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Brain, Behavior, and Immunity 80 (2019) 300–307

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Febrile and sleep responses to an immune challenge are affected by trait aggressiveness in rats

Rosario Statello[a], Luca Carnevali[a], Susanna Bianchi[b], Andrea Sgoifo[a], Luca Imeri[b,*]

[a] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy
[b] Department of Health Sciences, University of Milan Medical School, Milan, Italy

ARTICLE INFO

Keywords:
Stress
Lipopolysaccharide
Fever

ABSTRACT

Sleep is altered in response to an immune challenge: non-rapid eye movement (NREM) sleep is increased and fragmented, REM sleep is inhibited. Sleep and immune response are affected by stress: several stressors inhibit sleep and increase waking time; stress-induced cortisol secretion affects the immune response, with immunosuppressive effects. Different levels of trait aggressiveness are associated with specific patterns of neuroendocrine and autonomic stress responsiveness.

Aim of this study was to test the hypothesis that trait aggressiveness, by affecting response to stressors, modifies sleep alterations induced by the activation of the immune response. To this aim, rats were selected on the basis of their latency time to attack a male intruder in the resident-intruder test. Animals were instrumented for chronic recordings of sleep-wake activity and injected, intraperitoneally, with an immune challenge (250 μg/kg lipopolysaccharide – LPS, a component of gram-negative bacterial cell wall).

Here we report that high aggressive (HA) rats responded to an immune challenge with a 24-h long increase in cortical brain temperature. During the first 12 post-injection hours, HA rats also responded with a prolonged increase in NREM sleep amount, and a 5-h long and continuous inhibition of REM sleep. In HA rats, the LPS-induced increase in the amount of time spent in NREM sleep was due to an increase in the number of episodes of this sleep phase, without any change in the bout duration. The LPS-induced REM sleep inhibition observed in HA rats was due to a decrease in both the number and duration of REM sleep bouts. In HA rats, during REM sleep, LPS administration significantly reduced the power of the EEG theta band. In non-aggressive (NA) rats, in response to LPS administration, cortical brain temperature was increased only for two hours, NREM sleep was unaffected, and REM sleep inhibition was scattered along the first 8 post-injection hours. The LPS-induced changes in the number of NREM sleep bouts of NA rats were limited to few and scattered hours, with a change in bout duration only in a single hour. A combination of decreases, in few hours, in both REM sleep bouts and their duration contributed to the REM sleep inhibition observed in NA rats. In NA rats, the power of EEG theta band was not modified, during REM sleep, by LPS administration. Gross motor activity was inhibited in both HA and NA rats.

Results of this study show that trait aggressiveness affects febrile and sleep responses to an immune challenge.

1. Introduction

Links between the CNS and the peripheral immune system are now well established (Besedovsky and del Rey, 2007), and much is known about the mechanisms by which bidirectional communication occurs between these systems (Dantzer et al., 2008). As a result of neuro–immune interactions, sleep patterns and the immune response are reciprocally linked: on the one hand, the activation of the immune response (as it occurs, for instance, during an infection) alters sleep patterns: for example, non-rapid eye movements (NREM) sleep is increased and fragmented, whereas rapid eye movements (REM) sleep is inhibited (Imeri and Opp, 2009); on the other hand, alterations in sleep patterns affect the immune response: for example, sleep deprivation, both acute and semi-chronic, impairs the immune response to vaccination (Lange et al., 2003; Spiegel et al., 2002). Immune signalling molecules are present in the healthy brain, where they interact with neurochemical systems to contribute to the regulation of normal sleep. Animal studies have shown that interactions between immune signaling

* Corresponding author at: Department of Health Sciences, University of Milan Medical School, San Paolo University Hospital (building C, floor 7, room 708), Via A. Di Rudinì, 8, 20142 Milan, Italy.
E-mail address: luca.imeri@unimi.it (L. Imeri).

https://doi.org/10.1016/j.bbi.2019.04.007
Received 9 August 2018; Received in revised form 18 March 2019; Accepted 2 April 2019
Available online 03 April 2019
0889-1591/

molecules (such as the cytokine interleukin 1) and brain neurochemical systems (such as the serotonin system) are amplified during infection, indicating that these interactions might underlie the changes in sleep that occur during infection (Imeri and Opp, 2009).

Sleep and stress are also associated. While sleep inhibits the activity of the hypothalamic-pituitary- adrenal gland (HPA) axis, which mediates several facets of the response to most stressors, several stressors inhibit sleep and increase waking time and brain cortical arousal (Opp, 1995).

An immune challenge triggers a stress response. In this context, the major effector systems are the sympathetic nervous system (SNS) and HPA axis. SNS modulates immune response gene transcription leading to an increased pro-inflammatory state. Instead, under normal conditions, HPA axis activity leads to the release of glucocorticoids that, in turn, inhibit immune response gene transcription. However, a persistent glucocorticoid secretion, due to an excessive HPA axis activation (as in the case of chronic stress), leads to a reduction in immune cell sensitivity to the anti-inflammatory effects of cortisol ("glucocorticoid resistance") (Irwin and Opp, 2017; Slavich and Irwin, 2014).

A social conflict in rodents is a natural stressor causing robust short- and long-lasting autonomic, neuroendocrine and behavioral stress responses (Sgoifo et al., 2014). Like humans, the way most animal species cope with stressful situations shows a high variability in behavioral responses. In feral rodent populations, a major feature of behavioral coping is the individual tendency to exhibit aggressive intraspecific behaviors (de Boer et al., 2003). In line with the characterization of personality in many other animal species (Bell and Sih, 2007; Groothuis and Carere, 2005; Reale et al., 2007; Sih et al., 2004), high levels of aggression in rodents are considered an important indicator and component of a more general proactive coping style, whereas low levels of aggression are believed to be a reflection of a reactive coping style (Koolhaas, 2008; Koolhaas et al., 1999). These divergent behavioral coping styles have frequently been associated with different patterns of both autonomic nervous and endocrine (re)activity (de Boer et al., 2003; Koolhaas et al., 1999).

On the basis of the lines of evidence reviewed above (Besedovsky et al., 1991), the aim of this study was to test the hypothesis that sleep patterns are differentially affected by an immune challenge in animals that differ widely for aggression. To this aim, rats were selected on the basis of their latency time to attack a male intruder in the resident-intruder test (Koolhaas et al., 2013). Animals were instrumented for chronic recordings of sleep-wake activity and subjected to an immune challenge (lipopolysaccharide - LPS, a component of gram-negative bacterial cell wall).

2. Methods

2.1. Animals and ethical approval

All experimental procedures were conducted in accordance with the laws and policies for the care and use of experimental animals of the European Community Council (2010/63/UE L 276 20/10/2010; authorization 1/2013, Italian Ministry of Health, Department of animal welfare). In this study 4-month-old male Wild-type Groningen (WTG) rats (*Rattus norvegicus*) weighing 370–450 g were used. At time of surgery, body weight was 394.4 ± 5.8 g in NA rats (n = 8), and 435.3 ± 9.9 g in HA rats (n = 7). The difference between these weights is statistically significant ($p < 0.01$, Student's *t* test). This rat strain, originally derived from the University of Groningen (The Netherlands), was bred in our own animal facility under standard conditions, at ambient temperature of 25 ± 1 °C (humidity 55 ± 10%) and on a 12:12 light–dark cycle (light on at 02:00), with food and water available *ad libitum*.

2.2. Selection of HA and NA animals

Seventy WTG rats were assessed for their aggressive behavior toward male unfamiliar conspecific intruders using a standard resident-intruder aggression test (Koolhaas et al., 2013). Before the test, each rat was housed with a conspecific oviduct-ligated female partner for ten days to stimulate territorial behavior (Koolhaas et al., 1980). Fifteen min before the start of the test, the female partner was removed and an unfamiliar male Wistar rat (which was socially housed with other three cagemates) was introduced to the home cage of the experimental rat. The non-aggressive intruder rats weighed on average 250 g (3 months old). The test was repeated on three consecutive days, using a different intruder every time, in order to avoid familiarity between the opponents and obtain a reliable characterization of aggressive traits (de Boer et al., 2003). All tests lasted 10 min and the latency to the first attack toward the intruder (in s) was measured. The attack latency (average of 3 tests) was used as an index of individual aggressive behavior (Carnevali et al., 2013). As commonly seen in this feral rat strain (Carnevali et al., 2013; de Boer et al., 2003), individual male resident rats differed broadly in their level of aggression toward unfamiliar intruder males. We chose an arbitrary cut-off point of average attack latency of 100 s to identify high-aggressive (HA) rats, based on previous studies (Carnevali et al., 2014, 2013). Consequently, seven rats (average attack latency = 85 ± 6 s; average number of attacks = 7.7 ± 0.4) were classified as HA; eight rats did not attack the intruder during the 600-s confrontations and were classified as non-aggressive (NA) rats. HA and NA rats were then used for the following experimental procedures.

2.3. Surgery: preparation of animals for recording of sleep-wake activity in chronic conditions

Rats were anesthetized (5.0% isoflurane in O_2 during induction, then adjusted to 1.5–2% during surgery), positioned in a stereotaxic apparatus, and surgically prepared for chronic polygraphic recordings. Stainless steel screws placed over frontal, parietal, and occipital cortices served as electroencephalographic (EEG) recording and ground electrodes. A calibrated 30-kW thermistor (Omega Engineering, Stamford, CT, USA) was implanted between the dura mater and the skull over the parietal cortex to measure cortical brain temperature (Tcort). Insulated leads were routed from the screws and the thermistor to a plastic pedestal (Plastics One Inc., Roanoke, VA, USA) that was cemented in place with dental acrylic. The incision was treated topically with Polysporin (polymyxin B sulfate-bacitracin zinc) and animals were placed under heat lamps and monitored until recovery from anesthesia. On the surgical day, animals were injected with a broad spectrum antibiotic (benzyl penicillin, 5000 IU/rat) and an analgesic (flunixin 2.5 mg/kg). The analgesic treatment was repeated on the first post-surgical day, when the rats were connected to the recording apparatus (see later) via a flexible tether connected to a Teflon pedestal, which allowed relatively unrestricted movement within the cage. Then, rats were allowed one week of recovery from surgery before the beginning of baseline recordings.

2.4. Recording apparatus and data analysis

Gross body activity was detected using an infrared sensor housed in an observation unit that also contained a camera (BioBserve GmbH, Bonn, Germany). Movements detected by the infrared sensor were converted to a voltage output, the magnitude of which was directly related to the magnitude of the detected movements. Signals from the EEG recording electrodes and from the thermistor were fed into a Grass (Quincy, MA, USA) polygraph in the adjacent room. The EEG was amplified (factor of 3000) and analog bandpass filtered between 0.3 and 35 Hz (frequency response: ± 3 dB; filter frequency roll off: 12 dB/octave). These conditioned signals, as well as the voltage output from

the infrared sensor, were digitized with 12-bit precision at a sampling rate of 128 Hz (PCI-6023E; National Instruments, Austin, TX) and collected using custom software written in LabView (National Instruments). The digitized signals were stored as binary computer files until subsequent analyses. Movement values were integrated into 1-s bins. Post-acquisition determination of vigilance state was done by visual scoring of 12-s epochs using custom software, as previously described (Opp, 1997). Briefly, the rat's behavior was classified (by a scorer blind to the experimental condition) as either wakefulness (W), NREM sleep, or REM sleep. Wakefulness was characterized by low amplitude EEG and body movements. During NREM sleep, EEG amplitude was increased, the contribution of delta (1–4 Hz) frequency bands to the total EEG power increased, and body movements were absent or very brief. Complete absence of movements, and low amplitude EEG dominated by a regular theta rhythm (6–9 Hz) characterized REM sleep. EEG power densities were determined by fast Fourier transform. Values in the 0.5–4.0 Hz (delta) frequency range were collapsed and integrated for 12-s epochs, and used as measures of slow wave activity (SWA) during NREM sleep. A minimum of 20 NREM sleep epochs/hour/rat was used to calculate hourly averages for SWA during NREM sleep (NREM SWA). If these criteria for a minimum number of NREM sleep epochs/hour were not met, SWA values for that animal/hour were not included in subsequent analyses.

2.5. Substances

Lipopolysaccharide (LPS), a component of gram-negative bacterial cell wall (*Escherichia coli* serotype O111:B4), was purchased from Sigma, dissolved in isotonic pyrogen free saline (PFS, Abbot Laboratories, North Chicago, IL) as vehicle, and injected in a volume of 2 ml/kg intraperitoneally (ip).

2.6. Experimental protocol

Following recovery from surgery, polygraphic signals were recorded for 24 h during undisturbed baseline conditions. Then, according to a within-subject design, each experimental rat was injected with either PFS or LPS 250 μg/kg ip on different days immediately before the onset of the dark phase of the light/dark cycle. Experiments were scheduled randomly with an interval of at least 7 days between administrations. Polygraphic recordings were conducted for the 24 h that followed each administration.

2.7. Statistical analysis

Results are presented as means +/− SEM. Tests for statistical significance were performed using SPSS for Windows (IBM SPSS, version 24). Each of the variables was analyzed using two-way analysis of variance (ANOVA) for repeated measures followed by *post hoc t*-tests. A Greenhouse-Geisser correction was applied when the data distribution violated the assumption of sphericity. The individual rat was the subject variable, time (hours) was the repeated measure, and manipulation (vehicle vs. LPS) was the fixed effect. Experimental variables (amount of time spent in NREM sleep, REM sleep, wakefulness, Tcort values) were the dependent variables. An alpha level of $P < 0.05$ was used to indicate a statistically significant difference.

3. Results

3.1. First 12 post-injection hours

In the first 12 post-injection hours (corresponding to the dark portion of the light-dark cycle), in high aggressive (HA) rats (Fig. 1, panels E–H, and Table 1S), LPS administration induced a long lasting and biphasic increase in Tcort (panel E). In non-aggressive (NA) rats (Fig. 1, panels A–D, and Table 1S), Tcort increase induced by LPS administration was delayed and limited to post-injection hours 7 and 8 (panel A). In HA rats, LPS administration induced a significant and long lasting increase in NREM sleep, in comparison to PFS administration (panel F). No significant changes in NREM sleep were induced by LPS administration in NA rats (panel B). REM sleep was inhibited in both HA and NA rats, but with a different time course (panels C and G): in HA rats (panel G), REM sleep was obliterated in the first two post-injection hours, and then continuously inhibited until post-injection hour 5 included. In NA rats (panel C), REM sleep inhibition induced by LPS administration was delayed and scattered in time. Gross body activity (GBA) was significantly inhibited by LPS administration in both HA (panel H) and NA (panel D) rats.

In HA rats, the increase in the amount of time spent in NREM sleep induced by LPS administration (Fig. 1, panel F) was due to an increase in the number of episodes (bouts) of this sleep phase (Fig. 2, panel E, and Table 2S), without any change in the bout duration (Fig. 2, panel G). The LPS-induced REM sleep inhibition observed in HA rats (Fig. 1, panel G) was due to a decrease in both the number and duration of REM sleep bouts (Fig. 2, panels F and H). The changes induced by LPS administration in the number of NREM sleep bouts of NA rats were limited to few and scattered hours (Fig. 2, panel A), with a change in bout duration only in a single hour (Fig. 2, panel C). A combination of decreases, in few hours, in both REM sleep bouts and their duration (Fig. 2, panels B and D) contributed to the REM sleep inhibition observed in NA rats (Fig. 1, panel C).

In HA rats, LPS administration, beside inhibiting REM sleep amount (Fig. 1, panel G) and altering its architecture (Fig. 2, F and H), also affected its EEG hallmark in rodents, i.e. theta activity (Bland, 1986), whose amplitude was significantly and selectively reduced by LPS administration (Fig. 3, lower panel). No significant changes in EEG theta activity were observed in NA rats (Fig. 3, upper panel).

In baseline, undisturbed conditions (Fig. 1S and Table 1S), NA and HA rats did not sleep differently. Amount of time spent in both NREM and REM sleep, as well as EEG power in the delta waves range (an index of sleep drive and sleep depth) did not differ between the two strains. Tcort did also not differ between the two strains.

3.2. Second 12 post-injection hours (post-injection hours 13–24)

In the second 12 post-injection hours (corresponding to the light portion of the light-dark cycle, and the rest phase for rats and other rodents), in HA rats, following vehicle administration, Tcort (Fig. 1, panel E), amount of time spent in NREM (Fig. 1, panel F) and REM (Fig. 1, panel G) sleep, as well as GBA (Fig. 1, panel H) changed (in comparison to the previous dark period), as expected, according to their circadian rhythms (please see also Table 1S). In the same animals, following LPS administration (Fig. 1, panel E), T cort did not decrease, remaining significantly higher than following vehicle administration. Amount of time spent in NREM (Fig. 1, panel F) and REM (Fig. 1, panel G) sleep showed no differences between values recorded following vehicle and LPS administration. GBA (Fig. 1, panel H), already significantly inhibited by LPS administration during the previous dark phase, remained at the same low level, with no difference between control and treatment conditions.

As in HA rats, also in NA rats, following vehicle administration, Tcort (Fig. 1, panel A), amount of time spent in NREM (Fig. 1, panel B) and REM (Fig. 1, panel C) sleep, as well as GBA (Fig. 1, panel D) changed (in comparison to the previous dark period), as expected, according to their circadian rhythms. LPS administration did not alter Tcort and NREM and REM sleep amounts in comparison to vehicle condition, whereas LPS significantly decreased GBA.

During the light phase of the light-dark cycle, in both HA (Fig. 2, panels E–H) and NA (Fig. 2, panels A–D), LPS administration did not significantly affect (in comparison to vehicle administration) sleep architecture (i.e. number and duration of sleep bouts; Table 2S).

Area under the curve (AUC) was calculated for data shown in Fig. 1.

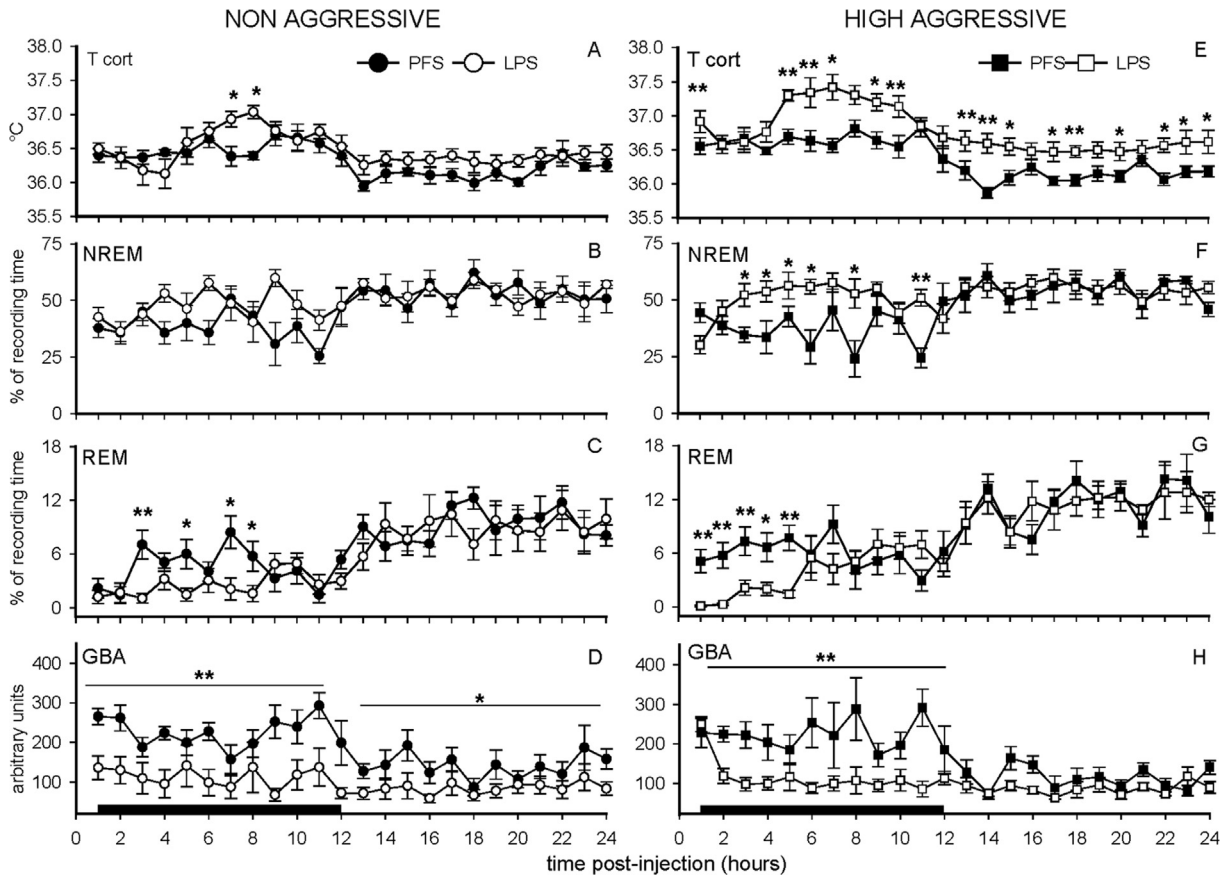

Fig. 1. Brain cortical temperature (Tcort; A and E), amount of time spent in NREM (B and F) and REM (C and G) sleep, and gross body activity (GBA; D and H) recorded following intraperitoneal administration of pyrogen free saline (PFS, as control vehicle; filled symbols) or lipopolysaccharide (LPS, 250 μg/kg – empty symbols) in non-aggressive (left panels, A-D; n = 8) and high-aggressive (right panels, E–H; n = 7; n = 6 for Tcort due to missing data) rats. Recordings began at dark onset. Bars on the x axis in the bottom (D and H) panels denote the 12-h dark portion of the light-dark cycle. Substances were administered immediately before dark onset. Symbols depict mean ± SEM. $^{*}p < 0.05$ vs. vehicle (PFS). Within strain comparisons by two-ways ANOVA and Student's *t*-test.

During the first 12 post-injection hours (i.e. the dark portion of the light-dark cycle), LPS-induced increase in Tcort was significantly higher in HA than in NA rats (Fig. 4).

4. Discussion

Findings in this study support the hypothesis that trait aggressiveness affects, in rats, sleep and febrile responses to an immune challenge. Specifically, they show that HA rats responded to an immune challenge with a 24 h long increase in Tcort, which spans across both the dark and light portions of the light-dark cycle. During the first 12 post-injection hours, HA rats also responded with a prolonged increase in NREM sleep amount, and a 5 h long, continuous and highly significant inhibition of REM sleep. In NA rats, in response to LPS administration, Tcort was increased only for two hours, NREM sleep was unaffected, and REM sleep inhibition was scattered along the first 8 post-injection hours. Gross body activity was inhibited in both HA and NA rats.

Aggressive behavior is evolutionarily conserved among various animal species, where it serves primarily to compete for territory, food and sexual partners. In humans, many different classification systems of aggressive behavior have been proposed (e.g. proactive and reactive, direct and indirect, emotional or social-cognitive, A and B, etc…) because of its heterogeneous nature (Hagenbeek et al., 2016). Moreover, this personality trait seems to affect other biochemical pathways and physiological functions, including the immune response (Coccaro et al., 2014, 2016; Hagenbeek et al., 2016; Koolhaas, 2008; Lindberg et al., 2003; Suarez et al., 2002).

The use of established animal models with high translational value is crucial to investigate the biological bases of both adaptive and excessive aggressive behavior and its implications for health (de Boer et al., 2017; Sgoifo et al., 2014; Takahashi and Miczek, 2014; Waltes et al., 2016). For example, male rodents show aggressive behavior in response to a reproducible threatening stimulus, such as the presence of an intruder conspecific into their territory (i.e., their home cage) (Miczek, 1979). In this context, adult male rats of the WTG strain exhibit forms of aggressive behavior that better resemble the human condition than those expressed by laboratory strains (de Boer et al., 2003). Indeed, similarly to humans, the WTG rat strain shows large individual differences in trait aggressiveness (de Boer et al., 2003). Members of this strain (which is derived from feral rats) differ widely in its propensity to engage in either an active or passive behavioral strategy of coping with various environmental challenges (de Boer et al., 2017). Previous studies (Carnevali et al., 2014, 2013; Sgoifo et al., 1996) using the WTG rat strain demonstrated that high levels of trait aggressiveness are associated with specific neuroendocrine, autonomic, breathing and behavioral patterns, including a larger catecholamine production and reduced vagal antagonism under stress conditions; a larger vulnerability to stress- and pharmacologically-induced cardiac arrhythmias; a higher respiratory rate at rest and increased incidence of sighs; and anxiety-like behaviors in the elevated plus maze test (Carnevali et al., 2014, 2013; Sgoifo et al., 1996). Importantly, these changes closely resemble those described in humans that exhibit exaggerated forms of aggressive behavior.

It has been hypothesized that changes induced in sleep by an immune challenge (increased and fragmented NREM sleep; decreased REM sleep) evolved to facilitate the generation of fever, promoting

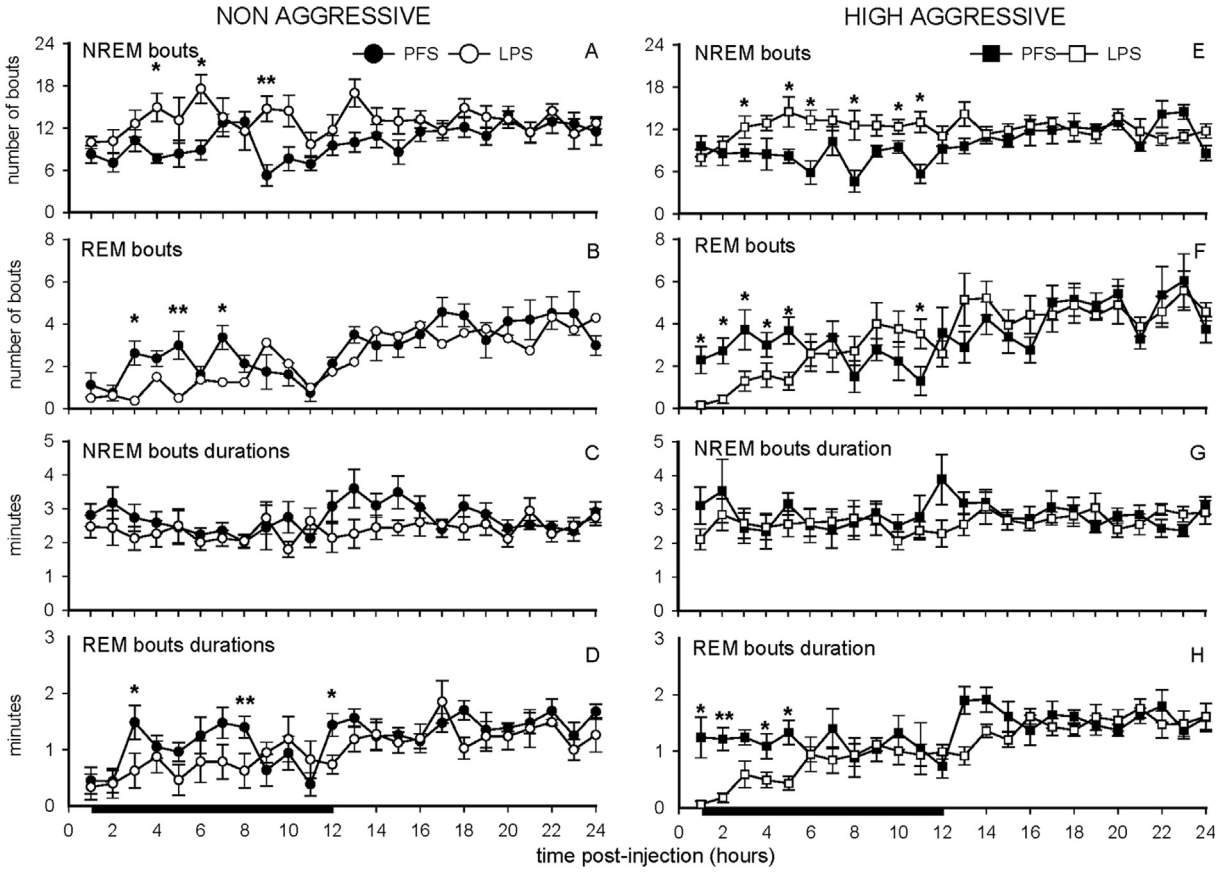

Fig. 2. Changes induced in the number (A and B, and E and F) and duration (C and D, and G and H) of NREM (A and E, and C and G) and REM (B and F, and D and H) sleep bouts by intraperitoneal administration of 250 μg/kg lipopolysaccharide (LPS; empty symbols) or pyrogen free saline (PFS, as control vehicle; filled symbols) in non-aggressive (left panels, A-D) or high-aggressive (right panels, E–H) rats. Recordings began at dark onset. Bars on the x axis in the bottom (D and H) panels denote the 12 h dark portion of the light-dark cycle. Substances were administered immediately before dark onset. Symbols depict mean ± SEM. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. vehicle (PFS). Within strain comparisons by two-way ANOVA and Student's *t*-test.

recovery (Imeri and Opp, 2009). Fever imparts survival value, when the host develops a moderate fever during bacterial or viral infection, because facets of the immune response are potentiated, and conditions for replications of pathogens are less optimal (Harden et al., 2015; Roth and Blatteis, 2014). On the other hand, generation of fever is energetically costly, because metabolism must be increased to raise body temperature, and because fever, as a brain-regulated elevation of body temperature that occurs during an inflammatory response (Saper et al., 2012), requires active thermoregulation (Kluger et al., 1996). The increase in the amount of time spent in NREM sleep induced by immune activation (as it occurs during infection) could reduce the energy expenditure that is associated with competing activities, such as locomotion, allowing to devote more energy to the generation of fever. Since brain and body temperature decrease during NREM sleep, fragmentation of this sleep phase induced by an immune challenge could reduce heat loss, facilitating the raise in body temperature. Finally, since thermoregulation is less efficient during REM sleep (Parmeggiani, 2003) and, for instance, shivering does not occur (whereas it is critical to generation of fever), inhibition of REM sleep amount induced by an immune challenge could help in mounting the febrile response. Whereas results of this study show that trait aggressiveness affects sleep and febrile responses to an immune challenge, they challenge key points of the hypothesis that changes induced in sleep by an immune challenge evolved to facilitate the generation of fever. In the first 12 post-injection hours, GBA, which includes locomotion, is significantly inhibited in both NA and HA rats, i.e. regardless of NREM sleep changes, suggesting that animals can move less without sleeping more. Moreover, results also suggest that the reduction in competing activity, such as locomotion, which is included in GBA and would make more energy available for the generation of fever is necessary, but not sufficient for febrile response, as it occurs in both strains tested in this study. Reduction in locomotion and exploratory activity could be independent of sleep changes and, on the other hand, a general key feature of sickness behavior (Dantzer et al., 2008), present also in NA rats.

In this study, HA aggressive rats respond to LPS administration with five, block hours of REM sleep inhibition, which starts with no delay. NA rats responded to the same treatment with an inhibition scattered over a longer time interval and which starts with a two hours delay. Such observations could suggest that REM sleep inhibition induced by an immune challenge could be more important in helping fever generation than NREM sleep enhancement.

It has been shown that old (24 months) Wistar rats respond to LPS (100 mg/kg, intraperitoneally) administration with a fever which is delayed, but prolonged in comparison to the fever induced in young (2 months) rats (Koenig et al., 2014). Sleep response to an immune challenge is affected by aging: in response to an immune challenge (interleukin-1, 2.5 ng, intracerebroventricularly), NREM sleep is not enhanced, whereas REM sleep is inhibited in old (25–27 months) Fisher rats (Imeri et al., 2004). It is possible to hypothesize that REM sleep inhibition allows old rats to mount a prolonged febrile response. Although it is difficult to compare the results of the two studies, because rat strains, immune challenges and routes of administration were different, it should be noted that febrile response was not different in old and young Fisher rats. Since ambient temperature plays a decisive role in the ability of old rats to mount febrile responses (Peloso et al., 2003),

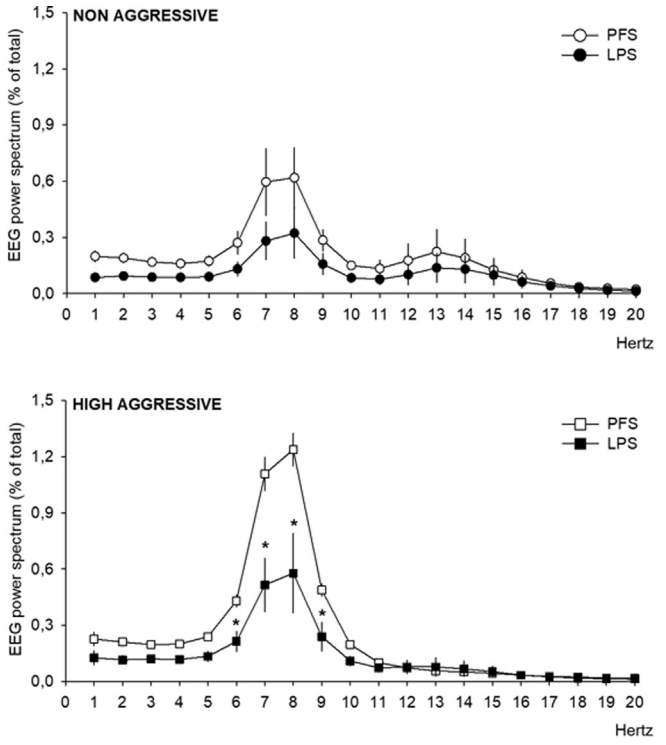

Fig. 3. In high aggressive rats, lipopolysaccharide administration significantly and selectively reduced the amplitude of EEG theta activity during REM sleep. 250 μg/kg lipopolysaccharide (LPS; filled symbols) or pyrogen free saline (PFS, as control vehicle; empty symbols) were injected intraperitoneally in non-aggressive (upper panel) or high-aggressive (lower panel) rats. REM sleep-specific EEG power spectra were obtained during the first twelve post-injection hours. Spectra were normalized as a percentage of total power across all frequencies within the 12 h of the dark phase, and are plotted as mean ± SEM for each frequency bin. Statistical analyses were performed on bins in the theta (6.0–9.0 Hz) frequency band. *: $p < 0.05$ vs vehicle. Within strain comparisons by two-way ANOVA [$F(1.732, 10.390) = 9.776$, $p = 0.005$] and Student's *t*-test [6 Hz: $t(6) = 3.438$, $p = 0.014$; 7 Hz: $t(6) = 3.694$, $p = 0.010$; 8 Hz: $t(6) = 3.692$, $p = 0.010$; 9 Hz: $t(6) = 3.72$, $p = 0.010$].

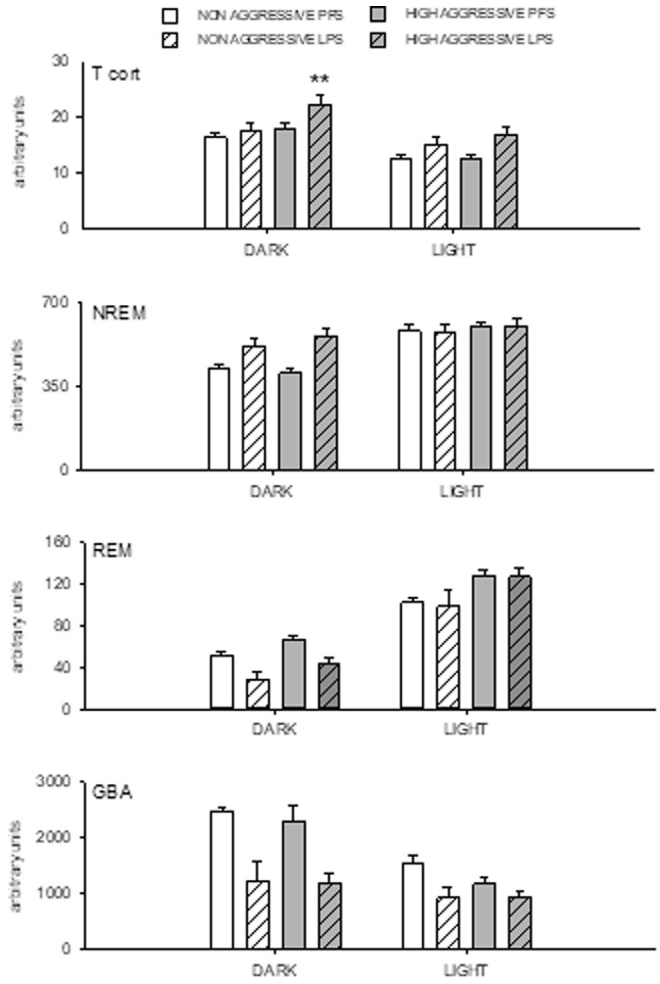

Fig. 4. Bars depict (in arbitrary units) the area under the curve (AUC) corresponding to data shown in Fig. 1. AUCs were calculated for each NA (n = 8) and HA (n = 7; n = 6 for Tcort due to missing data) rat, and then means and SE (shown here) were obtained. Rats were injected intraperitoneally, at the beginning of the dark period of the light-dark cycle with vehicle (pyrogen-free saline, PFS), and lipopolysaccharide (LPS), on different days (for further details, please see legend to Fig. 1, and Section 2.6). Bars show data for the first and second 12 h of the recording period, corresponding to the dark and light portions of the light-dark cycle, respectively. Two ways ANOVA (within each 12 h time block) [$F(1, 12) = 8.122$, $p = 0.015$], and Student's *t* test (when appropriated), **$p < 0.01$ vs. LPS in the other strain [$t(12) = -3.164$, $p = 0.008$].

observation that rats were kept at a sub-thermoneutral (21 ± 0.5 °C) ambient temperature should be taken into account when considering the results obtained by Imeri and colleagues (Imeri et al., 2004). As such, the exact role played by sleep changes in supporting the generation of fever needs to be further investigated.

Observation that, in HA rats, Tcort is increased in response to LPS administration also during the light phase (post-injection hours 13–24) suggest that, if any, sleep changes in response to an immune challenge could help in fever generation only when body temperature is already physiologically higher (i.e. during the dark and active phase in comparison to the light and rest phase) and an extra "boost" (i.e. sleep changes) is needed to increase body temperature above an already high value. On the other hand, during the light and rest phase, body temperature physiologically drops in comparison to the previous dark phase and in order to get an elevated temperature it is sufficient to not let body temperature decrease, which is energetically less costly. We would like to note that Tcort (recorded in the present study between dura mater and skull over the parietal cortex) is obviously lower than typical body core temperatures.

As reported in the Methods section (Section 2.1), HA rats were significantly heavier than NA rats. As such, the possibility that NA rats had less energetic resources to generate fever than HA rats should be taken into account.

Laboratory, outbred Sprague-Dawley rats that were not selected for trait aggressiveness respond to administration of an immune challenge, such as LPS, with typical and robust increases in NREM sleep and body temperature, accompanied by REM sleep inhibition (Imeri et al., 2006). By comparing those results and results of the present study, the outliers are the NA animals, which do not respond with increased NREM sleep amount and fever to LPS administration.

The inhibitory vagal activity is responsible for heart rate variability (HRV). Heart rate variability (HRV) provides an index of the cardiac vagal tone (Laborde et al., 2017). The observation that HRV is reduced in HA rats in comparison to NA animals (Carnevali et al., 2013) indicates that vagal, parasympathetic activity is higher in NA rats, than in HA animals. Data showing that parasympathetic activity inhibits pro-inflammatory cytokine release and inflammation (Tracey, 2002), suggest that the inflammatory response could be reduced in NA rats in comparison to HA rats and are in agreement with the up regulation of pro-inflammatory cytokines observed in proactive coping male rats (Kavelaars et al., 1999). It has also been suggested the existence of a positive correlation between cytokine levels and aggressive behaviour (Zalcman and Siegel, 2006). Enhanced vagal activity could result in a blunted immune response and in reduced production of pro-

inflammatory cytokines in NA rats. Since fever is mediated by pro-inflammatory cytokines and, in turn, prostaglandins (Saper et al., 2012), a blunted production of these molecules could account for the lack of a prolonged febrile response in NA rats reported in this present study. Further studies are needed to test this hypothesis.

The need for research on the link between different copying style and the immune response has been already underlined (Koolhaas, 2008). In addition, previous rodent studies have shown that social (dominance) hierarchies can influence the expression of sickness behaviour in response to an immune challenge. Specifically, dominant mice would prioritize recuperative behavior and, on the other hand, submissive mice would essentially focus on social defensive behaviors (Cohn and de Sa-Rocha, 2006; Cohn et al., 2012). Furthermore, repeated social defeat is known to promote immune dysregulation in rodents (Powell et al., 2009; Takahashi et al., 2018). However, the fact that subordinated animals can also show different levels of trait aggressiveness might indicate different strategies to cope with stressors also in this group. Therefore, further investigations are needed to clarify the role of aggressiveness and dominance in this context. It is worth noting that, as far as research on aggressiveness is concerned, most studies deal with increased up to pathological aggressiveness, maybe for the social burden and costs of these conditions, whereas little, if any attention has been paid to conditions of low or no aggressiveness.

Aggressive disposition differences could be due to individual variations in prefrontal cortical functioning, serotonergic signaling and HPA axis activity (Kamphuis et al., 2012). Thus, these putative differences between HA and NA rats may contribute to the modulation of sickness behavior in response to an immune challenge. Importantly, alterations in prefrontal cortical control of amygdala - which is thought to underlie the inhibition of aggressiveness and impulsivity (Takahashi and Miczek, 2014) - might be indirectly associated with immune dysregulation via HPA or autonomic activity changes (Cerqueira et al., 2008). For instance, chronic HPA hyperactivity could lead to the onset of glucocorticoid resistance, which in turn might modify the regulation of pro-inflammatory cytokine production (Avitsur et al., 2006a; Avitsur et al., 2006b).

Aggressiveness implicates not only different types of “benefits”, but also “costs”. Importantly, when this behavior leads to abnormal increases in “costs”, it becomes maladaptive and pathological (Georgiev et al., 2013; Takahashi and Miczek, 2014; Waltes et al., 2016). For example, as a component of Type A personality behavior pattern (Friedman and Rosenman, 1959), aggressiveness has traditionally been linked to an increased risk of cardiovascular disease (Betensky and Contrada, 2010; Lachar, 1993; Razzini et al., 2008; Smith et al., 2004). In this context, signs of cardiac autonomic impairment and increased arrhythmogenic susceptibility were previously described in WTG HA rats (Carnevali et al., 2013). If this is one of the costs of aggressiveness, would it be possible to hypothesize that a sleep response that can sustain the generation of a robust febrile response could represent one of the possible advantages of enhanced aggressiveness, at least when compared to no aggressiveness? It has been proposed that sleep (and specifically NREM sleep) provides a suitable condition that supports immune responses and pro-inflammatory activity (Besedovsky et al., 2012). Of course, a robust inflammatory (and febrile) response has its own costs, as shown by the observation that proactive coping male rats are far more vulnerable for the experimental induction of the autoimmune disease EAE (experimental allergic encephalomyelitis, (Kavelaars et al., 1999).

4.1. Limitations of the study

This study is merely observational, as no attempt was made to interfere with either immune response or sleep patterns. Moreover, the study does not provide data on possible mechanisms mediating the observed effects. On the other hand, the present results offer ground for future studies investigating such mechanisms using this animal model. However, since i) cortisol, dehydroepiandrosterone (DHEA) and a-amylase (assessing the hypothalamic-pituitary-adrenal gland – HPA – axis, and the sympathoadrenal system) can be reliably measured in human saliva splits (Ghiciuc et al., 2011), ii) aggressiveness in humans can be measured by mean of different psychometric inventories (Takahashi et al., 2018), and iii) common vaccinations have been used as a model of immune challenge (Lange et al., 2003; Spiegel et al., 2002), studies on the relationship between trait aggressiveness, sleep and immune response could be even designed in human subjects. Finally (but definitely not less important), in a direct comparison between NA and HA rats (Fig. 4), only the LPS-induced increase in Tcort (during the first 12 post-injection hours) was statistically significant.

4.2. Conclusions

In summary, this study suggests that trait aggressiveness affects febrile and sleep responses to an immune challenge in rats, although HA and NA animals do not sleep differentially under baseline conditions. In an evolutionary perspective, these results seem to be consistent with the facilitation of an effective protection against recurrent infections associated with higher risk of wound and inflammations in high aggressive animals (Takahashi et al., 2018). Although further investigations are needed to clarify the link between aggressiveness and physiological adaptations to an immune challenge, the present study bears translational value. It underscores the importance of evaluating also in humans – where the investigation of adaptive (non-pathological) aggressiveness is quite limited – the role of different levels of trait aggressiveness on immune function.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

5. Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2019.04.007.

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