Self-grooming, experimental anxiety and paradoxical sleep deprivation in rats

Helga Caputo Nunes¹, Fernanda Augustini Pezzato¹, Katsumasa Hoshino¹

ABSTRACT

Objective: Paradoxical sleep deprivation increases grooming behaviors in rats. Discordant data on the change promoted by such deprivation on the experimentally defined anxiety levels have made difficult to assess if the deprivation induced grooming may be used as an experimental model of repetitive behaviors, observed in different psychiatric disorders, due increased anxiety levels. In such context, the present study aims to report data gathering grooming and anxiety in rats. Methods: The degrees of tidiness displayed by female (n=26) and male (n=27) rats were evaluated after a period of maintenance in home-cages provided with wood shavings that have the property to dye animal’s fur. In the second study, the tidiness degrees were evaluated after 96 h of sleep deprivation in anxious male rats (n=6) and compared to non-anxious controls (n=6). Results: Females, that are believed to be more anxious than males, displayed higher tidiness (9% colored females x 96% colored males) in the first study. All anxious male rats (100%) that displayed wild running in response to intense acoustic stimulation showed better tidiness than their controls at the end of 96 h sleep deprivation. Conclusions: Our data suggest that self-grooming in rats is tightly related to anxiety and allows the interpretation that its increased occurrence in sleep deprivation results from its anxiolytic or tranquilizing property.

Keywords: animal, anxiety, compulsive behavior, grooming, models, rats, sleep deprivation.

INTRODUCTION

Frequent manifestation of a behavioral pattern is the most outstanding feature of some psychiatric disorders. Trichotillomania (the compulsive urge to pull out one’s own hair), washing the hands very often and bathing excessively are well-known examples. Nevertheless, available data on their causes, mechanisms and treatments are scarce. For this reason, the advancement of knowledge about these and other psychiatric disorders still depends on further research carried out through experimental models employing laboratory animals²,³.

Washing hands and bathing are self-grooming behaviors that have high adaptation value. Their excessive and unjustified manifestation, however, characterizes an anxiety disorder known as obsessive-compulsive disorder. Trichotillomania, despite being classified as a manifestation of habit and impulsive disorders, is also preceded by increasing and uncontrollable anxiety, which is relieved by pulling out the hair (ICD-10). The involvement of anxiety in both of these types of disorders suggests a common link between them, and this fact seems to justify classifying trichotillomania as a distorted manifestation of grooming, as proposed in the literature²,³.

The association of anxiety with grooming behavior disorders is supported by laboratory models employing animals, where it must be emphasized that anxiety is ethologically or experimentally defined. This allows experimental paradigms for the study of repetitive psychopathological behavioral patterns to be established. The available data show that rats manifest grooming ritually with distinguishable patterns and sequences and quantifiable intensities in several appetitive or aversive situations⁵. Each grooming modality (licking, scratching, etc.) seems to have a unique central mechanism⁶, and their manifestation in situations of anxiety or stress is well established⁷.

¹ Universidade Estadual Paulista “Julio de Mesquita Filho” – UNESP – Bauru (SP), Brasil.
Corresponding author: Katsumasa Hoshino. Biological Sciences Department. Sciences Faculty – UNESP - Av. Luiz Edmund Carrijo Coube, 14-01 - Bauru (SP), Brazil - CEP: 17033-360 - E-mail: kt.hoshino@gmail.com
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This relationship allows grooming time to be experimentally employed to evaluate levels of anxiety. From this perspective, increases in grooming as observed in rats subjected to paradoxical sleep deprivation suggest that this is a fitting paradigm for the study of repetitive behavior disorders. First, the grooming behaviors of different species seem to have similar evolutionary origins due to their high adaptation value. This suggests that grooming behaviors comply with the criterion of homology that is mandatory in experimental models. Second, paradoxical sleep deprivation is recognized as stressful, and it involves an increase in the level of anxiety with the demonstration of behaviors suggestive of anxiety. This relationship among anxiety, sleep deprivation and grooming can manifest in different ways. Deprivation increases the production of wild running in audiogenic seizures, which indicate an abnormal increase of anxiety because they can be viewed as episodes of panicking escape. Concordantly, escape behavior that can be evoked by high-intensity sound stimulation is expressed by the most anxious animals.

Interestingly, escape-inducing sound stimuli also evoke grooming. The anxiogenic property of sleep deprivation is also evident in the observation of the management of the aggression it induces. Fights induced by sleep deprivation can be diminished with the use of fluoxetine (an antidepressant drug used in the treatment of panic syndrome) and increased with the administration of hypertonic sodium lactate (this procedure is used to induce attacking to diagnose panic disorder). The fact that panic disorder is one of the anxiety disorders supports the hypothesis that sleep deprivation is anxiogenic. Concordantly, sleep deprivation exacerbates panic attacks in the population affected by this anxiety disorder. Finally, the proposal to use sleep deprivation and its effects as an experimental model for mania validates the use of grooming in rats as a paradigm for studying the excessive manifestation of behavioral patterns in some psychopathological disorders.

The literature is not limited to data agreeing with the relationships among sleep deprivation, anxiety and grooming in rats mentioned above. The same studies indicating an increase in the manifestation of grooming in paradoxical sleep deprivation also report that other anxiety-indicating behaviors, evaluated with the elevated-plus maze, do not occur. Moreover, and in contrast to what could be expected, the increase of the time that sleep-deprived rats spend in the open arms or the center of the elevated plus-maze leads to the conclusion that such deprivation is anxiolytic. Further, sleep deprivation decreases the manifestation of cleansing behavior, which is increased by intracerebral administration of enkephalinase inhibitors or opioid abstinence. This contradiction must be resolved before sleep deprivation–affected grooming can be used as an experimental model; it should be noted that this model has the advantages of being simple, quick and less expensive than models employing genetic manipulation and revealing the molecular mechanisms involved in the production of compulsive behaviors. The need to quantify grooming in order to support other data in knockout models also makes it important to confirm that grooming is a component of anxious behaviors.

The existence of contradictory data necessitates the evaluation of the validity of grooming as a paradigm for the investigation of pathologically repetitive behaviors. In this context, the difference in the levels of anxiety between male and female rats provides an opportunity to confirm the relationships between variables relevant to this matter. Like women, female rats also manifest more anxious behaviors than males. Therefore, it is likely that female rats will keep themselves cleaner than males under natural conditions due to their higher frequency of grooming behaviors arising from a higher level of anxiety. This hypothesis is backed by the higher rate of wild running in females in response to high-intensity sound stimuli, which are stressful, anxiogenic and grooming-inducing.

The hypothesis that greater body cleansing in females is determined by greater anxiety might be contradicted by the hypothesis that the degree of body cleansing results from a reduction in the chances to soil oneself due to a smaller rate of locomotor activity because of anxiety. The validity of any of these hypotheses can be tested through the use of male rats subjected to paradoxical sleep deprivation. Male rats manifesting wild running when subjected to high-intensity sound stimulation exhibit higher indexes of anxiety in the elevated plus-maze test. Because paradoxical sleep deprivation through the single-platform method is anxiogenic and restricts locomotion, the manifestation of grooming assessed by the degree of body cleansing is a function of the individual degree of anxiety.

Due to the relevance of this subject and its potential to improve our understanding of sleep deprivation and anxiety disorders, this study assessed the indices of body cleansing in male and female rats under natural living conditions and between anxious and non-anxious males subjected to paradoxical sleep deprivation.

MATERIAL AND METHODS

Ethical requirements
This study complied with the recommendations for the use of animals in experimentation, and it began only after obtaining the approval of the Research Ethics Committee of the institution with which the authors are affiliated.

Experimental subjects
Sixty-five Wistar rats were used; the animals were supplied by the Central Vivarium of Unesp, Botucatu. In the first study, which addressed intersexual differences in cleansing, 27 males and 26 females were used. The second study compared the degree of cleansing in 6 males that exhibited wild running induced by high-intensity sound stimulus with 6 males that did not exhibit this manifestation after 96 hours of sleep deprivation. Sensitive animals were selected from a sample of 45 male rats.

Evaluation of body cleansing in study 1
The animals of both sexes were received at 45 days of age and were housed in groups of 4 or 5 individuals of the same sex in conventional propyl propylene cages (32x40x18 cm) covered by metallic-grid lids. Commercial feed and potable water were...
given ad libitum. Cages were lined with pine (Pinus elliottii) trunk clippings that were changed every 2 days. This lining caused a yellowish hue of varying intensity in the hair of animals, which agrees with previous observations. Animals were kept in rooms with automatically controlled temperature (23–25°C) and light (7:00–19:00 h) until reaching the age of 100 days. Two evaluators with experience in handling animals classified each as having white, slightly yellow, average yellow or very yellow body hair. For this purpose, a series of digital photographs of the different degrees of hair color was taken against a black background. Disagreement between evaluators was resolved by a third evaluator. The number of animals exhibiting each color intensity was computed according to sex at the end of the study.

### Evaluation of sensitivity to high-intensity sound stimulus in study 2

Animals were individually placed in a transparent acrylic cage (30.5x30.0x18.5 cm) that was placed inside a sound isolation box (48.0x48.0x28.0 cm) with a glass window and 11-watt incandescent lamp inside to facilitate observation. Animals were allowed to explore the cage for 15 seconds before a 103-dB, 200-Hz sound stimulus was applied for 60 seconds. Wild running was operationally defined as explosive high-speed running inside the cage that could progress into a gallop and jumps, making the animal crash against the walls. Manifestation of this behavioral pattern was the criterion that defined an animal as susceptible to wild running, as previously reported\(^{15,16,29}\).

### Paradoxical sleep deprivation

The single platform technique was used to induce paradoxical sleep deprivation because it is the classical and commonly employed method. Briefly, it consists of keeping the rat on a circular 6-cm-diameter platform 1 cm above the surface of 5-cm-deep water in a plastic vessel (32x40x27 cm). The occurrence of muscular relaxation, which characterizes paradoxical sleep, made the animal’s snout touch the water or eventually fall down, which awakened them and suppressed this stage of sleep.

### Index of grooming in sleep deprivation

Grooming in sleep-deprived animals was evaluated by the same evaluators as in the first study. In this second stage, animals were classified on a progressive scale from the cleanest to the dirtiest. Disagreements in rating were decided by the third evaluator. At the end, the susceptibility of each animal to intense sound stimulation was disclosed, and the distribution of animals on each cleansing scale was evaluated using the Mann-Whitney test\(^{15}\).

### Data analysis

The difference in the frequencies of animals with different hair colors stratified by sex was calculated with the chi-squared test. The Mann-Whitney test was used to evaluate the dependence of grooming on the susceptibility to escape induced by high-intensity sound stimulation. The level of significance was established at 0.05 in both studies.

### RESULTS

The results of the first study are described in Table 1. All females maintained their white hair color, while males exhibited different intensities of yellow.

#### Table 1. Frequencies of animals with different degrees of yellow hair color due to housing in cages lined with pine (Pinus elliottii) clippings for 45 days. Males and females were significantly different (chi-squared test \(\chi^2\), \(p<0.05\)).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Very</th>
<th>Average</th>
<th>Slight</th>
<th>White</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>19</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Females</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>7</td>
<td>1</td>
<td>26</td>
<td>53</td>
</tr>
</tbody>
</table>

The second study showed that the cleanest rats were the ones that manifested wild running, while the rats resistant to sound stimulation were the dirtiest. As seen in Table 2, the animals that manifested wild running induced by sound stimulation received all of the lowest (cleanest) scores on the cleanliness scale, while the rats that were resistant to this effect all had the highest (dirtiest) scores.

#### Table 2. Sorting of rats according to the degree of body cleansing

(1=cleaner, 12=less clean). The evaluation was carried out after 96 hours of paradoxical sleep deprivation in rats resistant (R) and susceptible (S) to the induction of wild running by high-intensity sound stimulation. A significant difference in scores was observed (Mann-Whitney, \(p<0.05\)).

<table>
<thead>
<tr>
<th>Cleanliness score</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild running</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

### DISCUSSION

#### Obtained data

The data obtained in this study indicate that females exhibited a higher degree of body cleansing and that the males with the highest levels of anxiety manifested this trait to a lesser degree.

#### Support of methodological validity

In this study, grooming was evaluated globally only once, which differs from the classic quantification method of repeated counting in standardized observation time windows. Therefore, it is legitimate to ask whether the degree of body cleanliness of the animals could be attributed to a higher frequency of grooming activities. Originally, this question involved the assumption that there are different types of grooming, namely efficacious and ineffectual, that promote body cleansing. The literature does not reflect this assumption, which seems rather logical given that ineffective activities must necessarily result from topographic differences in their execution; in this case, they would no longer be grooming behaviors. The subjective nature of our evaluation of body cleansing might be considered inappropriate. However, our results should still be valid because the procedures complied with established principles of psychometric quantification\(^{15}\) and the evaluation exhibited a high degree of interobserver agreement.
Another question relevant to our results concerns the possibility of females having hair chemically and structurally different from males, which confers less adhesiveness to dirt. The literature suggests microstructural differences in hair as a function of aging and sex, thereby giving support to this possibility. Our preliminary studies showed that keeping rats for 24 hours in cages lined with red, iron oxide–rich soil stains the hair of males and females strongly and equally. Moreover, the possibility of an intersexual difference in cleansing resulting from hormonal differences can be ruled out because these differences are restricted to the genital area, whereas this study focused on the full surface of the body.

**Function of grooming**

Grooming has the adaptive function of caring for the surface of the body in order to diminish the risk of infection and infestation. The greater degree of cleansing in females may be attributed to the need to guarantee hygiene in the process of procreation. Despite the likelihood of this tendency, the observation of higher degrees of cleansing also in males susceptible to wild running indicates that at least a part of grooming is due to higher levels of anxiety. This causal relationship suggests the possibility that grooming has other adaptive functions besides body cleansing. Indeed, grooming is manifested in both aversive and appetitive situations, such as new environments, social encounters, stressors and exploratory and feeding behaviors. Previous observations in such situations indicate that rodents groom when their level of alertness is decreased, which suggests that grooming reduces anxiety, that is, it has anxiolytic effects, as shown in primates. This function gives coherence to and supports the data obtained in this study because the increase of cleansing can be attributed to an increase of the activities aimed at reducing high levels of anxiety. This interpretation is supported by the blockade promoted by anxiolytic agents on the grooming induced by brain intraventricular administration of adrenocorticotropic hormone or bombesin. The notion of the evolutionary incorporation of new adaptive functions into grooming is viable because grooming has a well-known thermolytic function, that is, it decreases body temperature through the evaporation of saliva, which is mirrored by the activity of grooming all over the body.

**Anxiolytic mechanism of grooming**

The idea that the higher degree of body cleansing observed in this study was due to grooming’s anxiolytic function leads one to question how this behavior promotes the reduction of anxiety. The answer to this question might be found in the high dependence of rats on the detection of sensory information, mainly through touch and olfaction, for their activities that are predominantly nocturnal. To soil oneself or to have odors impregnating the body reduces or distorts the acuity of the sensory channels, reducing their ability to detect and analyze information. Risky situations are anxiogenic, and activities that unblock and improve the acuity of the sensorial channels decrease anxiety. This process shows that the anxiolytic function of grooming observed in stressful or aversive situations derives from its original cleansing function. This interpretation supports the assertion that grooming activities are stereotyped and always end in body cleansing, even when their manifestation might have a different function at that moment, such as the regulation of temperature.

The observation that grooming manifests in moments of decreased alertness in stressful situations seems logical. The rise in the level of alertness signals the need to manifest defensive behaviors that have higher priority in the face of danger or threats. An example is the need to become immobile to avoid detection by a predator, which has the first priority, while the activity of grooming must be inhibited until the danger goes away. This episodic trait leads one to believe that the higher degree of cleansing of females under normal living conditions might be attributed to their higher sensitivity to stimuli, which increases the frequency of experienced anxiogenic episodes (state anxiety) or to their high basal anxiety rate (trait anxiety). How much the grooming manifested by females depends on the processes of learning through reinforcement generated by the relief of anxiety is a matter that still requires clarification.

The observation that male rats manifesting wild running kept themselves cleaner when deprived of paradoxical sleep contradicts the hypothesis that body hygiene is determined by the reduction of the possibility of soiling through the inhibition of locomotor activity caused by higher levels of anxiety. Therefore, the association of panicked running with cleanliness confirms the notion that anxiety is directly related to the level of cleanliness. However, rats are awoken by wetness at the onset of paradoxical sleep in the platform method. Wetting rats by sprinkling water on them is stressful and induces grooming. This means that paradoxical sleep deprivation through the use of the platform method imposes an additional chronic anxiogenic factor, and this perhaps is the main reason for the increased grooming observed in this situation, a connection that has been previously hypothesized. This interpretation makes it necessary to re-evaluate contradictory data describing sleep deprivation as anxiolytic or having no influence on anxiety-indicating behaviors in rats. In this regard, it is important to consider that the anxiolytic property manifested in the elevated plus-maze depended on the use of the multiple-platform method to induce sleep deprivation in rats.

Finally, it should be noted that the observations of a higher degree of cleansing associated with the highest levels of anxiety in female rats and also in the most anxious male rats when subjected to sleep deprivation, when analyzed together with other data reported in the literature, indicate that the aim of the present study, namely, to increase the available data on the validity of the use of this behavioral manifestation as an experimental model for psychopathological repetitive behaviors, was fulfilled.

**REFERENCES**


Self-grooming, experimental anxiety and paradoxical sleep deprivation in rats


