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EFFECT OF RAPID EYE MOVEMENT SLEEP DEPRIVATION ON RAT BRAIN MONOAMINE OXIDASES

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Abstract—Monoamine oxidase, monoamine oxidase-A, and monoamine oxidase-B activities were compared in free moving, rapid eye movement sleep-deprived, recovered, and control rat brains. The activities were estimated in the whole brain, cerebrum, cerebellum, whole brainstem, medulla, pons, and midbrain. The flowerpot method was used for continuing deprivation for one, two, or four days. Monoamine oxidase activity decreased significantly in the cerebrum and the cerebellum of the sleep-deprived rats, whereas monoamine oxidase-A and monoamine oxidase-B were differentially affected. Medullary MAO-A was the first to be affected, showing an increase after just one day of rapid eye movement sleep deprivation, while longer deprivation decreased its activity. The activity of monoamine oxidase-B was not significantly affected in any brain areas of the deprived rats until after two days of rapid eye movement sleep deprivation. All the altered enzyme activities returned to control levels after recovery. Control experiments suggest that the decrease was primarily caused by the rapid eye movement sleep deprivation and was not due to nonspecific effects.

These findings are consistent with past studies and may help to explain earlier observations. The results support the involvement of aminergic mechanisms in rapid eye movement sleep. The plausible reasons for the changes in the activities of monoamine oxidases, after rapid eye movement sleep deprivation, are discussed.

Norepinephrine (NE) may be involved in the regulation of rapid eye movement (REM) sleep.[12,18,19,30,34,38] In one study the level of NE was found to decrease, [44] while in another it remained unaltered[40] after REM sleep deprivation. While, REM sleep deprivation has been suggested to enhance NE synthesis;[39,40] an NE synthesis inhibitor has been found to increase REM sleep.[10] Inhibition of the monoamine oxidase (MAO), in an attempt to increase the level of NE, led to the reduction of REM sleep.[22,49] Aminergic neurons are known to alter their firing rates during REM sleep[12,18,36] and REM sleep deprivation.[25] Alterations in the release or levels of NE might affect MAO activity in the rat brain or vice versa.[14,45] However, MAO is known to have two forms, MAO-A and MAO-B, [17] and the former is primarily responsible for the breakdown of NE. REM sleep has also been reported to be influenced by several small peptides and hormones[15,31] that are known to influence MAO-A activity.[42,43,52] On the other hand, MAO-B in rat brain, is a nonspecific enzyme for the breakdown of amines in general.[6,42] Therefore, it is possible that REM sleep deprivation might influence MAO-A and MAO-B selectively or to different extents.

Noradrenergic neurons in the rat brain are found mainly in the locus coeruleus, [16] which is the primary site for supplying noradrenergic input to the rat brain. However, its projections are not uniformly distributed throughout the brain.[5,46] Recently, it has been shown that brainstem areas also receive dense inputs from noradrenergic neurons located in areas other than the locus coeruleus.[7] The pontine region is likely to be the REM generator[37] and REM deprivation has been reported to affect other enzyme activities in restricted areas of the rat brain.[9,26] Hence, we studied the effect of REM sleep deprivation on rat brain MAO, MAO-A, and MAO-B activities in the cerebrum, cerebellum, brainstem, midbrain, pons, and medulla.

EXPERIMENTAL PROCEDURES

Experiments were conducted on male albino rats (Wistar) weighing 220–280 g. The rats were maintained in the animal house under a 12/12 h light/dark cycle. REM sleep deprivation was continued for one, two, or four days using the flowerpot technique as described earlier.[41] In brief, experimental (E) rats were maintained on a 6.5-cm diameter island projecting above a pool of water. In addition to free moving controls (FMC), where rats were maintained in normal rat cages, a second control group, large platform controls (LPC), were maintained under identical condition as the E rats, except that the platform size was a little larger (12.5 cm). The size of the platform (for the weight group of rats taken in this study) was decided as per the available reports.[29,51] To eliminate the effect of movement restriction (RM), rats were maintained individually on normal litter for 15 days in 12.5-cm diameter cages to restrict their movement. For the recovery study, REM sleep-deprived rats spent a period similar to that of deprivation in normal cages.

Mitochondrial preparation

The cerebrum, cerebellum, brainstem, midbrain, pons, and medulla were dissected within three min of decapitation following spinal dislocation.[8,53] After removing the brain,

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Abbreviations: E, experimental; FMC, free moving control; LPC, large platform control; MAO, monoamine oxidase; NE, norepinephrine; REM, rapid eye movement; RM, restricted movement control.

the cerebellum was first dissected out. The brain stem was separated from the cerebrum by a dorsoventral cut through the anterior border of the superior coliculli dorsally and the posterior end of the corpus mammillary ventrally. The brainstem was further dissected into the midbrain, pons, and medulla. The brainstem from the anterior border to ~ 3 mm (anterior to that of nucleus trigeminus corresponding approximately to the level of nucleus trochlearis) was taken as midbrain. Thereafter, up to the anterior margin of the corpus trapezoideum was taken as the pons. The anterior border of the pons would pass anterior to that of locus coeruleus. The remaining brainstem to the posterior border of the nucleus hypoglossus was taken as medulla. Thus, as shown by Zeman and Innes,[53] in this study the medulla corresponds approximately to the transection levels between A3 and A8, the pons to levels A8–A11, and the midbrain to levels A11–A14.

Areas from two rats were pooled for each assay and homogenized in 0.32 M sucrose buffer containing 12.5 mM Tris–HCl (pH 7.4). The homogenate was centrifuged at 3000 *g* (5000 r.p.m.) for 10 min, the pellet discarded, and the supernatant was again centrifuged at 27,000 *g* (15,000 r.p.m.) for 30 min to pellet out the crude mitochondria.[50] The pellet was rehomogenized in an appropriate quantity of 0.32 M sucrose buffer so that the final protein concentration was 4–10 mg/ml of the crude mitochondrial homogenate for the whole brain, cerebrum, cerebellum, and brainstem. In the case of the medulla, pons, and midbrain, the protein concentration was 1 mg/ml. This crude mitochondrial preparation was used for MAO assay.

Estimation of monoamine oxidase, monoamine oxidase-A and monoamine oxidase-B in different areas of the rat brain

MAO estimation was based on the spectrophotometric method reported earlier[4,28] using 4-hydroxyquinoline as the standard. In brief, the reaction mixture contained 0.44 mM Tris–HCl buffer (pH 7.4), 0.08 mM $MgCl_2$, 0.22 mM kynuramine dihydrobromide (final concentration), and the crude mitochondrial extract (0.4–1 mg protein) as the source of enzyme. To estimate the activities of MAO-A and MAO-B, 1 μmol (final concentration) of either (−)deprenyl (MAO-B inhibitor) or clorgyline (MAO-A inhibitor), respectively, was added to the reaction mixture.[27] The reaction was carried out for 90 min at 37°C and then terminated by 0.2 ml of 0.5 N NaOH and 0.4 ml of 10% $ZnSO_4$. The mixture was vortexed and heated in a water bath for 10 min, cooled, and centrifuged at 4300 *g* (6000 r.p.m.) for 10 min to remove the precipitate. The clear supernatant was then read at 330 nm (Shimadzu UV 260).

Estimation of monoamine oxidase, monoamine oxidase-A and monoamine oxidase-B in medulla, pons, and midbrain

Since the protein content in the medulla, pons, and midbrain was low, the enzyme activities were estimated by spectro-fluorometric methods.[21,27] In brief, the reaction mixture contained 20 μM kynuramine dihydrobromide (final concentration), 100 mM phosphate buffer, and 100–200 μg protein (crude mitochondrial pellet as source of enzyme). For the estimation of MAO-A and MAO-B, 1 μmol (final concentration) of either (-)deprenyl (MAO-B inhibitor) or clorgyline (MAO-A inhibitor), respectively, was added to the reaction mixture. The mixture was incubated at 37 C for 10 min and the reaction was terminated with 400 μl 10% trichloroacetic acid and then centrifuged at 10,000 *g* (10,000 r.p.m.) for 10 min. Eight hundred microlitres of the supernatant was then added to 2 ml of 1 N NaOH and the fluorescence was read (Shimadzu RF 540) at 315 nm (excitation), 380 nm (emission). In case of a blank, 400 μl of trichloroacetic acid was added before the addition of Kynuramine. 4-OH Quinoline was used as a standard.

The protein concentration was estimated by the method of Lowry *et al.*[24] Data was collected from six to 16 rats in each group. The results from different groups (FMC, LPC, E, RM and recovered groups) were compared by applying ANOVA, and those that showed a significant difference were subjected to Scheffe's *post hoc* test to determine the level of significance of the group/s with respect to the FMC animals.

RESULTS

The E rats could stand or sit on the smaller platform but could not assume the posture necessary for REM sleep without falling into the water surrounding the platform resulting in waking. On the other hand, rats on the larger platforms could maintain the posture required for REM sleep. Although no quantitative tests were done, after four days of REM sleep deprivation the E rats were more sensitive to touch and noise and grooming was reduced compared to LPC, RM and recovered rats.

Regional distribution of monoamine oxidase, monoamine oxidase-A and monoamine oxidase-B activity in control animals

The enzyme activities (mean ± S.D.) in different regions of the rat brain are shown in Tables 1–4. In normal free moving rats, MAO and MAO-A activities were highest in the cerebrum and cerebellum, and lowest in the brainstem (Tables 1, 3). Within the brainstem the activities were maximum in the midbrain while they were comparable in medulla and pons (Tables 2, 4). Activity of MAO-B was lowest in the brain stem (Table 3), but comparable in the medulla, pons, and midbrain (Table 4).

Effect of rapid eye movement sleep deprivation on wholebrain monoamine oxidase activity

Four days of REM sleep deprivation did not induce a significant change in the MAO activity in

Table 1. Monoamine oxidase activity (μmol/90 min per mg protein) in the rat brain in different groups of animals

Groups	Specific activity of MAO			
	Whole brain	Cerebrum	Cerebellum	Brainstem
FMC	0.340 ± 0.028 (16)	0.378 ± 0.054 (16)	0.356 ± 0.051 (16)	0.306 ± 0.039 (16)
E4	0.299 ± 0.053 (18)	0.288 ± 0.040 (18)**	0.301 ± 0.029 (18)**	0.260 ± 0.048 (18)
E2	—	0.350 ± 0.038 (6)	0.327 ± 0.055 (6)	0.297 ± 0.013 (6)
R	0.340 ± 0.056 (14)	0.347 ± 0.020 (14)	0.342 ± 0.037 (14)	0.291 ± 0.048 (14)
LPC	0.324 ± 0.050 (14)	0.357 ± 0.041 (14)	0.333 ± 0.052 (14)	0.302 ± 0.052 (14)
RM	—	0.389 ± 0.011 (6)	0.350 ± 0.017 (6)	0.320 ± 0.023 (6)

No. of animals is shown in parentheses under respective group; **$\alpha = 0.01$.

Table 2. Monoamine oxidase activity (μmol/90 min per mg protein) in different regions of rat brainstem in separate groups of rats

Groups	Specific activity of MAO		
	Medulla	Pons	Midbrain
FMC	0.027 ± 0.006 (12)	0.030 ± 0.003 (12)	0.042 ± 0.005 (12)
E1	0.032 ± 0.002 (12)	0.029 ± 0.003 (12)	0.039 ± 0.003 (12)
E2	0.030 ± 0.003 (8)	0.021 ± 0.002 (8)**	0.037 ± 0.003 (8)
R	0.028 ± 0.001 (8)	0.026 ± 0.006 (8)	0.039 ± 0.005 (8)
LPC	0.030 ± 0.006 (12)	0.030 ± 0.005 (12)	0.040 ± 0.008 (12)

No of animals is shown in parentheses under respective group; **$\alpha = 0.01$.

sleep deprived, LPC or recovered animals. However, there was an insignificant decrease in enzyme activity in wholebrain of the sleep deprived rats (Table 1).

Effect of rapid eye movement sleep deprivation on regional monoamine oxidase activity

After four days of REM sleep deprivation there was a significant decrease in MAO activity in the cerebellum ($F_{(4,63)} = 4.39$; $P < 0.01$; Scheffe's *post hoc* test $\alpha = 0.01$) and cerebrum ($F_{(4,63)} = 14.06$; $P < 0.001$; Scheffe's *post hoc* test $\alpha = 0.01$) in experimental compared to FMC animals. The brainstem did not show any significant change, although there was a tendency for a decrease. The decreased MAO activity returned to the baseline level after recovery (Table 1). The enzyme activities in different brain areas of LPC, RM and FMC rats were comparable.

Two days of REM sleep deprivation did not induce a statistically significant change in MAO activity in any of the regions of the rat brain, although there was a tendency for a decrease (Tables 1,2). Relative changes in the regional distribution of MAO activity on REM sleep deprivation, as compared to that of the FMC taken as 100%, are shown in Table 5.

Effect of rapid eye movement sleep deprivation on regional monoamine oxidase-A activity

After four days of REM sleep deprivation there was a significant decrease in MAO-A activity in the brainstem ($F_{(4,59)} = 5.25$; $P < 0.01$; Scheffe's *post hoc* test $\alpha = 0.05$), cerebellum ($F_{(4,59)} = 5.25$; $P < 0.01$; Scheffe's *post hoc* test $\alpha = 0.05$), and cerebrum ($F_{(4,59)} = 25.98$; $P < 0.001$; Scheffe's *post hoc* test $\alpha = 0.01$) compared to FMC animals. The cerebrum showed the maximum reduction in enzyme activity. The decreased MAO-A activity returned to the baseline after recovery (Table 3). The regional enzyme activities in LPC, RM, and FMC rats were comparable. MAO-A activity was not affected by two days of REM sleep deprivation. Changes in enzyme activity after REM sleep deprivation, compared to FMC taken as 100%, are shown in Table 5.

Effect of rapid eye movement sleep deprivation on regional monoamine oxidase-B activity

Neither two nor four days of REM sleep deprivation produced a significant change in MAO-B activity in the cerebrum, cerebellum, or whole brain stem. The activity of MAO-B in LPC, recovered, RM, and FMC was comparable (Table 3).

Since certain regions in the brain stem are reported to be involved in REM generation, [36,37] the effect of short-term REM deprivation on the activity of MAO, MAO-A, and MAO-B was studied in the medulla, pons, and midbrain.

Effect of rapid eye movement sleep deprivation on monoamine oxidase activity in brainstem regions

One day of REM sleep deprivation did not lead to any significant change in MAO activity in the medulla, pons, or midbrain (Table 2).

Table 3. MAO-A and MAO-B activities (μmol/90 mn per mg protein) in different regions in control and experimental rat brain

Groups	Specific activity of MAO-A			Specific activity of MAO-B		
	Cerebrum	Cerebellum	Brainstem	Cerebrum	Cerebellum	Brainstem
FMC	0.189 ± 0.035 (16)	0.176 ± 0.036 (16)	0.154 ± 0.029 (16)	0.195 ± 0.036 (16)	0.190 ± 0.032 (16)	0.167 ± 0.015 (16)
E2	0.179 ± 0.002 (6)	0.176 ± 0.013 (6)	0.170 ± 0.015 (6)	0.181 ± 0.013 (6)	0.190 ± 0.004 (6)	0.174 ± 0.006 (6)
E4	0.112 ± 0.024** (14)	0.140 ± 0.018* (14)	0.119 ± 0.029* (14)	0.177 ± 0.031 (14)	0.175 ± 0.023 (14)	0.155 ± 0.036 (14)
R	0.176 ± 0.009 (14)	0.180 ± 0.024 (14)	0.148 ± 0.022 (14)	0.172 ± 0.015 (14)	0.174 ± 0.019 (14)	0.158 ± 0.016 (14)
LPC	0.173 ± 0.014 (14)	0.155 ± 0.030 (14)	0.158 ± 0.031 (14)	0.206 ± 0.023 (14)	0.181 ± 0.032 (14)	0.175 ± 0.036 (14)
RM	0.169 ± 0.013 (6)	0.174 ± 0.013 (6)	0.163 ± 0.016 (6)	0.211 ± 0.014 (6)	0.194 ± 0.014 (8)	0.177 ± 0.023 (6)

No. of animals is shown in parentheses under respective group; *$\alpha = 0.05$; **$\alpha = 0.01$.

Table 4. MAO-A and MAO-B activities (μmol/90 min per mg protein) in different regions in control and experimental rat brainstem

Groups	Specific activity of MAO-A			Specific activity of MAO-B		
	Medulla	Pons	Midbrain	Medulla	Pons	Midbrain
FMC	0.008 ± 0.003 (12)	0.009 ± 0.003 (12)	0.016 ± 0.005 (12)	0.020 ± 0.006 (12)	0.020 ± 0.003 (12)	0.025 ± 0.003 (12)
E1	0.016 ± 0.002** (12)	0.008 ± 0.002 (12)	0.018 ± 0.001 (12)	0.021 ± 0.002 (12)	0.022 ± 0.001 (12)	0.028 ± 0.002 (12)
E2	0.011 ± 0.004* (8)	0.009 ± 0.003 (8)	0.015 ± 0.003 (8)	0.018 ± 0.002 (8)	0.012 ± 0.001** (8)	0.022 ± 0.001 (8)
R	0.009 ± 0.004 (8)	0.009 ± 0.004 (8)	0.016 ± 0.005 (8)	0.019 ± 0.002 (8)	0.018 ± 0.003 (8)	0.024 ± 0.002 (8)
LPC	0.008 ± 0.003 (12)	0.011 ± 0.003 (12)	0.016 ± 0.004 (12)	0.019 ± 0.003 (12)	0.018 ± 0.006 (12)	0.023 ± 0.004 (12)

No. of animals is shown in parentheses under respective group; * $\alpha = 0.05$; ** $\alpha = 0.01$.

However, after two days of REM sleep deprivation MAO activity was reduced significantly (Table 2) in the pons ($F_{(3,36)} = 8.39$; $P < 0.001$; Scheffe's *post hoc* test $\alpha = 0.01$), while there was no change in the medulla or midbrain. The altered activity returned to the baseline after the recovery. The enzyme activity in LPC rats was not changed. The enzyme activity after REM sleep deprivation, expressed as percent change compared to that of FMC taken as 100%, is shown in Table 5.

Effect of rapid eye movement sleep deprivation on monoamine oxidase-A activity in brainstem regions

After one day of REM sleep deprivation the MAO-A activity increased in the medulla ($F_{(3,40)} = 41.07$; $P < 0.001$; Scheffe's *post hoc* test $\alpha = 0.01$), whereas it did not change significantly in the pons, or midbrain (Table 4).

Two days of REM sleep deprivation significantly increased MAO-A activity in the medulla ($F_{(3,36)} = 4.81$; $P < 0.01$; Scheffe's *post hoc* test $\alpha = 0.05$), compared to FMC animals. However, the activity was insignificantly reduced (Table 4) compared to one day REM sleep deprived rats. The altered activity returned to the baseline after recovery. The enzyme activity was normal in LPC rats. There was no significant change in the pons or midbrain in any group of animals. Changes in the enzyme activity after REM sleep deprivation, compared to FMC taken as 100%, are shown in Table 5.

Effect of rapid eye movement sleep deprivation on monoamine oxidase-B activity in brainstem regions

After one day of REM sleep deprivation there was no significant change in the enzyme activity in any brainstem region (Table 4). However, after two days of REM sleep deprivation there was a significant decrease in MAO-B activity in the pons ($F_{(3,36)} = 7.88$; $P < 0.01$; Scheffe's *post hoc* test $\alpha = 0.01$), while there was an insignificant decrease in the medulla and midbrain compared to FMC animals (Table 4). The altered activity returned to the baseline after recovery. The enzyme activity was not affected in control rats. The distribution of the enzyme activity in brain-

Table 5. Regional relative distribution of MAO, MAO-A and MAO-B activities in the control and experimental rat brains compared to FMC group taken as 100%

	Groups	Cerebrum	Cerebellum	Brainstem	Medulla	Pons	Midbrain
MAO	E1	—	—	—	122.22	90.00	95.24
	E2	90.44	91.85	97.06	111.11	70.00	92.10
	E4	75.93	85.11	84.97	—	—	—
	R	89.66	96.07	95.10	103.70	76.67	92.86
	LPC	92.25	93.54	98.70	111.11	99.07	95.24
	RM	105.20	98.31	95.50	—	—	—
MAO-A	E1	—	—	—	200.00	88.89	112.50
	E2	94.71	100.00	110.39	137.50	100.00	93.75
	E4	59.26	79.54	77.27	—	—	—
	R	93.12	102.27	96.10	112.50	100.00	100.00
	LPC	91.53	88.07	102.60	100.00	122.22	100.00
	RM	89.42	98.86	105.80	—	—	—
MAO-B	E1	—	—	—	105.00	110.00	112.00
	E2	92.82	100.00	104.19	90.00	60.00	88.00
	E4	90.77	92.11	92.81	—	—	—
	R	88.21	94.61	94.61	95.00	90.00	96.00
	LPC	105.64	95.26	104.47	95.00	90.00	92.00
	RM	108.21	102.10	105.99	—	—	—

Abbreviations mentioned in the text. — indicates experiments not done.

stem regions after REM sleep deprivation, compared to FMC taken as 100%, is shown in Table 5.

DISCUSSION

Although results of this study showed that four days of REM sleep deprivation caused a decrease in rat brain MAO and MAO-A activities, MAO-B activity did not change significantly. However, MAO-B was affected by two days of REM sleep deprivation. In the majority of instances, the largest changes were seen in MAO-A activity. After short-term (one and two days) deprivation the enzyme activity was not affected in the midbrain. The medulla was the first site to be affected where MAO-A activity increased even after 24 h of REM sleep deprivation, and pons was the only area/site where the MAO-B activity showed a decrease, but only after two days of deprivation. The change in the enzyme activity was proportional to the number of days of deprivation. However, whenever the medulla was affected it showed an increase while pons, if affected, showed a decrease. Whenever MAO-B activity was affected it showed a decrease. The enzyme activities did not change in the control rats and the deprivation-induced alteration in the enzyme activities returned to the baseline after recovery.

The changes in the enzyme activities were unlikely due to restriction of movement or stress, if any, caused by the experimental set up. To rule out the possibilities of the nonspecific effects in causing the decrease, in addition to the FMC and recovered experiments, LPC and RM experiments were carried out. The LPC rats served as control for environmental nonspecific factors while the RM rats served as control for the immobilization stress, if any, involved due to restriction of movement to the E animals. Movement was restricted for as long as a fortnight. Neither the LPC nor the RM rats showed statistically significant alteration in the enzyme activities. Recently, it has been proposed[35] that REM sleep may be a response to stress. Thus, the effect observed in this study might be due to REM pressure built up in the system in response to REM sleep deprivation. It is unlikely that the observed effects are due to stress induced by REM sleep deprivation since immobilization stress, which is reported to cause a decrease in the activity of MAO, [1] was ineffective in this study. However, long-duration immobilization has also been reported to cause habituation[35] and, therefore, the effects observed in this study are likely to be the result of REM deprivation and not stress. How REM sleep deprivation was best achieved in this study must also be examined. The flowerpot method, widely used[11,48] and probably the ideal method for REM deprivation,[32,47,48] worked well in this study. The method has been reported to cause an insignificant reduction in non-REM sleep duration, [29,47] and hence it is unlikely that the observed effects were due to the small reduction in sleep other than REM. Though similar reduction in non-REM sleep has been reported on LPC as well,[29,47] the enzyme activities did not change significantly in that group of rats in this study. We used this method earlier for REM sleep deprivation in cats and rats.[25,41] The sizes of the platforms for the weight group of the rats used in this study were chosen based on earlier reports.[29,51] The effects of REM sleep deprivation observed in this study correspond to earlier reports.[26,41]

The significant decrease in MAO activity in rat brain on REM sleep deprivation may be explained and supported by earlier studies. MAO activity in the rat brain may be directly decreased by REM sleep deprivation or the effect may be mediated through the changes in the release or levels of NE,[14,45] which are reported to be affected by REM sleep deprivation. Though it is tempting to say that MAO activity may be altered in parallel with NE release, there are reports suggesting that the activity of MAO is unlikely to be related to the release of NE.[6,42] The deprivation might cause an increase in endogenous MAO inhibitor, namely tribulin (or alike), which is known to increase with stress.[1,2] Though the mechanism of decrease is yet to be investigated, an earlier report found that MAO inhibitors and monoamine uptake blockers, which are likely to increase the level of NE, are known to decrease REM sleep.[49] This study found that MAO activity decreased in brain areas containing relatively more NE-ergic neuronal projections. The NE-ergic neurons are located in the pontine region, with a maximum concentration in the rat locus coeruleus, [16] and the cerebrum/cortex receives a bulk of its projections.[5,46] The pontine NE-ergic neurons (REM-OFF) are also affected by REM sleep deprivation.[25] Therefore, the MAO activity in the cerebrum and the pons is probably maximally affected. Though the MAO activity in the brainstem decreased, it was just short of being statistically significant. An increase in the MAO-A activity in the medulla and a decrease in the MAO-B activity in the pons, after short-term REM deprivation probably did not allow a statistically significant change in the brainstem MAO activity. On short-term deprivation, the MAO activity showed a statistically nonsignificant increase in the medulla even after 24 h of REM sleep deprivation.

The alterations in MAO-A and MAO-B activities on REM deprivation are interesting. The stronger influence (a decrease) of REM on MAO-A supports the involvement of norepinephrinergic mechanism in REM sleep.[12,18,19,36] It is difficult to determine if the deprivation-induced alteration in the MAO-A activity is a primary effect, which ultimately affects the level of NE or is secondary to changes in the turnover of NE.[40] The MAO-A activity is known to be affected by small peptides and hormonal levels.[42,43,52] Since REM deprivation is known to affect hormonal levels[23] and is likely to affect the levels of sleep substances,[15,31] the changes in the MAO-A activity may be a secondary effect.

The decrease in MAO-B activity reflects the probable effect of REM sleep deprivation on the system's biogenic amines in general.[6,42] Earlier reports agree that the MAO-B activity increases in Alzheimer's disease[33] where the REM increases.[3] It has been found that the MAO-A and MAO-B are affected differentially. Short-term REM deprivation influenced either the MAO-A or the MAO-B activity within the different regions of the brainstem (midbrain, pons, and medulla). This may possibly be due to local interaction among different neurotransmitters and small peptides, namely somatostatin, VIP, substance P, etc., [18] which have been shown to co-exist in the brainstem neurons[13] and to influence REM sleep.[15,31]

Short-term (one and two days) REM deprivation affected the medulla first; MAO-A activity showed a tremendous increase which was reduced and reversed on longer deprivation. Similar short-term deprivation (24 h) has been reported to increase the AChE activity in the medulla only.[26] These results suggest that the integration of the aminergic and cholinergic mechanisms for the generation of REM sleep[12] may be taking place somewhere in the medullary region of the brain stem and short interneurons containing low-molecular weight peptides may possibly be involved in the modulation of such a process. The medullary REM-ON neurons[20,36] might be involved in mediating and/or initiating such a response. Normally, cessation of firing of the NE-ergic REM-OFF neurons during REM sleep probably upregulates and/or resensitizes the NE receptors.[38] Reduction of firing of those REM-OFF neurons is probably a step towards compensatory mechanism.[25] Since the REM-OFF neurons do not stop firing on REM deprivation, it is likely to cause a continuous release of NE. Increased MAO-A activity in the medulla may attempt to reduce the NE level to move towards a normal situation. However, when that fails due to continued deprivation, the activities of MAO-A as well as MAO-B decrease throughout the brain, though the former is affected more. Alternatively, the changes seen in the MAO activity may reflect a different time-course of NE release by the REM-OFF NE-ergic neurons in relation to REM sleep deprivation.

CONCLUSIONS

The findings of this study support the involvement of aminergic mechanism in REM sleep regulation[12] and suggest that pontomedullary region is probably the site for integration of REM sleep. An initial increase in the enzyme activity in the medulla is likely to be a compensatory physiological response which fails on longer deprivation. However, any differential effect of deprivation on synaptosomal and extra-synaptosomal MAO-A and MAO-B activities remains to be determined.

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