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Research Report

Chronic stress-induced changes in REM sleep on theta oscillations in the rat hippocampus and amygdala

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ABSTRACT

The present study investigated the effect of Chronic Immobilization Stress (CIS) on theta oscillations in the hippocampus and amygdala during Rapid Eye Movement (REM) sleep. Adult male Wistar rats were subjected to 2 h of CIS daily for 10 days. Polysomnographic recordings with electroencephalogram (EEG) from hippocampus (CA3 and CA1 subregion) and lateral nucleus of amygdala (LA) were carried out after termination of CIS protocol on the 7th, 14th and 21st day. The results showed a bimodal distribution on the total REM sleep duration in CIS rats: group of rats exhibited increased REM sleep duration considered as a stress-enhanced REM (SER) and rats with reduced REM sleep as stress-reduced REM sleep (SRR) group. The bimodal distribution in REM sleep was continued to exhibit even after 21 days of termination of stress, showing increased REM sleep in SER and reversible REM sleep in SRR rats. In addition to changes in sleep, increased REM sleep in SER rats was associated with attenuated theta activity in the hippocampus and amygdala, while the SRR rats did not show attenuated theta activities during the stress recovery period. Thus, the study demonstrates the dependence of synchronized amygdalo-hippocampal theta activity with the CIS-induced enhanced REM sleep duration. This raises the possibility that CIS-induced manifestations of the anxiety may be associated with synchronized theta oscillations in the hippocampus and amygdala.

1. Introduction

It is well documented that stress is a biologically powerful contributory factor in the cause and progression of many psychiatric diseases (Breslau et al., 2004; Habukawa et al., 2007; Mellman et al., 2002; Reist et al., 1995; Ross et al., 1994). Recent studies in rodents suggest that repeated stress that produces degenerative changes in the hippocampus impairs hippocampal-dependent learning (McEwen and Sapolsky, 1995; Sapolsky, 1996), while dendritic hypertrophy in the basolateral amygdala (BLA) facilitates aversive learning and anxiety-like behavior (Liang et al., 1994; Shors and Mathew, 1998; Vyas et al., 2002, 2006). In humans, stress-induced anxiety disorders are of serious concern because augmented anxiety is postulated to

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Abbreviations: CA1, CA1 subregion of the hippocampus; CA3, CA3 subregion of the hippocampus; CIS, Chronic Immobilization stress; EEG, Electroenecephalogram; EMG, Electromyogram; EOG, Electrooculogram; LA, Lateral amygdala; EEG, Electro encephalogram; PTSD, Post traumatic stress disorder; REM, Rapid eye movement sleep; SER, Stress enhanced REM sleep; SRR, Stress reduced REM sleep; SWS, Slow wave sleep; NREM, Non-REM sleep; TST, Total sleep time; TWT, Total wake time; W1, Active wake; W2, Quiet wake

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play a major role in inducing sleep disturbances (Mellman and Uhde, 1989; Reynolds et al., 1983; Ross et al., 1994). Such stress-induced damage is thought to be related to an increase in cortisol levels and glucocorticoids associated with stress (Bremner, 1999).

Evidence from both human and animal studies suggest that sleep facilitates information processing and synaptic remodeling (Hill et al., 2008; Lopez et al., 2008). REM sleep, in particular, has been identified as an important stage of sleep in memory consolidation (Hellman and Abel, 2007; McNamara et al., 2009; Stickgold et al., 2001; Stickgold and Walker, 2007). Further investigations into the behavioral basis of rhythmical slow wave activity (theta) from the hippocampus suggest the presence of two types of theta activity in most animal species (Bland, 1986; Montoya and Sainsbury, 1985; Sainsbury, 1970; Vanderwolf et al., 1988). Type 1 theta has a frequency range of 6–12 Hz, is present during voluntary movements such as running, rearing and swimming. Type 2 theta activity usually has a frequency range at 4–9 Hz and occurs during alert immobility, REM sleep and sensory processing (Bland, 1986; Leblanc and Bland, 1979).

The existences of rhythmic theta oscillations in the hippocampus and amygdala are essential for normal physiological functions during sleep (Popa et al., 2010; Wilson and McNaughton, 1994) and abolishing them will result in severe behavioral deficits (Winson, 1978; Winson and Abzug, 1978). We have also reported the changes in REM sleep and theta activity following exposure to acute immobilization stress (Hegde et al., 2008). Furthermore, behavioral and morphological changes induced by stress suggest that stress impairs memory through both hippocampal and non-hippocampal mechanisms (Conrad et al., 1996; Vyas et al., 2002, 2006). Amygdala dependent enhancement in the anxiety-related behavior was reported until 21 days after termination of stress (Vyas et al., 2004), whereas hippocampal dependent behavioral impairment returned to normal when left undisturbed under standard home conditions (Conrad et al., 1999; Luine et al., 1994).

The above mentioned studies clearly suggest the deleterious effect of stress on sleep–wake behavior and theta activity. It may be plausible to corroborate that the stress-induced changes in REM sleep may be one of the major factors responsible for the genesis of anxiety leading to changes in theta rhythm in the hippocampus and amygdala. The present experiment was designed to further investigate the relationship between the chronic stress and the amygdalo-hippocampal theta activity during REM sleep.

2. Results

2.1. *Evaluation of sleep architecture*

Fig. 1B shows the site locations of the electrodes in the different brain regions such as CA1, CA3 regions of the hippocampus and LA. Polysomnographic recordings from the Control rats ($n=11$) showed that rats spent predominant time in Slow Wave Sleep (SWS) and Rapid Eye Movement (REM) sleep, that is, 51.53±2.49% and 10.52±1.433% of the total 6 h of recording time respectively. The total wake time (TWT) of the animals was 37.41±2.198% as shown in Fig. 1C. The distribution of the sleep–wake cycle was comparable to that observed in the previous study in the laboratory (Raol and Meti, 1998). Fig. 1D shows representative sleep architecture from Control and CIS rats after 21 days of termination of stress.

As compared to control animals, the CIS rats showed significant changes in the sleep architecture. This was mainly due to the changes in REM sleep. The CIS ($n=13$) rats showed a wide range of variations in REM sleep states; from 4.549±1.09% to 16.14±1.192 % of the total recording time. Accordingly, the rats were further categorized as stress-enhanced REM (SER) group ($n=6$) with a total of 16.14+1.192% REM sleep and stress-reduced REM group ($n=7$) with 4.549±1.09% of REM sleep. By means of categorical variable analysis, CIS group of rats showed a very clear dichotomy in the distribution of REM sleep duration ($t_{11}=7.080$, $p<0.0001$), and no such dichotomy was seen in Control group ($t_9=1.970$, $p=0.0843$) as shown by Student *t*-test analysis. Two way ANOVA with Bonferroni post-hoc comparison test revealed (ANOVA: $F_{2,53}=19.56$, $p<0.0001$) that total duration of REM sleep was increased ($p<0.05$) in SER group and reduced ($p<0.05$) in SRR group when studied after 7 days of termination of CIS (see Fig. 2A). Though the REM sleep has come back to normal by day 14 (*T*-test: $t=1.140$, $p>0.05$), but showed an increasing trend on day 21 ($t=1.527$, $p>0.05$) (Fig. 2A). Additionally, the SER rats did not display any significant changes in the total number of REM episodes as compared to Control group (Fig. 2B).

SRR group, on the other hand, showed a decreased number of REM episodes ($p<0.05$) as compared to Control rats after 7 and 14 days of termination of stress (ANOVA: $F_{2,23}=8.806$, $p<0.001$) (Fig. 2B). While the SER rats showed increased REM sleep, SRR group showed a statistically significant decrease in total REM duration on day 7 ($t=2.956$, $p<0.01$) (Fig. 2A), day 14 ($t=3.890$, $p<0.001$) and returned to normal levels on day 21 after CIS termination ($t=1.613$, $p>0.05$) (Fig. 2A). Similarly, REM episodes in SRR group also showed a significant decrease on day 7 (*T*-test: $p<0.008$, $t_{1,15}=3.064$) (Fig. 2B) and day 14 (*T*-test: $p<0.006$, $t_{1,15}=3.197$). The REM latency did not show any significant differences across recording sessions.

In contrast to REM sleep, CIS did not affect the total wakefulness of SER rats. While, the SRR group of rats showed statistically significant increase in TWT (*T*-test: $p<0.008$, $t_{1,15}=3.057$) and significant decrease in TST ($t_{1,15}=3.508$, $p<0.003$) on day 14 after CIS in comparison with Control rats (Fig. 3A and C). In both SER and SRR groups, the TSWS remained unaffected at all the three time points of sleep recordings (Fig. 3B). These results indicate that CIS-induced increase in REM sleep did not alter the TST, however, CIS-induced reduction in the REM sleep was accompanied with reduced TST specifically 14 days after termination of CIS.

2.2. *Theta activity from hippocampus and amygdala*

The impact of chronic stress on theta modulation during stress recovery period was evaluated by FFT analysis. In comparison to rhythmic theta activity in the controls, CIS rats showed profound changes in theta activity of both hippocampal (CA1 and CA3) and LA (Figs. 4, 5) neurons, specifically 21 days after termination of CIS. The change in theta activity during REM sleep was very prominent in SER group when compared to SRR and Control group of rats.

Spectral analysis from Control and SRR rats showed a predominant distribution of signal power over theta frequency range (4–8 Hz) during REM sleep (Fig. 4). It was observed