Assessment of impulsivity in REM-sleep deprived rats

Avaliação da impulsividade em ratos privados de sono REM

Fernanda A. Pezzato1, Drielly T. L. Silveira2, Diego B. Novais2, Katsumasa Hoshino3

ABSTRACT
The progress of knowledge in the area of mental health still depends, at least in part, on the development of experimental paradigms using laboratory animals. In such context, sleep deprivation and its effects were proposed as an animal model of mania. Although being a prominent manifestation of mania, the increased impulsivity in sleep deprived rats seems to be not experimentally confirmed, albeit its occurrence seems to be due to the short lasting duration of the behavioral effects of sleep deprivation, which seems to hinder long-lasting evaluations. The present study, therefore, demonstrated an alternative way to operationally apprehend impulsivity and used it to confirm the higher impulsivity levels in sleep deprived rats. The latency, number of episodes, and the total time displayed by rats to move from a safe platform to narrow runways, where the risk to drop into a water tank was high, showed to lower after experiencing fall, indicating an increase in consequence evaluation. Sleep deprived rats showed a significantly greater number of displacements to strait runways and a greater time spent in this risk situation. It is concluded that the method used is reliable and allows confirming the occurrence of enhanced impulsivity in sleep deprived rats.

Keywords: animal model, behavioral methods, impulsivity, mania, sleep deprivation.

INTRODUCTION
Impulsivity can be considered “a predisposition towards rapid, unplanned reactions to internal or external stimuli, with decreased sensitivity to the negative consequences of the action”(1,2) or simply “taking action without assessing the consequences”(3). Impulsivity manifests itself in different psychopathologies such as bipolar disorders, personality disorders, substance abuse disorder, attention deficit hyperactivity disorder (ADHD) and impulse control disorders that are exemplified by pathological gambling(4,5). The evaluation of impulsivity for the purpose of study or clinical work is very diverse because of the various operational definitions(6) and has been the subject of several reviews(3,6,7,8). However, the approaches to evaluate impulsivity depend on three behavioral aspects: motor inhibition/aversion, the ability to evaluate risks before making a decision(5). Research on animal impulsivity usually uses one of the following three procedures: delay of reinforcement, differential reinforcement of low-rate schedules (DRL) or autoshaping(19). Previous studies have aimed to answer questions related to the effects of genetic factors, neural circuitry, different drugs and environmental conditions on impulsive behavior(9,10,11). Some authors suggest that impulsivity can be explained by deficits in behavioral inhibition, and therefore, impulsivity involves less capacity for adopting passive, waiting positions, both in aversive and reinforcing situations(12,13).

Impulsivity, when characterized by increased self-exposure to risk situations, appears to increase with sleep deprivation in humans(14,15). These results associate sleep deprivation with a behavior observed in manic disorders and are corroborated by studies on experimental animals. Gessa and colleagues(16) report that rats that are sleep deprived for 72 hours exhibit various behaviors including insomnia, hyperactivity, irritability, aggressive behavior and hypersexuality. Some of these behavioral manifestations, such as hyperactivity and insomnia, which are similar to those observed clinically in manic disorders, are reduced using lithium treatment(14,16,17). These similarities led to the suggestion that sleep-deprived rats can serve as experimental models for mania(18,19), which is one of the states of bipolar disorder. How-
ever, the literature indicates that this increase in impulsive manifestations in sleep-deprived animals has not yet been effectively demonstrated. This gap appears to result from the temporal limitation imposed by the short duration of the deprivation effects, which is estimated to be approximately 30 minutes\(^{(15)}\), and the longer evaluation time required for the existing methods.

The evidence that sleep deprivation in experimental animals leads to increased levels of impulsivity has heuristic importance. First, this evidence supports the model of mania based on sleep deprivation. Second, advances in the knowledge of psychopathological problems still partially depend on studies conducted in animal models\(^{(20)}\). Therefore, the current study devised and evaluated a relatively rapid method to assess variations in the level of impulsivity in rats and, in a second independent study, demonstrated the capacity of this method to detect increased impulsivity in sleep-deprived rats.

**METHODS**

**Ethical commitment**

This project was first submitted to the Research Ethics Committee of the Faculty of Sciences (Universidade Estadual Paulista - UNESP), and the experimental activities were initiated only after its approval (Protocol no 6171/46). All experimental activities and manipulations followed the recommendations in the literature\(^{(21)}\).

**Experimental subjects**

Adult male albino Wistar rats were used in this study and were provided by the Central Animal Facility (UNESP - Botucatu, SP). The animals were adapted to laboratory conditions from the 45\(^{th}\) to 100\(^{th}\) day of life and received commercial feed and water ad libitum. They were kept in groups of five in conventional polypropylene cages, each with an automatically controlled light cycle (7:00 am to 7:00 pm) and temperature (23-25°C). Cage cleaning and bedding replacement was performed every two days.

**Study 1: Design and evaluation of the measurement of impulsivity**

The definition of impulsivity as taking an action without assessment of the consequences was used in our experimental design, aiming the detection of differences in the assessment of consequences and, therefore, in the decision-making process. The Consequence Evaluation Aquatic test (CEAT) was created according to the assumption that information from a prior significant experience affects the decision-making time when animals encounter similar situations. The idea for this experimental tool was based on the well-known elevated plus maze, from which impulsive animals jump or fall\(^{(22)}\) and experience pain from the fall to the ground. The designed test was conducted in a circular plastic tank (1 m diameter; 0.6 m high) with a 500 L capacity, containing enough water (24-25°C) to reach a depth of 40 cm. The tank contained a wooden platform (14 x 15 cm) set at 1.5 cm above the water surface in the center of the tank. Four narrow radial lanes extended (2.5 cm wide; 35 cm length) from this platform and were symmetrically arranged and ended in the oblique fold of the tank (5 cm wide and 45°C inclination), whose function was to confer resistance to the deformation. The transit of animals through this inclined section of the tank invariably leads to their fall into the water. Figure 1 presents photographs of this apparatus.

![Figure 1. Photographs of the Consequence Evaluation Aquatic Test (CEAT). A: Overview of the central platform that provides greater security over the water surface and the four radial narrow lanes, where the risk of falling is greater. B: Overview of the tank. The dimensions are specified in the text.](Image)

The animals were individually placed on the platform, which is relatively more secure than the narrow lanes because it offers less risk of falling in the water. Next, the latency of the first exit, the total number of exits and the total time away from the platform were evaluated for five minutes by two observers equipped with timers and standard annotation sheets. The animals that spontaneously fell into the water were immediately removed and returned to the tank’s central platform, and the observation resumed until the end of the observation period. Animals that had no spontaneous fall until the end of the observation were gently pushed into the water, which aimed to provide the experience of falling resulting from walking on the narrow lanes.

The possibility that the CEAT might be suitable for detecting variations in impulsivity was evaluated by comparing the three parameters selected obtained in the first evaluation (test) with those reassessed in the same animals 24h later (retest). It was expected to observe test parameters changes compatible with the incorporation of the aversive information from the previous fall into the water into the new decision-making process. Twenty animals were evaluated for this purpose.

**Study 2. Assessment of impulsivity after sleep deprivation using the CEAT**

The assessment of impulsivity after sleep deprivation was designed to compare the levels of impulsivity of sleep-deprived animals with those of control animals without sleep deprivation. Ten animals were sleep deprived for 96 hours using the single platform method\(^{(23)}\). This method consists of keeping the rat on a 6-cm diameter circular platform, which is placed 1 cm above 5-cm deep water stored in a plastic tank (32 x 40 x 27 cm). The occurrence of muscle relaxation that characterizes paradoxical sleep causes the animal to touch its nose to the water, or even fall, waking the animal and suppressing this sleep state. The eight control animals were kept in individual nursery cages during the sleep deprivation period, as the method requires social isolation.

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Assessment of the impulsivity levels using the CEAT was performed immediately after withdrawal of the animals from the sleep deprivation platform or removal from the nursery cages for the control animals. The evaluations were performed in a random sequence and without knowledge of the experimental treatment applied to the animal. As in the previous study, all CEAT evaluations were performed between the hours of 4:00 pm and 7:00 pm.

Data analysis

The data analysis of the first study was performed by repeated measures design for two samples (paired t-test). Each parameter’s values (latency, the number of exits and time spent in the narrow lanes) in the test and the retest were compared independently. For the second study, the assessment of the mean differences between the two groups (sleep deprived and control) was performed using an independent t-test, with each parameter evaluated separately. In all of the comparisons, a significance level of $p < 0.05$ was adopted. All of the analyses were performed using the software STATISTICA® v.10 Statsoft).

RESULTS

The results obtained in the first study are presented in Table 1. The number of exits and the time spent away from the platform are significantly reduced, while the latency time of the first exit to the narrow lanes increases after experiencing a fall into the water (latency $t(19) = -3.722; p = 0.001$; number of exits $t(19) = 3.107; p = 0.006$ and time away from the platform $t(19) = 3.508; p = 0.002$).

Table 1. Assessment of the proposed method for the measurement of impulsivity in rats.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Latency of the first exit from the platform</th>
<th>Number of exits from the platform</th>
<th>Total time away from the platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>23.4 ± 14.6*</td>
<td>4.5 ± 0.7</td>
<td>82.1 ± 13.5</td>
</tr>
<tr>
<td>Retest</td>
<td>137.1 ± 31.6*</td>
<td>2.1 ± 0.5*</td>
<td>31.7 ± 3.1*</td>
</tr>
</tbody>
</table>

Means and standard errors (..) difference (t-test, $p<0.05$)

The results of the second study are presented in Table 2. Data obtained indicate that the sleep-deprived animals exit more times and remain away from the platforms for longer times compared with the control animals without sleep deprivation (number of exits $t(16) = 2.26; p = 0.038$; time away from the platform $t(16) = 2.4; p = 0.029$). The latency of the first exit from the platform was equivalent in both groups ($t(12.45) = 0.25; p = 0.80$).

Table 2. Impulsivity of sleep-deprived rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Latency of the first exit from the platform</th>
<th>Number of exits from the platform</th>
<th>Total time away from the platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep-deprived</td>
<td>124.3 ± 35.7*</td>
<td>5.4 ± 1.9*</td>
<td>70.7 ± 4.4*</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>139.1 ± 48.8*</td>
<td>1.0 ± 1.1</td>
<td>10.5 ± 12.3</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values (...) differences observed (t-test, $p<0.05$).

DISCUSSION

The data obtained in the first study indicate that there is a significant increase in latency, reduction in the number of exits and decrease in length of stay in the narrow lanes when the animals had the previously aversive experience of falling into the water. These results are consistent with the interpretation that the rats are more reluctant to leave a safe situation to another when they had a prior aversive experience, similar to what is observed in the evaluation paradigms of avoidance learning and memory.

This reluctance implies that the learned aversive consequences become incorporated in assessing the situation when the animal encounters it again. As impulsivity is defined by the degree of evaluation of consequences, it can be concluded that the behavioral parameters evaluated by the method used in this study have the capacity to detect impulsivity. This conclusion agrees with the concept of impulsivity as a multifaceted construct with three fundamental axes: the inability to delay gratification, disinhibition and inattention.

The early and delayed exits of animals from risky situations in the method devised for and used in the present study may result from the inability to inhibit a prepotent response in favor of a better or correct response, which characterizes the impulsivity of the disinhibition axis. Moreover, risk exposure is a common characteristic of impulsivity.

The evaluation of the magnitudes of the parameters obtained using the CEAT demonstrates that the test values of normal animals in the first study are indicative of increased impulsivity compared with the control animals in the second study. Although the comparison is not recommended because the data were obtained in two independent studies, it is interesting to consider that the most recent evaluations performed in our laboratories demonstrate that this impulsivity is not as pronounced in the control groups. This fact leads us to assume that the sample obtained for the first study, even though it was drawn at random, exhibited a predominance of animals with some special feature. Previous studies in our laboratory indicate that the rat strain that we used consisted of animals that are genetically more anxious and manifest wild running, with an incidence (14%) near that described for other colonies.

Because anxiety encourages impulsivity by requiring rapid decisions, the levels of impulsivity may be higher if a relatively larger number of anxious animals are included in the sample. However, because the test and retest data were obtained with the same animals, the conclusion regarding the incorporation of information to assess the consequences and decision making process when encountering the same situation does not appear to be affected. Another plausible explanation is the methodological difference of the imposition of social deprivation before the assessment of impulsivity in rats from the second study, which was absent in the animals from the first study.

This social condition appears to change the characteristics of the effects induced by sleep deprivation and should not be disregarded when comparing the data from the studies.
A possible alternative interpretation of the results of the study is that the reduction in the number of exits from the platform is due to the motivational loss of exploratory activity due to habituation or loss of novelty of the situation. This possibility is untenable based on the observation that the animals still exhibited exploratory behavior in the retest and indecision behavior between exiting or staying on the platform (go, no-go conflict), which were observed concurrently with the data collection. Apparently, the act of exiting the platform is still motivated by the anxiety experienced by rats when remaining in open areas where they are most vulnerable to predation\(^\text{29}\), as observed in the open field test\(^\text{28}\), while the act of staying on the platform is motivated by aversion to falling into the water. In contrast, our interpretation that the CEAT retest data are due to the reduction of impulsivity and not to the reduction of exploratory motivation is supported by our more recent study that evaluated rats with median raphe nucleus lesions\(^\text{31}\). These animals exhibited indices of increased impulsivity compared with controls and maintained the same levels in the retest, while animals with sham lesions exhibited reduced impulsivity. Inactivation of the nucleus renders the rats more impulsive, a fact demonstrated by the anxiety experienced by rats when remaining in open areas that are most vulnerable to predation\(^\text{29}\), as observed in the open field test\(^\text{28}\), while the act of staying on the platform is motivated by aversion to falling into the water. In contrast, our interpretation that the CEAT retest data are due to the reduction of impulsivity and not to the reduction of exploratory motivation allows to conclude that this property determined also the values obtained in the retest of the animals in the present study.

The acceptance of the parameters used in this study as valid for evaluating impulsivity allows to conclude that this study supports the assumption that sleep deprivation induces an increase in impulsivity in rats. As previously demonstrated, the number of exits and the time spent away from the platform were significantly higher in the sleep-deprived animals, which indicated a reduction in the consequence analysis of the situation. The absence of a significant difference between the latencies for the first exit from the platform observed in the present study appears to be due to the prevalence of self-grooming behavior, which is one of the rat's first manifestations after the end of sleep deprivation, as observed during the data collection stage and repeatedly demonstrated in sleep-deprivation studies\(^\text{29}\). Another important aspect to emphasize, considering the acceptance of the CEAT parameters as being a valid method for measuring impulsivity, is the methodological advantage of allowing assessments in a simple and rapid manner and in cases of ephemeral changes in impulsivity.

The evidence that sleep deprivation increases impulsivity allows for this method to be considered as a valid animal model of impulsivity. Although the evidence corroborates the acceptance of the sleep deprivation model for studying mania in animals, it does not allow extrapolation of the validity for the disorder in its entirety. In fact, impulsivity is manifested in other disorders, as noted earlier, and this fact indicates a low translational exclusivity for a framework consisting of numerous other manifestations, some of which are highly specific. In this context, the ideal model of mania should consider other clinical manifestations of this pathology and be free of contradictions, such as the issue of mania having a long duration\(^\text{33}\) and affecting sleep\(^\text{34}\) in humans, while in animal models, the duration of the manic episode is brief\(^\text{35}\) and derives from sleep deprivation\(^\text{38}\), suggesting a reversal of the cause-effect relationship. Moreover, the existence of this type of contradiction should not discourage the search for animal models. Our studies, which use animals with median raphe nucleus lesions\(^\text{31}\), suggest that the manic manifestations in rats may result directly from an increased excitability of the central mechanisms connected to vigilance or from failure of the inhibitory mechanisms, which disinhibit the excitatory mechanisms. In this framework, the increase in excitation may, through circuits of reciprocal inhibition, strengthen the excitation by reducing or inactivating the activity of the inhibitory brake, and in turn, the inactivity state promoted by the reduction of the excitatory activity would be further accentuated by the release of the inhibitory mechanisms. This mechanism supports the proposition made by Wehr and colleagues\(^\text{35}\) that the relationship between sleep deprivation and mania is bidirectional and self-intensifying. The understanding of these etiological mechanisms appears to be essential for explaining the translational inconsistencies of the model, such as detecting increased impulsivity in studies of the effects of sleep deprivation in humans\(^\text{19}\), although certain studies have shown the opposite results\(^\text{39}\).

Finally, and in a broader perspective, it can be asked to what extent this lack of sleep exacerbates impulsivity in our daily lives, as it is already known that humans have the propensity to take risks when deprived of sleep\(^\text{37}\) and that this deprivation is certainly a feature of contemporary life\(^\text{8,29}\).

REFERENCES


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