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Research report

Social stress does not interact with paradoxical sleep deprivation-induced memory impairment

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Abstract

Extensive evidence has linked both paradoxical sleep (PS) and stress to memory processing. The purpose of the present study was to examine the effect of social instability stress on memory and to verify whether this stress interferes with the amnesic effect of PS deprivation using the modified multiple platform method. In addition to the PS-deprived group (put onto narrow platforms inside the deprivation tanks) two control groups were used: one of them remained in its home-cages and the other was placed inside the deprivation tanks, onto a grid that contained large platforms on it. All groups were subdivided in socially stable and unstable conditions. Immediately after 96 h of sleep deprivation, the animals were trained in three different memory tasks: inhibitory avoidance, classical fear conditioning to a discrete stimulus and contextual fear conditioning. Twenty-four hours after training, the animals were tested in order to assess task acquisition. The results showed that social instability did not impair the performance of animals nor interacted with PS deprivation in any of the tasks. Grid control animals presented a selective impairment in the inhibitory avoidance task and contextual, but not in the classical, fear conditioning task, compared to cage control rats. This finding could be due to the stress to which grid control animals were exposed (humidity and luminosity) during the manipulation period. PS-deprived animals exhibited poorer performance than the other groups in all tasks. As they also showed an increased threshold to shock-induced vocalisation, but not to flinch response, it is not possible to completely rule out a decreased response to noxious stimulation as a contributing factor for the present results with PS deprivation.

Keywords: Social stress; Sleep deprivation; Contextual fear conditioning; Inhibitory avoidance; Classical fear conditioning

1. Introduction

Several studies have suggested a relationship between sleep and memory. Sleep, especially paradoxical sleep (PS), appears to be important for the consolidation of recently acquired information [36]. Some studies report augmented acquisition of tasks related to memory and learning processes following a period of PS [9,15,37] and others have shown post-training PS deprivation-induced deleterious effects [10,30,35]. Pre-training PS deprivation also seems to interfere with learning processes [10], i.e. it can induce anterograde amnesia. In fact, there are some studies investigating the pre-training PS deprivation effects. These studies led to conflicting results. Some authors, for instance, have found an impairment of acquisition of one-way avoidance [4,5,14,38], whereas others have failed to report such impairment [1,17]. Although classical conditioning is not affected by pre-training PS deprivation [5], a go no-go discrimination task is reported to be impaired [38].

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Changes in the cholinergic system impair acquisition of several tasks [2,3,11]. It is believed that cholinergic blockade affects mainly acquisition of hippocampal-dependent tasks [11], which might explain some of the inconsistencies, since PS deprivation has been shown to produce neurochemical alterations in this system [5,26,34]. Therefore, the nature of the task may be a determinant factor for PS deprivation-induced memory impairment.

Nonetheless, other factors may concur for the variability of results reported in the literature, such as the animal strain [10] and the method employed to produce PS deprivation. The present study focused on the latter possibility. PS deprivation procedures are known to be stressful, making difficult to distinguish between the effects of PS deprivation per se and those of PS deprivation-induced stress [16]. On the one hand, the platform (flower pot) technique to induce PS deprivation results in stress-related physiological alterations, such as body weight loss, reduced thymus and augmented adrenal weights [6,40], in addition to elevated basal and ACTH-stimulated corticosterone plasma levels [29,40]. On the other hand, stressful situations are also reported to produce memory impairment [7,13,25]. It is possible, therefore, that memory impairment results from PS deprivation per se and/or from the adverse stimuli associated to the technique.

Stress effects depend on the type, intensity and duration of the stimulus. Moreover, it is known that different stressors can induce different physiological responses [23,31,42]. Social stress, such as social defeat, results in intense physiological alterations [19]. Recently, Zelena et al. [42] and Fuchs et al. [12] observed that social stress induced by contact between unacquainted dominating males or by placement of an intruder in a dominating male cage, alters stress-related physiological indices. Moreover, it has been shown that this type of social stress also alters the performance of animals in behavioural tests, interfering with spatial memory [20].

Since stressors are associated with the platform technique to induce PS deprivation, some modifications of the original single platform technique were introduced in order to reduce such associated stressors. The multiple platform technique was designed as an attempt to reduce immobilisation stress [41]. In order to exclude the stress of social isolation this technique was further modified in such a way that several animals are sleep-deprived together in a deprivation tank containing several platforms [40].

Using the modified multiple platform (MMP) technique, Suchecki and Tufik [40] investigated the influence of social instability during PS deprivation, by sleep deprived animals that were raised together since weaning (socially stable) or mixing unacquainted animals in the deprivation tank (socially unstable). The results show that socially unstable animals, whether sleep-deprived or not (cage-control), present exacerbated stress indices, such as augmented ACTH and corticosterone levels.

Although stressful stimuli may produce memory deficits [7,13,25], it has been reported that this effect depends on the type of task [13]. Thus, the first purpose of the present study was to examine whether social instability per se could cause impairment in three different aversively motivated learning tasks, namely passive avoidance, classical conditioning and fear contextual conditioning.

It has been shown that different types of stress produce differential alterations in the sleep pattern of rats. If the stress contained in the socially unstable condition in the MMPM were responsible for interfering even further on the animals' sleep, then removal of social instability could alter the memory impairment observed after PS deprivation [28]. Previous studies from our laboratory examined the amnesic effects of PS deprivation using socially unstable animals [4,5]. It is possible then that the memory impairment observed in these experiments would result from the interaction between PS deprivation and social instability. Thus, the second purpose of this study was to investigate the influence of social instability on the performance impairment produced by 96 h of PS deprivation in rats. In order to do so, animals were PS-deprived under socially stable or unstable conditions and submitted, after the deprivation, to the same memory tests mentioned above. To the best of our knowledge there is no published study investigating the interaction of social stress with sleep deprivation on memory.

2. Methods

2.1. Subjects

Wistar male rats aged 2.5–3 months, maintained in groups of six animals per cage (30 cm long × 16 cm wide × 18 cm high), from the animal facility of the Department of Psychobiology of the Universidade Federal de São Paulo, were used. Before the beginning of the experiments, the animals were familiarised with the sleep deprivation room under a controlled 12 h light–dark cycle (lights on at 7:00 a.m.) and room temperature (23 ± 2 °C), with free access to rat chow and tap water.

2.2. PS deprivation tanks

Animals were PS-deprived in tilled tanks (150 cm long × 44 cm wide × 45 cm high) filled with water until 1 cm of the upper surface of the platform or the grid. PS deprivation was accomplished by placing the ani-

mals onto 14 narrow platforms (6.5 cm in diameter), located approximately 10 cm apart from each other. Environmental control groups were placed onto a stainless steel grid (149 cm long × 43 cm wide × 9 cm high) that contained on its surface 14 platforms of 14 cm in diameter and 1 cm height. The rods of the grid were spaced 2.3 cm apart from each other, allowing the animals to lie down without any possibility of falling in the water. Rat chow and tap water bottles were made available to the animals through a grid located on the top of the tanks. Water inside the tanks was changed daily [40]. Animals remained in the deprivation tanks for a period of 96 h. The animals were submitted to either one of the manipulations only once.

2.3. Training apparatus

A passive avoidance apparatus consisting of two acrylic boxes, each measuring 30 × 21 × 30 cm, and connected by a sliding door was used. The clear box (white acrylic) was the safe compartment, whereas the box where the animals received the shock (dark compartment) was made of black acrylic containing some white squares on its walls. The ceiling was made of transparent acrylic. The floor of the apparatus was made of parallel metallic rods that conduct the electrical current. Each rod was 0.4 cm in diameter, located 1.2 cm apart from each other and connected to a Ugo Basile/Passive Avoidance Controller CAT 7551 Model electric shock generator.

2.4. Testing chamber

For testing of classical conditioning a chamber consisting of a cylindrical transparent recipient measuring 35 cm in diameter and 60 cm in height was used. A tone generator (80 dB), used as the conditioning stimulus, was attached to the passive avoidance apparatus and to the testing chamber.

3. Experimental procedures

3.1. Groups

Groups of six animals were removed from their home-cages, placed in separate tanks at 10:00 a.m. or maintained in the home-cage for a period of 96 h. Groups were composed according to (1) assemblage: socially stable (animals were raised together from weaning until the end of the experiment) or socially unstable (three animals from one cage were mixed with three animals coming from another cage) and (2) environment: cage control, grid control, PS deprivation. Thus, six groups were used: stable and unstable cage control (SCC and UCC); stable and unstable grid control (SGC and UGC); stable and unstable PS deprivation (SPS and UPS).

3.2. Experimental design

The memory tests were performed on two consecutive days (training and testing days). Immediately after the end of 96 h of PS deprivation, the animals were trained and then placed back in their home-cages for 24 h (during this period the animals did not return to the deprivation tanks). After the 24 h period, animals were tested for retention of the tasks.

3.3. Experiment 1: one-trial passive avoidance

It was performed on two consecutive days (training and testing days). Immediately after the end of 96 h of PS deprivation, each animal, including cage control, was dried with a towel and placed in the clear compartment of the apparatus, with the sliding door closed. Ten seconds later, the door was opened. As soon as the animal crossed to the dark compartment the door was closed, the latency to enter was recorded, and the animal received five footshocks of 1 mA/1 s, with a 30 s interval between shocks (training day). After the training, the animal was removed from the apparatus and placed in its original home-cage. Twenty-four hours later (test day), each animal was placed again in the clear compartment of the apparatus. The sliding door was opened 10 s later and, again, the latency to cross to the dark compartment was recorded. The animal was given 540 s to cross to the dark compartment. If it did not do so, it was removed from the apparatus and a latency of 540 s was attributed.

3.4. Experiment 2: classical fear conditioning to a discrete stimulus

It was carried out on two consecutive days. Immediately after the end of the PS deprivation period each animal was dried and confined to the dark compartment of the passive avoidance apparatus, and 30 s later, a series of five tone-shock pairings was presented (training day). The tone (conditioned stimulus—CS) lasted 5 s and during the last second a 1 mA footshock (unconditioned stimulus—UCS) was delivered. The 30 s interval between pairings was maintained. Thirty seconds after the last footshock, the animals were removed from the apparatus and placed back in their original home-cages. Twenty-four hours later (test day), each animal was placed inside the cylindrical test chamber for a period of 8 min. During the fourth and fifth minute, CS was presented five times, beginning at the end of the third minute, and each presentation lasted 5 s with a 30 s interval between tones. The freezing time was recorded minute by minute during the first 3 min

(before the tone) and during the last 5 min (after the tone).

3.5. Experiment 3: contextual fear conditioning

Immediately after the end of the sleep deprivation period, each animal was dried and placed in the dark compartment of the passive avoidance apparatus and freezing time was recorded for 5 min. After this period, a series of five footshocks of 1 s duration each was started (training day). At the end of the series, the animals were removed from the apparatus and placed back in their original home-cages. On the day of testing (24 h later), contextual conditioning was assessed by placing the animal in the same training context, i.e. in the dark compartment of the apparatus. The sliding door remained closed and no stimulus (tone or shock) was presented. Freezing time was recorded minute by minute for 5 min.

3.6. Experiment 4: response to footshock

This experiment was included to examine whether the manipulation interfered with shock sensitivity. Immediately after the sleep deprivation period, the animals were dried and placed in the dark compartment of the avoidance apparatus with the sliding door closed. A series of progressively increasing footshocks was delivered, with an interval of 30 s between shocks, beginning with a 0.1 mA shock and increasing the shock intensity by 0.1 mA until the animal flinched (coordinated movements of paw withdrawal) or vocalised. The intensity of shock necessary to produce any of the behaviours was recorded.

4. Statistical analysis

Latency in passive avoidance (Experiment 1) was analysed by a three-way repeated ANOVA, with a 2 (Condition [stable, unstable]) × 3 (Environment [cage control, grid control, PS deprivation]) × 2 (Day [repeated measure: training, test]) design. Data from Experiment 2 were analysed by a three-way repeated ANOVA with a 2 (Condition [stable, unstable]) × 3 (Environment [cage control, grid control, PS deprivation]) × 2 (Tone [repeated measure: before, after]) design. The time of freezing displayed by the animals in Experiment 3 was analysed by a two-way ANOVA with a 2 (Condition [stable, unstable]) × 3 (Environment [cage control, grid control, PS deprivation]) design. Finally, data from Experiment 4 were analysed by a two-way ANOVA with a 2 (Condition [stable, unstable]) × 3 (Environment [cage control, grid control, PS deprivation]) design. When necessary, post hoc analyses were performed by the Duncan Multiple Range Test, with the level of significance set at $P \leq 0.05$.

5. Results

5.1. Experiment 1: one-trial passive avoidance (Fig. 1)

A main effect of Environment ($F_{(2,87)} = 10.9523$; $P \leq 0.05$) and an interaction between Environment and Day ($F_{(2,87)} = 15.3898$; $P \leq 0.05$) were detected. Post hoc analysis revealed that on Day 1 (training), all animals behaved similarly; however, on Day 2 (test), cage control animals displayed a longer latency than grid control animals, which in turn, took longer than PS-deprived animals to enter the dark compartment of the apparatus. No main effect of Condition ($F_{(1,87)} = 0.2026$), nor an interaction between Condition and Environment ($F_{(2,87)} = 0.3601$), Condition and Day ($F_{(2,87)} = 0.0993$) or a three-way interaction was observed ($F_{(2,87)} = 0.5545$).

5.2. Experiment 2: classical fear conditioning to a discrete stimulus (Fig. 2)

ANOVA revealed a main effect of Environment ($F_{(2,99)} = 23.71$; $P \leq 0.05$) and an interaction between Environment and Tone ($F_{(2,99)} = 24.8843$; $P \leq 0.05$). Post hoc analysis of this interaction showed that on Day 2 (test) both cage and grid controls presented an equally longer freezing time than PS-deprived animals, i.e. the latter group exhibited a poorer performance than control rats.

Moreover, there was a main effect of Tone ($F_{(1,99)} = 270.4332$; $P \leq 0.05$). No differences between environments were observed before tone presentation; however, after the tone, all animals increased the freezing time.

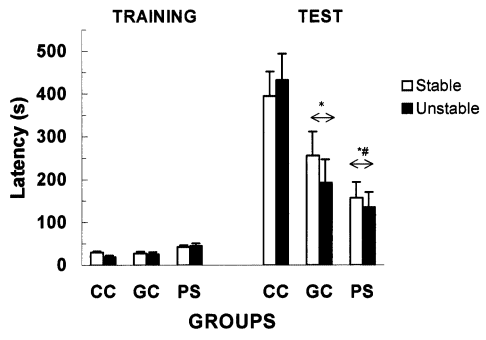

Fig. 1. Effects of pre-training paradoxical sleep deprivation and/or social tress on the latency (s) to enter the dark compartment of the passive avoidance apparatus during one trial training and retention test of the inhibitory avoidance task. Training was conducted immediately after sleep deprivation. Test was conducted 24 h after training. CC = cage control; G = grid control; PS = PS deprivation. Data are presented as mean ± s.e.m. of 10–18 animals/group/condition. *—different from CC group; # —different from GR group.

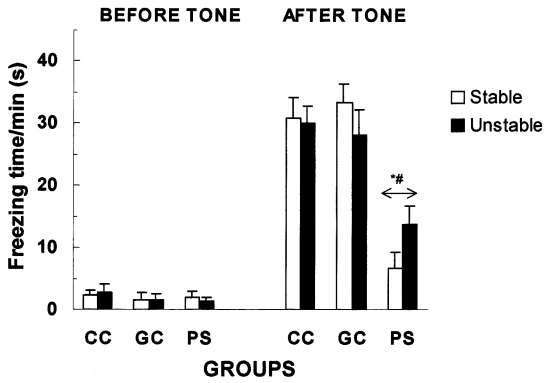

Fig. 2. Effects of pre-training paradoxical sleep deprivation and/or social stress on the freezing response of rats in the classical fear conditioning test before and after tone. Training was conducted immediately after sleep deprivation. Test was conducted 24 h after training. CC = cage control; GC = grid control; PS = PS deprivation. Data are presented as mean ± s.e.m. of 10–12 animals/group/condition. *—different from CC group; # —different from GR group.

In regard to environment, PS-deprived animals displayed shorter freezing time, compared to cage control and grid control groups, which, in turn, did not differ from each other. There were no main effects of Condition ($F_{(1,99)} = 0.009$), Condition × Tone ($F_{(1,99)} = 0.02$), Environment × Tone ($F_{(2,99)} = 1.39$), or Condition × Environment × Tone ($F_{(2,99)} = 2.01$) interactions.

5.3. Experiment 3: contextual fear conditioning (Fig. 3)

Statistical analysis showed a main effect of Environment ($F_{(2,61)} = 32.954$; $P \leq 0.05$) but no interaction between Environment and Condition ($F_{(2,61)} = 1.267$; $P > 0.05$). Post hoc analysis revealed that animals from the cage control group presented longer freezing time than animals from the grid control group, which, in turn exhibited a longer freezing time than PS-deprived animals.

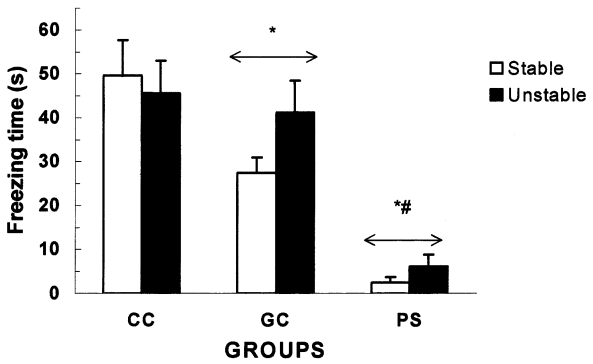

Fig. 3. Effects of pre-training paradoxical sleep deprivation and/or social stress on the freezing response of rats in the contextual fear conditioning test. Training was conducted immediately after sleep deprivation. Test was conducted 24 h after training. CC = cage control; GC = grid control; PS = PS deprivation. Data are presented as mean ± s.e.m. of 17–18 animals/group/condition. *—different from CC group; # —different from GR group.

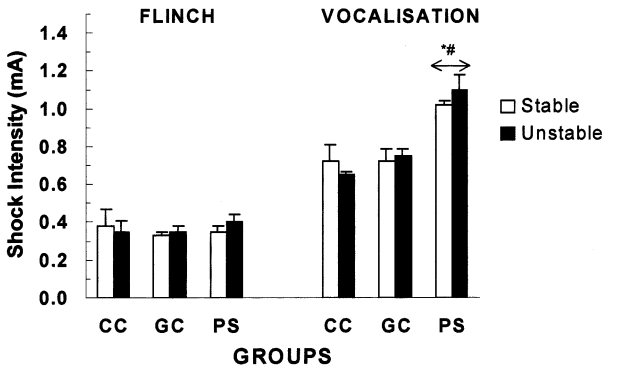

Fig. 4. Effects of pre-test paradoxical sleep deprivation and/or social stress on the threshold for shock-induced flinch or vocalisation behaviours. CC = cage control; GC = grid control; PS = PS deprivation. Data are presented as mean ± s.e.m. of 4–7 animals/group/condition. *—different from CC group; # —different from GR group.

No significant effect of Condition was revealed, i.e. stable animals behaved similarly to unstable rats ($F_{(1,61)} = 0.993$; $P > 0.05$). None of the interactions involving Condition was statistically significant.

5.4. Experiment 4: response to footshock (Fig. 4)

No significant differences were observed in regard to threshold for flinch behaviour. Regarding vocalisation, however, the only significant main factor observed was the Environment ($F_{(2,29)} = 9.185$; $P \leq 0.05$), where threshold for vocalisation was higher in PS-deprived than in all other animals.

6. Discussion

In the present study we showed that social instability did not, by itself, lead to impaired performance on either passive avoidance, classical or contextual fear conditioning, insofar as cage control animals, regardless of being socially stable or unstable, performed similarly in all tasks. In addition, social instability did not interfere with the behaviour of PS-deprived animals, since socially unstable and socially stable groups performed similarly.

Gamaro et al. [13] observed that restraint stress impairs performance in some memory and learning tasks and Krugers et al. [20] showed that social stress (exposure to a dominant rat) interferes with spatial memory. Previous studies from our laboratory using the same rat colony have shown augmented stress indices, such as higher ACTH and corticosterone plasma levels, in socially unstable PS-deprived and non-deprived animals [40]. Therefore, we expected a learning impairment in unstable non-deprived control groups. Nonetheless, the present results did not show any impairment due to social instability stress.

It is well known that stress stimuli may differ in terms of intensity, frequency and duration, in addition to their predictability and controllability [19]. Moreover, any of these characteristics, solely or in combination, may have distinct effects on any of the different paradigms to which the animals are exposed, resulting in different behavioural outcomes [13,42]. In addition, a possible dissociation between physiological and behavioural responses to a given stressor is frequently observed [21]. In our specific case, there might have been an effect of social instability on ACTH and corticosterone plasma levels and in relative adrenal weight, without any alteration on the neural circuitry involved in the performance of memory tasks. Supporting the idea that stress may have different outcomes depending on the evaluated end-point, animals PS-deprived by the flower pot technique or by the MMP technique showed exactly the same corticosterone response to a mild stress, but behaved differently in the elevated plus-maze (unpublished data), suggesting that stress-related hormone profile does not always reflect stress-related behavioural performance.

Another finding from the present study was the distinct responses of grid control animals in each of the three memory tasks employed. The grid is an alternative environmental control for the large platform, since the animals are able to sleep, despite being exposed to a situation (humidity and illumination) similar to PS-deprived rats. In fact, previous studies from our laboratory have shown that animals placed onto the grid do not show sleep rebound [39], a compensatory phenomenon resulting from sleep deprivation. In the passive avoidance and contextual fear conditioning tests, grid control animals exhibited poorer performance than cage control rats, which was not the case in the classical fear conditioning, where both groups presented an identical performance. Thus, it is possible that differences in performance between cage and grid control animals result from the greater pre-training stress to which the latter group was exposed during the manipulation period [40]. The lack of difference between these groups in the classical fear conditioning may be related to the neural circuitry involved in this task, since passive avoidance and contextual fear conditioning are hippocampus-dependent, whereas classical fear conditioning is a hippocampus-independent task [18,32]. Therefore, the stress contained in the grid environment, independent from the stress produced by PS deprivation, may be already sufficient to interfere with the hippocampus and other hippocampal-related brain regions.

Compared to grid control, PS-deprived animals exhibited a poorer performance in all three tests. Since both groups were submitted to the same environment, impairment of the animal's performance can be attributed to the PS deprivation per se.

The present study employed the same PS deprivation procedures used by Bueno et al. [4], in which 96 h of PS deprivation resulted in an impairment of passive avoidance learning, but not of classical fear conditioning. The present results, however, confirmed only in part the above-mentioned study, in which classical fear conditioning was not impaired by PS deprivation. Discrepancy between the present and the previous study may be explained by the fact that in the former, animals were supplied by a different animal colony. Distinct rat strains can differ in their abilities to learn fear-motivated tasks [33]. In addition, genetic differences of mice were reported to influence response to novelty, spatial memory ability and fear potentiated startle [8,24]. Thus, it is likely that different genetic characteristics of the rats used in the two studies might have produced the differences observed.

It is possible that pre-training PS deprivation interferes with task acquisition and not with retrieval. During the 24 h period between training (acquisition) and testing (retrieval), PS-deprived animals were certainly experiencing a rebound effect [22]. It should be expected that animals exhibiting increased PS rebound would present a better retention of the information since post-training PS is believed to promote memory retention [9]. Yet they did not do as well as the other two groups. This argument favours an encoding impairment interpretation. However, all tasks employed in the present experiments involved fear-based motivation, i.e. the animal learns because it receives a noxious stimulation (footshocks). Since PS deprivation altered the animals' performance in all tasks, it is not possible to state beyond doubt that these animals exhibited learning or memory impairment, instead of a drive or a motivation problem. For instance, if the experimental manipulation reduced the animal's reaction to shock or its capacity to feel fear, the net result would be about the same as memory impairment because they would not fear to enter the dark compartment of the apparatus. To address this issue, we carried out Experiment 4 and observed no differences in flinch response among the groups; however, the threshold for vocalisation was augmented after PS deprivation, compared to both cage and grid control groups. This ambiguous result does not allow us to completely rule out the possibility that performance of PS-deprived animals resulted from a reduction of sensitivity to footshock (at least at the 1 mA shock range). However, work from others [27] and from our laboratory (unpublished data) have shown increased, instead of decreased, reaction to noxious stimuli (paw pressure and heat) after PS deprivation. An alternative explanation might be that PS-deprived animals were somewhat dizzy during the test, because they slept more during the recovery period. However, this seems unlikely since it was observed in a previous study that, 24 h after PS deprivation, animals display a

higher ambulation score than cage-controls [4], not showing any sign of being sleepy or disoriented. In that previous study, increased ambulation did not prevent freezing reaction to occur in response to an aversive conditioned stimulus suggesting that increased motor activity per se does not impair acquisition of conditioned fear [4]. But as we observed in the present study a generalised impairment of performance, the possibility that this impairment is secondary to an increased motor activity cannot be fully discharged.

In conclusion, the present data showed that social instability did not produce nor did interfere with PS deprivation-induced performance impairment. Environmental control (GC) animals displayed a poorer performance than cage-controls in a hippocampus-dependent task, presumably as a result of being previously exposed to a more stressful environment. Finally, PS deprivation resulted in the poorest performance, which could be explained either by memory impairment or by reduction of sensitivity to the footshock. Whether this alteration is solely responsible for the decreased performance in PS-deprived animals could be answered by appetitive or non-fear motivated tasks.

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