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ORIGINAL INVESTIGATION

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Anxiogenic effect of sleep deprivation in the elevated plus-maze test in mice

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Abstract *Rationale:* Several clinical studies demonstrate that the absence of periods of sleep is closely related to occurrence of anxiety symptoms. However, the basis of these interactions is poorly understood. Studies performed with animal models of sleep deprivation and anxiety would be helpful in the understanding of the mechanisms underlying this relationship, but some animal studies have not corroborated clinical data, reporting anxiolytic effects of sleep deprivation. *Objectives:* The aim of the present study was to verify the effects of different protocols of sleep deprivation in mice tested in the elevated plus-maze and to assess the effect of chlordiazepoxide and clonidine. *Methods:* Three-month-old male mice were sleep-deprived for 24 or 72 h using the methods of single or multiple platforms in water tanks. Mice kept in their home cages were used as controls. Plus-maze behavior was observed immediately after the deprivation period. *Results:* Mice that were sleep-deprived for 72 h spent a lower percent time in the open arms of the apparatus than control animals. This sleep deprivation-induced anxiety-like behavior was unaffected by treatment with chlordiazepoxide (5.0 and 7.5 mg/kg IP), but reversed by an administration of 5 or 10 μg/kg IP clonidine. *Conclusion:* The results indicate that under specific methodological conditions sleep deprivation causes an increase in anxiety-like behavior in mice exposed to the elevated plus-maze.

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Introduction

Sleep disturbances are often paired with anxiety, and sleep problems are diagnostic criteria for anxiety disorders (American Psychiatry Association 1994). Sleep deprivation was reported to produce anxiety in normal humans (Wyatt et al. 1971; Peeke et al. 1980; Dinges et al. 1997) and to aggravate symptoms of anxiety disorders (Roy-Byrne et al. 1986). Although sleep deprivation has been proposed as a treatment for some psychiatric disorders, such as depression (Wyatt et al. 1971; Van den Burg and Van den Hoofdakker 1975; Svendsen 1976; Gerner et al. 1979) and even to ameliorate anxiety symptoms in depressed patients (Roy-Byrne et al. 1986), it does not represent an effective treatment of anxiety disorders (Roy-Byrne et al. 1986; Labatte et al. 1998).

Although the relationship between sleep and anxiety is accepted, the basis of these interactions is less well understood (Bourdet and Goldenberg 1994). Therefore, it was thought that additional studies in sleep-deprived animals, using frequently used animal models of anxiety, might help to further elucidate potential links between sleep (deprivation) and anxiety. This is especially important because previously performed animal studies have pointed to a paradoxical anxiolytic-like effect of sleep deprivation (Pokk and Zharkoversusky 1995, 1997, 1998; Pokk et al. 1996; Suchecki et al. 2002): in these studies rats and mice spent more time on the open arms of the elevated plus-maze. In contrast, in our own unpublished data, while using a specific experimental protocol for sleep deprivation, a clear-cut anxiogenic-like effect in sleep-deprived mice was observed.

The aims of the present study were: (1) to confirm the above mentioned anxiogenic effect in the elevated plus-maze; (2) to extend this finding to another animal model of anxiety (open field test); (3) to evaluate the influence of methodological variations on the sleep deprivation-in-

duced anxiogenesis in the elevated plus-maze; and (4) to try to modulate the anxiogenic effect of sleep deprivation by treating the mice with chlordiazepoxide or clonidine.

Materials and methods

Subjects

Three-month-old Swiss EPM-M1 male mice were housed (since weaning) under conditions of controlled temperature (22–23°C) and lighting (12 h light, 12 h dark; lights on 7 a.m.) in 33×40×17 cm cages, six per cage. Food (Purina lab chow) and filtered water were available ad libitum throughout the experiments. All animals were naive to the experimentation. All behavioral parameters were recorded based on direct observation with manual counters and stopwatches, between 3 and 5 p.m. In all experiments, animals were observed in a counterbalanced order for sleep condition and treatment.

Sleep deprivation procedure

The animals were sleep-deprived or maintained in their home cages (control group) in the same room. In most of the experiments, the method of sleep deprivation used was an adaptation of the multiple platform method, initially developed for rats (Nunes and Tufik 1994) on the basis of the original single platform method (Jouvet et al. 1964, for cats; Mendelson et al. 1974, for rats). Groups of 4–5 animals were placed in water tanks (41×34×16.5 cm), containing 12 platforms (3 cm in diameter) each, surrounded by water up to 1 cm beneath the surface (4 cm depth), for 72 h. In this method, the animals are capable of moving inside the tank, jumping from one platform to the other. Food and water were made available through a grid placed on top of the water tank. Mean (±SE) body weights were 30.8±0.4 g (controls) and 29.4±0.3 g (sleep-deprived) and 30.9 ±0.4 g (controls) and 29.3±0.3 (sleep-deprived) before and at the end of sleep deprivation, respectively. Two-tailed Student's *t*-test for paired samples showed no differences between the weight of the mice before and after sleep deprivation, and none of the mice died during the sleep deprivation procedure.

The applied protocol of sleep deprivation was shown to prevent the occurrence of both slow-wave and paradoxical sleep (Silva et al. 2004). Indeed, in this previous study, the duration of paradoxical sleep and slow-wave sleep was evaluated for efficiency of the adopted sleep deprivation method. The states of wakefulness, paradoxical sleep and slow-wave sleep were identified and scored by a combination of electrocorticography, electromyogram and standard criteria (Timo-Iaria et al. 1970) during the 72 h sleep deprivation period. Slow-wave sleep was reduced by 82% and paradoxical sleep was reduced by 95% relative to basal values (Silva et al. 2004).

Behavioral procedures

Elevated plus-maze test The plus-maze consisted of two open (28.5×7 cm) and two closed (28.5×7×14 cm) arms, arranged perpendicularly, and was elevated 50 cm above the floor. Each mouse was placed in the center of the apparatus and number of entries and time spent per open and closed arms, respectively, were recorded. Number of entries into closed arms and percent time spent and entries in the open arms (time or entries in open/time or entries in open+time or entries in closed arms) were compared by two-tailed Student's *t*-test (experiments I and III) or by analysis of variance (ANOVA) (experiments IV, V and VI). The trial lasted for 5 min.

Open-field test Each mouse was placed in the center of a circular arena (40 cm in diameter) which was divided into 19 squares and surrounded by a 40 cm high wall. Peripheral (in the 12 squares close to the wall) and central (those seven divisions not contiguous to the wall) locomotion frequencies were quantified by counting the number of inter-square lines crossed. An entry into a square was counted by an observer, sitting close, once the mouse had entered a new square with all four paws. Peripheral, central, total locomotion (peripheral plus central) and locomotion ratio (central/total) were compared by two-tailed Student's *t*-test. The trial lasted for 5 min.

Experimental design

Experiment I: effects of 72 h of sleep deprivation on anxiety-like behavior in the elevated plus-maze

This experiment was performed to confirm the anxiogenic effect of sleep deprivation in the elevated plus-maze. Mice were sleep-deprived for 72 h (n=8) or maintained in their home cages (control group, n=9) in the same room. After the deprivation period, control and sleep-deprived animals were exposed to the elevated plus-maze.

Experiment II: effects of 72 h of sleep deprivation on anxiety-like behavior in the open-field arena

This experiment looked at whether the anxiogenic effect of sleep deprivation would be shown in the open field arena. Mice were sleep-deprived for 72 h (n=8) or maintained in their home cages (control group, n=8) in the same room. After the deprivation period, control and sleep-deprived animals were exposed to the open-field arena.

Experiment III: effects of 24 h of sleep deprivation on anxiety-like behavior in the elevated plus-maze

Since previous studies on effects of sleep deprivation in mice were carried out within a 24-h sleep deprivation protocol, we evaluated here whether the length of the sleep deprivation period would modify the anxiogenic effect of sleep deprivation. Mice were sleep-deprived for 24 h (n=12) or maintained in their home cages (control group, n=12) in the same room. After the deprivation period, animals were tested in the elevated plus-maze.

Experiment IV: effects of 72 h of sleep deprivation by the single platform method on anxiety-like behavior in the elevated plus-maze

Sleep deprivation can be achieved by different methodologies. We therefore investigated the behavior of mice which were sleep-deprived by using the single platform method, i.e. the animal was individually placed in one single platform in a water-filled compartment.

At first, we compared the behavior of mice exposed to the single versus multiple platforms techniques during the sleep deprivation procedure. Mice were sleep-deprived by the single or the multiple platform methods for 72 h (n=6). During the last 30 min of each deprivation day (1, 2 and 3), the animals were observed in the sleep deprivation cages. The number of times the animals jumped from one platform to another (in multiple platforms cage) or the number of times the animals left the single platform was recorded. In addition, duration of complete immobilization and rearing frequency (number of times the animal stood on its hind legs) were recorded.

Subsequently, other mice were sleep-deprived by the single platform method for 72 h (n=10, one animal per cage, with one platform) or maintained in their home cages alone (isolated group, n=10) or in group (control group, n=10) in the same room. After the deprivation and/or isolation period, animals were tested in the

elevated plus-maze. Number of entries in the closed arms and percent time spent and entries in the open arms were compared by one-way ANOVA followed by Duncan's test.

Experiment V: effects of chlordiazepoxide, given following 72 h of sleep deprivation on anxiety-like behavior in the elevated plus-maze

This experiment was performed to evaluate whether chlordiazepoxide would modify the anxiogenic effect of sleep deprivation by the multiple platforms method.

Mice were sleep-deprived for 72 h or maintained in their home cages (control group) in the same room. At the end of the deprivation period, control and sleep-deprived animals were treated with saline or 2.5, 5.0 or 7.5 mg/kg IP chlordiazepoxide (n=8–11) 30 min prior to the beginning of the elevated plus-maze test. Chlordiazepoxide hydrochloride (RBI, diluted in saline solution) and saline were given in a volume of 10 ml/kg. The number of entries in the closed arms and percent time spent and entries in the open arms were compared by separate two-way ANOVAs which were followed by Duncan's test.

Experiment VI: effects of clonidine, given following 72 h of sleep deprivation on anxiety-like behavior in the elevated plus-maze

This experiment was performed to evaluate whether clonidine would modify the anxiogenic effect of sleep deprivation by the multiple platforms method.

Mice were sleep-deprived for 72 h or maintained in their home cages (control group) in the same room. At the end of the deprivation period, control and sleep-deprived animals were treated with saline or 5, 10, 50 or 100 μg/kg IP clonidine (n=9–11) 15 min prior to the beginning of the elevated plus-maze test. Clonidine hydrochloride (Sigma, diluted in saline solution) and saline were given in a volume of 10 ml/kg. The number of entries in the closed arms and percent time spent and entries in the open arms were compared by separate two-way ANOVAs which were followed by Duncan's test. Planned comparisons with Student's t-test were also applied in order to clarify ANOVA results.

Results

Experiment I: effects of 72 h of sleep deprivation on anxiety-like behavior in the elevated plus-maze

Sleep-deprived animals had a significantly lower percent time spent on the open arms (t=2.32; P<0.05; Fig. 1), whereas sleep-deprived and control mice differed neither in the percent of open arms entries (45.6±3.5 and 35.2 ±6.2, respectively) nor in the number of closed arms entries (9.7±0.3 and 10.2±2.2, respectively).

Experiment II: effects of 72 h of sleep deprivation on anxiety-like behavior in the open-field arena

Results are shown in Table 1. Central and total locomotion frequencies were significantly lower in sleep-deprived animals when compared to control group (t=5.03 and 4.47, respectively; P<0.05). No differences were found in peripheral locomotion frequency. Central/total locomotion ratio was significantly decreased in the sleep-deprived group (t=6.78; P<0.05).

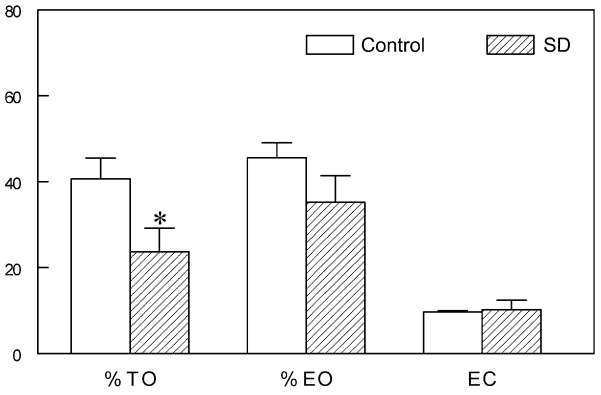

Fig. 1 Percent time spent in the open arms (*%TO*), percent number of entries in the open arms (*%EO*) and number of entries in the closed arms (*EC*) (mean±SE) presented by mice deprived of sleep (*SD*) for 72 h or mice kept in their home cage (*Control*) in the elevated plus-maze. *P<0.05 compared with control group (Student's t-test)

Experiment III: effects of 24 h of sleep deprivation on anxiety-like behavior in the elevated plus-maze

No differences were found in percent time in open arms (19.1±4.2 and 23.1±6.2), percent of open arms entries (30.8±3.9 and 30.6±4.9) and in number of entries in the closed arm (10.6±0.9 and 9.0±1.5, for control and sleep-deprived groups, respectively).

Experiment IV: effects of 72 h of sleep deprivation by the single platform method on anxiety-like behavior in the elevated plus-maze

The only difference found in the comparison of the behavior of mice exposed to the single versus multiple platforms techniques during the sleep deprivation procedure was in locomotion features. The mean (±SE) number of times the animals exposed to the single platform method left the platform during the last 30 min of days 1, 2 and 3 of sleep deprivation were 1.7±0.5, 1.0±0.6 and 1.6 ±0.4, respectively. The mean (±SE) number of times the animals placed in the cage with multiple platforms jumped from one platform to another on the 30-min period of days

Table 1 Mean (±SE) peripheral (*PLF*), central (*CLF*) and total (*TLF*) locomotion frequencies and central/total locomotion ratio (*LR*) presented by mice deprived of sleep for 72 h or mice kept in their home cage (*Control*) in an open field arena

Group	n	PLF	CLF	TLF	LR
Control	8	104.8±08.1	61.1±5.7	160.5±13.6	0.38±0.04
Sleep-deprived	8	086.8±13.3	23.4±4.8*	109.8±17.7*	0.21±0.05*

*P<0.05 compared to control group (Student's t-test)

1, 2 and 3 of sleep deprivation were 32.8±4.9, 48.5±21.7 and 26.1±10.4, respectively. Significant differences were detected in all observations by the Student's *t*-test (t=6.27, 2.38 and 2.35, respectively; P<0.05).

Percent time spent [F(2,27)=11.49; P<0.001] and percent of entries [F(2,27)=9.57; P<0.001] in the open arms presented by both isolated groups (sleep-deprived or not) were significantly decreased when compared to those presented by control animals (Fig. 3). The number of entries in both closed arms (Fig. 2) presented by isolated animals was significantly decreased when compared to control animals [F(2,27)=4.70; P<0.05].

Experiment V: effects of chlordiazepoxide, given following 72 h of sleep deprivation on anxiety-like behavior in the elevated plus-maze

The two-way ANOVA revealed a significant drug effect [F(3,64)=7.70; P<0.005] and a significant sleep condition-effect [F(1,64)=45.36; P<0.001] but no drug×sleep condition-interaction [F(3,64)=0.63; P>0.05] for percent time spent in the open arms. Post hoc analysis with Duncan's test indicated that 5.0 or 7.5 mg/kg chlordiazepoxide-treated control and sleep-deprived mice spent significantly more time in the open arms than the saline-treated groups. In addition, sleep-deprived groups treated with saline, 5.0 or 7.5 mg/kg chlordiazepoxide presented percent time spent in the open arms significantly decreased when compared to the respective non-sleep-deprived groups (Fig. 3a).

The two-way ANOVA for the percent of open arm entries indicated only a significant sleep deprivation-effect [F(1,64)=17.48; P<0.001] but no significant drug effect [F(3,64)=0.14; P>0.05] or sleep×drug interaction [F(3,64)=1.21; P>0.05]. Indeed, post hoc analysis revealed that sleep-deprived animals treated with 5.0 or 7.5 mg/kg chlordiazepoxide had a significantly lower percent number of entries onto the open arms than their respective controls (Fig. 3b).

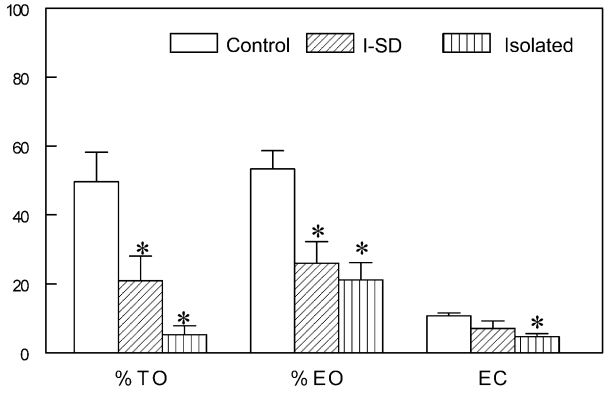

Fig. 2 Percent time spent in the open arms (*%TO*), percent number of entries in the open arms (*%EO*) and number of entries in the closed arms (*EC*) (mean ± SE) presented by mice isolated and deprived of sleep (*I-SD*) for 72 h or mice kept in their home cage alone (*Isolated*) or in group (*Control*) in the elevated plus-maze. *P<0.05 compared with control group (one-way ANOVA followed by Duncan's test)

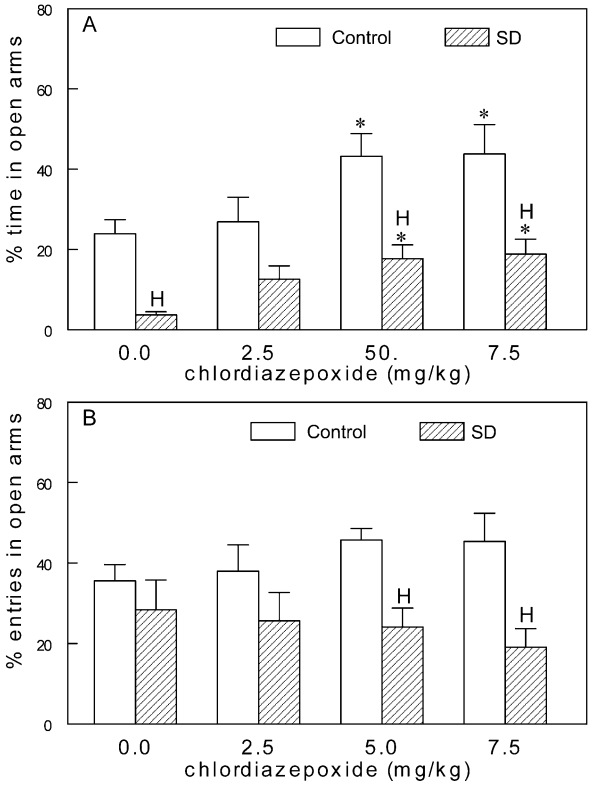

Fig. 3 Mean (±SE) percent time spent **a** and percent number of entries **b** presented by saline (0.0) or 2.5, 5.0 or 7.5 mg/kg chlordiazepoxide-treated mice that were deprived of sleep (*SD*) for 72 h or mice kept in their home cage (*Control*) in the elevated plus-maze. *P<0.05 compared with respective saline-treated group; ^{H}P<0.05 compared to respective control group (two-way ANOVA followed by Duncan's *t*-test)

For the total number of closed arms entries, the two-way ANOVA indicated significant effects of sleep condition [F(1,64)=54.96; P<0.001], drug effect [F(3,64)=4.43; P<0.01] and sleep condition×drug interaction [F(3,64)=5.17; P<0.005]. Post hoc analysis performed with Duncan's test revealed that: (1) chlordiazepoxide-treated control animals were not significantly different from saline-treated controls; (2) saline-treated or 5.0 or 7.5 mg/kg chlordiazepoxide-treated sleep-deprived animals presented total number of entries significantly lower when compared to respective control animals and (3) 2.5 mg/kg chlordiazepoxide-treated sleep-deprived animals presented a significantly higher total number of entries than saline-treated sleep-deprived animals (Table 2).

Experiment VI: effects of clonidine, given following 72 h of sleep deprivation on anxiety-like behavior in the elevated plus-maze

Two-way ANOVA revealed a significant drug effect [F(4,85)=3.93; P<0.005], sleep condition-effect [F(1,85)=7.24; P<0.01] and drug×sleep condition-interaction [F

Table 2 Mean (±SE) number of entries in closed arms in the elevated plus-maze presented by mice sleep-deprived (*SD*) for 72 h or kept in their home cage (*Control*). Control and SD mice were treated with saline (*SAL*), 2.5, 5.0 or 7.5 mg/kg chlordiazepoxide (*CDZ*: experiment V) or 5, 10, 50 or 100 μg/kg clonidine (*CLO*: experiment VI)

	Control	*n*	SD	*n*
Experiment V				
SAL	10.0±0.8	11	04.8±0.8¬	10
CDZ2.5	10.4±1.1	8	09.7±1.5*	8
CDZ5.0	11.1±0.9	9	2.7±0.4¬	10
CDZ7.5	10.0±1.3	8	4.1±0.6¬	8
Experiment VI				
SAL	08.0±1.1	11	06.0±1.2	11
CLO5	11.9±0.6*	9	05.9±0.9¬	9
CLO10	07.1±0.6	9	07.3±1.1	9
CLO50	04.9±1.1*	10	02.9±0.6*	9
CLO100	04.1±0.8*	9	01.9±0.3*	9

*$P<0.05$ compared with respective to saline-treated group; ¬$P<0.05$ compared with respective to control group (two-way ANOVA followed by Duncan's *t*-test)

(4,85)=2.54; $P<0.05$] for percent time spent in the open arms. Post hoc analysis with Duncan's test indicated that: (1) control animals treated with 50 μg/kg clonidine presented higher percent time in open arms than saline-treated controls; (2) sleep-deprived animals treated with 10 μg/kg clonidine presented higher percent time in open arms than saline-treated sleep-deprived animals and (3) sleep-deprived mice treated with 50 μg/kg clonidine presented lower percent time spent in the open arms than control animals treated with the same dose (Fig. 4a). In addition, planned comparisons with Student's *t*-test revealed that sleep-deprived animals treated with saline presented significantly lower percent time in open arms than control saline-treated animals ($t=2.14$; $P<0.05$). Control animals treated with 100 μg/kg clonidine spent significantly lower percent time in open arms than control animals treated with saline ($t=2.13$; $P<0.05$).

The two-way ANOVA for the percent of open arm entries indicated significant effects of sleep condition [F(1,85)=5.58; $P<0.005$], no drug effect [$F(4,85)=1.65$; $P>0.05$] but a sleep condition×drug interaction [$F(4,85)=3.05$; $P<0.05$]. Indeed, post hoc analysis indicated that clonidine-treated control animals were not significantly different from saline-treated controls, while sleep-deprived animals treated with 5 or 10 μg/kg clonidine presented a higher percent number of entries in the open arms than saline-treated sleep-deprived mice. In addition, sleep-deprived animals treated with 100 μg/kg clonidine presented a lower percent number of entries in the open arms than controls treated with the same dose, as well as than the saline-treated sleep-deprived animals (Fig. 4b).

For the total number of closed arms entries, the two-way ANOVA indicated significant effects of sleep condition [F(1,85)=15.54; $P<0.001$], drug effect [$F(4,85)=12.33$; $P<0.001$] and sleep condition×drug interaction [$F(4,85)$

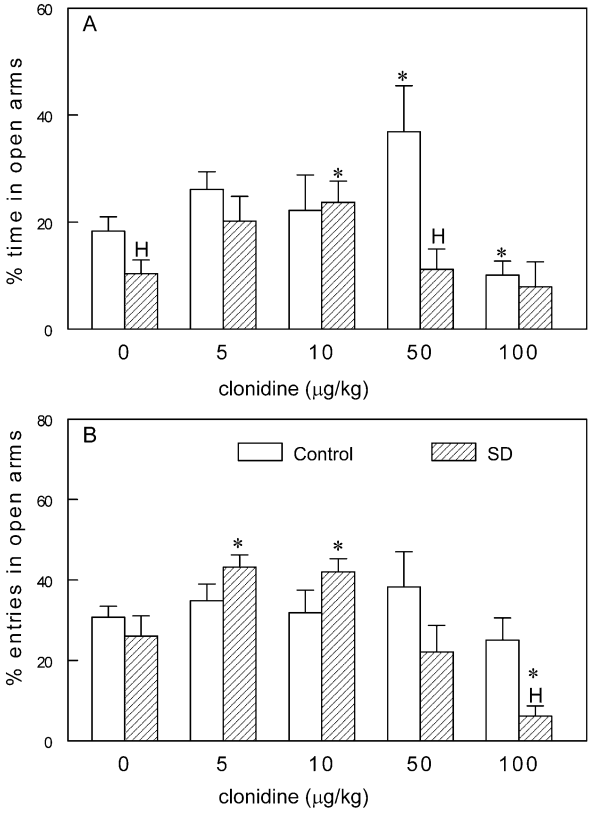

Fig. 4 Mean (±SE) percent time spent **a** and percent number of entries **b** presented by saline (0) or 5, 10, 50 or 100 μg/kg clonidine-treated mice that were deprived of sleep (*SD*) for 72 h or mice kept in their home cage (control) in the elevated plus-maze. *$P<0.05$ compared to respective saline-treated group; $^{H}P<0.05$ compared with respective control group (two-way ANOVA followed by Duncan's *t*-test or Student's *t*-test)

=2.53; $P<0.05$]. Post hoc analysis performed with Duncan's test indicated that: (1) control and sleep-deprived mice treated with 50 or 100 μg/kg clonidine presented significantly lower number of entries in the closed arms than the respective saline-treated groups; (2) control animals treated with 5 μg/kg clonidine presented a higher number of entries in the closed arms when compared to saline-treated controls and (3) sleep-deprived animals treated with 5 μg/kg clonidine presented a lower number of entries in the closed arms when compared to control animals treated with the same dose (Table 2).

Discussion

Rats and mice clearly prefer the peripheral region of an open field as well as the closed arms of an elevated plus-maze. These preferences are potentiated by anxiogenic drugs and counteracted by anxiolytic compounds or in less emotionally reactive strains and/or species (Handley and Mithani 1984; Pellow et al. 1985; Gentsch et al. 1987; Lister 1987; Goto et al. 1993; Frussa-Filho et al. 1999; Pereira et al. 1999; Silva and Frussa-Filho 2000, 2002; Frussa-Filho and Ribeiro 2002; Silva et al. 2002). The results presented here suggest an increased level of

anxiety-like behavior in 72 h sleep-deprived mice when compared with control mice, given that both absolute and percent of central locomotion in the open field and the time spent on the open arms of the elevated plus-maze were attenuated. In the few previously published studies dealing with the effect of sleep deprivation using well established models of anxiety (Pokk and Zharkoversusky 1995, 1997, 1998; Pokk et al. 1996; Suchecki et al. 2002) an increased time spent on the open arms was verified, indicating an anxiolytic effect. Whether the discrepant findings in our own and those studies depend on a difference in animal species (rats in the study by Suchecki et al. versus mice in the present experiments) remains at present unclear. It is also important to note that in the previous studies (Pokk and Zharkoversusky 1995, 1997, 1998; Pokk et al. 1996), although sleep parameters records were not mentioned, it was stated that the mice were specifically paradoxical sleep-deprived. The method used in the present work leads to similar deprivation of both slow-wave and paradoxical sleep (Silva et al. 2004), and this condition induced increased anxiety-like behavior. These data are in accordance with clinical studies reporting anxiety after total sleep deprivation (Roy-Byrne et al. 1986; Labbate et al. 1998).

Pokk and co-workers (Pokk and Zharkoversusky 1995, 1997, 1998; Pokk et al. 1996) had used, though in mice, sleep deprivation for 24 h only. Our experiment III aimed at assessing the potential effect of the duration of sleep deprivation. The fact that sleep deprivation of 24 h did not affect the level of anxiety-like behavior in our mice either (Fig. 2) indicates that the duration of sleep deprivation is critical. Since previous studies (Pokk and Zharkoversusky 1995, 1997, 1998; Pokk et al. 1996) have used a single platform method, and given that this might add additional stressful factors to that imposed by sleep deprivation, this study also compared (with our experiment IV) the effect of 72 h of different housing and sleep deprivation on the behavior in the elevated plus-maze test. Our data in experiments I and IV indicate that housing per se had no prominent influence. One may elect to view the methodology from a different perspective and realize that social isolation plus immobilization are inherent to the single platform approach when it is used for sleep deprivation. These factors are known to cause alterations in several central and peripheral indices of stress (Coenen and Van Hulzen 1980; Patchev et al. 1991; Ledoux et al. 1996). It was exactly the interplay of these various effects which led to the development of the multiple platform method (Van Hulzen and Coenen 1981; Nunes and Tufik 1994), as applied here. Under the present conditions, social stability in the multiple platform tank was maintained as all animals originated from the same home cage. These animals show an attenuated release of stress hormones such as plasma corticosterone and ACTH (Suchecki and Tufik 2000). Ultimately, however, the stress/sleep deprivation interdependence might exist as evidenced by many common effects (Tufik et al. 1987, 1995; Kushida et al. 1989; Lourenzi et al. 1993; Seabra and Tufik 1993; Koolhaas et al. 1997).

Besides the effect on anxiety-like behavior, sleep-deprived mice also showed significantly attenuated locomotion in the open-field (experiment II) and a decreased number of entries in the closed arms of the elevated plus-maze (experiments V and VI). This finding is not in line with previous reports in which sleep deprivation in rodents had been shown to increase spontaneous motor activity (Albert et al. 1970; Hicks and Adams 1976; Pokk and Zharkoversusky 1995; Pokk and Vali 2001). In these previous studies, however, the animals were deprived of sleep by the single platform technique, being strongly inhibited in their activity (see experiment IV). It has been suggested that this immobilization during the deprivation period, rather than sleep deprivation per se, would be responsible for the increase in locomotor activity (Van Hulzen and Coenen 1981). Although in the present study mice submitted to the single platform method did not show decreased locomotion, mice that were only socially isolated showed decreased number of entries in the closed arms (see Fig. 3). Thus, it seems that sleep deprivation by the single platform method attenuated the hypolocomotion induced by social isolation.

With regard to hypolocomotion induced by sleep deprivation, it is important to mention that a decreased number of entries into closed arms was observed in experiments V and VI, only, in studies in which IP injections had been used. The behavior of rodents in the elevated plus-maze can be modified by changes in experimental procedures, such as the kind of manipulation before testing (Hogg 1996).

The increase in percent time in the open arms reported by previous studies (Pokk and Zharkoversusky 1995, 1997, 1998; Pokk et al. 1996; Pokk and Vali 2001) was accompanied by a significant increase in the total number of entries in all the arms of the apparatus. Changes in both percent entries into the open arms and total number of entries made by rats in a plus-maze, as opposed to modifications only in percent entries or time in the open arms, have been interpreted by Moser (1989) to reflect a non-specific effect which is not related to a change in the anxiety level. In the present work, although locomotor activity in the open field was decreased in sleep-deprived mice, the total number of entries presented by these animals was not different from that of control animals in experiment I (data not shown). The number of closed arms entries, suggested to be a more suitable measure of locomotion in the plus maze (File et al. 1993), was also unchanged (Fig. 1). Thus, the anxiogenic effect observed here seems to be specific.

Results from experiment V showed that the administration of the anxiolytic chlordiazepoxide (5.0 or 7.5 mg/kg) increased percent open arms time in both control and sleep-deprived animals. However, the effects of sleep deprivation were similar in chlordiazepoxide and saline-treated mice. Indeed, percent time in the open arms by sleep-deprived mice treated with 5.0 or 7.5 chlordiazepoxide was decreased when compared to respective control animals. These data suggest that sleep deprivation-induced anxiety was unaffected by chlordiazepoxide,

indicating that the anxiogenic effect of sleep deprivation may not be directly related to GABAergic neurotransmission.

Alternatively, the effects following clonidine (experiment VI) suggest that the anxiogenic effect of sleep deprivation might be mediated via noradrenergic transmission; in control animals, 50 μg/kg clonidine induced an anxiolytic effect while the 100 μg/kg dose caused increased anxiety. Such a biphasic action by clonidine on the elevated plus-maze behavior has already been reported (Söderpalm and Engel 1988), and these authors had suggested that clonidine's anxiolytic (at lower doses) and anxiogenic effect (at higher doses) are mediated via alpha-2 and alpha-1 adrenoceptors, respectively. In sleep-deprived mice, we found that 5 and 10 μg/kg clonidine (doses which had no effect in non-deprived control mice) were anxiolytic, whereas 50 and 100 μg/kg were anxiogenic. Since sleep deprivation have been shown to modify plasticity of central noradrenergic transmission (Hipólide et al. 1998), it might be speculated that alpha-2 and alpha-1 adrenoceptor supersensitivity might have caused the dose–response leftward displacement concerning anxiolytic and anxiogenic clonidine effects in sleep-deprived animals, respectively. Although also speculative, the hypothesis that the prevalence of the supersensitivity of one of these receptors could lead to the manifestation of anxiogenic (reported here) versus anxiolytic (shown in other reports) effects of sleep deprivation should be considered. Of course, further experimentation is necessary to address this issue.

In conclusion, the data reported here indicate that sleep deprivation in mice had caused an increase in anxiety-like behavior. Although in some discrepancy with previous pre-clinical studies (which had used different species and/or deprivation methodologies) the present findings seems of very important clinical relevance, not only because increased anxiety is an important behavioral consequence in situations of reduced or absent sleep (Wyatt et al. 1971; Peeke et al. 1980; Gottileb et al. 1993; Dinges et al. 1997; Dahl and Lewin 2002), but also due to the proposal of sleep deprivation as a therapy for depression (Larsen et al. 1976). Indeed, although there is some evidence that this procedure ameliorates anxiety symptoms in depressed patients (Roy-Byrne et al. 1986), the observation that the efficacy of sleep deprivation is considerably reduced in depressed patients with significant anxiety symptoms (Vovin and Fakturovich 1985) should be taken into account.

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