Sleep Science

ORIGINAL ARTICLE

Prevalence of flight response in female rats: does it explain the reported gender difference in sleep deprivation related aggressiveness?

Prevalência da resposta de fuga em ratas: isto explica a diferença de gênero na agressividade da privação de sono?

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ABSTRACT

Objective: Rapid Eye Movement Sleep Deprivation (RSD) increases aggressiveness and is manifested in male rats as fighting episodes. The determination of the role played by such fights should constitute an important contribution toward assessing the adaptive function of this type of sleep. Nevertheless, female rats do not exhibit such behavior. Recent data indicate that RSD-induced fighting and wild running flights are panic manifestations, thus advancing the hypothesis that female rats do not fight because their prevalent response in panic situations is flight. **Methods:** Therefore, 10 male and 12 female rats were acoustically kindled at 14 days of age, and their susceptibility to display wild running was evaluated at 30, 60 and 90 days of age. **Results:** 6 males and 10 females produced respective totals of 12 and 30 wild running episodes (chi-square test, p=0.0003), with mean (± standard error) of 0.60±0.21 and 1.41±0.24 episodes/rat. (ANOVA/MANOVA, p=0.0169). **Conclusions:** The results validate the hypothesis and are concordant with the higher frequency of panic attacks observed in women as compared to men.

Keywords: Sleep, REM; Sleep deprivation/physiopathology; Aggression/physiology; Panic/physiology; Rats

RESUMO

Objetivo: A privação do sono REM aumenta a agressividade expressa pelos ratos machos em forma de episódios de brigas. O esclarecimento do papel funcional dessas brigas pode ser uma contribuição importante para a determinação da função desse tipo de sono. Apesar desse fato, as fêmeas não apresentam esse tipo de brigas. Dados recentes indicam que as brigas induzidas pela RSD e as fugas por corridas selvagens (*wild running*) dos ratos são manifestações de pânico. Com base nesse dado, objetivou-se testar a hipótese de que as fêmeas não manifestam brigas induzidas por essa privação pelo fato de a fuga ser a manifestação prevalente de pânico. **Métodos:** 10 ratos machos e 12 fêmeas foram abrasados acusticamente aos 14 dias de idade e sua sucetibilidade para manifestar as corridas selvagens foram avaliadas com 30, 60 e 90 dias de idade. **Resultados:** 6 machos e 10 fêmeas apresentaram, respectivamente, os totais de 12 e 30 episódios de corrida (teste qui-quadrado, p=0,0003), com média (± erro padrão) de 0,60±0,21 e 1,41±0,24 episódios/rato (ANOVA/MANOVA, p=0,0169). **Conclusões:** Os resultados validam a hipótese testada e são concordantes com a maior frequência de ataques de pânico que se observa nas mulheres em relação aos homens.

Descritores: Sono REM; Privação do sono/fisiopatologia; Agressão/fisiologia; Pânico/fisiologia; Ratos

INTRODUCTION

Sleep, composed of two different functional states of the central nervous system in mammals and birds, seems to play some important adaptive roles because total or selective deprivation of the state manifested by desynchronized waves in the electroencephalogram (paradoxical or REM-sleep) have been reported, among other effects, to increase aggressiveness which is expressed as anti-social behaviors in humans[1,2]. In male rats, such aggressiveness induced by REM-sleep deprivation (RSD) is expressed as an increase in the number of fighting episodes spontaneously displayed[3] or induced by electrical footshocks[4] or dopamine agonistic drugs[5]. The assessment of the nature and role played by such fights constitutes a key point for ascertaining the adaptive role of REM-sleep. Nevertheless, it was reported[6] that such increase in aggressiveness does not occur in female rats treated with apomorphine at doses inducing fighting in male animals. Hormonal differences were advanced as the possible mechanism responsible for this intersexual difference. A substantial volume of data has documented that in males aggressiveness is expressed with greater intensity due to testosterone, whereas females

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display aggressiveness at similar intensities only in the early period of lactation[7].

Fighting in rats may be offensive or defensive and can be determined by specific behavioral patterns assessed ethologically[8]. Ethograms of the fights induced by RSD in male rats have been shown to be always defensive, while some of them were found to initiate without a detectable environmental cause, suggesting that this behavior may be related to panic[3]. This possibility was supported by the reduction of fights with fluoxetine[9], an antidepressant drug used in panic disorder treatment[10], and by their increase promoted by lactate administration[11], a procedure used to trigger panic attacks and diagnose panic disorder[12]. The possibility of RSD-induced fights as panic manifestation makes it difficult to explain why female rats do not display it. The possibility of females being exempt from panic may be discarded because women reportedly display panic crises at a higher frequency than do men[13].

Panic is expressed as flight or fight behavior. Male rats that fight when submitted to RSD are those that display wild running (WR), a flight response induced by intense acoustic stimulation[14]. WR episodes sometimes end with a tonic-clonic seizure, a fact that has lead to their classification as pre-convulsive manifestations of audiogenic epilepsy[15]. Given that panicolytic procedures reduce this flight response while panicogenic ones augment it[16,17], it seems that brainstem activation promotes both panic and convulsive manifestations when activation becomes excessive[18,19]. Accordingly, RSD was observed to facilitate panic attacks in panic disorder bearers[20] and to increase WR episodes proportionately to the sleep deprivation period in rats[21]. The conception of WR as a panic expression leads to the hypothesis that a female rat does not fight when subjected to RSD because its prevalent panic response is flight.

Aiming to find supportive data for this hypothesis, the present study evaluated whether female rats display some difference in the expression of wild running flight, compared to males, when submitted to an equivalent panicogenic condition. For this, male and female rats were submitted to a high intensity acoustic stimulation (acoustic kindling) during the lactation period in order to render them prone to an audiogenic crisis. Wild running parameters displayed in both sexes were evaluated at three moments during their development.

METHODS

Subjects

All procedures recommended by the *Colégio Brasileiro de Experimentação Animal* (COBEA) were followed, and the study started only after approval by the Local Ethics Committee.

Twenty-two Wistar rats were used. They were obtained by reproductive crossings of adult males and females provided by the Central Animal Breeding House (Biotério Central) of Universidade Estadual Paulista "Júlio de Mesquita Filho"(UNESP), Botucatu Campus. These adults had been previously tested for audiogenic crisis proneness and only the WR-resistant rats were bred. Only newborns rats weighing 15 g or more at 14 days old were selected for kindling. The animals were marked for individual identification, kindled and returned to the cage of their respective dam. They started to live in conventional polypropylene cages after weaning (21 days old), in groups of four animals with proximal ages. These cages were maintained in a silent room, with cyclic light (7:00 a.m. - 7:00 p.m.) and controlled temperature (22-24°C). Food and potable water were kept *ad libitum* while cages were cleaned and wood shavings replaced on alternate days.

Kindling

Each animal was moved into a wire mesh cage (38x18x18 cm) of acoustic kindling when 14 days old and were kept inside a soundproof wooden box (44.5x38.5x33.0 cm) with a fluorescent lamp (40W) and a 125 dB, 10 KHz, sound-generating speaker. All these devices were maintained inside another soundproof cage. The continuous acoustic stimulation for 8 minutes was started 15 seconds after placing the animal in the cage and closing the soundproof wooden boxes. Acoustic stimulation parameters were chosen based on a kindling study reported in the literature[22]. Ten males and 12 females were submitted to this acoustic kindling stimulation.

Audiogenic crisis evaluation

Audiogenic crisis was determined at 30, 60 and 90 days of age. Such evaluation was made in a translucent plexiglass cage (30.5x30.0x18.5 cm) maintained inside a soundproof wooden cage (48.0x48.0x28.0 cm) provided with a glass window and illuminated by an 11-watt incandescent light bulb for behavioral observation and video recording. Once inside the test cage, 15 seconds were allowed for exploration and a 103 dB, 200 Hz acoustic stimulation was presented for 60 seconds, as previously described[14]. WR was operationally defined as explosive high-speed running around the cage that evolves to galloping and then to jumping and collisions against cage walls. Seizures were defined as a generalized muscular contraction with postural hind-limb extension (tonic manifestation) and by intermittent strong kicking movements (clonic manifestation).

Data analysis

WR frequencies computed in the male and female groups were compared by the chi-square test while means were compared by ANOVA/MANOVA. The statistic program from Statsoft was used at 0.05 significance level.

RESULTS

All animals were found healthy at the end of observations, thus indicating that they tolerated the acoustic stimulation applied during kindling. The WR patterns were similar in males and females. The number of animals displaying WR increased progressively through evaluation moments; 4, 4 and 9 WR in the female group, respectively, at 30, 60 and 90 days of age, with many of them manifesting two or more running episodes in the same test. Corresponding numbers in the male group were 0, 2 and 4 episodes, respectively, at the same ages.

As shown in table 1, the proportions of WR-prone males (60%) and females (80%) were statistically equivalent. Twelve WR episodes were observed in all tests performed on male rats, whereas 30 WR episodes were counted in the 36 evaluations (3 tests *versus* 12 animals) of the female group. This difference was found to be significant (p=0.0003) and determined by the relatively large number of females that displayed WR at two or more different ages and in two or more episodes under the same test. The mean number of WR episodes displayed by males was significantly lower than the value found in the female group. Convulsive seizures at the end of WR episodes occurred in both sexes with equivalent frequencies and were not related to WR frequencies.

Table 1: Wild-running parameters in male and female rats acoustically kindled at 14 days of age§

	Males	Females
Number of animals	10	12
Number and % of WR prone rats	6 (60%)	10 (83%)
Total number of WR episodes / number of evaluations	12/30	30/36*
Mean number of WR in the group (± standard error)	0.60±0.21	1.41±0.24**
Number of rats with WR plus seizures	2 (20%)	4 (33%)

§Wild-running (WR) evaluations were made at 30, 60 and 90 days of age using high intensity acoustic stimulation; *significant (chi-square test, p=0.0003); **significant (ANOVA/MANOVA, F=6.2178; p=0.0169).

DISCUSSION

The acoustic kindling imposed in the present study can be considered effective because 60% of males and 83% of females were found to be WR-prone. These percentages of WR incidence are higher than those of spontaneous incidence that reach at maximum 20% of the population of rat colonies around the world[23]. As these high incidences do not differ significantly between males and females, it may be concluded that male and female rats display equivalent susceptibility to acoustic kindling. Loss of internal cochlear capillary cells and disorganization of external ones, which are highly sensitive to acoustic stimulation[24], seem to be part of the mechanism responsible for the acoustic kindling presently observed. The concomitant involvement of the inferior colliculus in this process should be highlighted, because its lesion or chemical blockade antagonizes audiogenic crisis manifestation, whereas chemical or electrical stimulation facilitates it[25]. The reason why acoustic stimulation for kindling was not effective in all animals submitted to it is the other aspect of the question concerning effectiveness. Acoustic kindling depends on a critical postnatal period of susceptibility in which tonotopic bands of frequencies develop in the inferior colliculus[26]. Inter-individual differences in the development rate are common facts and explain non-kindled animals as stimulated when they were not in the critical period of susceptibility.

The main finding in the present study is the significantly higher number of WR episodes displayed by female rats compared to males submitted to the same procedures of kindling and evaluations. If one considers this behavioral pattern as a panic flight, as discussed before, it seems possible to state that, once rendered prone to panic manifestation and kept in similar panicogenic conditions, females display running crises more frequently than males. In other words, WR seems to be the most used defensive manifestation of female rats in threatening conditions that increase anxiety and culminate in panic. Prevalence of manifestation seems to be an adequate term, because greater frequency of use does not imply absence of the alternative manifestation. In fact, female rats fight aggressively during the first *post-partum* days[7] or when grouped and submitted to electrical footshocks [K.H., personal observations]. Such prevalence seems to involve lower triggering thresholds of the neural circuitries, which promote readiness to flight and inhibit the fighting manifestation. These aspects of female rats seem to derive from an evolutionary selection conditioned by the presence of aggressive males. This may explain why female rats do not fight when deprived from REM sleep. This property seems to play a secondary adaptive role after the first lactation days, when litter movements and demands impose REM-sleep fragmentation and reduction[27]. On the other hand, male rats that evolved under a selective pressure based on aggressiveness seem to have lower triggering thresholds for fighting, but higher ones for flight. When faced with overcrowding, specifically the sleep fragmentation and reduction imposed by its high competition level, increased aggressiveness may be advantageous.

The finding that female rats exhibit WR more frequently than males is concordant with some previous data demonstrating their higher susceptibility to an audiogenic crisis[28]. These data also corroborate the higher frequency of panic disorder crisis in women[13]. This aspect leads us to consider female rats prone to WR as a potential valuable model to study the intersexual difference in panic disorder manifestation.

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