 2.5 cm depth) were located in each corner, 6 cm away from the sidewalls. The apparatus was placed in a room exposed to a 60 db background noise and a light centred over the apparatus provided a 20 lx intensity at the position of the apparatus floor. The apparatus was cleaned with ethanol 95%, then with water before each behavioral test. Photocells placed in each hole allowed to measure the number of head dips in each hole without any experimenter's interference.

2.3.1. Serial Spatial Discrimination (SSD) task

The experimental design of the SSD task allows investigating spatial memory, only based on the use of spatial allocentric cues (Fig. 1A).

- *Acquisition:* In room A, mice were first placed at the centre of the board in a PVC tube for 15 s and then learned two successive discriminations for 6 min each. During this acquisition phase, the same floor (grey colour) was used for the two successive diagonally opposite discriminations D1 and D2, separated by a 2-min time interval during which the mouse was placed in its home cage in room B. For D1, ten food pellets (20 mg) were available only in one of the four holes of the board. The baited hole for D1 was chosen at random. For D2, ten food pellets were systematically located in the diagonally opposite hole as compared to D1. Subjects which did not eat at least 8 out of the 10 pellets at D1 and D2 within the 6 min-period were discarded from analysis. The environmental cues were made of coloured and stripped paper sheets stuck on the walls of the room, and positioned 1 m above the floor. These allocentric cues remained at the same place for the two successive discriminations. Thus, in this task, the learning of both discriminations D1 and D2 is based on the use of external allocentric cues only. At the end of the acquisition phase, mice were replaced in the animal room for 24 hr, and were then assigned to the sleep deprivation protocol, as described below.
- *Memory test phase:* Thirty-four hours after the acquisition phase, and 30 min after the end of the sleep deprivation period, mice were repositioned on the grey floor used for the acquisition phase, however without any pellet in the apparatus. They were allowed to freely explore the apparatus for 6 min. In this test phase, the memory of D1 or of D2 is evaluated simultaneously on the same mouse. Performance was assessed by the percentage of correct responses, i.e. the number of head dips in the previously rewarded holes/by the total number of head dips × 100.

Twenty-six mice were used, randomized between two groups: non sleep-deprived (NSD) controls ($n = 14$) and sleep-deprived (SD) animals ($n = 12$).

2.3.2. Contextual Serial Discrimination (CSD) task

The experimental design of the CSD task allows investigating contextual memory based on the use of internal cues (floors) and spatial memory based on the use of spatial allocentric cues (Fig. 1B).

- *Acquisition:* As compared to the previous task, we evaluated the effects of varying the internal context for each discrimination. Overall, the procedure is similar to the one described above for the SSD task, except that the two serial discriminations D1 and D2 differed by the colour and texture of the floor (white and smooth versus black and rough). Further, the sequence of the two different floors in the series (first versus second discrimination) has been systematically alternated from one mouse to another within each group. At the end of the acquisition phase, the mice were replaced in the animal room for 24 hr, and were then submitted to the sleep deprivation protocol, as described below. At the end of the 10-hr

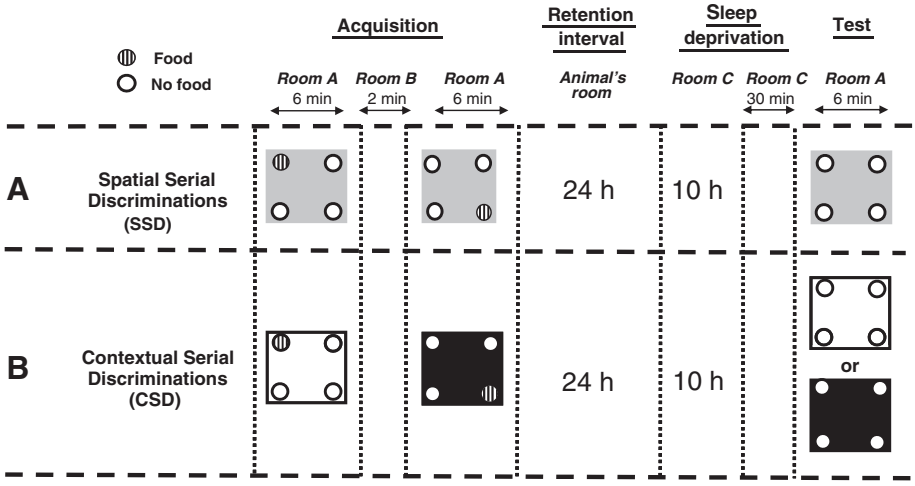

Fig. 1. Memory tests. (A): Serial Spatial Discrimination (SSD). (B): Contextual Serial Discrimination (CSD). This task was performed on independent groups of mice insofar as we need to change the floor between D1 and D2 discriminations. In the second CSD experiment, modafinil or vehicle was administered after the sleep deprivation period, 30 min before the memory test phase.

sleep deprivation period, animals were then tested for memory of the previously learned discriminations.

- *Memory test phase:* Thirty-four hours after the acquisition phase, and 30 min after the end of the sleep deprivation period, mice were placed either on the floor of D1 or on the floor of D2 and were allowed to freely explore the apparatus. Thus, a retrieval test was carried out on independent groups of mice for D1 and D2 insofar as we need to change the floor between D1 and D2 discriminations. Performances were assessed by measuring the exploration for each hole for 6 min without any pellet in the apparatus.

Using this procedure, two parameters were measured: i) the percentage of correct responses, rated by the number of head dips in the previously rewarded hole/by the total number of head dips × 100; ii) the percentage of interfering responses, rated by the number of head dips in the diagonally opposite rewarded hole of the other discrimination/total number of head dips × 100. This procedure also allowed calculating an indirect index of spatial memory by adding the percentage of correct responses and the percentage of interfering responses, that is to say head dips into the two previously baited holes regardless of the internal context of the apparatus (Celerier et al., 2004).

- *In the first CSD experiment*, we investigated the effect of a total 10-hr sleep deprivation on contextual serial memory. For this purpose, 39 mice were randomized between a non sleep-deprived (NSD) group ($n = 19$) and a sleep-deprived (SD) group ($n = 20$). In the NSD group, 10 animals were tested for D1 and 9 for D2. In the SD group, 10 mice were tested for D1 and the 10 others for D2.
- *In the second CSD experiment*, we investigated the effect of modafinil on the CSD task following sleep deprivation. Five experimental groups were implemented (24 mice each): non sleep-deprived (NSD) group, sleep-deprived (SD) group, and 3 sleep-deprived groups treated by modafinil at the doses of 16, 32 and 64 mg/kg (SD16, SD32 and SD64 respectively). Half of the animals were tested for D1, the other half for D2.

2.4. Modafinil

Given our results showing impairments after SD in the CSD but not the SSD task, we intended to evaluate the corrective effect of modafinil in the CSD task only.

Modafinil, insoluble in water, was suspended in a 0.5% Arabic gum solution (vehicle) and i.p. injected in mice at the doses of 16, 32 or 64 mg/kg. Modafinil or vehicle alone (0.1 ml/10 g b.w.) was administered 30 min before testing.

2.5. Plasma corticosterone assay

Mice were decapitated either immediately after sleep deprivation or immediately after the CSD task to collect trunk blood. They were compared to control mice located for 10 hr in the sleep deprivation room, without any food in their cage to allow a comparison with sleep-deprived animals. After centrifugation at 3000 rpm for 10 min, the supernatant was stored at −80 °C until assay. Plasma corticosterone was analyzed by way of the HPLC-fluorimetry method (adapted from Mason et al., 1992 and validated by AFNOR XPT 90-210 standard). The sensibility of the assay was 25.98 nmol/l. Therefore, baseline sample concentration was more than 3-fold superior than the sensitivity threshold.

2.6. Statistical analyses

Statistical analyses were performed using the Statview 5.0 software. Two-way analysis of variance (ANOVA) was performed to assess the effects of SD and modafinil on the animals' memory performance and corticosterone level. Further comparisons between individual groups were performed with the Fisher post-hoc test.

3. Results

3.1. Effects of sleep deprivation on memory

3.1.1. SSD task: spatial memory

For D1, the percentage of correct responses rates 35.8 ± 5.7% in SD mice *vs* 38.5 ± 3.8% in control NSD animals. For D2, the percentage of correct responses reaches 45.5 ± 5.2% in SD mice *vs* 34.3 ± 4.0% in NSD animals. Global ANOVA evidences that these results are not significantly different ($F(3,48) = 1.097$; NS). Since the first and second baited holes were equally explored by both groups during the test phase, the performance by each group (NSD and SD) was pooled for further analysis of spatial memory performance. The results are presented in Fig. 2. Overall, the percentage of correct responses amounts to 72.8 ± 2.2% in NSD mice and 81.3 ± 3.3% in SD animals. These values are significantly different ($p < 0.0001$) from chance level (50%) and between them ($F(1,24) = 4.988$; $p < 0.05$).

3.1.2. First CSD experiment

3.1.2.1. Contextual serial memory

- *Correct responses:* The results are presented in Fig. 3. Global ANOVA evidences that the percentage of correct responses (visits of the hole

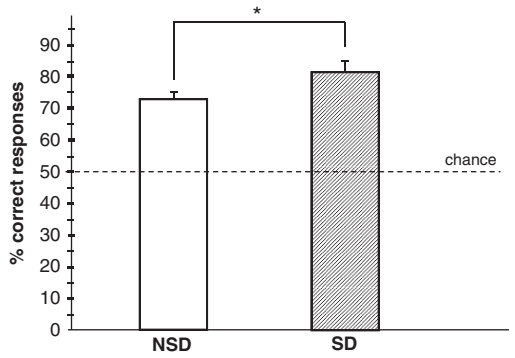

Fig. 2. Effect of sleep deprivation on spatial memory (SSD task). NSD: non sleep-deprived; SD: sleep-deprived. Results are expressed as the percentage of *correct responses*. *: $p<0.05$.

previously rewarded in the same context the day before) is significantly different among groups ($F(3,35)=10.04$; $p<0.0001$). Individual comparisons using the Fisher post-hoc test display the following features:

i) the percentage of correct responses for D1 is significantly higher than for D2 in the NSD mice ($51.5\pm3.6\%$ *vs* $29.7\pm4.9\%$; $p<0.001$), as well as in the SD mice ($37.6\pm4.3\%$ *vs* $24.1\pm2.0\%$; $p<0.05$),

ii) the percentage of correct responses for D1 in the SD mice is significantly lower as compared to the NSD animals ($37.6\pm4.3\%$ *vs* $51.5\pm3.6\%$; $p<0.05$), whereas there is no significant difference for D2 between the NSD and SD groups ($24.1\pm2.0\%$ *vs* $29.7\pm4.9\%$, respectively).

- *Interfering responses:* The results are presented in Fig. 4. Global ANOVA evidences that the percentage of interfering responses (visits of the hole previously rewarded the day before, but in the other context) is significantly different among groups ($F(3,35)=6.265$; $p<0.01$). Individual comparisons using the Fisher post-hoc test show that:

i) the percentage of interfering responses for D1 in the NSD mice is significantly lower as compared to D2 ($28.0\pm3.5\%$ *vs* $47.4\pm4.4\%$; $p<0.01$), whereas D1 and D2 responses are not significantly different in the SD mice ($46.3\pm6.2\%$ *vs* $52.6\pm2.3\%$; NS),

ii) the percentage of interfering responses for D1 in the SD mice is significantly higher as compared to the NSD animals ($46.3\pm6.2\%$ *vs* $28.0\pm3.5\%$; $p<0.01$), whereas there is no significant difference between both the NSD and SD groups ($52.6\pm2.3\%$ *vs* $47.4\pm4.4\%$; NS).

3.1.2.2. Spatial memory. Results are summarized in Fig. 5 which provides the spatial memory scores obtained by the sum of the percentages of correct and interfering responses obtained in the first CSD experiment. Global ANOVA showed that spatial memory scores were significantly different from chance level (50%) for each group ($p<0.001$), but not significantly different between them ($p>0.10$ in all comparisons).

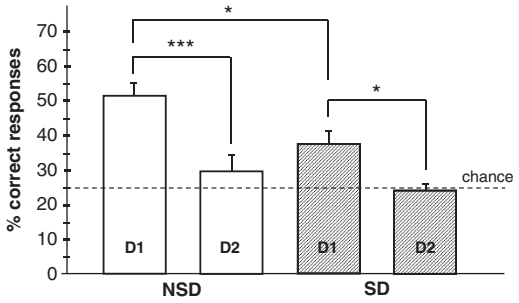

Fig. 3. Effect of sleep deprivation on contextual serial memory (first CSD experiment). NSD: non sleep-deprived; SD: sleep-deprived. Results are expressed as the percentage of *correct responses*. *: $p<0.05$; ***: $p<0.001$.

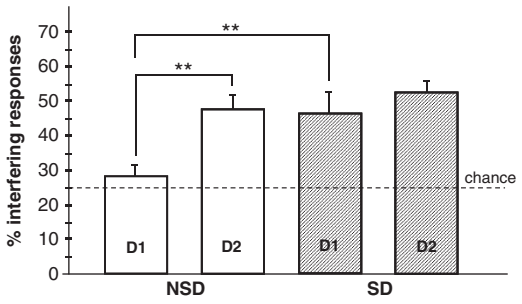

Fig. 4. Effect of sleep deprivation on contextual serial memory (First CSD experiment). NSD: non sleep-deprived; SD: sleep-deprived. Results are expressed as the percentage of *interfering responses*. **: $p<0.01$.

3.2. Effects of sleep deprivation and behavioral testing on plasma corticosterone

Results are summarized in Fig. 6. The ANOVA performed on the overall groups showed a significant difference ($F(5,46)=11.6$; $p<0.0001$). More specifically, the SD control group ($n=6$) exhibited a significant increase in plasma corticosterone concentration as compared to NSD controls ($n=7$; 0.225 ± 0.019 μg/ml versus 0.133 ± 0.018 μg/ml respectively; $p<0.05$). In addition, plasma corticosterone concentration was also increased by behavioral testing in the CSD task in NSD animals for D1 ($n=10$; 0.240 ± 0.014 μg/ml; $p<0.01$) as well for D2 ($n=9$; 0.305 ± 0.026 μg/ml; $p<0.001$) as compared to NSD controls not submitted to behavioral testing (0.133 ± 0.018 μg/ml). Interestingly, the test-induced increase in corticosterone concentration was greater in SD animals as compared to NSD animals also submitted to behavioral testing. Indeed, in SD mice tested for D1 ($n=10$), the concentration of corticosterone was significantly higher as compared to NSD mice (0.334 ± 0.024 μg/ml versus 0.240 ± 0.014 μg/ml; $p<0.01$). This was also observed in SD mice tested for D2 ($n=10$), as compared to NSD mice also tested for D2 (0.374 ± 0.034 μg/ml versus 0.305 ± 0.026 μg/ml; $p<0.05$).

3.3. Effect of modafinil on memory after sleep deprivation

In this second CSD experiment, we intended to evaluate the retrograde corrective effect of modafinil on the retrieval of D1, previously impaired by the sleep deprivation. Results are represented in Fig. 7.

- *SD group:* We confirmed that the sleep deprivation induced a decrease in D1 retrieval, as compared to the NSD group ($37.3\pm3.5\%$ *vs* $52.3\pm2.8\%$; $p<0.05$).
- *SD16 group* (Fig. 7A): On the one hand, D1 response is not significantly different as compared to the SD group ($37.2\pm3.5\%$ *vs* $37.3\pm3.5\%$; NS), but decreased as compared to the NSD group ($37.2\pm3.5\%$ *vs* $52.3\pm2.8\%$; $p<0.01$). On the other hand, D2 response is increased as compared to the NSD group (42.7 ± 5.0 *vs* $30.1\pm3.1\%$; $p<0.01$) and SD group ($42.7\pm5.0\%$ *vs* $26.0\pm1.5\%$; $p<0.001$).

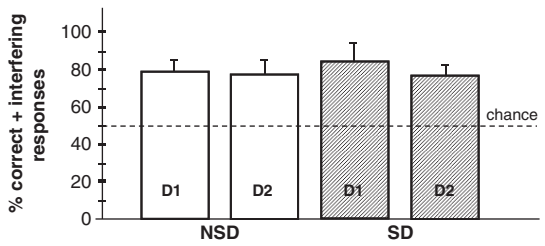

Fig. 5. Effect of sleep deprivation on spatial memory (First CSD experiment). NSD: non sleep-deprived; SD: sleep-deprived. Results are expressed as the percentage of *(correct + interfering) responses*, as an index of spatial memory. No effect was evidenced.

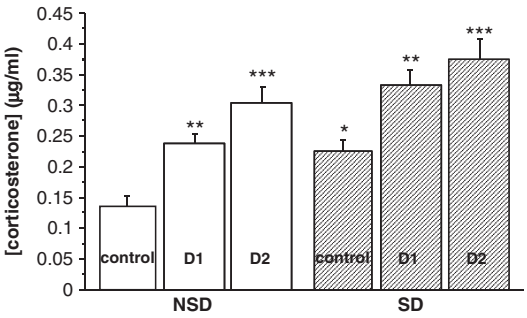

Fig. 6. Plasma corticosterone concentrations (µg/ml). Values are means ± SEM. NSD: non sleep-deprived animals (open bars); SD: sleep-deprived animals (striped bars). Control animals were not submitted to behavioral testing. Comparisons to control NSD animals. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

- *SD32 group* (Fig. 7B): On the one hand, D1 response is significantly higher than in the SD group (47.0 ± 3.0% *vs* 37.3 ± 3.5%; $p<0.05$) and not significantly different from the NSD group (47.0 ± 3.0% *vs* 52.3 ± 2.8%; NS). On the other hand, D2 response is not significantly different as compared to the NSD group (32.0 ± 2.5% *vs* 30.1 ± 3.1%; NS) and SD group (32.0 ± 2.5% *vs* 26.0 ± 1.5%; NS).
- *SD64 group* (Fig. 7C): As for the SD32 group, the D1 response is higher than the D2 response (46.2 ± 4.4% *vs* 27.4 ± 1.9%; $p<0.0001$). On the one hand, the percentage of D1 response is not statistically different as compared to the NSD group (46.2 ± 4.4% *vs* 52.3 ± 2.8%; NS), but was significantly above the performance of the SD group (46.2 ± 4.4% *vs* 37.3 ± 3.5%; $p<0.05$). On the other hand, the D2 response was not statistically different as compared to the NSD mice (27.4 ± 1.9% *vs* 30.1 ± 3.1%; NS) and SD animals (27.4 ± 1.9% *vs* 26.0 ± 1.5%; NS).

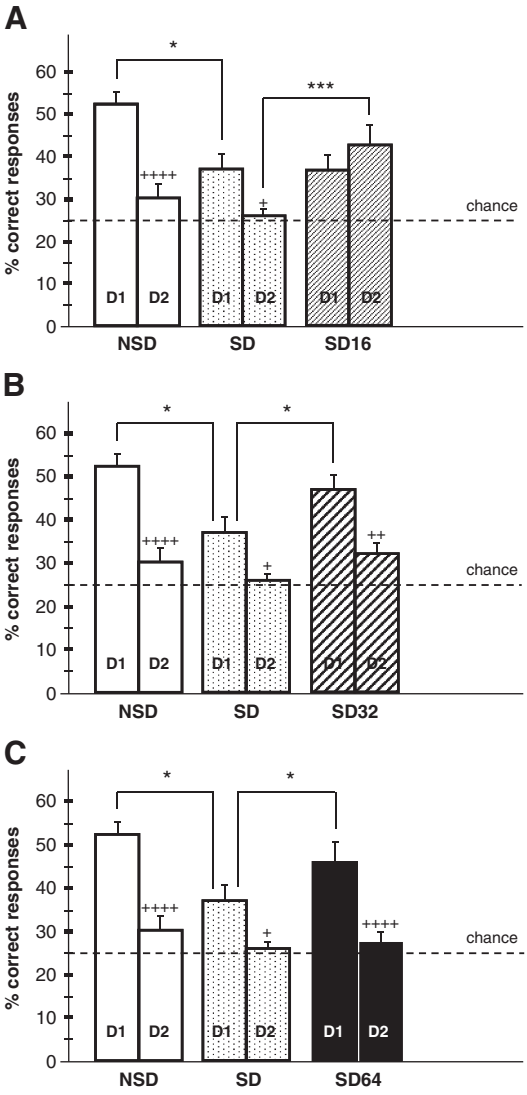

Fig. 7. Effect of modafinil on contextual serial memory after sleep deprivation (second CSD experiment). A: 16 mg/kg; B: 32 mg/kg; C: 64 mg/kg. Results are expressed as the percentage of *correct responses*. NSD: non sleep-deprived; SD: sleep-deprived; SD16, SD32, SD64: sleep-deprived mice, i.p. injected with modafinil after the sleep deprivation period, at the doses of 16, 32 and 64 mg/kg respectively. Intergroups comparisons: *: $p<0.05$; ***: $p<0.001$. Intragroups comparisons: +: $p<0.05$; ++: $p<0.01$; ++++: $p<0.0001$.

4. Discussion

Our findings may be summarized as follows. We showed that a 10-hr total sleep deprivation impaired contextual memory in the CSD task, whereas in contrast, spatial memory was either increased in the SSD task or remained unaffected in the CSD task. Moreover, the impairment of contextual memory was dose-dependently corrected by modafinil administration. Indeed, the lowest modafinil dose (16 mg/kg) increased memory retrieval of the second discrimination, whereas the two other modafinil doses (32 and 64 mg/kg) increased the memory retrieval of the first discrimination, *i.e.* restored a memory retrieval pattern identical to the NSD animals.

4.1. Sleep deprivation and memory performance

As a major leading result within our study, spatial memory is either unaffected (CSD task) or further enhanced (SSD task) by sleep deprivation. Such a finding may seem unexpected at first sight, since some studies (including ours) evidenced that sleep deprivation often disrupted hippocampal-dependent tasks involving a spatial component (Guan et al., 2004; Hairston et al., 2005; Pierard et al., 2007; Hagewood et al., 2010). However, the discrepancy among existing studies to date may be due to procedural difference implemented either to induce sleep loss and/or used to evaluate spatial memory. Indeed, on the one hand, the sleep deprivation procedure greatly differed among the studies referenced here. As evidenced by plasma corticosterone measurements in the present study, SD induced a significant increase in corticosterone as compared to NSD controls, but which was similar to the one resulting from behavioral testing only, in NSD animals submitted to the CSD task (D1 or D2). Thus, we can assume that our automated sleep deprivation system ("water box") induces only low stress levels. On the other hand, in our behavioral SSD and CSD paradigms, allocentric spatial cues remained available for 12 min during the acquisition phase: hence the spatial learning may recruit larger neural networks, allowing thereby a compensation for any potential hippocampal dysfunction. In agreement with such a hypothesis, we already showed that in the very same tasks, neurotoxic lesions of the hippocampus also spared spatial memory while affecting the contextual one (Chauveau et al., 2008). Moreover, the sparing or enhancement of the spatial memory observed in the present study could be a compensatory mechanism following an impairment of the contextual memory. Indeed, we already showed that ageing induced an important hippocampal-dependent impairment of contextual memory while enhancing the use of allocentric spatial memory strategies (Tronche et al., 2010a). Thus, the impairment of the more flexible form of memory (contextual memory processing) could be compensated by the use of a more stable one (spatial reference memory) in sleep-deprived subjects as observed previously in middle-aged mice.

In the CSD task, sleep deprivation affects the retrieval of the first discrimination (D1) but not the retrieval of the second one (D2). Contrariwise in the SSD task, sleep deprivation does not affect the retrieval of serial order for D1 and D2, since both baited holes were equally explored. Thus, the introduction of the change of the internal

context by the use of two different floors in the 4-hole board modifies the expression of the serial memory retrieval pattern.

It is noteworthy that we already demonstrated that the retrieval of D1 in the CSD task is specifically sustained by the hippocampus (Chauveau et al., 2008, 2010) which is known to be involved in the treatment of contextual memory processes (Eichenbaum, 2000). Such earlier findings suggest therefore that the sleep deprivation-induced contextual memory impairment presently observed in the CSD task would be a consequence of a hippocampal dysfunction mainly as regards the observed increase of plasma corticosterone in SD animals submitted to the D1 test session. Indeed, we already showed that direct injection of corticosterone into the hippocampus induced a memory retrieval deficit similar to the one observed here in SD subjects (Chauveau et al., 2010). Other mechanisms can also account for the memory deficit induced by SD. Thus, several studies show that sleep restriction impairs hippocampal-dependent memory (Ruskin et al., 2004; Walker, 2008), maybe by suppressing neurogenesis into the hippocampus (Hairston et al., 2005) or by affecting hippocampal synaptic plasticity (Mc Dermott et al., 2003; Romcy-Pereira and Pavlides, 2004; Mirescu et al., 2006; Tadavarty et al., 2009). On the other hand, a recent study showed that sleep selectively enhances hippocampus-dependent memory in mice (Cai et al., 2009).

In addition, the difference between SD and NSD groups in the CSD task may rely on the emergence of interference between the two previously learned discriminations. Indeed, it has been shown that sleep deprivation could impair retrieval functions by increasing false memories (Diekelmann et al., 2008). Accordingly, our behavioral results in SD animals exhibit two opposite profiles of correct versus interfering responses on retrieval curves (Figs. 3 and 4). More precisely, when tested on the D1 context, SD animals recall better the second discrimination learned the day before, and inversely when they are tested for D2. Thus, we may consider that sleep deprivation increases intrusions in our context-dependent CSD paradigm. Another interpretation to explain the observed intrusions at the time of retrieval, could be that sleep deprivation induces a conflict between a contextual recall strategy (based on the colour and texture of the floors) versus a spatial recall strategy (based on invariant allocentric cues), so that mice explored the previously baited holes regardless of the internal contexts (floors) of the acquisition.

4.2. Effects of modafinil on memory impairments induced by sleep deprivation

We already showed that modafinil administration was able to increase the working memory performance in NSD animals (Beracochea et al., 2001) and also to restore working memory performance previously impaired by a 10-hr total sleep deprivation (Pierard et al., 2007). More specifically, the memory facilitation induced by modafinil was associated with a recovery of c-Fos activity in several brain areas including the frontal cortex, the hippocampus and the amygdala, i.e. in brain areas known to be involved in memory, emotion and attention (Pierard et al., 2007).

Since this enhancing effect of modafinil on working memory was observed only at the dose of 64 mg/kg (Pierard et al., 2007), we aimed at determining in the present study if the potential cognitive-enhancing effect of modafinil in the CSD task could occur for the same or even lower doses. Indeed, we found in SD subjects that modafinil restored a normal contextual memory at a lower (32 mg/kg) dose as compared to working memory. Hence, the following hypothesis, namely: higher doses of modafinil are required to restore performance in memory tasks involving a more important cognitive load, such as the T-maze task as compared to the CSD task.

As regards the effects of modafinil in the CSD task, our data show more specifically that the lowest modafinil dose (16 mg/kg) increased memory retrieval in the second discrimination (D2) whereas the two higher modafinil doses (32 and 64 mg/kg) increased the memory retrieval within the first discrimination (D1), *i.e.* restored a memory retrieval pattern identical to the NSD animals. Thus, modafinil induced a modulation of the retrieval pattern in a dose-dependent manner. Our earlier findings as regards brain neural networks sustaining either D1 or D2 retrieval in the CSD task (Chauveau et al., 2008, 2009, 2010; Tronche et al., 2010b) tend to corroborate the following hypothesis, namely that the low dose (16 mg/kg) of modafinil would act preferentially on the frontal cortex activity which has been found to sustain the retrieval of D2 (Chauveau et al., 2009), probably via an increase in attention (Waters et al., 2005). In contrast, the two higher doses of modafinil (32 and 64 mg/kg) restored a memory retrieval pattern similar to that observed in the NSD animals. This memory-enhancing effect on D1 could be mediated by an enhancement of the activity of the hippocampus, as shown by c-Fos immunochemistry (Pierard et al., 2007). The latter hypothesis is congruent with recent studies showing that modafinil is more selective and specific to hippocampus-dependent memory, as compared to other vigilance-enhancing drugs such as amphetamine (Pierard et al., 2007) or cocaine (Shuman et al., 2009). Further, existing studies to date reveal that modafinil can also activate subcortical as well as cortical sites in sleep-deprived states (Thomas and Kwong, 2006). Whereas its specific neurochemical mechanism of action still remains partially unaccounted for (Saper and Scammel, 2004), modafinil has been proven however to enhance wakefulness by acting on both norepinephrine and dopaminergic systems (Boutrel and Koob, 2004; Wisor and Eriksson, 2005; Madras et al., 2006). In addition, our team showed that modafinil could act via corticosterone, mainly in stress conditions (Pierard et al., 2006), via excitatory amino acids or via an enhancement of energy metabolism in cerebral cortex (Pierard et al., 1995, 1997).

5. Conclusion

The present study evidenced that a total 10-hr sleep deprivation disrupted contextual but not spatial memory processes. The contextual memory deficit induced by sleep deprivation in the CSD task mainly occurred for the retrieval of the first discrimination which is hippocampus-dependent. Moreover, modafinil is able to compensate the sleep-induced contextual memory deficit at the doses of 32 and 64 mg/kg. As shown by the inversion of normal retrieval patterns with the lower modafinil dose (16 mg/kg), but not with higher ones, modafinil most definitely acts on different brain areas, as a function of the administered dose. Thus, further neurobiochemical studies are needed to evidence the differential involvement of brain areas as a function of either the nature of the cognitive task and/or of the administered dose of modafinil.

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