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Grooming analysis algorithm: Use in the relationship between sleep deprivation and anxiety-like behavior

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ABSTRACT

Increased anxiety is a classic effect of sleep deprivation. However, results regarding sleep deprivation-induced anxiety-like behavior are contradictory in rodent models. The grooming analysis algorithm is a method developed to examine anxiety-like behavior and stress in rodents, based on grooming characteristics and microstructure. This study evaluated the applicability of the grooming analysis algorithm to distinguish sleep-deprived and control rats in comparison to traditional grooming analysis. Forty-six animals were distributed into three groups: control (n = 22), paradoxical sleep-deprived (96 h, n = 10) and total sleep deprived (6 h, n = 14). Immediately after the sleep deprivation protocol, grooming was evaluated using both the grooming analysis algorithm and traditional measures (grooming latency, frequency and duration). Results showed that both paradoxical sleep-deprived and total sleep-deprived groups displayed grooming in a fragmented framework when compared to control animals. Variables from the grooming analysis algorithm were successful in distinguishing sleep-deprived and normal sleep animals regarding anxiety-like behavior. The grooming analysis algorithm and traditional measures were strongly correlated. In conclusion, the grooming analysis algorithm is a reliable method to assess the relationship between anxiety-like behavior and sleep deprivation.

1. Introduction

Anxiety is a classic effect of sleep deprivation. This effect was first observed by Dement (1960), who reported that anxiety, in association with irritability and concentration deficits, is one of the most important neuro-behavioral consequences of rapid eye movement (REM) sleep deprivation. In addition, anxiety also is related to total sleep deprivation (Labbate et al., 1998; Sagaspe et al., 2006).

Animal models are a valuable experimental tool for research on anxiety. However, the same applicability has not been observed with sleep deprivation paradigms. Results of elevated plus-maze studies (the gold standard technique for anxiety-like behavior in animal models) in paradoxical sleep-deprived animals are contradictory. In most of these cases an anxiolytic-like behavior is observed (Alvarenga et al., 2008; Suchecki et al., 2002), in contrast to the anxiogenic response seen in humans (Labbate et al., 1998; Sagaspe et al., 2006). The relationship between total sleep deprivation and anxiety-like behavior in animal models remains poorly investigated. The applicability of classic behavioral models, based mostly on latency, frequency and total duration of specific behavioral parameters, is questionable because they do not mimic human behavioral manifestation. It is important to note that, besides anxiety, sleep deprivation also induces a mania-like behavior (Gessa et al., 1995; Young et al., 2011). Hence, the anxiety-like behavior induced by sleep deprivation is somehow peculiar, since the anxiogenesis is accompanied by a mania-like episode. This peculiar condition should be the reason by which classic parameters on the elevated plus-maze are not able to assess the sleep deprivation-induced anxiety.

One of the behavioral parameters widely used for quantifying anxiety in animal models is self-grooming. Self-grooming is an ancient and innate behavior (Spruijt et al., 1992) that plays an important role in animal behavioral repertoire (Berridge and Whishaw, 1992; Feusner et al., 2009). From an ethological perspective, grooming serves to a broad variety of purposes (Feusner et al., 2009). Specifically in rodents, this behavior is strongly associated with stress, both in high and low levels, as well as to self-cleaning (Kalueff, 2000; Katz and Roth, 1979). According to Feusner et al. (2009), grooming is closely related to adaptive behaviors, such as the stress response. In this case, a pathological or abnormal grooming is an adaptive response, being the result of excessive degrees or distortions of a primary and normal behavior. However, despite of the strong association between grooming and stress, the precise role of grooming in rodents' stress or anxiety is not well understood and still requires further examination (Homberg et al., 2002; Komorowska and Pellis, 2004). Grooming has already been used as an anxiety-related behavioral parameter in studies involving paradoxical sleep deprivation (Andersen et al., 2005; Pires et al., in press). However, this behavior is

Abbreviations: CTRL, control group; GH, gentle handling; IR, interquartile range; PSD, paradoxical sleep deprivation; REM, rapid eye movement; SD, standard deviation.

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heightened in a stage-dependent framework. Self-grooming is performed in low anxiety animals, mostly in the transition between sleep and wakefulness, as well as in highly anxious and stressed animals (Kalueff, 2000). Thus, the classic analysis of grooming (based on latency, frequency and duration) can be biased, as it cannot distinguish between states of low and high anxiety (Kalueff, 2000; Kalueff and Tuohimaa, 2005).

Kalueff and Tuohimaa (2004, 2005) proposed the grooming analysis algorithm to discriminate levels of anxiety in rats based on grooming characteristics and microstructure. This model is based on the observation that low anxiety rodents present a well-ordered and uninterrupted cephalocaudal pattern of grooming, with the behavior beginning with licking of the forepaws and ending with tail/genital grooming. Conversely, animals presenting prominent anxiety-like behavior display self-grooming in a chaotic and fragmented progression. Self-grooming analysis, when compared to classic models to assess anxiety-like behavior, presents marked advantages. Investigating anxiety-like behavior through grooming analysis does not require specific equipment like the elevated plus maze or open field protocols. Moreover, the grooming analysis algorithm allows the acquisition of data about grooming microstructure that could not be assessed in traditional behavioral measures.

Considering the difficulty in reproducing the sleep deprivation-induced anxiety observed in humans in classic behavioral animal models, the grooming algorithm analysis may be a reliable method to investigate anxiety-like behavior. The aim of the present study was to evaluate the applicability of the grooming analysis algorithm to discriminate between sleep-deprived and normal rats in comparison to traditional grooming analysis.

2. Methods

2.1. Animals

Ninety-day-old male Wistar rats were used. All animals were from the Center for Development of Experimental Models for Medicine and Biology (CEDEME — São Paulo, Brazil) and were kept in monitored rooms with controlled temperature (22 ± 1 °C) and a 12 h light–dark cycle (lights on at 07:00 AM). All animal procedures were performed in accordance with ethical standards and the experimental protocol was approved by the institutional research ethics committee.

2.2. Experimental procedure

The animals were distributed into 3 groups: 1) control group ($n = 22$), not subjected to any protocol before behavior assessment; 2) paradoxical sleep-deprived group ($n = 10$), subjected to 96 h of paradoxical sleep deprivation through the multiple modified platform method and; 3) total sleep-deprived group ($n = 14$), subjected to 6 h of total sleep deprivation through the gentle handling method. The multiple modified platform method consisted of keeping the animals in a tiled water tank ($110 \times 41 \times 30$ cm), which contained 14 platforms (6.5 cm in diameter) rising 1 cm above the water surface. This method makes use of the muscular atonia characteristic of paradoxical sleep to promote its deprivation. Thus, by means of this method, the animals are able to freely behave, move and interact with other animals during wakefulness, as well as to sleep almost normally in what regards to slow wave sleep (the first phase of a rodent's sleep). However, every time when the animal enters into REM sleep, due to the muscle atonia, they fall from the platform or, more commonly, touch the snout in to the water, consequently waking. The gentle handling method consists of gently manipulating the animals with a soft brush at any behavioral sign of sleep. These methods and the durations of each were chosen since they are extensively used for paradoxical and total sleep deprivation, respectively. Further details concerning both techniques are reviewed elsewhere (Mallick and Singh, 2011; Nunes and Tufik, 1994).

Immediately after the end of sleep deprivation, the animals were individually placed in cylindrical polypropylene cages (diameter: 25 cm; height: 45 cm) and their behavior was recorded for 5 min. Behavioral recordings were performed between 2:00 PM and 6:00 PM. The animals subjected to the platform method and thus at risk of becoming wet were dried with cotton towels before behavioral recording.

2.3. Behavioral analysis

The grooming microstructure was analyzed based on definitions provided by Kalueff and Tuohimaa (2004). The self-grooming behavior was categorized in stages, as follows: 0) no grooming; 1) forepaw licking; 2) nose, snout and face grooming; 3) head washing (semicircular grooming behind the ears and over the top of the head); 4) body grooming/scratching (including body scratching with hind paws); 5) leg licking and; 6) tail/genital grooming. Through a grooming transition matrix (Kalueff and Tuohimaa, 2004), the frequency of each grooming category, the number of correct and incorrect transitions, and the number of interrupted grooming bouts were quantified. Correct grooming transitions were defined as transitions between two subsequent grooming stages (e.g.: 0–1; 3–4; and 5–6). Incorrect grooming transitions were defined as those between two non-adjacent grooming stages or a reverse sequence (e.g.: 0–6; 1–4; and 6–5). Lastly, interruptions were defined as a pause of at least 5 s, determining the end of a grooming bout. The total transition frequency, defined by the sum of correct and incorrect transitions, and the transitions ratio, defined as the ratio between correct and incorrect transitions, were both calculated. A transitions ratio higher than one indicates that the animal displays more correct than incorrect transitions, while a transition ratio lower than one indicates that the animal performs more incorrect than correct transitions. Calculation of the total transition sequence was intended to estimate the total time spent in grooming activity. To measure traditional behavioral parameters, latency, frequency and duration of grooming and rearing were quantified (Fig. 1).

2.4. Statistical analysis

Every variable was compared through Kruskal–Wallis' test, followed by Games–Howell's test, when appropriate. Variables from the grooming analysis algorithm were correlated with those from traditional measures by a Spearman's correlation matrix. For all tests, a p value lower than 0.05 was defined as statistically significant.

3. Results

Mean body weight was measured at the beginning of the experiment and was not significantly different among groups (control group: $396.7 \text{ g} \pm 24.8$ g; paradoxical sleep-deprived group: $373.9 \text{ g} \pm 28.5$ g; total sleep-deprived: $391.9 \text{ g} \pm 25.4$ g; $F(2;43) = 2.73$; $p > 0.05$).

Regarding the traditional measures of grooming, the data showed that the latencies to display self-grooming were similar among the groups ($p < 0.05$; non-significant at post hoc), but the paradoxical sleep-deprived animals presented a higher frequency ($p < 0.01$) and duration ($p < 0.001$) of grooming behavior when compared both with control and total sleep-deprived groups. Moreover, control and total sleep-deprived animals did not statistically differ in any traditional measure of grooming. In rearing measures, total sleep-deprived animals presented a higher frequency (control group: 18.7 ± 7.8; paradoxical sleep-deprived group: 17.2 ± 8.3; total sleep-deprived: 26.1 ± 6.5; $p < 0.01$) and duration (control group: 53.4 ± 33.8; paradoxical sleep-deprived group: 39.8 ± 18.6; total sleep-deprived: 84.9 ± 25.7; $p < 0.001$), while there were no significant differences in latency for this behavior (control group: 5.3 ± 4.4; paradoxical sleep-deprived group: 8.5 ± 15.0; total sleep-deprived: $2.4 \pm 1.7p > 0.05$).