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CYTOMORPHOMETRIC CHANGES IN RAT BRAIN NEURONS AFTER RAPID EYE MOVEMENT SLEEP DEPRIVATION

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Abstract—Rapid eye movement sleep plays a vital role in the survival of animals. Its deprivation causes alterations in brain functions and behaviors including activities of important enzymes, neurotransmitter levels, impairment of neural excitability and memory consolidation. However, there was a lack of knowledge regarding the effects of rapid eye movement sleep deprivation on neuronal morphology that may get affected much earlier than any permanent damage to the neurons. In the present study, some of these issues have been addressed by studying the effects of rapid eye movement sleep deprivation on various morphological parameters viz. neuronal perimeter, area and shape of neurons located in brain areas known to regulate rapid eye movement sleep and as a control in other brain areas which do not regulate rapid eye movement sleep. The results showed that rapid eye movement sleep deprivation differentially affected neurons depending on their physiological correlates of rapid eye movement sleep and neurotransmitter content. The effects could be reversed if the animals were allowed to recover from rapid eye movement sleep loss or by applying alpha1-adrenergic antagonist, prazosin. The findings in rats support reported data and help explaining previous observations.

Key words: Na-K ATPase, neuronal excitability, neuronal morphometry, neuronal size and shape, norepinephrine, REM sleep deprivation.

Rapid eye movement (REM) sleep is a physiological phenomenon that is unique for more than one reason. The body compensates for the lost REM sleep (experimental or otherwise) by proportionately enhancing it during the post-deprivation recovery sleep phase. Ontogenetic studies revealed that it is maximum in the animals who are born immature e.g. kangaroos; it is higher in the pre-term (immature) babies followed by in the newborn babies and gradually reduces with advancing age (Roffwarg et al., 1966). REM sleep deprivation reduces brain maturation and memory consolidation (Youngblood et al., 1997). Some of the recent related studies have shown that REM sleep may play a significant role in development of neuronal connectivity in the lateral geniculate nucleus (Pompeiano et al., 1995; Oksenberg et al., 1996; Shaffery et al., 1998, 1999). Although REM sleep has not been classified as a vital function, its prolonged loss may have cumulative effect increasing the risk of hypertension as well as heart attack and even may become fatal (Zenko et al., 2000), rendering it effectively essential for life. The precise function(s) of REM sleep and its mechanism(s) of action are not known and there are no known underlying unified common cellular mechanism(s) that can possibly explain such REM sleep loss-induced effects. Rapid eye movement sleep deprivation (REMSD) modulates the level of norepinephrine (NE) (Porkka-Heiskanen et al., 1995; Shouse et al., 2000) and enzyme(s) regulating its breakdown (Thakkar and Mallick, 1993) as well as synthesis (Basheer et al., 1998; Majumdar and Mallick, 2003). It also affects neuronal responsiveness (Mallick et al., 1990, 1991) and excitability (Cohen and Dement, 1965; Cohen et al., 1970; Mallick et al., 1999, 2002; McDermott et al., 2003). Further, it has been shown that activities of ATPases (Gulyani and Mallick, 1993; Mallick and Gulyani, 1993), calcium ion concentration (Mallick and Gulyani, 1996), and neuronal membrane fluidity (Mallick et al., 1995) alter after REM sleep deprivation and alterations in either or all of these parameters could mediate changes in neuronal excitability. Notwithstanding, these changes at the cellular level are also known to modulate, among other factors, neuronal size (Glyn, 1985), growth (Spafford et al., 2004), excitability (Kandel et al., 2000) and synaptic potential (Vaillend et al., 2002).

Based on the facts mentioned above, it was proposed that one of the functions of REM sleep is to maintain neuronal integrity. Since factors like neuronal excitability, changes in Na-K ATPase (sodium potassium ATPase), etc. may lead to structural changes in neurons (Henneman, 1957; Glyn, 1985; Kandel et al., 2000), one of the initial important consequences of REM sleep loss is likely to be changes in neuronal size and structure. Since cells have a normal repair mechanism, such changes may or may not be reversible depending on the intensity of insult, duration of REM sleep loss in this case. Hence, in this study rats were deprived of REM sleep and the effects were studied on neuronal perimeter, area and shape. The results of this study showed that REM sleep deprivation caused differential changes in neuronal sizes depending on the anatomic location, REM sleep-related function and neurotransmitter content of the neurons. Furthermore,

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Abbreviations: ABC, avidin–biotin–peroxidase complex; ChAT, choline acetyltransferase; DPX, distrene plasticizer xylene; FF, soma form factor; FMC, free-moving control; GAD, glutamic acid decarboxylase; IgG, immunoglobulin; i.p., intraperitoneal; LC, locus coeruleus; LDT, laterodorsal tegmentum; LPC, large platform control; LS, lateral septum; MPO, medial preoptic area; Na^+, sodium ion; Na-K ATPase, sodium potassium ATPase; NE, norepinephrine; PBS, phosphate-buffered saline; PPT, pedunculopontine tegmentum; PRZ, prazosin; REC, recovery; REM, rapid eye movement; REM-ON, rapid eye movement ON; REMSD, rapid eye movement sleep deprivation; TH, tyrosine hydroxylase.

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Table 1. Mean area (±S.E.M.) of neurons after Nissl staining from LC, LDT/PPT, MPO and LS in three individual experiments show no intra-group variation in data (significant values given in the text)

Regions	Experiment 1 area (μm^2)					Experiment 2 area (μm^2)				
	FMC	REMSD	LPC	REC	PRZ	FMC	REMSD	LPC	REC	PRZ
LC	266.3±5.9	300.2±4.7	257.2±5.8	243.8±4.4	253.5±5.2	251.6±6.7	308.7±4.4	243.5±4.4	250.8±3.1	249.7±7.2
LDT/PPT	275.8±3.6	227.3±6.9	274.6±1.4	272.1±3.2	269.8±5.6	272.4±2.6	220.3±2.1	276.7±4.1	273.7±2.9	272.5±2.3
MPO	153.2±1.1	120.1±1.4	151.9±5.5	151.3±4.3	159.9±1.4	160.8±8.8	124.4±6.3	159.0±3.5	159.4±3.1	162.4±4.1
LS	127.2±6.4	133.2±4.9	137.6±2.7	138.5±4.2	140.1±5.3	135.9±3.4	140.4±1.8	140.8±2.1	142.4±1.3	148.7±1.2

Experiment 3 area (μm^2)				
FMC	REMSD	LPC	REC	PRZ
253.8±3.6	299.3±5.4	249.6±6.0	252.6±3.6	250.8±2.6
263.6±3.2	224.1±2.8	268.1±2.4	265.8±5.9	272.6±0.7
164.2±2.5	128.5±1.4	148.3±3.4	156.8±3.3	155.5±2.9
143.5±1.1	143.3±1.2	148.9±3.4	145.8±1.1	142.6±0.2

REM sleep recovery and alpha-1 adrenoceptor antagonist reversed these changes.

EXPERIMENTAL PROCEDURES

Experiments were approved by the Institutional Animal Ethical Committee (IAEC) that conformed to National Institutes of Health, USA guidelines on the ethical use of animals. Efforts were made to minimize the number of animals used and their suffering. Experiments were conducted on inbred male Wistar rats (250–300 g obtained from University Animal Facility) maintained with food and water *ad libitum* under 12-h light/dark cycle. Free-moving control (FMC) rats were maintained in their normal dry home cages. Experimental rats were REM sleep deprived by the classical flowerpot method for 6 days (Jouvet et al., 1964). In a pilot study, we had deprived the rats for 4, 6 and 10 days and found that after 4 days of REMSD there was no significant change in neuronal size compared with controls, however, significant changes were observed only after six and 10 days of REMSD. To avoid unnecessary discomfort to the rats, we opted for 6 days' REM sleep deprivation uniformly in this study. For REMSD, rats were kept on a 6.5 cm diameter platform surrounded by water. To rule out non-specific effects another group of control rats was maintained on a larger (diameter 13 cm) platform (LPC) surrounded by water i.e. except for diameter of the platform, all other conditions remained identical to that of the experimental animals. A fourth recovery control group (REC) included those animals that had been REM sleep deprived and then allowed to live in normal cages for 3 days to recover from lost REM sleep. Thus, in each set there was one rat each of FMC, REMSD, LPC and REC and three such sets were carried out for all the studies. Additionally, in another separate set of experiments prazosin (PRZ) (an alpha-1 adrenergic blocker, 2 mg/kg body wt) was intraperitoneal (i.p.) injected once a day in rats during the last four days of REMSD. At the end of the experiment the control as well as the experimental rats was anesthetized with high dose (i.p. 45 mg/kg) of sodium pentobarbital (Sigma, St. Louis, MO, USA). The brains were intracardially perfused with 0.1 M phosphate-buffered saline (PBS) and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and fixed overnight in the same fixative. After fixation, the brains were cryoprotected in 30% sucrose in PBS. Thereafter, 40 μm cryostat (Leica, Solms, Germany) sections were collected in vials containing PBS and stored at 4 °C. Alternate sections through locus coeruleus (LC), laterodorsal and pedunculopontine tegmentum (LDT/PPT), medial preoptic (MPO) and lateral septum (LS) were processed for Nissl and immuno-staining.

Histochemistry

For Nissl, the sections were stained with 1% Cresyl Violet and 0.1% Thionin in acetate buffer, dehydrated in ethanol and coverslipped with distrene plasticizer xylene (DPX). The immuno-staining procedure has been reported earlier in detail (Majumdar and Mallick, 2003). In brief, sections were blocked in 10% normal goat serum for 2 h and then incubated in primary antibody for 3 days at 4 °C. The antibody was diluted in 0.1 M PBS containing 5% normal goat serum and 0.5% Triton X-100. The primary antibodies used were tyrosine hydroxylase (TH, 1:5000 dilution), choline acetyltransferase (ChAT, 1:2000), glutamic acid decarboxylase (GAD, 1:3000) (all from Chemicon, Temecula, CA, USA). After washes in PBS, the sections were incubated in biotinylated goat anti-rabbit immunoglobulin (IgG) (rabbit IgG, Elite Vectastain ABC (avidin–biotin–peroxidase complex) kit, Vector Laboratories, Burlingame, CA, USA) at 1:200 for 18 h at 4 °C. Following this secondary antibody step, the sections were washed and placed in preformed avidin–biotin–peroxidase complex (Elite Vectastain ABC kit, Vector Laboratories) at 1:400 dilution for 2 h at room temperature. For visualization of the reaction sites, the sections were treated with the chromogen 3,3′diaminobenzidine (DAB; without nickel chloride) and hydrogen peroxide (DAB Kit, Vector Laboratories) for 2–3 min. Finally, the sections were rinsed in distilled water, mounted onto gelatin-coated slides, dehydrated in ethanol and coverslipped with DPX.

Analysis

All the experiments were repeated on three sets, every set consisting one animal each of FMC, REMSD, LPC and REC. The areas of individual neurons located within the anatomical boundaries of LC, LDT/PPT, MPO and LS, Paxinos and Watson (1998), were estimated in Nissl-stained brain sections from each group of rats. To understand if the changes in neuronal sizes were induced by increased NE after REMSD, in separate sets of rats the areas of the neurons were also estimated in REMSD rats treated with PRZ, an α-1 adrenoceptor antagonist. Further, to explore if the changes in areas varied with the type of neurotransmitter possessed by the neurons, estimations were carried out in neurons whose identity of neurotransmitter type was predetermined by immuno-staining them with antibodies against TH for aminergic,