

Intake of sucrose solution and paradoxical sleep deprivation in the rat

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Abstract

Background: Paradoxical sleep deprivation (PSD) is known for its antidepressive property, both in humans and experimental animals. The small platform method used to promote PSD in rats, however, involves stress similar to that observed in the methods used to induce depressive-like state.

Objective and methods: As sucrose solution intake is validated as a depressive-state marker in the rat, the present study evaluated such parameter before, during and after PSD carried out by the small platform method.

Results: The daily mean sucrose solution (15%) ingested by a group of 11 adult male Wistar rats was progressively reduced with PSD. The daily fall in sucrose intake was statistically significant (t-test, $p < 0.05$) from baseline, except in the second day of PSD. The development of taste aversion does not explain the reduction in sucrose intake, since its consumption, 2 days after the end of 4 days of PSD ($n = 5$), was similar to baseline levels. PSD until the animals ceased to stay on the platform ($n = 6$) showed to promote deaths in the recovery period with low consumption of sucrose.

Conclusion: The relationship of the data obtained with the development of anhedonia and carbohydrate metabolism opens new interesting aspects for investigation.

Keywords: sleep deprivation, method, stress, rats, sucrose intake, anhedonia.

Introduction

Stress, sleep and depression maintain important relationships. Depression is marked by changes in sleep architecture (1) among which shortened latency for paradoxical sleep occurrence is the most known aspect (2). Inversely, paradoxical sleep deprivation (PSD) has antidepressive effect (3) and most of the effective antidepressant agents suppress this state of sleep (4). On one hand, some

kinds of stress promote paradoxical sleep rebound (5,6) and, on the other hand, sleep deprivation per se is perceived as stressful by persons who experienced it. This stress seems sufficient to potentiate the psychogenic-like gastric ulcers and promote death in rats submitted to the lesion of the median raphe nucleus (7). The most accepted methods to induce depressive-like state in experimental animals involve stress. This is particularly true in the chronic mild stress (8), inescapable shocks (9), and forced swimming (10) meth-

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ods. In addition, depressive states may display autonomic signs of stress, as high glucocorticoid secretion (11).

Many aspects of sleep involvement in the relationships mentioned above were disclosed using the classical small platform method for PSD in rats. This method was first described for cats and was adapted subsequently for the rat (12). The animal, in this method, is maintained on top of a small circular platform emerged above the water surface in a tank. The muscular atonia of paradoxical sleep promotes rostrum touch in the water, or even animal's fall, awakening and suppressing this state of sleep. The additional stress promoted by this method was recognized in earlier studies (13), remaining a subject of concern until the present day (14).

The involvement of two opposite properties in the small platform method used for PSD does not seem to have been considered before. The method is efficient to promote the aimed deprivation. It increases cerebral excitability (15) and has antidepressive effect, as mentioned before. At the same time, the method involves stress and an inescapable condition, sometimes for long periods, as it occurs in some methods used to induce experimental depressive states. The development of depressive-like state during the PSD in the platform is, therefore, possible. In fact, some rats cease to stay on the platform and remain immobile in the upright position in the water when deprivation is long. Hypothermia may develop by the 7th–8th day of deprivation, and some of the animals may even die when removed back to their home-cages (16).

PSD and its associated stress impose a high energy demand condition to the animals. Hence, it is adaptive that they increase food intake (17), mainly carbohydrate-rich diets, as observed in rats (18). However, it is possible to think that daily sucrose solution intake (DSSI) is not particularly increased during PSD. The stress involved in such deprivation may induce a depressive-like state and anhedonia characterized by the loss of interest on rewarding stimuli. Change in sucrose solution intake was validated as a reliable marker of depressive states in the rat (19). In addition, sleep modulates carbohydrate metabolism, including insulin secretion and glucose clearance (20,21). Sleep deprivation is also reported to affect glucose tolerance (22,23). These facts may be related to development of anhedonia and to affect DSSI in PSD. The demonstration that exogenous insulin suppresses food intake supports such supposition (24).

The present paper reports the data obtained in a study that evaluated DSSI by rats submitted to PSD by the small platform method, since the occurrence of depressive-like state may be an important factor to be considered in sleep deprivation studies.

Methods

Eleven male Wistar adult rats, from the Central Colony of the Universidade Estadual Paulista, Botucatu, were used. All procedures and recommendations stated by Federação de Sociedades de Biologia Experimental (FeSBE) were followed. Adaptation to our laboratory conditions was allowed until they completed 6 months of age. For this purpose, they were maintained in groups of 5 animals per cage, with commercial food (Purina) and potable water ad libitum. The laboratory was kept under natural light and

temperature (22–25°C).

Animals were weighed and placed in individual propylene cages with wooden sawdust (38x40x16cm) and DSSI measured for 3 consecutive days. Commercially available sugar cane sucrose was weighed and dissolved in potable water using chemical glassware. Sucrose concentration was 15%, chosen based on previous studies (24). A volume of 250 ml of potable water and sucrose solution was offered, daily, in conventional water dispensers, placed. Their places in the cage were changed randomly every day. Commercial food pellets were made available ad libitum.

Two days later, the animals were submitted to PSD maintaining the potable water and DSSI measurements. The single small platform method was used for sleep deprivation following the procedures described before (16). The animal was placed on a circular platform measuring 6 cm in diameter and 6 cm in height. The platform was fixed in the plastic cage used before, filled with water sufficient to be 5cm deep.

Five rats were submitted to 4 days of PSD. Two days after the end of deprivation, their DSSI was measured in their home cages once again, to test the development of taste aversion. Six animals were sleep deprived until they ceased to stay on the platform (5th day for 3 animals, and 6th day for the remaining rats). DSSI was monitored during the 5 successive days of recovery in this subgroup.

Consumption measurements were made at 14:00–15:00h. In this occasion, the cages were cleaned, and food, sucrose solution and potable water, replaced. Care was taken to minimize disturbances to the animal.

The daily volume of sucrose solution ingested was transformed in grams of sucrose and, subsequently, in grams consumed per kg of body weight. Values are reported as mean \pm standard error. T-tests for dependent samples were used, adopting the level of significance at 0.05. The mean of the sucrose amounts ingested in the three baseline days was used for statistical comparisons.

Results

The mean volumes of sucrose solution drunk during the three baseline days were respectively 108.4 ± 1.7 , 102.4 ± 1.5 and 104.8 ± 1.5 ml/rat. These volumes corresponded approximately to 16 grams of sucrose ingested/rat/day, or to 27 g/kg of body weight per day; confidence limit 22–34 g/kg. The maximum volume of sucrose solution ingested in a day was 232 ml, observed only once in the baseline period. Daily volume of potable water consumed was 59.3 ± 1.2 ml/rat/day when furnished alone in their home-cages. However, offered concomitantly with sucrose solution, this mean lowered to 8.7 ± 0.7 ml. Absence of potable water intake during 24 hours was frequently observed when sucrose solution was present.

The average amounts of DSSI throughout PSD are shown in Figure 1. As shown, PSD promoted a progressive decrease in sucrose intake. T-test for dependent samples showed a significant reduction in DSSI at the end of the first day of PSD ($p < 0.05$). Mean sucrose intake in the subsequent day was reduced comparatively to the day before, however, it was not statistically significant, due the remarkable increase in the amount ingested by 3

animals (34.9, 39.0 and 43.3 g/kg, meanwhile the remaining animals showed amounts ranging from 0.7 to 28.0 g/kg, with mean value of 14.44. Such peak-values were also observed at the 4th and 5th day of sleep deprivation in two different animals. Sucrose intake was reduced to less than 1.5 g/kg of body weight when the animals ceased to stay on the platform. The average amount of potable water consumed during the PSD period ranged from 0.8 to 23.8 ml/day/rat, being the greater values resulting from one exceptional animal that drunk 105 and 60ml respectively in the 3rd and 5th days of PSD.

The animals ingested 32.2 ± 1.7 g/kg of sucrose in the test designed to evaluate the development of taste aversion. This value was not significantly different from those obtained in the baseline. All animals from this subgroup survived. Four of 6 animals, sleep deprived until they ceased to stay on the platform, died on the 1st, 2nd, 4th and 5th days of the recovery period, although they were removed from the platform in apparent good health conditions. Low ingestion of sucrose (less than 5g/100g of body weight) and potable water preceded death. No remarkable correlation was found between deaths and sucrose amounts ingested during the baseline period or with the days of sleep deprivation necessary to reduce sucrose intake. The two remaining animals returned to consume baseline volumes of sucrose on the 2nd and 4th days of recovery. DSSI mean value returned to baseline values on the 2nd day of recovery, as indicated by the absence of significant differences comparatively to baseline values (statistical test was not applied in the last recovery day due to the low number of animals).

The mean weight during baseline was 603.3 ± 2.3 g, which was reduced to 474.2 ± 2.1 g at the end of PSD. This 21.2% weight loss was statistically significant (t-test, dependent samples, $p < 0.05$).

Discussion

The results of the present study indicate that PSD in the small platform promotes an initial reduction in the DSSI, fol-

lowed by a transitory recovery in some of the animals, and by a subsequent progressive fall until a minimum, when rats cease to stay on the platform. This evolution corresponds to the classical stages of alarm, resistance and exhaustion of the general adaptation syndrome to stress, postulated by Selye (25). The significant reduction of sucrose intake observed in the first day of PSD seems to be determined by the trials to escape from the stressful situation, exploratory activity, body drying, feeding and even trials to sleep. The rise in sucrose intake, observed thereafter in some animals, seems to be due to the change in the strategy of coping and grant energy reposition and economy. Increased voluntary intake of sucrose activates also the stress coping mechanisms, which are triggered by glucocorticoids (26). Subsequent fading of homeostatic mechanisms seems to impede the emission of normal food intake. The existence of these stages during PSD deserves care, at least in studies in which the platform method is used, since straight linearity may not exist in its other effects.

The significant reduction in potable water intake, when sucrose solution was concomitantly offered confirms the high rewarding aspect of the solution employed. Reduction of its consumption in PSD, therefore, may be considered as loss of pleasure experienced in its intake, a condition that characterizes anhedonia. Indeed, operant behavior measurements indicates changed motivational or reward mechanisms in PSD rats (27). Consequently, it is possible to state that PSD in the small platform involves stress sufficient to induce depressive-like state, as other depression-like inducing methods do. In fact, chronic mild stress was shown to reduce sucrose intake and alter paradoxical sleep manifestation (28). The persistence of low ingestion of sucrose in the recovery period seems to corroborate such interpretation. In fact, anhedonia is described to persist for a week in other methods used to induce depressive-like state in experimental animals (10).

The alternative explanation for the present results, based on the development of taste aversion, can be discarded since sucrose ingestion returned to baseline levels in many animals after the end of PSD. In a similar way, the hypothesis of motivational competition

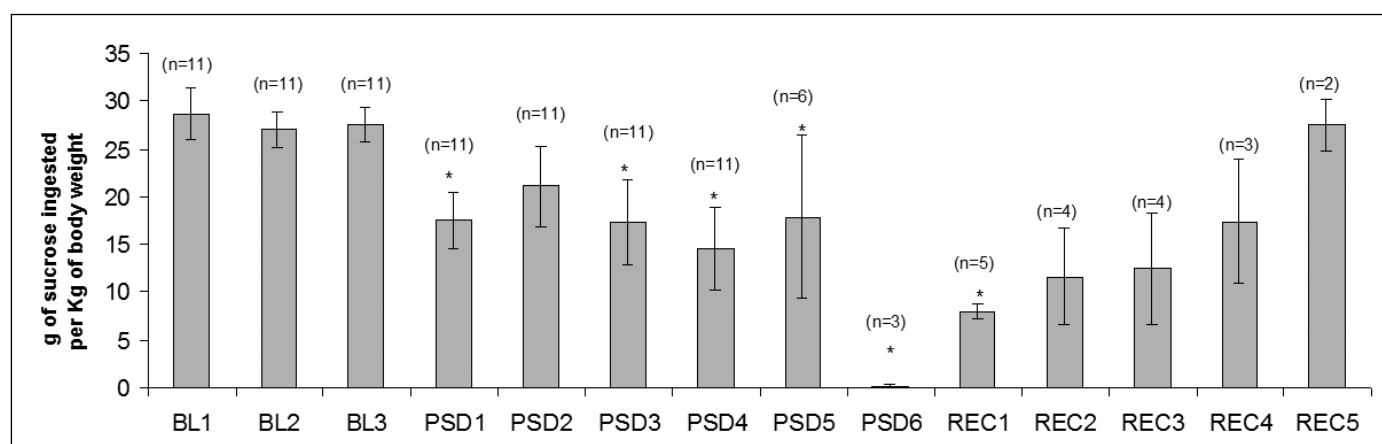


Figure 1. Mean (+ SEM) of sucrose consumption (g of sucrose/kg of body weight) throughout the days. BL columns (1 to 3) show measures in three baseline days, PSD columns (1 to 6) indicate measures in six days of paradoxical sleep deprivation, and REC columns (1 to 5) represent measures taken in five recovery days after PSD.

* Statistical difference from BL values according to T-test for dependent samples ($p < 0.05$).

seems not plausible. According to this hypothesis, the progressive increase in the time spent asleep imposed by the growing need to compensate paradoxical sleep, would surpass appetite and reduce sucrose intake. This possibility does not stand up since sucrose ingestion in some animals was higher than baseline levels in the last day of PSD. Moreover, 3 animals ingested normal amounts of sucrose in the first two days of recovery, even if they died subsequently.

Deaths in the recovery period after PSD, as observed in the present study, are not common according to previous studies (16). Such occurrence may be attributed to the development of depressive-like state itself. Death is common among depressive patients and such fact led to consider depression a life threatening disorder. However, another important factor may be involved in the case of sleep-deprived rats. Sucrose ingestion may induce satiety and abolish adequate nutritional reposition. Deficient or insufficient nutritional state may cease subsequent food intake, as in the anorexia induced by forced food restriction (29), mainly when associated to increased activity (30).

Although many etiologic mechanisms may be involved in anhedonia development and reduction in sucrose intake in PSD, insulin seems to participate at least partly. It is well known that glucocorticoid secretion is increased in stressful conditions due the activation of the hypothalamic-pituitary-adrenal axis. Glucocorticoids and related substances, as dexamethasone (31), seem to increase insulin secretion in vivo. Such increase may reinforce the secretion induced by the ingestion of sugar in large amounts. In response, down-regulation of insulin receptors may occur and central mechanisms may halt ingestion in response to elevated insulinemia. Intracerebral administration of insulin was shown to halt food intake (23), probably due to the action on IRS-1 and ISR-2, the intracellular proteins that mediate the central action of this hormone (Saad MS, personal communication).

Sleep deprivation or sleep-debt induce metabolic disturbances and impair insulin action, lower glucose tolerance, and increase the risk to develop diabetes (22,23,32). In addition, high sucrose, fructose and glucose diets result in impairment of carbohydrate and lipid metabolism that may culminate in diabetes and its associated angiopathy in genetically prone rats (33). Both mechanisms may explain the deaths observed in the rats submitted to the PSD. The age of the animals used in the present study may be supposed to have contributed to deaths, as old-aged animals are more susceptible to develop anorexia and metabolic disorders in response to stress (34). However, six months old rats cannot be considered as old-aged in a species that has a life span longer than two years.

Stressful conditions demands high energetic cost and increase in food intake is a well known fact. Observations using young-adult (100 days old) indicated that PSD days are characterized by a significant increase in sucrose solution intake (35,36). This difference from present results may suggest that animal's strain is an important variable because our rats drunk high amounts of sucrose solution in baseline days, whereas animals of the reported study displayed low intake levels in this period before PSD. This importance seems to be reflected in the inconsistencies of data relative to the amount of sucrose ingested in the assessment of depressive states induced by chronic mild stress (28).

At last, it may be considered that the present results and these considerations open many new questions for investigation, including those made in a broader context. The data indicate that the first period of resistance to PSD as adaptive to overcome common and short-lasting periods of unfavorable conditions. Anhedonia and depression, developed subsequently, may be thought as having another function. Overcrowding in rats is stressful (37), reduces sleep (38) and increases the risk of depression (39). In the hamster, it facilitates the development of depressive-like state in the forced swimming method (40). All of these facts lead to the speculation that if, despite the damage for individuals, it does not contribute to regulate population density.

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