Annexure-IX
PROJECT COMPLETION REPORT

Title of the project:

To alleviate cognitive deficits in the offspring induced by sleep loss during pregnancy by administering alpha-asarone: A study in an animal model

File No: DST No: SR/CSRI/102/2014

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(No of copies: TEN)

1. Title of the project: **To alleviate cognitive deficits in the offspring induced by sleep loss during pregnancy by administering alpha-asarone: A study in an animal model**

2. Principal Investigator(s) and Co-Investigator(s):

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3. Implementing Institution(s) and other collaborating Institution(s):

Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum

4. Date of commencement: **26.08.2015**

5. Planned date of completion: **25.08.2018**

6. Actual date of completion: **27.08.2019**

7. Objectives as stated in the project proposal:

   1. To evaluate the effects of daily administration of alpha-asarone (10 mg/kg body wt) during normal pregnancy on cognition and emotional behavior in offspring and maternal anxiety profiles.
   2. To investigate the effect of alpha-asarone (10 mg/kg body wt) in sleep deprived pregnant rats on maternal anxiety profiles and mother-pup bonding.
   3. To quantify the effects of administration of alpha-asarone (10 mg/kg body wt) in sleep deprived dams on offspring's emotional changes through ultrasonic vocalizations, elevated plus maze, and open field test during different stages of development.
   4. To evaluate the effects of alpha-asarone (10 mg/kg body wt) in sleep deprived dams on learning and memory in the offspring using radial arm maze.
   5. To investigate effect of alpha-asarone (10 mg/kg body wt) in sleep deprived pregnant rats on neuronal interaction in the offspring by measuring theta activity coherence from hippocampus, amygdala and prefrontal cortex.

8. Deviation made from original objectives if any, while implementing the project and reasons thereof: None

(# Project staff status: 24/11/2015 to 23/11/2016 (JRF, Neelima); 19/10/2017 to 27/06/2016 (JRF, Anjuprabha); 01/02/2019 to 27/08/2019 (JRF, Athira): Total 2.2 years

In the Group Monitoring Workshop held on 18 Aug 2017, request for one more project staff again re-appropriation of consumables was approved in meeting but was never materialized. This lead to problems in implementation of the project during later years. It takes almost 4-6 months to train new person in electrophysiological studies. Out of the approved 44 lakhs, we could use only 32 lakhs with battling with difficulties due to lack of timely and sufficient staff)

9. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs:

The study was carried out in Wistar rats bred and reared in the Animal Facility of the SCTIMST, Trivandrum. Female rats were implanted with electrodes (described below) for recording sleep-wakefulness (S-W). After post surgical recovery, S-W recorded for 24 hrs, on three days, was taken as control values. Following mating, pregnancy confirmed female rats were kept in separate cages and their body weights were monitored throughout the period of gestation. Animals were housed in a room having lights on from 6:00 to 18:00 hours (12:12 light: dark cycle), controlled temperature (26 ± 1ºC) with *ad libitum* supply of food and water. During pregnancy, S-W (24 hr) recordings in dams were taken on various gestational days (GD 2, 8, 15, 17 and 19). After parturition, recordings were taken on days 2, 3, 6, 10 and 18 (PD 2-18). Two post-weaning recordings (PW) were taken at an interval of one week (Fig.1. Setup for maternal sleep). Pups born these females (no treatment) served as control and their S-W were studied on 5 postnatal days. Maternal anxiety were assesses in elevated plus maze (EPM) test and pups emotional behaviour was tested through recording ultrasonic vocalizations (USV) in isolation paradigm.

Sleep deprivation was conducted during third trimester of pregnancy using two methods, deprivation of total component of sleep (TSD) by gentle handling and REM sleep deprivation by standard platform method. Effects of oral administration of alpha-asarone in pregnant rats undergoing total sleep deprivation of 5 h (TSDX5h) during 3rd trimester were studied taking doses 40, and 10mg/g b wt. Age matched sham control were given vehicle on similar days prior to sleep deprivation (another set of rats). Rats were sleep deprived for 5 h (from 9 am to 2 pm) in the TSD group by gentle handling during the gestational days 15-20. Whenever animal showed signs of sleepiness (assessed on the basis of changes in electrophysiological parameters), it was woken up by gently shaking the cage and disturbing the cage.

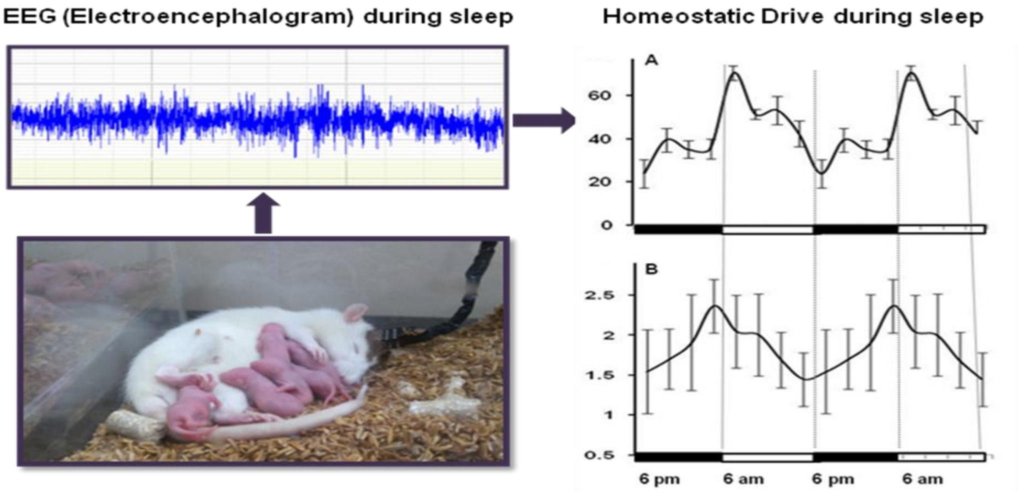
Effects of TSDX5h during 3rd trimester of pregnancy were tested on Ultrasonic vocalizations (USVs) of pups in isolation paradigm and S-W of pups from post natal days 1-20. Isolation paradigm consists of separating pups from mother and littermates for recording USVs for 2 minutes at 28 ºC. Maternal behaviour was monitored from postnatal days 1-7. Body weights of pups were noted everyday till weaning.

Some of the offspring born to these dams were tested for the S-W and anxiety, learning during middle age (when they reach age of 8 – 18 months).

Surgery for implantation of electrodes for recording sleep-wakefulness: For chronic implantation of EEG and EMG electrodes (to assess the state of vigilance), anesthetized adult female rats (250-280 gm) were fixed on the stereotaxic apparatus, (Ketamine (50 mg/kg b wt, i.m.) and Xylazine (5 mg/kg bwt, i.m.)). Rats were given 2 weeks for a complete recovery from surgical trauma. For deep brain recordings, micro-electrodes were implanted into pons, amygdala, prefonrtal cortex and hippocampus sterotaxically

In neonates, sleep was recorded using radio transmitters (F50-EEE) placed externally (Data Sciences International, USA), where the electrodes in the radio-transmitter were implanted under isoflurane anaesthesia for recording of EEG and EMG. After 2-3 hrs of recovery from anaesthesia, their sleep was recorded for 2-3 hrs, keeping them in humidified chamber at their thermoneutral temperature (34.5ºC for day 1-7; 33.5ºC for day 8-14; 32.5ºC for day 15-18; 30ºC for day 20). The ethical clearance from Institutional Animal Ethics Committee of Sree Chitra Tirunal Institute for Medical Sciences and Technology was obtained. All procedures were conducted in accordance with the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Fig.1 Maternal sleep recording postpartum, representative EEG and analyzed delta power for homeostatic drive



Changes in NREM sleep (A) and Delta power (B) during day and night in rat

Fig.2. Setup for recording ultrasonic vocalizations in pups

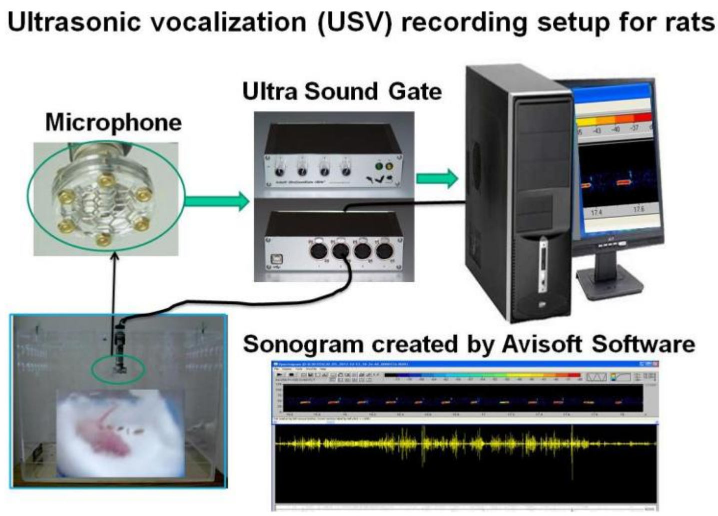


Fig. 3. Set-up for recording neonatal sleep

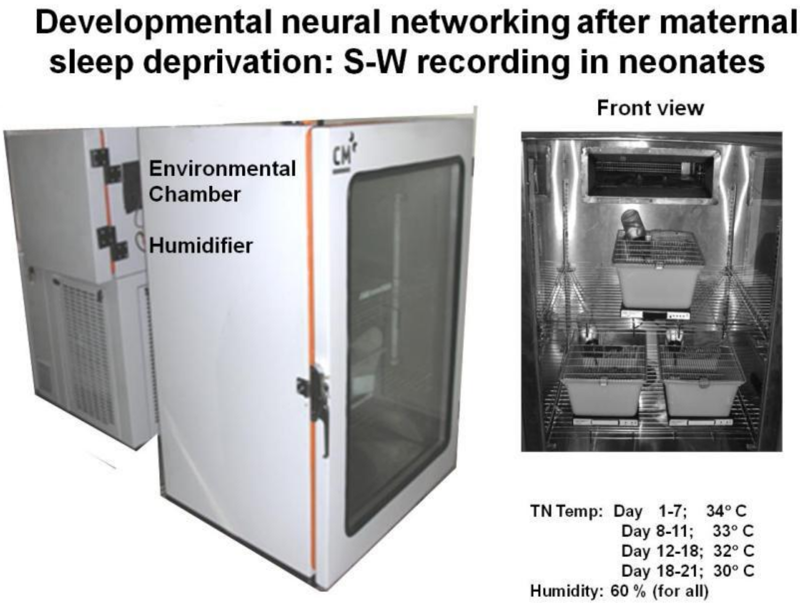
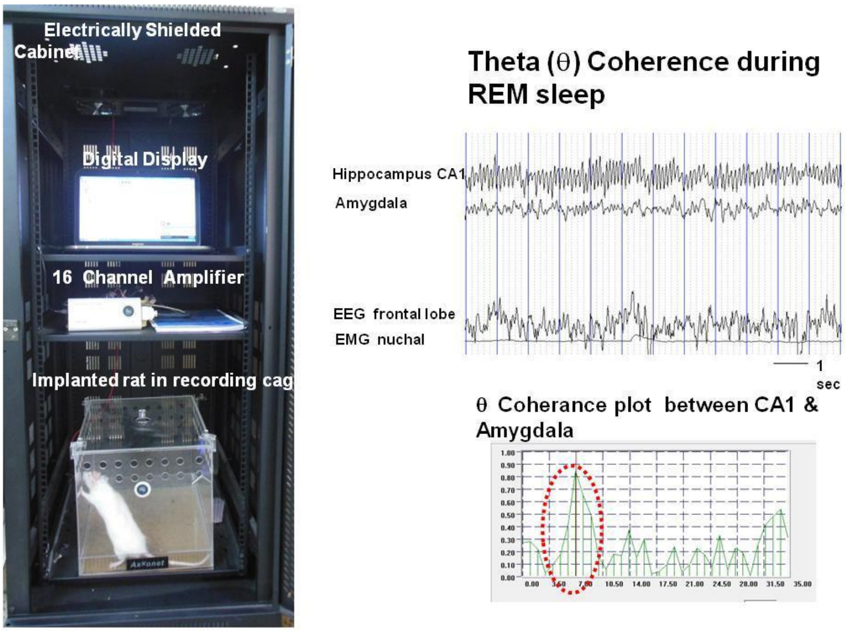


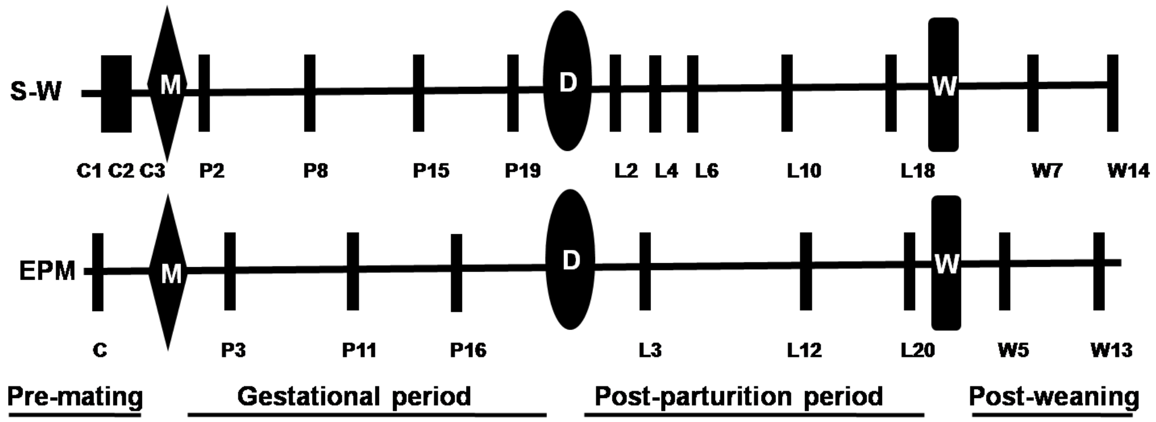
Figure 4 Experimental set up for the deep brain recordings and sleep (16 channel Brain Electrical Scanning system)



10. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:

We developed an animal model for studies on peri-partum sleep disorders, sleep patterns in female Wistar rats during pregnancy, post-partum and after weaning, and assessed associated adaptive changes in their anxiety as per protocol shown in Fig.5. During pregnancy, sleep increased primarily due to increase in light non-REM sleep during dark period (Fig. 6). There was an increase in non-REM sleep delta power after parturition, though the sleep was fragmented, especially during daytime (Fig.7, 8, 9). Simultaneous behavioural recording showed increased anxiety during third trimester of pregnancy and gradual reversal of it after parturition. This is the first report where diurnal and nocturnal variations in S-W and delta power, along with adaptive changes in anxiety, were studied before, during and after pregnancy. This study also provides an animal model for drug trials and studies on sleep disorders during peri-partum window.

Fig.5. Experimental protocol for recording S-W and anxiety before mating, during pregnancy, post-partum and post-weaning



Sleep-wakefulness (S-W) for 24 h (depicted by bars) were recorded during pre-mating (C1, C2, C3); after mating (M) on gestational days 2, 8, 15 and 19; after parturition (D) on days 2, 4, 6, 10, 18; and after weaning (W) on days 7 and 14. The testing in the elevated plus maze (EPM) was performed before mating (C), during gestational period (days 3, 11, and 16), post parturition period (days 4, 12 and 20) and post-weaning (days 5 and 13).

Fig.6. Changes in total sleep time (TST) over day and night during pregnancy and after parturition in rats

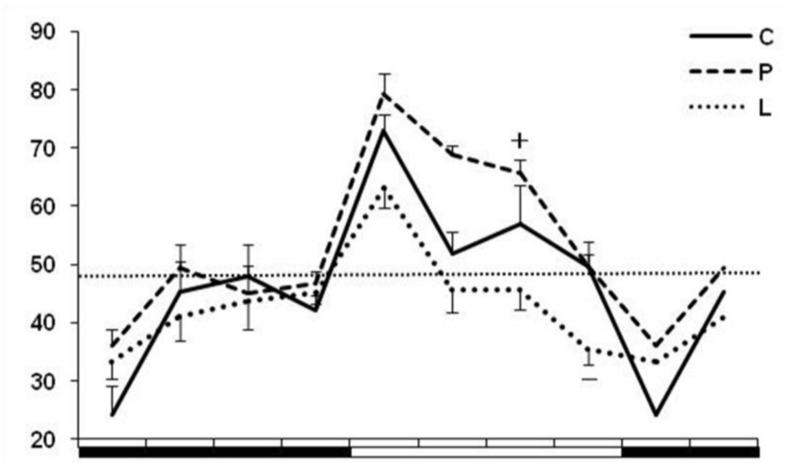


Fig. shows 3 hourly bins across night and day on the horizontal axis and total sleep time in % is shown on the vertical axis. C (black line) refers to the mean of three control values (pre-mating), and P (orange line) denotes average of all the gestational days and L (green line) refers to average of all post-parturition days. + and - symbols denotes increased and decreased significance respectively in comparison to C in each time bin. The data is represented as Mean ± SEM. The level of significance is +/- $p<0.01$.

Fig.7. Pattern of NREM sleep (A) and delta power (B) over day and night during pregnancy and after parturition in rats

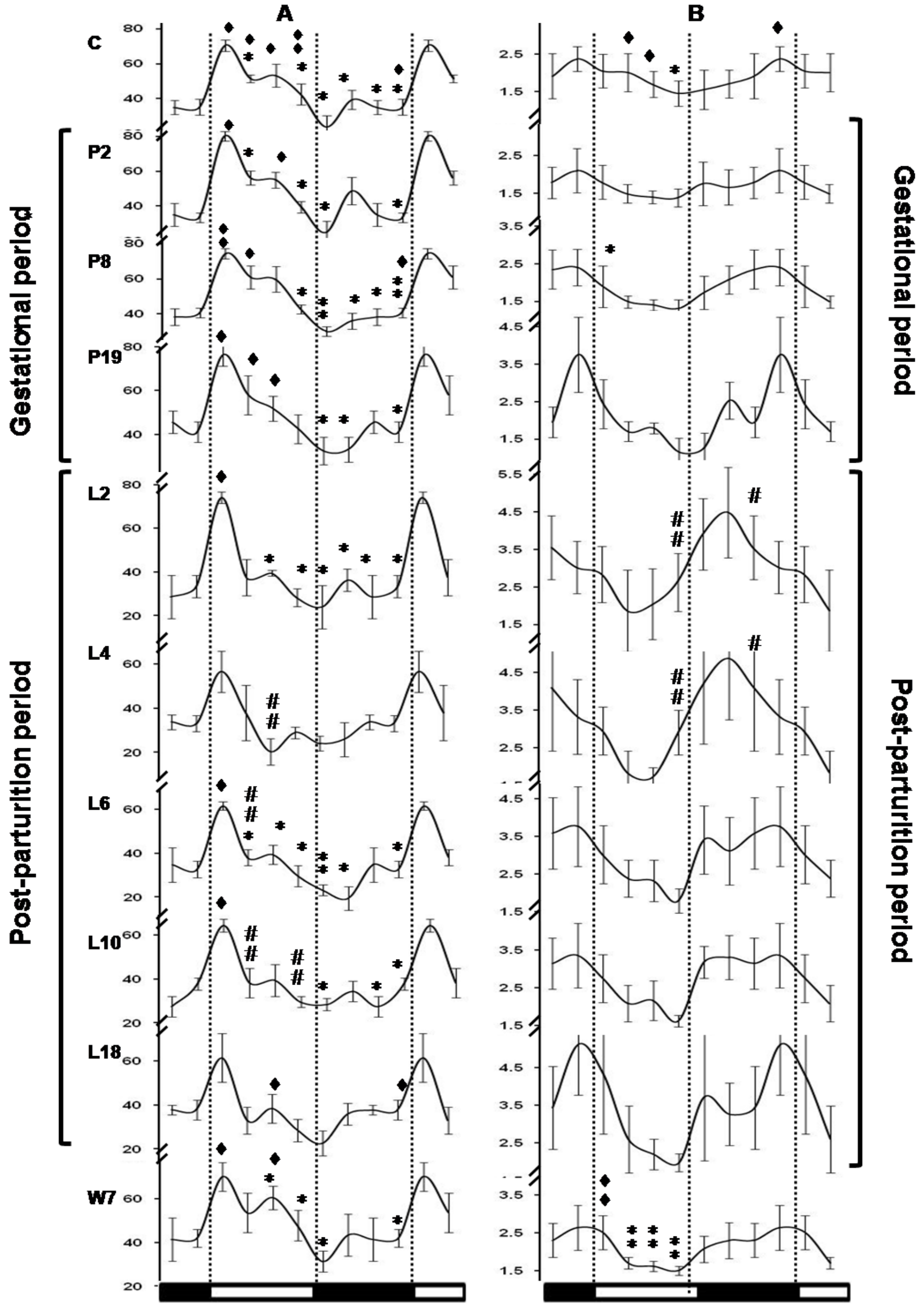
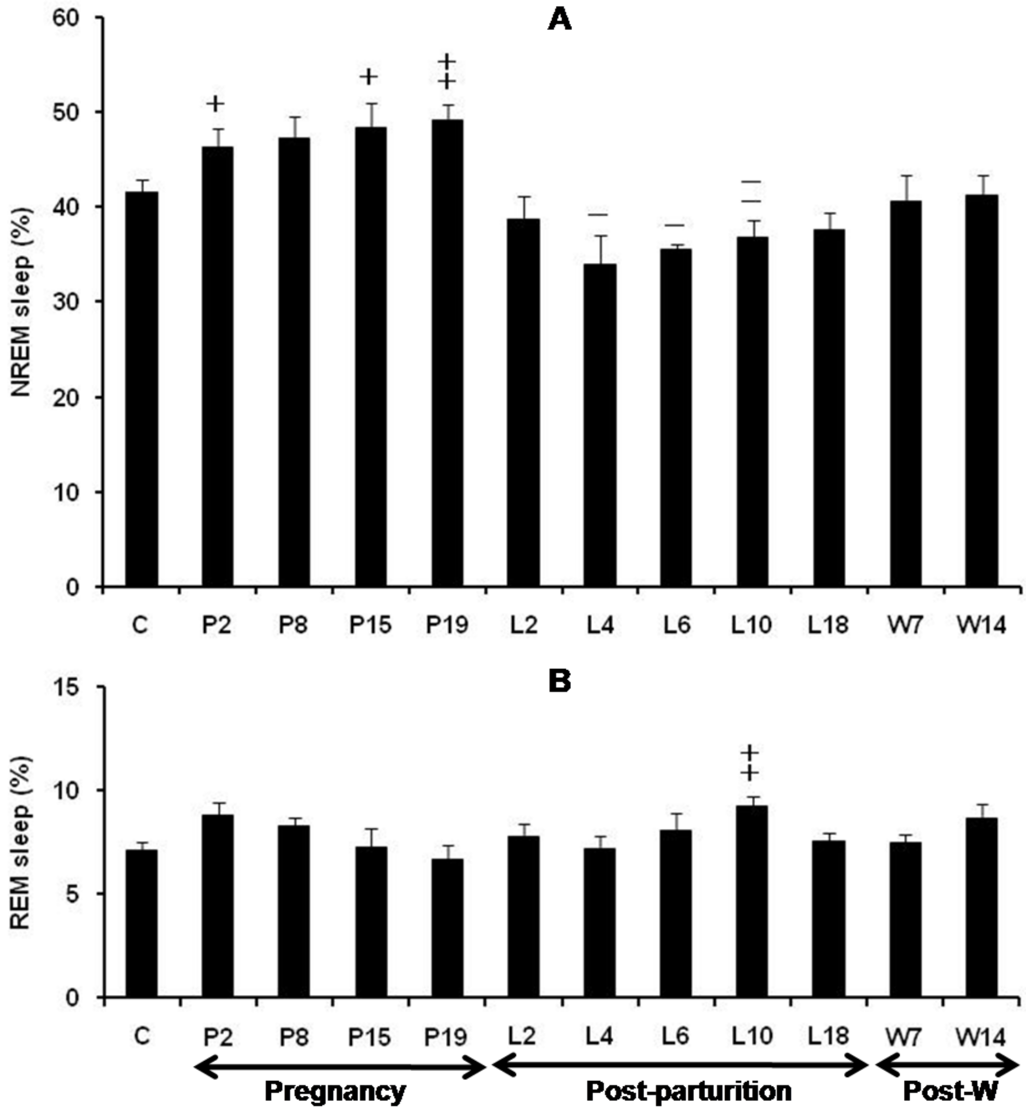


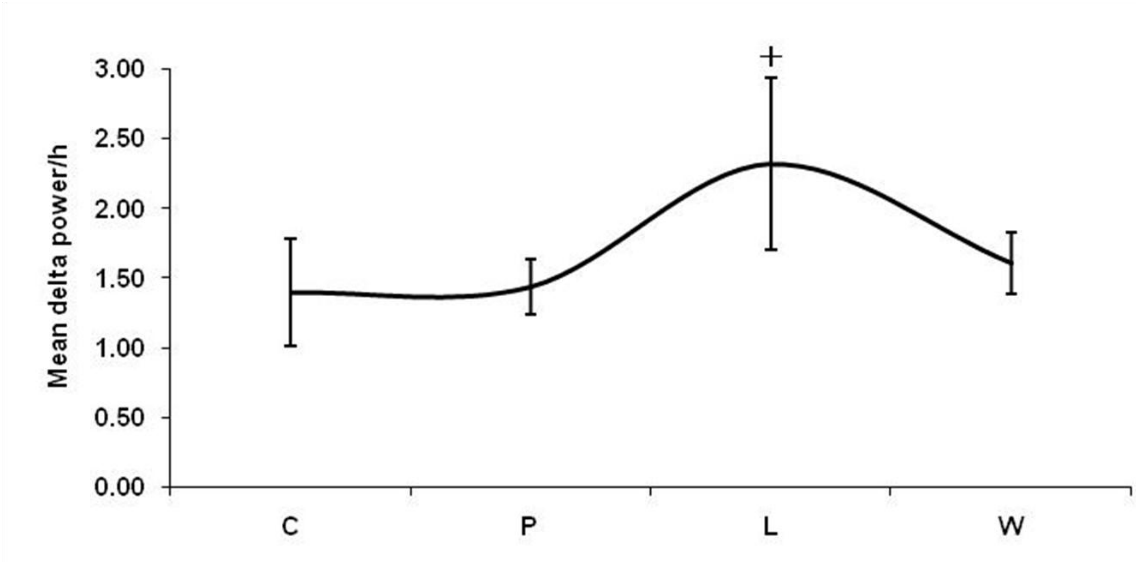
Fig. 7A shows NREM sleep (%) in 3 hourly bins across day and night for control (C), gestational day 1 (P1), 8 (P8), 19 (P19), post-delivery days 2 (L2), 4 (L4), 6 (L 6), 10 (L10), 18 (L18) and post-weaning day 7 (W7). In horizontal axis, the dark bar denotes dark period beginning at 6 pm and light bar shows light period beginning at 6 am. In Fig. 7B, normalized delta power is shown for respective bins. * symbol denotes significance on comparison of values with peak, ♦ with lowest values (trough); # denotes comparison of values on different days with C in the respective bins (C compared with P, L, and W days). The level of significance is *,♦, # $p<0.01$ and **,♦♦, ## $p<0.001$. The data is from four rats.

Fig.8. Percentage of NREM and REM sleep (24 h) during various days of pregnancy and post-partum



In horizontal axis, C refers to the mean of three control values (pre-mating), P denotes gestational days, L denotes post-parturition days and W denotes post-weaning days. Changes in NREM sleep are shown in A whereas the changes in REM sleep are shown in B. The data is represented as Mean ± SEM. + and - symbol denotes increase and decrease in significance respectively on comparison of C with P, L, W days; The level of significance is +,- p<0.01 and ++,-- p<0.001.

Fig. 9. Values of the averaged 'area under curve' (AUC) for NREM delta power during control, gestation, after parturition and after weaning



In vertical axis, AUC (the averaged NREM delta power/h) is shown during control, gestation (P), after parturition (L) and after weaning (W). The data is represented as Mean ± SEM. The level of significance is + $p<0.01$.

Conclusion:

This study provided deep insight into the changes in the architecture of non-REM sleep that primarily takes place during pregnancy and lactation. Increased delta power during the period of lactation, compensates for the decrease in light non-REM sleep and fragmentation of sleep. Increased anxiety during third trimester of pregnancy and gradual reversal of it after parturition are biological necessities that may not contribute towards the sleep alteration. This is the first in-depth study of S-W along with assessment of anxiety through pregnancy-post-partum continuum in rat which is an altricial species. This study provides a baseline for the sleep profile of the rats in the peri-natal period, which is similar to the changes during the periods of pregnancy, gestation and lactation in a woman. With this rat model, we can better study sleep disorders during the peri-natal period in humans.

Details are provided in this manuscript:

Sivadas N, Radhakrishnan A, Aswathy BS, Kumar VM, Gulia KK (2017). Dynamic changes in sleep pattern during post-partum in normal pregnancy in rat model. *Behav Brain Res.* 320: 264-274. (Impact Factor: 3.002)

Effects of REM sleep deprivation in pregnant dams on neonatal brain

Sleep loss during pregnancy is an emerging concern that affects the development of the growing fetus and babies. After parturition, the sleep-wakefulness (S-W) in neonates undergoes age-specific changes during the postnatal days (PNDs) which are indicative of developmental milestone. In this study the effects of deprivation of rapid eye movement (REM) sleep during the third term of pregnancy on the sleep-wake profiles of neonates in the Wistar rat model were evaluated. Sleep-wake patterns were assessed through

electrophysiological measures and behavioral observations, during postnatal days 1 to 21, on pups born to REM sleep deprived dams and control rats.
The gestational period were comparable in both the control (22.3±0.5 days) and the REMSD groups (22.7±0.5 days). The various parameters of control group pups showed the normal developmental profile during the PNDs. On postnatal developmental point D1, EEG amplitude was very low (10-20 μvolts, discontinuous at times) throughout the recording when pups were either into W, AS or QS. However, EMG changes were stage-specific. Even though the amplitude of EEG increased to 20-30 μvolts on D5, it remained undifferentiated. Same was the case during D9 when the EEG amplitude was 20-100 μvolts, with intermittent appearances of delta waves. Though EEG was also recorded on D1-D9, EMG was the primarily signal used for staging the S-W during this period. During D15 and 20, stage specific changes in EEG were evident and the amplitude also increased to 150 μvolts on D20.

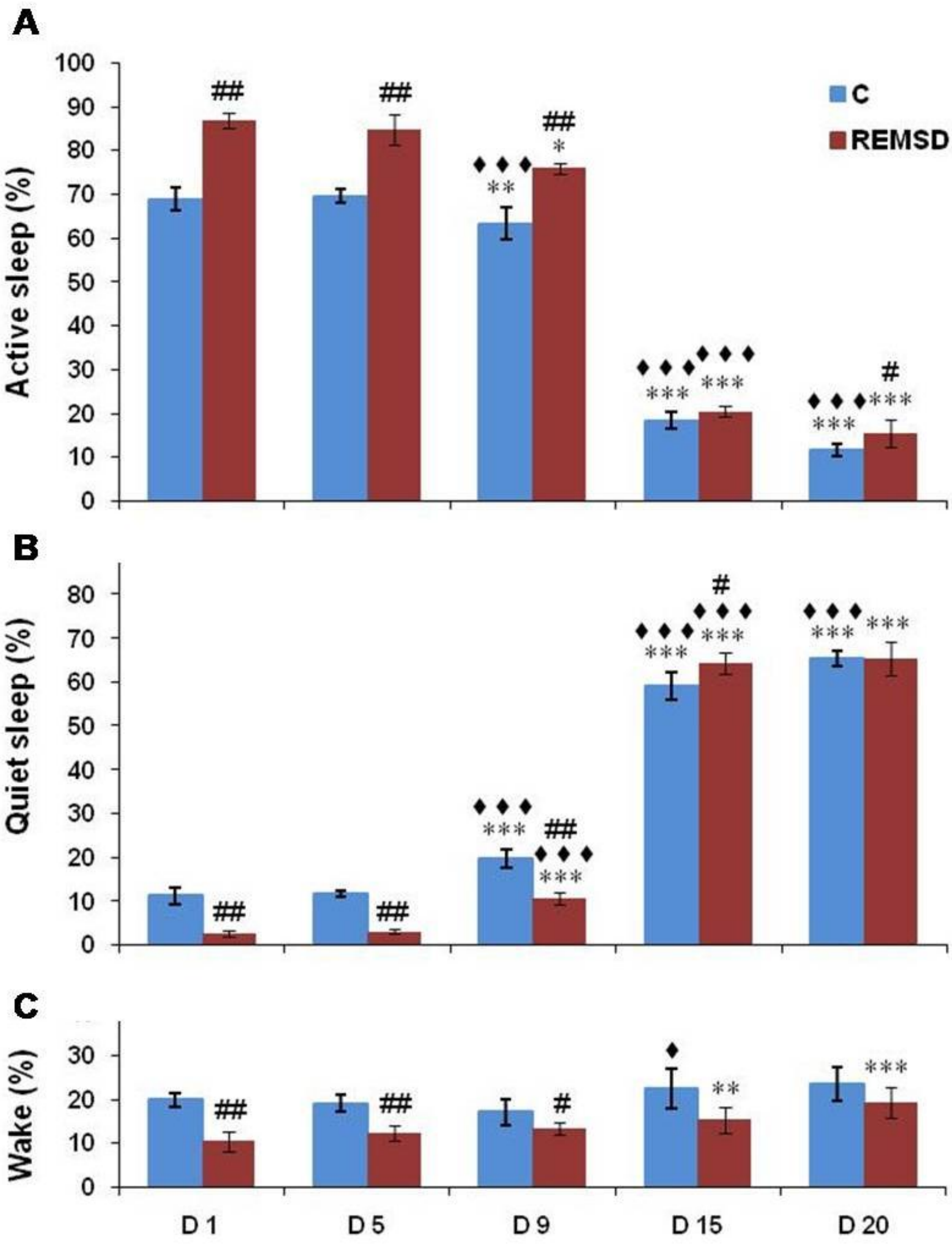
The percentage of AS was high whereas QS and W were low on D1 and D5 in the control group neonates (Fig. 10 A, B, C). The AS started decreasing on D9, and the decrease was marked thereafter on D15 and D20 (Fig. 10A). The QS increased significantly on D9 in comparison to D1 and D5, and an intensified build-up was observed on D15 and 20 (Fig. 10B). W percentage was low during D1 to D9, and increased only on D15 (Fig. 10C).

The frequency of AS stage was high on D1 (63.89±10.76), but decreased significantly from D9 (37.3±2.8) until D20 (7.6±1.9). The QS frequency, which was 58.6±10.3 on D1, decreased (26.6±2.1) on D15 (Fig. 11A, B). Wake frequency followed the same pattern as QS (Fig 11C). The duration of AS bouts on D1 was high (38.9±5.2), whereas durations of QS (10.1±1.8) and W (10.8±2.1) bouts were low (Fig. 12A). The bout durations of AS increased till D15 (78.6±2.7) and then decreased on D20 (62.2±2.6), whereas these durations of QS increased markedly after D15 (91.6±4.5). The bout duration of W (25.5±5.2) significantly increased after D15 (Fig. 12C).

Neonates in control group displayed polycyclic pattern of sleep immediately after birth, spending nearly 80.1±1.6 % of time in sleep with high frequency of S-W cycles (64.0±10.3) of short durations (44.7±3.2 s) on D1. This trend continued till D9. The frequency of the S-W cycles gradually decreased until D20, reducing to nearly half (23.1±2.0) in comparison to the value on D1 (Fig 13). However, durations of the S-W cycles increased substantially during D15 and D20. In comparison to control, the AS percentages in the pups from REMSD dams were higher during D1, D5, D9 and D20. The QS in these pups was significantly lower than the control pups during D1 to D9. However on D15, a higher percentage of QS was observed in these pups. The percentage of wake time in these pups was significantly lower on all the PNDs.

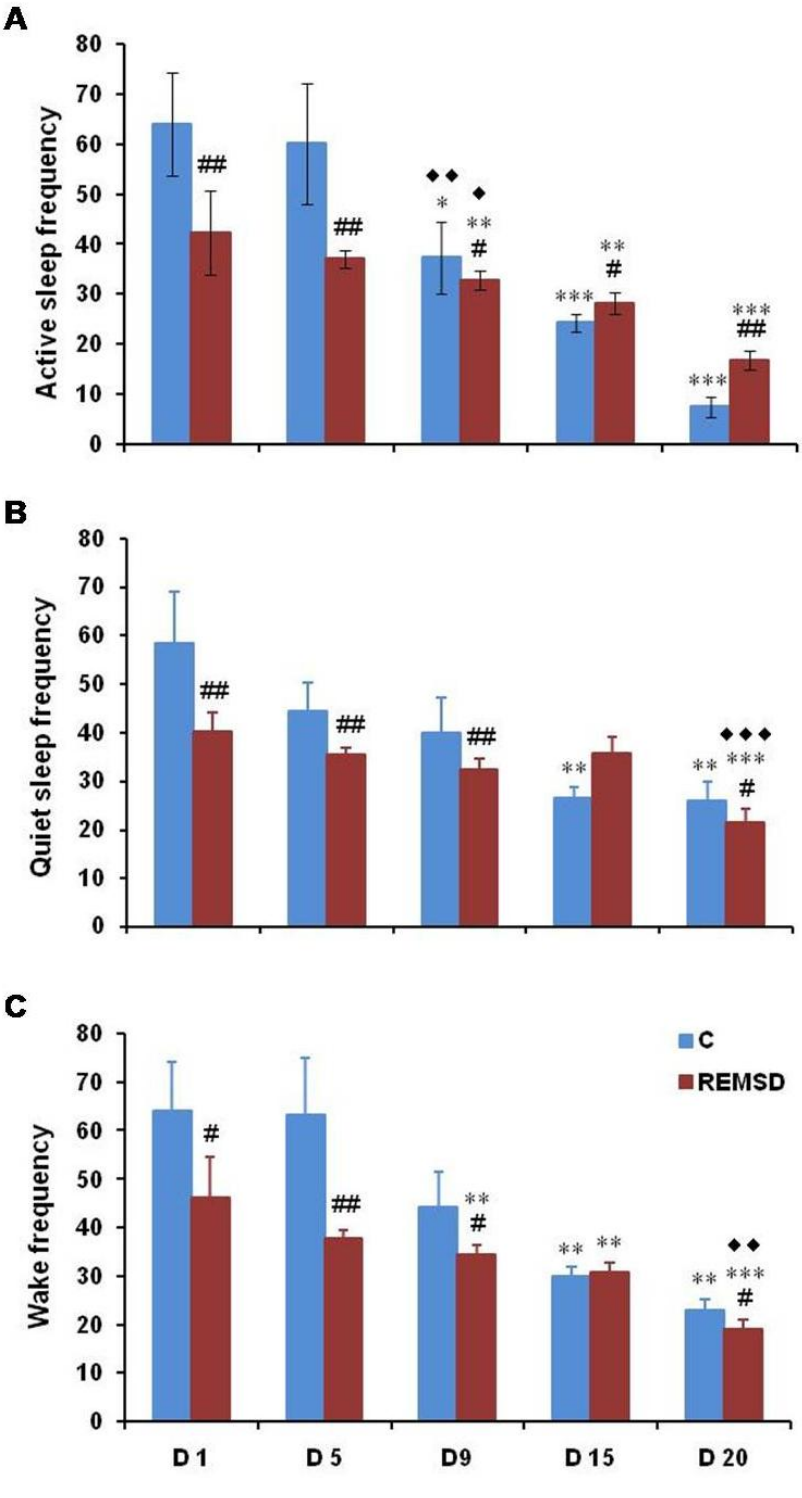
The bout frequencies of AS were lower on D1, D5, and D9 in comparison to control values (Fig. 11A). However on D15 and D20 a higher frequency of AS was observed. The frequencies of QS and W were lower than the control on D1, D5, D9 and D20 (Fig. 11B, C). Bout lengths of AS were longer in comparison to control on D1 to D9, but decreased on D15 and D20 (Fig. 12A). The lengths of the QS were similar during D1 to D9 in both groups but it became higher in the REMSD group on D15 and D20. The bout length of W was lower on D20 (Fig. 12C). Latency to REM sleep in pups born to REMSD dams was lower than the control group during D15 and D20 (Fig. 14). Moreover, durations of the S-W cycles were comparatively longer (Fig. 4) and their frequencies of S-W lower on all the PNDs.

Fig. 10. Changes in active sleep (A), quiet sleep (B) and wake (C) percentage in pups born to Control and REMSD dams on different postnatal days.



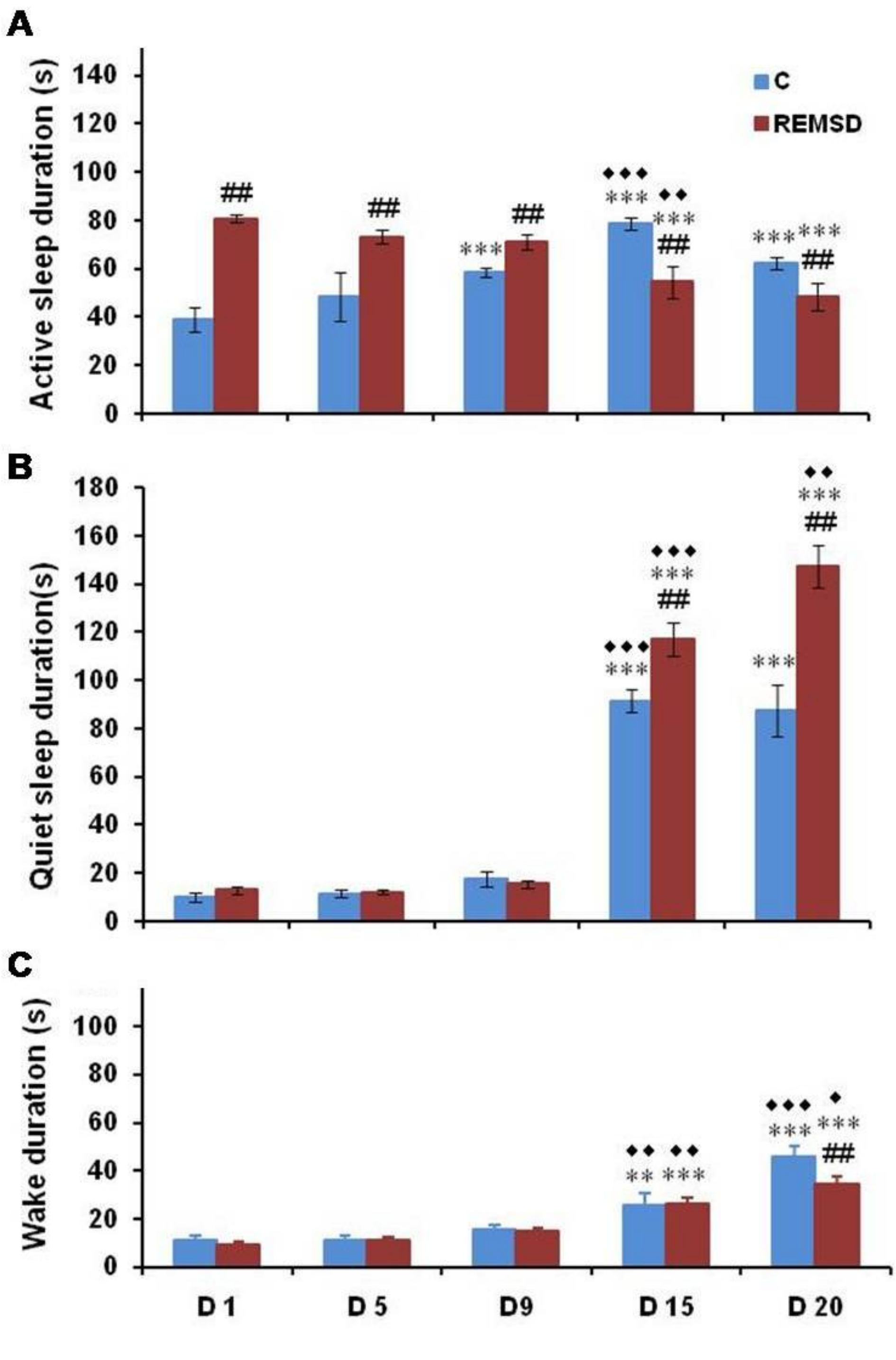
Y-axis represents averaged percentage of time spent/h in AS (A), QS (B) and wake (C) on different PNDs (X-axis) in control and REMSD group pups. Data is expressed as Mean±SD. The comparison of D1 values with other PNDs in each group is shown in *, the comparison of two subsequent D is depicted in ♦ and comparison between two groups is depicted in #. The level of significance is shown as *,♦,# $p\leq0.05$; **,♦♦,## $p\leq0.01$ and ***,♦♦♦ $p\leq0.001$.

Fig. 11. Frequencies of the episodes of active sleep (A) quiet sleep (B) and wake periods (C) in pups born to REMSD and Control dams on different postnatal days.



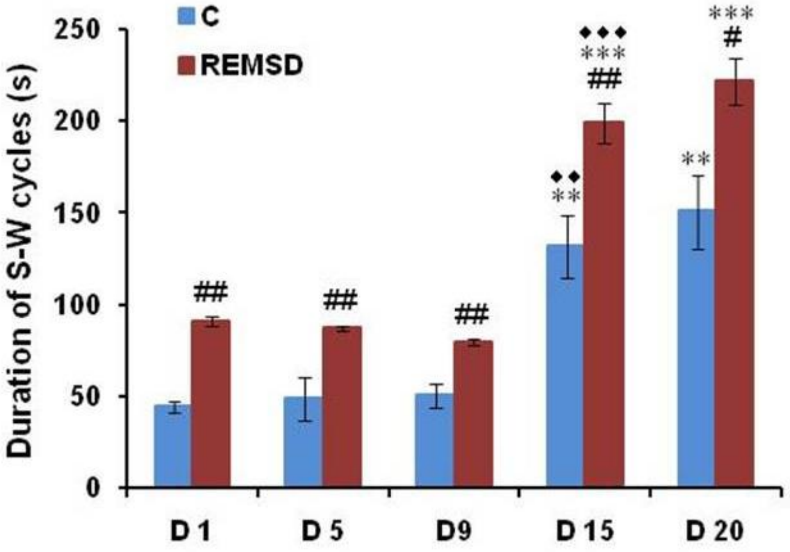
Y-axis represents averaged frequencies of the AS (A) quiet sleep (B) and wake periods (C) in REMSD and control on different PNDS (X-axis). Data is expressed as Mean±SD. The comparison of D1 values with other PNDs in each group is shown in *, the comparison of two subsequent D is depicted in ♦ and comparison between two groups is depicted in #. The level of significance is *,♦,# $p \leq 0.05$; **,♦♦,## $p \leq 0.01$ and ***,♦♦♦ $p \leq 0.001$.

Fig. 12. Bout durations of active sleep (A), quiet sleep (B) and wake (C) episodes in pups born to control and REMSD dams on different postnatal days.



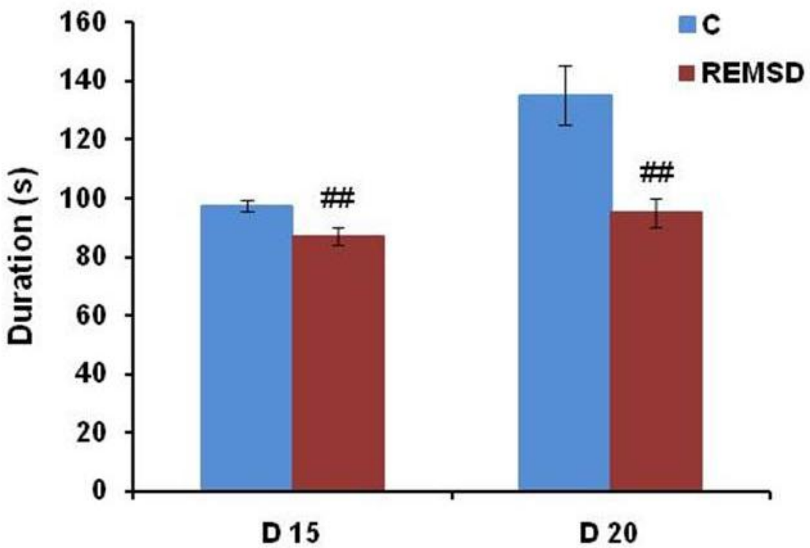
Y-axis represents averaged bout durations (s) of AS (A), QS (B) and wake in REMSD and control pups on different PNDS (X-axis). Data is expressed as Mean±SD. The comparison of D1 values with other PNDs in each group is shown in *, the comparison of two subsequent D is depicted in ♦ and comparison between two groups is depicted in #. The level of significance is *,♦ $p \le 0.05$; **,♦ ♦,## $p \le 0.01$ and ***,♦ ♦ ♦ $p \le 0.001$

Fig. 13. Changes in duration of S-W cycles in pups born to REMSD and control dams on different postnatal days.



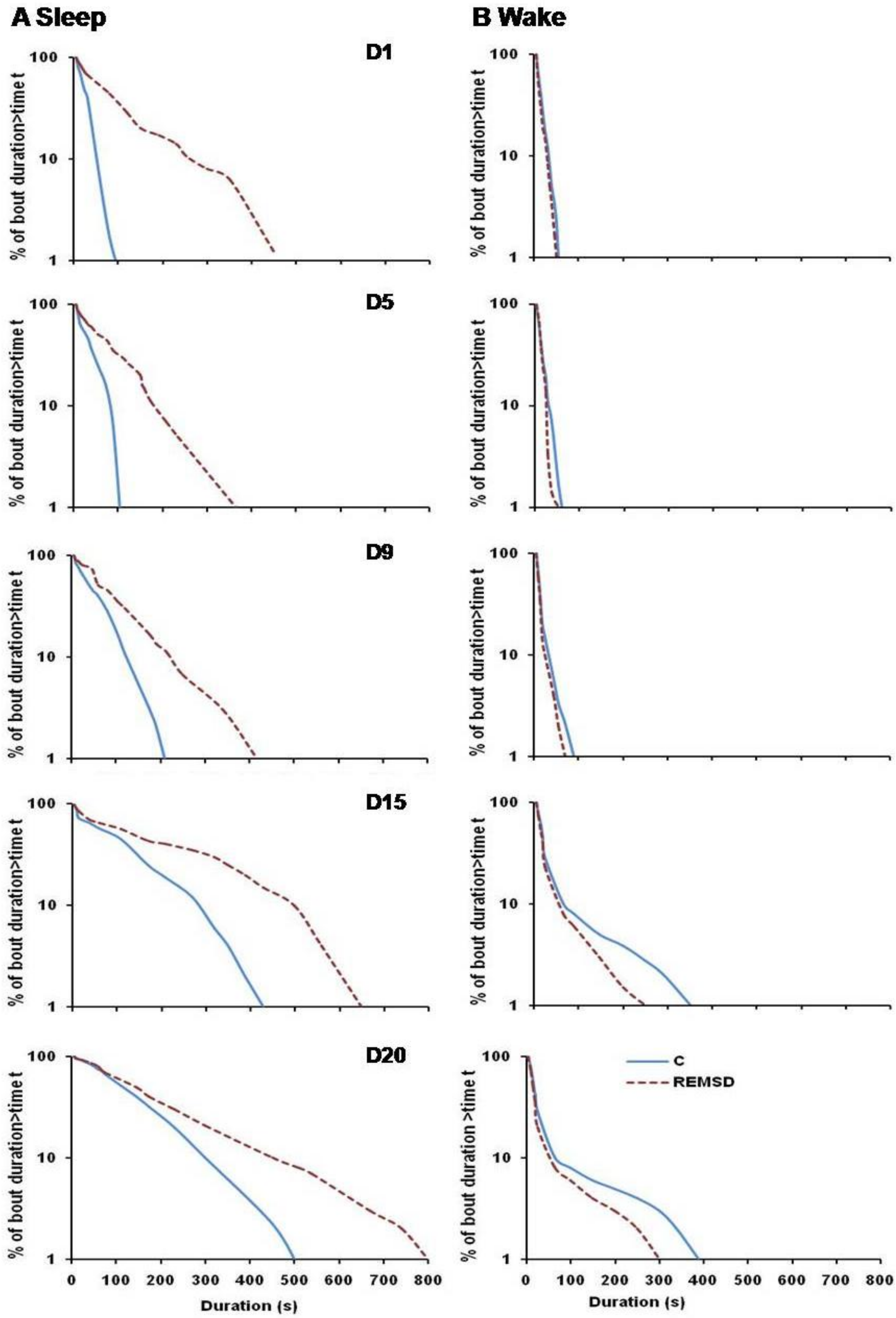
Y-axis represents averaged duration (s) of S-W cycles in REMSD and control pups (C) from D1-20 (X-axis). Data is expressed as Mean±SD. The comparison of D1 with other PNDs is shown in *, two subsequent PNDSs is depicted in ♦ and between C with REMSD group is shown in #**.** The level of significance is *,♦,# $p \leq 0.05$; **,♦♦,## $p \leq 0.01$; ***,♦♦♦ $p \leq 0.001$

Fig. 14. Latency to AS/REM sleep in pups born to control and REMSD dams on D15 and 20.



Y axis shows the latency to AS/REM sleep in REMSD group and control pups on D15 and 20 (X-axis). Data is expressed as Mean±SD. The comparison between REMSD and C group is depicted by symbol #. The level of significance is ## $p \leq 0.01$

Fig. 15. Survivor plots for sleep and wake bouts in pups born to control and REMSD dams on different postnatal days.

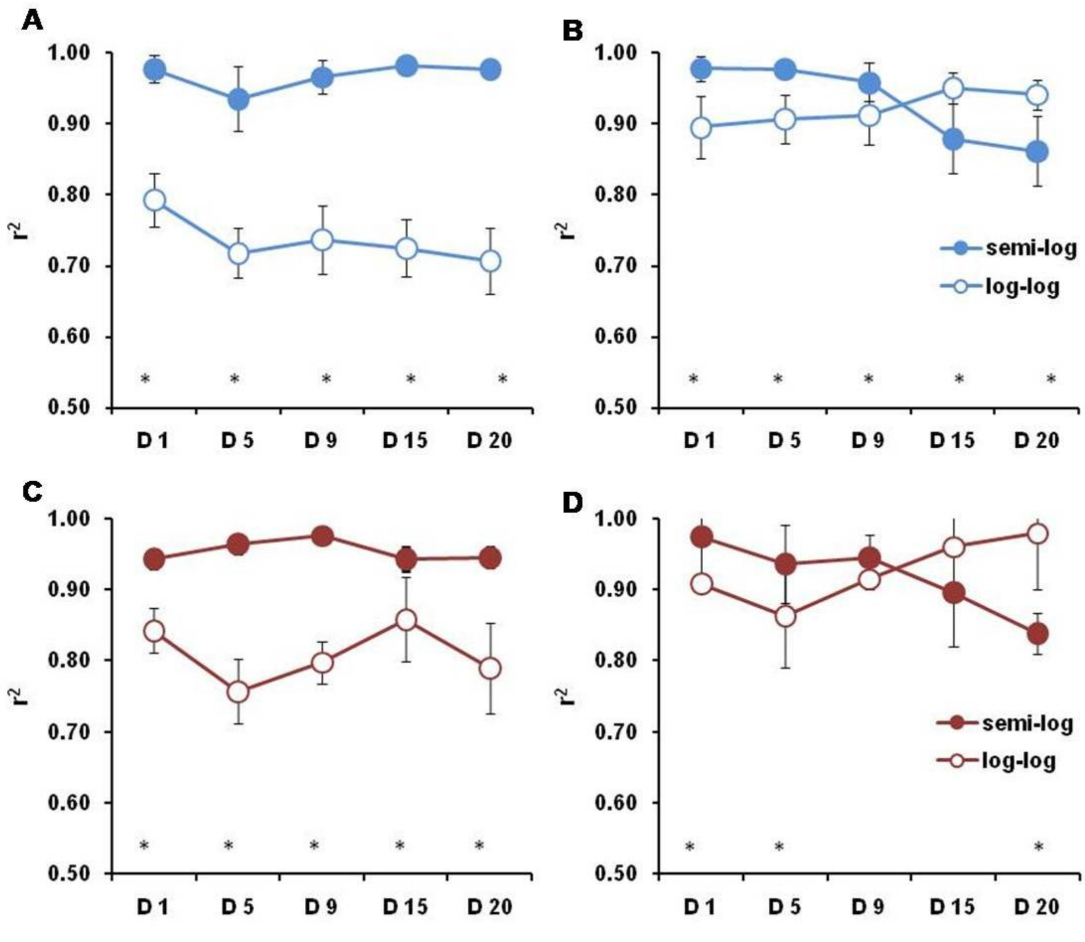


Log-survivor plots of sleep (A) and wake (B) bout distributions for rats on D1, 5, 10, 15 and 20 for the control and REMSD. Each plot was constructed from the pooled data. The Y-axis represents the percentage of bout duration and X-axis represents the duration (s).

The survivor plots of S bouts showed altered slope of the curve in the REMSD pups during all the recorded days in comparison to control, whereas the changes in W were evident during D15 and D20 only (Fig. 15). Regression analysis of S-W bouts indicated that the sleep followed exponential distribution during all recorded days in both the groups, while W followed power law only during D20 in comparison to D15 and 20 in control (Fig. 16).

Pups of REM sleep deprived dams had active sleep that was not only markedly higher in percentage during all the studied days, but also had reduced latency during later postnatal days 15 to 21. Quiet sleep and wake periods were lower. These factors, along with less frequent but longer sleep-wake cycles indicated maturational delay in the sleep-wake neural networks. Disruption of time bound growth of sleep-wake neural networks was further substantiated by the decreased slope of survival plots in the sleep bouts.

Fig. 16. Regression analysis of the sleep and wake bouts in pups born to control (A,B) and REMSD (C,D) dams on different postnatal days.



For regression analysis, r^2 values (Y-axis), for the degree of the fit to data derived from the exponential and power law are plotted during different developments Ds (X-axis) for sleep bouts (A), wake bouts (B) in control, and sleep bouts (C) and wake bouts (D) for the REMSD group. Data is expressed as Mean±SD. * $p \leq 0.05$

This study provided detailed effects of restriction of REM sleep during pregnancy in the development of the S-W patterns in the offspring in the rat model as there were no reports on the S-W patterns of neonates if their mothers are sleep deprived during pregnancy. Further this study provided model of changing sleep architecture during early development, S-W stage transitions in pups from control and REM sleep deprived dams using survivor plots.

Details are provided in this manuscript:

Aswathy BS, Kumar VM, Gulia KK (2018). The effects of rapid eye movement sleep deprivation during late pregnancy on newborns's sleep. *J Sleep Res.* 27: 197-205. (Impact Factor: 3.432)

Effects of total sleep restriction (TSR) in pregnant dams on neonatal brain

In this study, the pregnant rats were subjected to TSR for 5 h only by the gentle handling method. This technique is much less stressful in comparison to the platform method of sleep deprivation. The study was conducted on the 70 pups born to the 14 pregnant Wistar rats, raised in standard laboratory conditions. The ethical clearance for this study was obtained from the Institutional Animal Ethics Committee of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala (SCT/ABS/IAEC-87/5). All procedures were performed in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals of Government of India**.**

The efficiency of the sleep deprivation procedure of the mother rats, by gentle handling, was 98%. The gestation period was slightly longer in the TSR dams (23.3 ± 0.5 days) in comparison to the control group (22.4 ± 0.5 days).

Quantitative changes in S-W of pups born to control and sleep deprived dams

AS was high (D1: 69.1 ± 2.6 %; D5: 70.8 ± 3.8 %), whereas QS (D1: 10.67 ± 2.4 %; D5: 11.1 ± 1.7 %) and W (D1: 20.2 ± 1.7 %; D5: 18.1 ± 2.8 %) were low during the initial PNDs in the pups of the control group (Fig. 17). As the age progressed, AS was reduced (D15: 18.1 ± 1.9 %; D20: 11.2 ± 1.6 %), and QS increased (D15: 60.2 ± 3.9 %; D20: 66.1 ± 2.4 %) in control pups. However, in pups of the TSR group, AS percentage was significantly higher than in the control group, while the QS percentage was significantly lower than that observed in the control pups during D1 to D9 (Fig. 17). Time spent in W in these pups from the TSR group was also lower during the same period. But, in the pups of the TSR group, the AS, QS and W reached the control group values on D20. AS was reduced and QS increased on D15, in the pups of the TSR group. The AS/QS ratio was significantly higher from D1 to D9 (Fig. 18A) while the W/AS ratio remained lower during D1 to D9 in the TSR group, in comparison to the control group (Fig. 18B).

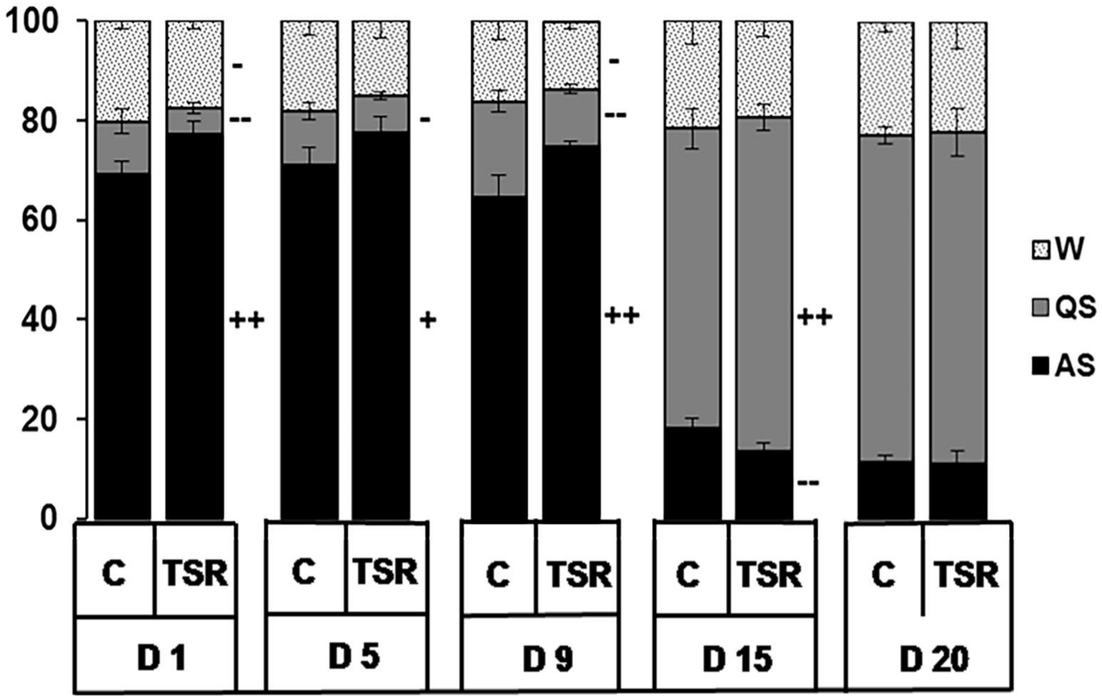
Changes in S-W (AS, QS and W) frequencies and episode duration in pups born to control and sleep restricted dams

In comparison to control pups, the neonates in the TSR group had lesser number of AS and QS episodes during D1 to D9 (Fig. 19A, B). The frequency of wake episodes was lower till D15 in the sleep deprived group as compared to the control group. The AS frequency increased only on D20. In comparison to the values from the control group, the neonates in the TSR group displayed longer AS bout length during initial D1 to D15, and it decreased only by D20 (Fig. 20A). Bout durations of QS were similar to those of the control group during D1 to D9, but the bout durations increased tremendously during D15-20 (Fig. 20B). Bout durations of W of the experimental group, at the time of birth were shorter during D1 and continued to remain shorter than those of the control group during D9 and D20 (Fig. 20C).

The durations of S-W cycle in the TSR group were comparatively longer than the values of the control group on all PNDs (Fig. 21) whereas its frequencies were reduced on D1 to D15. The arousal frequencies in the TSR group were significantly reduced on all days as compared to those in the control group (Fig. 22).

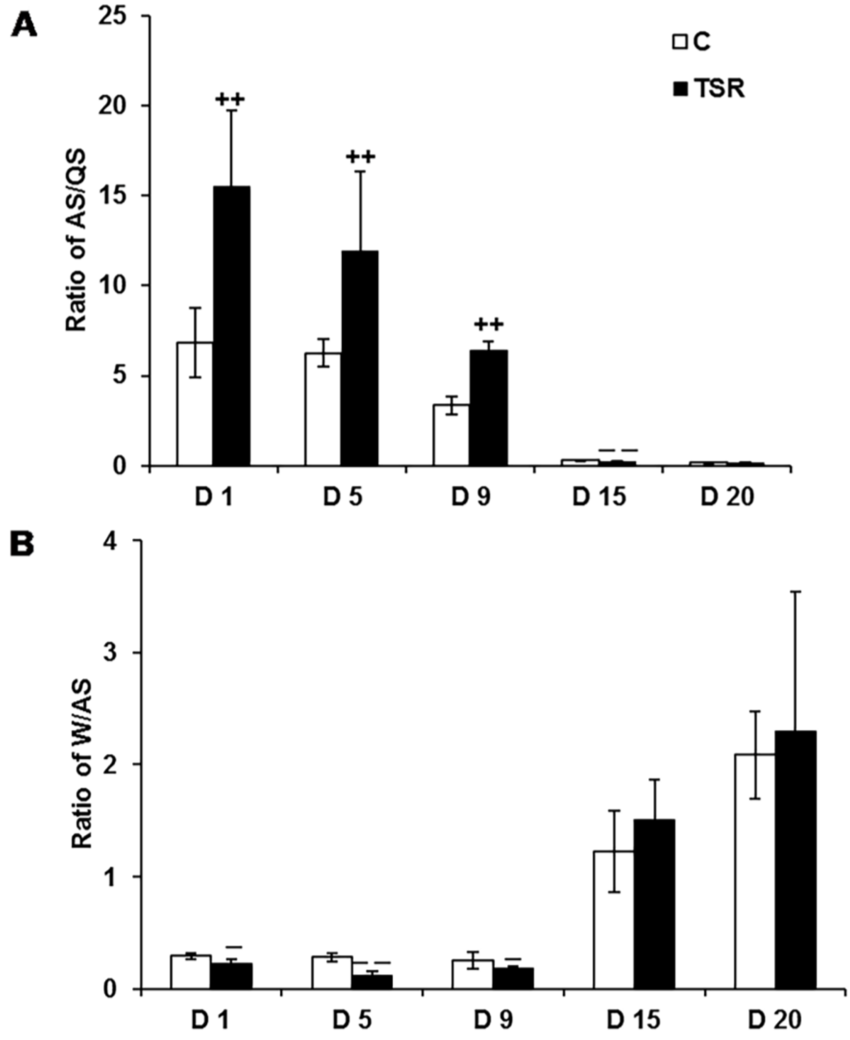
On D15, the TSR group had lower delta power in the NREM sleep traces and higher power on all other frequencies (alpha, beta and theta) as compared to the control group (Fig. 23). On the other hand, the trend was reversed on D20.

Fig.17. Changes in sleep-wakefulness on different post natal days.



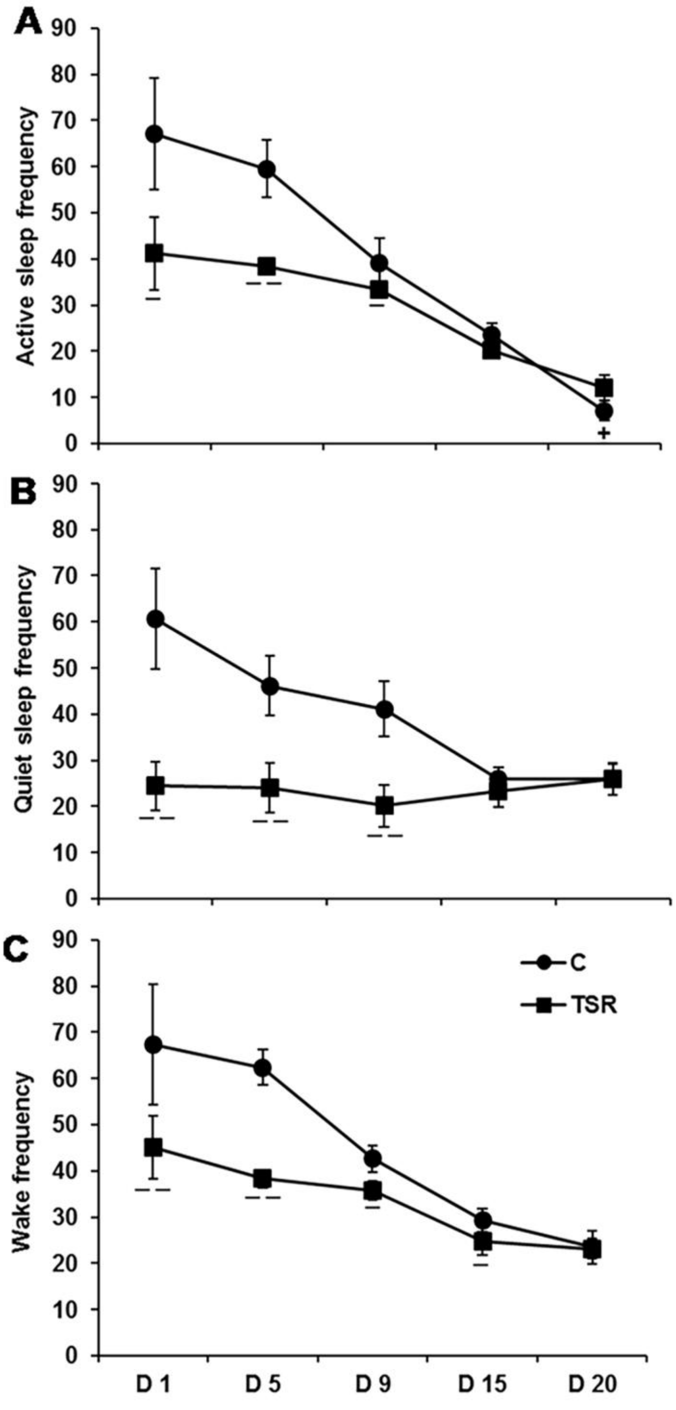
Y axis represents averaged percentage of time spent/h in active sleep (AS), quiet sleep (QS) and wake (W) on different post natal days in pups born to the control (C) and the TSR dams. X axis shows different post natal developmental days from D 1 to D 20. Data is expressed as Mean ± SD. The comparison of S-W parameters between C and TSR groups on different Ds is depicted as increase (+) or decrease (–). The level of significance is $p \leq 0.05$ for +,- , and $p \leq 0.01$ for ++,--.

Fig. 18. The ratio of the AS/QS (Fig. 18A) and W/AS during D1 to D20 in the TSR group in comparison to the control group



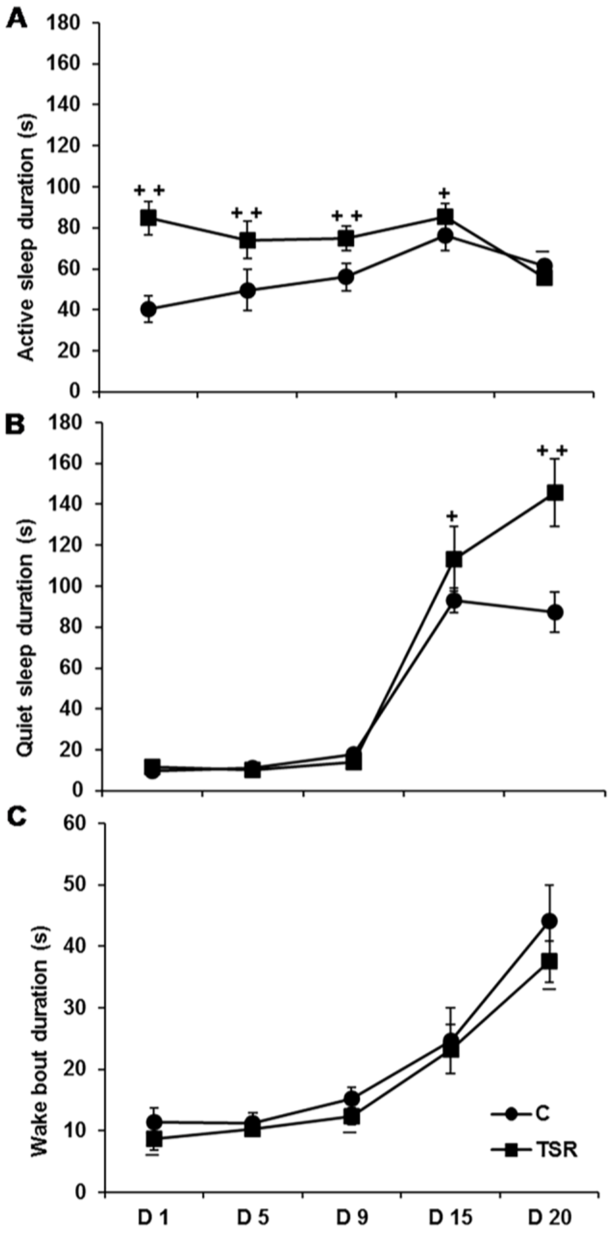
Y-axis represents various developmental days. Data is expressed as Mean±SD. The comparison of S-W parameters between C and TSR group on different Ds is depicted as increase (+) or decrease (–). The level of significance is $p \leq 0.05$ for +,- , and $p \leq 0.01$ for ++,--.

Fig. 19. Frequencies of the episodes of active sleep (A), quiet sleep (B) and wake (C) in pups born to control (C) and TSR dams on different post natal days.



Y axis represents frequencies of active sleep (A), quiet sleep (B) and wake (C) on different post natal days (D) in TSR group and C group pups. Data is expressed as Mean ± SD. Comparison of the TSR group with the control group on different Ds is depicted as increased + whereas decrease is shown in symbol -. The level of significance is shown as +, - $p < 0.05$; - - $p < 0.01$.

Fig.20. Bout durations of active sleep (A), quiet sleep (B) and wake (C) episodes in pups born to control (C) with total sleep restricted (TSR) dams on different post natal days.



Y axis represents averaged bout durations (in s) of active sleep in A, quiet sleep in B and wake in C and TSR group pups on different from postnatal days (D). Data is expressed as Mean ± SD. The comparison between TSR and C group is shown in symbol + (increase) and – (decrease). The level of significance is shown as +, - $p < 0.05$; + +, - - $p < 0.01$

Fig. 21. Changes in duration of S-W cycles in pups born to control (C) and TSR dams on different post natal days.

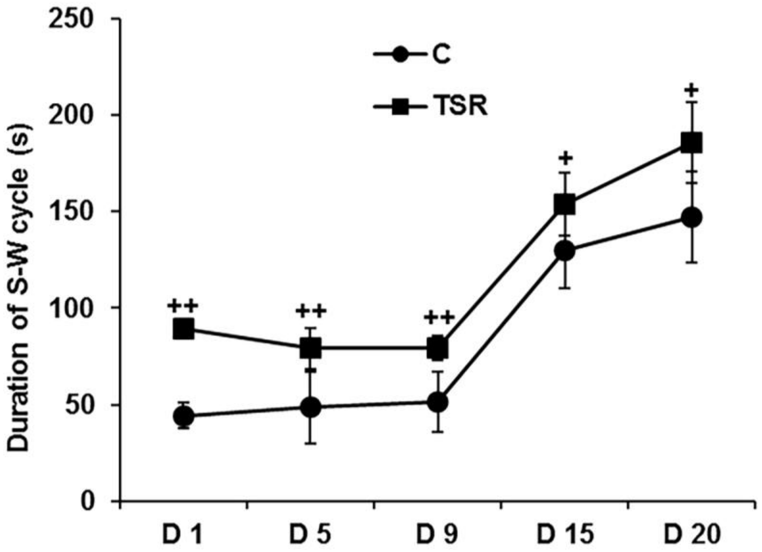


Figure shows S-W during post natal days (D1 - 20) in pups born to control and TSR dams. Y axis represents bout durations (in s). Data is expressed as Mean ± SD. The comparison between TSR and C group is shown in symbol + (increase). The level of significance is shown as + $p < 0.05$; + + $p < 0.01$.

Fig. 22. Arousal frequencies in pups born to control (C) with TSR dams on different post natal days.

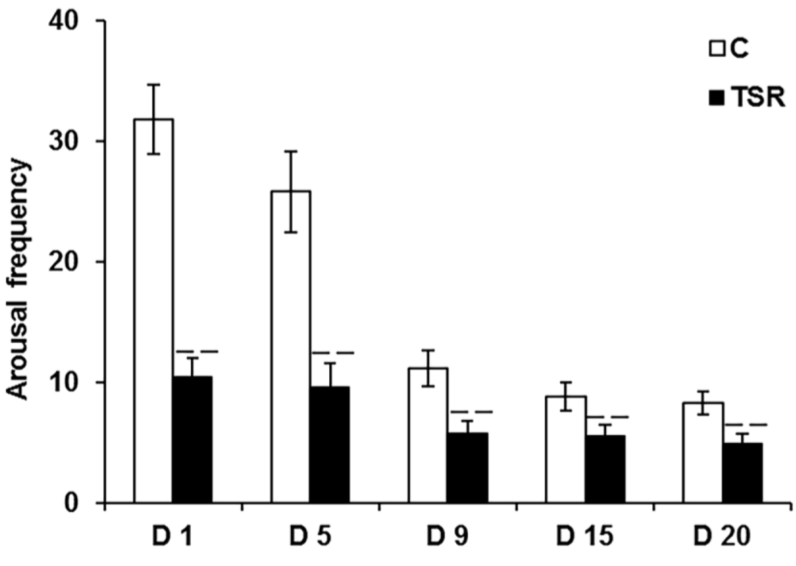
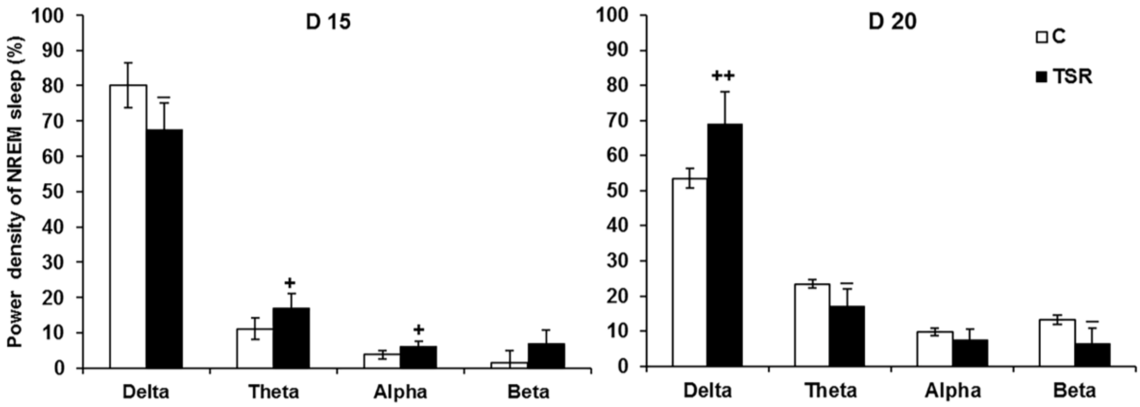


Figure shows arousal frequency in TSR group and control pups from post natal day 1 to 20 (D on X axis). Y axis represents arousal frequency. Data is expressed as Mean ± SD. The comparison between the pups born to control and TSR dams is shown in symbol - for decrease. The level of significance is -- $p<0.01$.

Fig. 23. Power density of different bands of EEG



The figure shows the mean power of individual frequency bands on D15 and D20 in pups of control (C) and TSR group during NREM sleep. The level of significance is -,+ $p \leq 0.05$, ++ $p \leq 0.01$

Details are provided in this manuscript:

Aswathy BS, Kumar VM, Gulia KK (2018). Immature sleep pattern in newborn rats when dams encountered sleep restriction during pregnancy. International J Developmental Neurosci. 69: 60-67. (Impact Factor: 2.025)

Effects of sleep deprivation on the USV in pups (isolation paradigm) and grown up adults (during mating test)

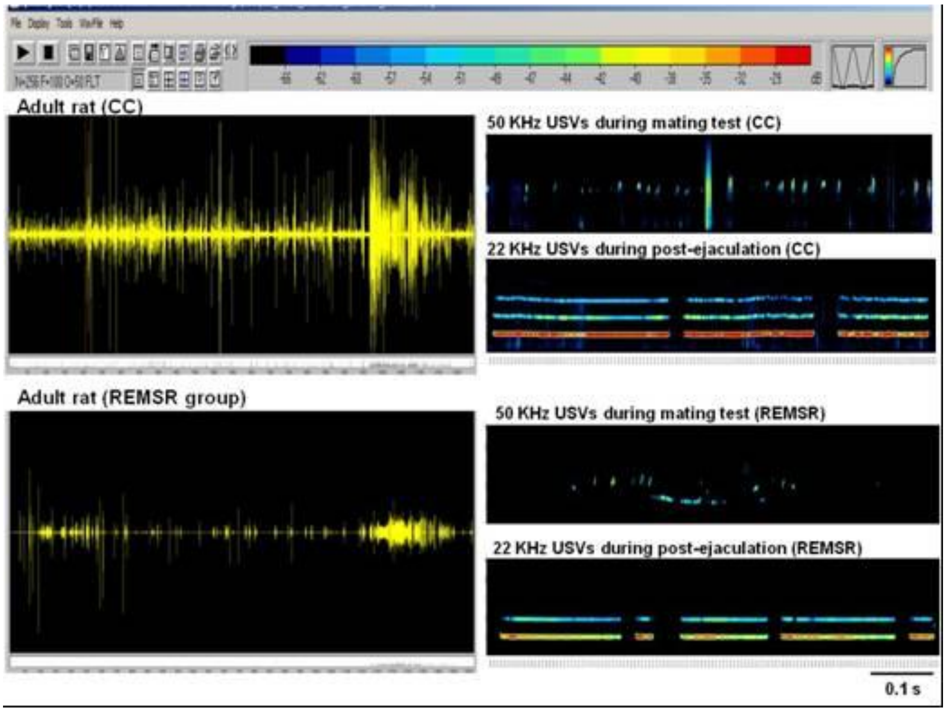
The effects of two types of sleep deprivation on various behaviors are shown in Table 1

Table 1

Parameters	Total sleep restriction X 5h/d	Total REM sleep restriction X 22h/d
Maternal body wt	Not affected	Initial drop in body wt at onset of sleep restriction
PP maternal care	Maternal care not changed	Maternal care impaired during initial 3 pnds
Babies birth weight	Babies with low birth wt	Babies with low birth wt
PP growth of babies	Body wt of babies recovered by pnd 3	Body wt remained low on pnds 1-21
Crying profiles of babies	Increased crying rate during inds 1-9 Advanced onset in the peak USVs (pnd 9) Disturbed developmental profile in USVs	Reduced crying rate in babies during pnds 1-11 Peak USVs on pnds 9 Disrupted temporal profile in USVs
PW wt profiles of babies	Decreased body wt after weaning	Decreased body wt after weaning
	Increased mobility in EPM test	Decreased mobility in forced swim test
PW behavioral profile	Persistent hyperactivity	Depression like symptoms
	Increased risk taking behavior in babies	Impaired social communication (mating song)
	Some symptoms of the ADHD	Deficits in male sexual behavior (mating test)

Abbreviations: h/d, hour/ day; wt, weight; pnds, post natal days; PP: postpartum; PW, post weaning; EPM, elevated plus maze; ADHD, attention deficit and hyperactivity disorder

Fig. 24. Traces of USV in adult showing stress calls



Left panel shows raw sonograph of the USVs during mating test in adult rats from control (upper left) and REMSR (lower) dams. Right panel shows a representative 50 KHz mating song and 22 KHz calls post ejaculatory vocalization in each group.

More details are provided in this book chapter:

Gulia KK, Kumar VM (2018). Ultrasonic vocalizations and behavior of rat pups born from sleep-deprived dams. ***In Handbook of Behavioral Neuroscience Series, Handbook of Ultrasonic Vocalizations-A Window into the Emotional Brain.*** **Edited by Stefan M Brudzynski, Chapter 44, Vol 25, pages 467-478. (Available online 27 April 2018)**

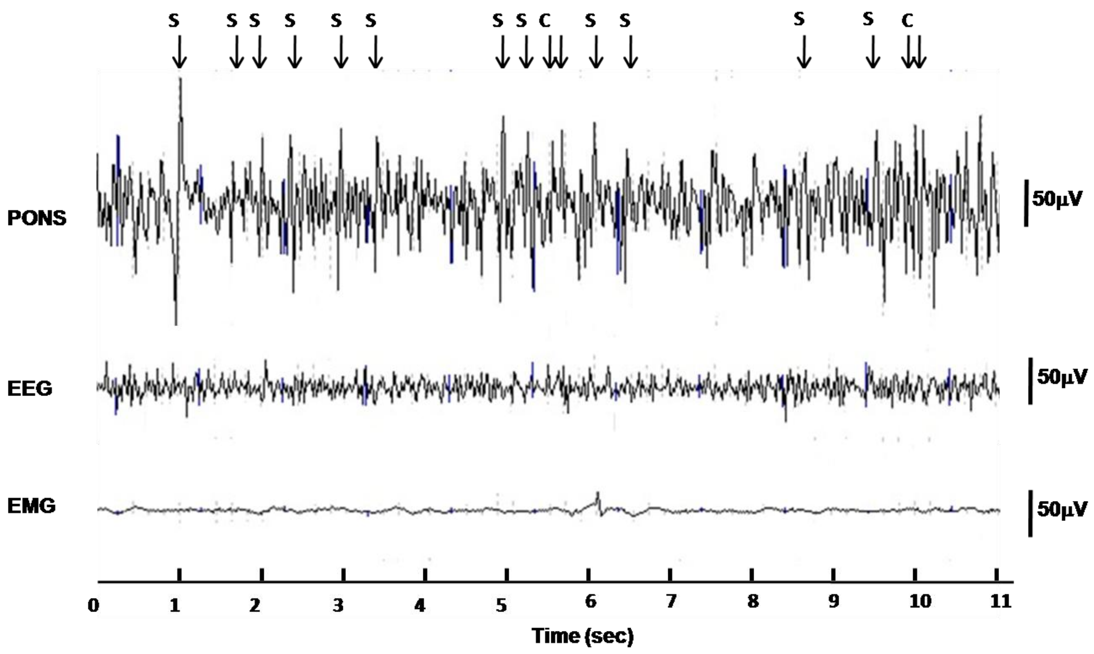
Effects of total sleep deprivation of 24 hrs on brain

It is widely accepted that sleep deprivation affects various components of cognition. There are several evidences to indicate that sleep deprivation disturbs cognition, working memory and attention. However, studies on the effects of total sleep deprivation (SD) and on theta coherence among HPC, Amyg and PFC are not available. Moreover, it is also essential to correlate these changes in coherence with memory deficits and P waves to understand the mechanism involved in cognitive deficits resulting from SD. Therefore, in this study, changes in the P waves and theta coherence between the HC, Amyg and PFC, were studied before and after exposing the animals to SD for 24 h, along with evaluation of reference and working memory.

Theta waves and ponto-geniculo-occipital (PGO) waves are characteristic features of REM sleep, in addition to desynchronized EEG, muscle atonia, rapid eye movements and many other physiological changes. The PGO waves, as the name indicates, originate from pontine area and spread to geniculate and occipital areas, and are well demonstrated in cats. In rats, these were recorded well from the pontine area only, and so they are referred to as P waves (Fig. 25). These P waves are also shown to be associated with learning and memory.

The theta waves have been reported to facilitate the neuronal communication between the limbic regions including the hippocampus (HC) and amygdala (Amyg) for memory-retrieval and synaptic remodelling. Apart from the HC and Amyg, the prefrontal cortex (PFC) is also involved in encoding spatial information generated in the HC. Theta coherence is considered an important indicator of brain network activity during learning. It may assist in communication between brain areas by encoding episodic memories, essentially using working memory. Coherence of theta oscillation between the HC and PFC is reported to be a key in the working memory process. Theta coherence between the Amyg, PFC, and HC during REM sleep is suggested to be involved in memory consolidation of aversive experiences.

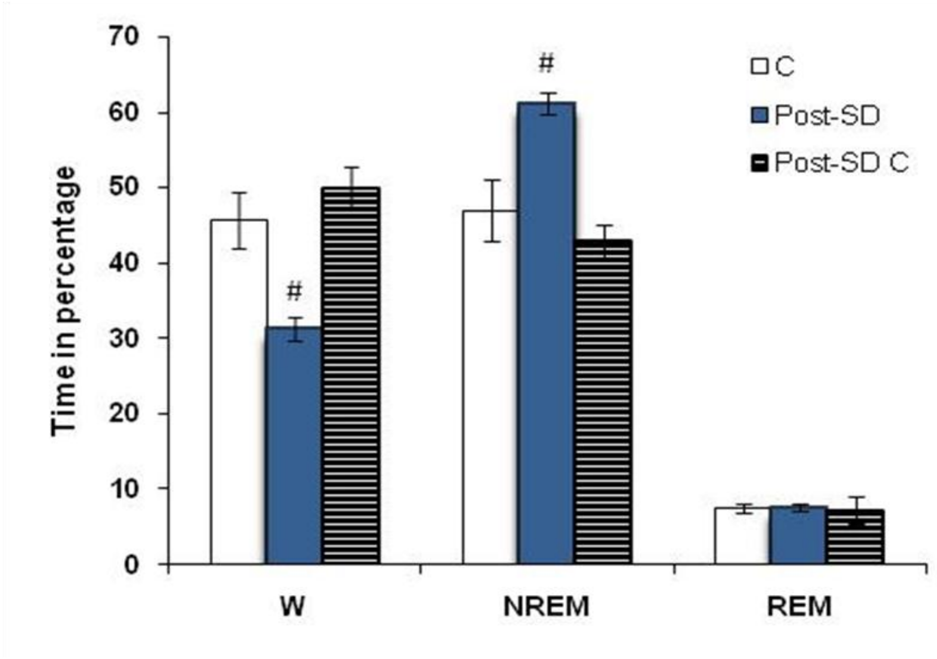
Fig. 25. Representative traces of the P waves (singlet and clusters) in the pons during REM sleep



Concomitant with a rebound increase in non-REM sleep after total sleep deprivation (Fig. 26), there was a decrease in the coherence of theta waves between all the studied areas, especially between hippocampus and amygdale (Fig. 27, 28). The ratio of P wave cluster to singlet was reduced. Representative figure for electrode placement in different brain areas is shown in Fig. 29.

There was a decrease in ‘correct memory performance’ after sleep deprivation (Fig. 30). This study showed that after sleep deprivation, there was a correlation in the cognitive deficits and decrease in the coherence of theta waves, and the ratio of P waves for cluster to singlet. Impairments in brain function, especially cognition, after sleep loss could be attributed to changes in functional connectivity between these neural networks. This study highlighted important functions of sleep in maintaining an appropriate synchrony between various neuronal networks during REM sleep.

Fig. 26. The sleep-wakefulness profiles (%) before (C) and after sleep deprivation (Post-SD and Post-SD C)



Levels of significance are # $p < 0.05$ and ## $p < 0.01$ The data is represented in the form of Mean ± SEM.

Fig. 27. Field potential traces from HC, Amyg, PFC along with EEG and EMG during REM sleep

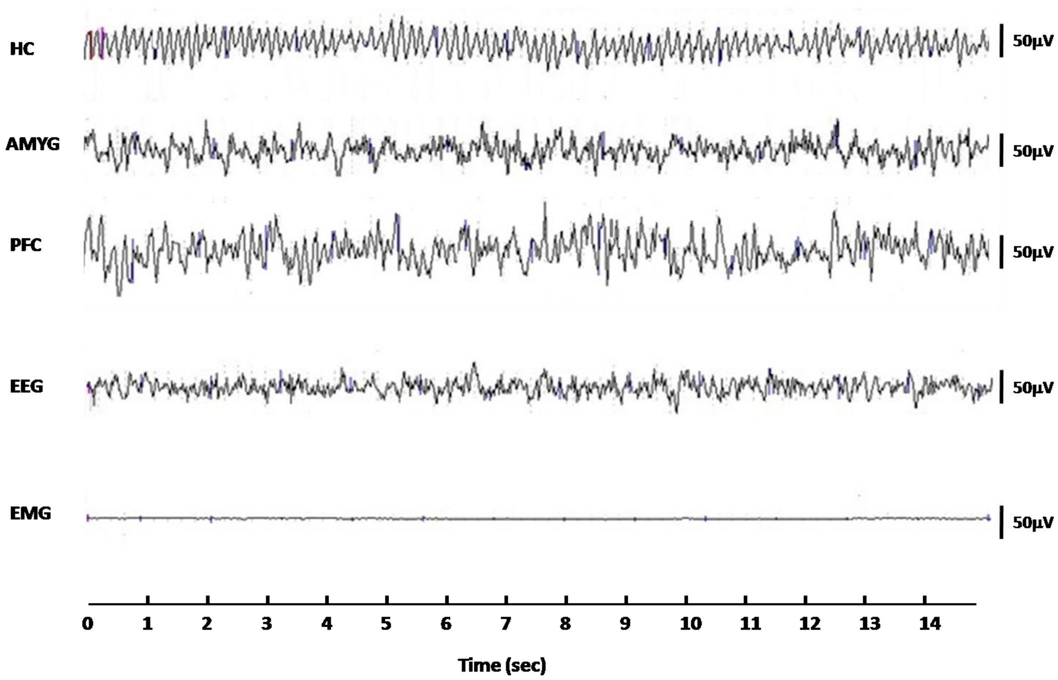
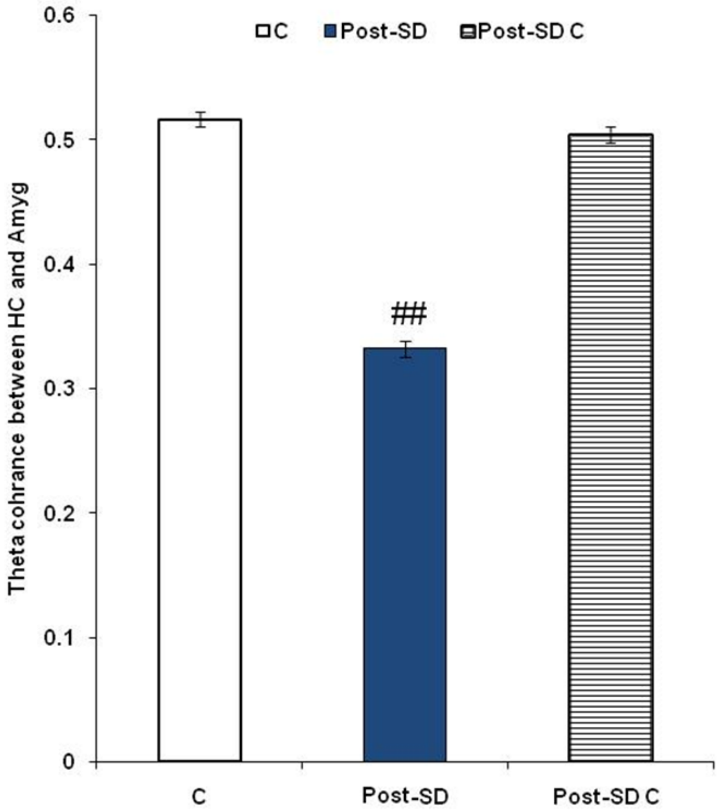


Fig. 28. Effect of sleep deprivation on the theta coherences between HC and Amyg.



The data is presented as Mean ± SEM. Symbol # indicates significance on comparison of C with Post-SD and Post-SD C values. Level of significance is represented by $^{\#}p < 0.05$, $^{\#\#}p < 0.01$

Fig. 29. Sites of localization of electrode tip in HC, Amyg, PFC and Pons

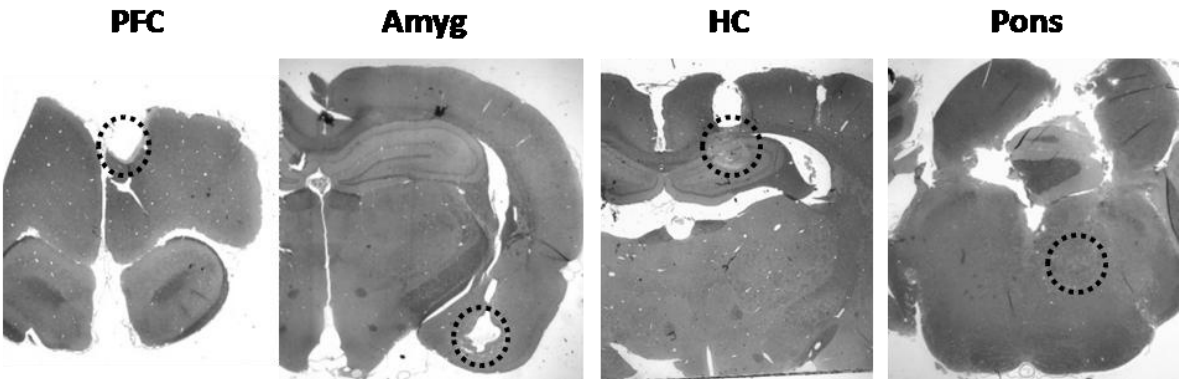
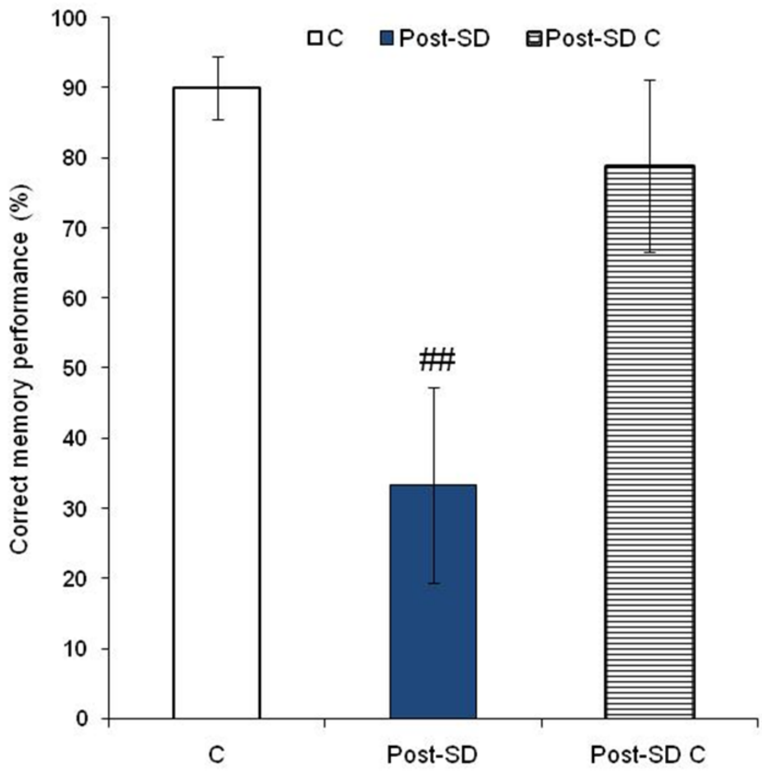


Fig. 30. Effects of sleep deprivation on correct memory performance



Correct memory performance (%) in the RAM test. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ significance of comparison of C with Post-SD and Post-SD C. The data is presented as Mean ± SEM.

Details are provided in this manuscript:

Gulia KK, Sivadas N, Kumar VM (2017). Reduced Theta Coherence and P Wave Ratio Linked to Memory Deficits after Sleep Deprivation in Rat Model. ***Sleep Vigilance*** **1: 21-29. DOI: 10.1007/s41782-017-0005-x**

Effects of alpha-asarone during pregnancy associated sleep deprivation

It is evident from above described results of the experiments that these rigorous longitudinal studies involved monitoring of pregnant animals in 1) control conditions (normal pregnancy), 2) sleep deprivation condition (two types of sleep deprivation techniques, REMSD and deprivation of total sleep) and 3) sleep restricted dams. Further, the pregnancy outcomes in pups were evaluated by taking various parameters including their sleep-wakefulness, emotional behaviour (USV, EPM, OFT) and learning and memory (RAM test). These effects of sleep deprivation during pregnancy were tested after administration of α-asarone.

In our previous studies, 10 mg/ k wt of α-asarone in male rats was found optimal for improving sleep quality when given through intra peritoneal (ip) route (Radhakrishnan et al., 2017#). In the current study, in initial experiments, we tried with ip adminstration of α-asarone, however due to practical difficulties, we resume oral administration.

Daily oral administration of 40 mg/ kg bwt in sleep deprived pregnant rats from gestational day 15 produced restlessness and intermittent paroxysmal activity in EEG immediately after injection in two rats. However, in another two animals no abnormal behaviour changes were noticed. Results with 10 mg/ kg bwt oral administration were not conclusive.

It is suggested that further experiments should be conducted by administering the α-asarone via the oral route at doses of 20, 30 mg/ kg bwt. It is suggested to use the folic acid in combination.

Radhakrishnan A, Jayakumari N, Kumar VM, Gulia KK (2017). Sleep promoting potential of low dose α-Asarone in rat model. *Neuropharmacology* 125: 13-29.

11. Conclusions summarising the achievements and indication of scope for future work:

We have developed an animal model for drug trials and study of sleep disorders during pregnancy and postpartum. This model will be beneficial to study effects of various herbal preparations on various pregnancy associated anomalies like gestational diabetes, preeclamsia, intrauterine growth restriction etc. Besides this, sleep was recorded successfully in neonates, infants and adolescents. This will provide an important insight into early window of critical development to understand the mechanism and role of sleep in neural networking crucial for cognitive development. Examination of altered sleep-wake patterns during early development may provide crucial information about deranged neural development in the offspring. We provided the first report to show that maternal sleep deprivation during pregnancy can delay and impair the development of sleep-wake profile in the offspring. Changes in USVs and delayed development of S-W patterns in neonates could be early markers of deranged neural development.

This extensive study provided crucial information on role of sleep during pregnancy in shaping brain for effective cognitive abilities during prenatal to postnatal life. The key results are that due to sleep fragmentation during last trimester of pregnancy and post-partum, Non-REM sleep delta power was increased during late pregnancy and after parturition but Post-partum sleep and anxiety was reduced in contrast to ante-partum

This is probably the first study highlighting the importance of prenatal sleep for the appropriate development of S-W networks of the growing newborns which has immense translational importance. It would be interesting to study the development of S-W of these newborns after subjecting them to post natal sleep deprivation.

- Sleep disturbance of pregnant rats results in disruption of sleep in newborn
- Pups of sleep restricted dams had longer active sleep and shorter quiet sleep
- Higher ratio of active to quiet sleep implied maturational delay of brain
- Lower ratio of active sleep to wakefulness also indicated delayed brain maturation

Long Term application potential

This study provided preclinical evidences for management of sleep loss during pregnancy. In the light of the pertinent risk involved in maternal sleep deprivation, and lack of information on various mechanisms that may be operative in the prenatal periods, the finding of this study will address sleep associated alteration in neuronal milieu, early in life for a cognitive development, later in life. Sleep architecture and efficiency profile of mothers during and after pregnancy will provide us important information that will be guiding factors in appraising and defining the optimal sleep for a better mother-child health and appropriate cognition. The comparison of sleep profiles in offspring of sleep challenged mothers with the control ones will provide us the changes in neural network and a possible early electrophysiological marker for a normal development.

12. S&T benefits accrued:

i. List of Research publications

S No	Authors	Title of paper	Name of the Journal	Volume	Pages	Year

1. Gulia KK and Kumar VM (2016). Sleep Deprivation during Pregnancy: The Cost of Ignorance!. *SM J Sleep Disord*. 2(1): 1004.
2. Gulia KK, Kumar VM (2016). Sleep Medicine in Ayurveda. *Sleep Med Rev*. 25:131.
3. Gulia KK, Sivadas N, Kumar VM (2017). Reduced Theta Coherence and P Wave Ratio Linked to Memory Deficits after Sleep Deprivation in Rat Model. *Sleep Vigilance* 1: 21-29. DOI: 10.1007/s41782-017-0005-x
4. Sivadas N, Radhakrishnan A, Aswathy BS, Kumar VM, Gulia KK (2017). Dynamic changes in sleep pattern during post-partum in normal pregnancy in rat model. *Behav Brain Res.* 320: 264-274.
5. Aswathy BS, Kumar VM, Gulia KK (2018). Immature sleep pattern in newborn rats when dams encountered sleep restriction during pregnancy. International J Developmental Neurosci. 69: 60-67.
6. Gulia KK, Kumar VM (2018). Sleep disorders in the elderly: A growing challenge. Psychogeriatrics, 18: 155-165.
7. Gulia KK, Kumar VM (2018). Proper sleep during pregnancy for mental health of new-born: An evidence based appeal to policy makers. Sleep Vigilance. 2: 97-98.
8. Aswathy BS, Kumar VM, Gulia KK (2018). The effects of rapid eye movement sleep deprivation during late pregnancy on newborns's sleep. *J Sleep Res.* 27: 197-205.

Book chapter

Gulia KK, Kumar VM (2018). Ultrasonic vocalizations and behavior of rat pups born from sleep-deprived dams. *In Handbook of Behavioral Neuroscience Series, Handbook of Ultrasonic Vocalizations-A Window into the Emotional Brain.* Edited by Stefan M Brudzynski, Chapter 44, Vol 25, pages 467-478. (Available online 27 April 2018)

Reports/ abstracts in Journals/ Proceedings

1. Gulia KK (2018). Sleep restriction during pregnancy a risk for neuro-cognitive development of offspring in rats: Recent evidences from animal model. Sleep Biol Rhythms 4: 36.
2. Gulia KK (2017). Neonatal sleep in normal and prenatal stress conditions. Sleep Vigilance, 1, 124.
3. Gulia KK (2017). Sleep deprivation induced memory deficit are attributed to disrupted communications between amygdala and hippocampus. Sleep Med, 40: e121.
4. Gulia KK (2017). An animal model for studying sleep during pregnancy-postpartum continuum. Sleep Med 40: e121.
5. Aswathy BS, Kumar VM, Gulia KK (2017). Sleep loss during pregnancy delays and disrupts the development of sleep-wake networks in neonates: Novel evidences from rat model. *Proceedings of the Science Fete*, SCTIMST, Trivandrum.
6. Mary SLJ, Gulia KK (2017). Rats born to rapid eye movement sleep deprived dams during pregnancy show fragmented sleep during the middle age. *Proceedings of the Science Fete*, SCTIMST, Trivandrum.
7. Gulia KK (2017). Sleep for integrated life: changing concepts in the Workshop on Integrative Biology at University of Kerala on 21 Feb 2017.
8. Gulia KK, Kumar, VM. Ultrasonic vocalizations and behavior of rat pups born from sleep-deprived dams. Chapter 11.4, In Handbook of Ultrasonic Vocalization Edited by Stefan M Brudzynski published by Elsevier. 2018 (In Press)
9. Aswathy BS, Kumar VM, Gulia KK (2017). Sleep loss during pregnancy delays and disrupts the development of sleep-wake networks in neonates: Novel evidences from rat model. *Proceedings of the Science Fete*, SCTIMST, Trivandrum.
10. **Aswathy BS won First prize in the Applied Sciences in Science Fete held on 15 July 2017.**
11. Mary SLJ, Gulia KK (2017). Rats born to rapid eye movement sleep deprived dams during pregnancy show fragmented sleep during the middle age. *Proceedings of the Science Fete*, SCTIMST, Trivandrum.
12. Gulia KK (2016). Prenatal sleep disruptions and early cognitive development. In proceedings of 34th Annual meeting of IAN in NBRC, Manesar.
13. Gulia KK (2016). Sleep during pregnancy-postpartum in women and its development in children in Proceedings of the APPICON meeting, Patna. Gulia KK (2016). Prenatal sleep disruptions and early cognitive development. In *Proceedings of the 34th Annual meeting of IAN* in NBRC, Manesar.
14. Gulia KK (2016). Sleep during pregnancy-postpartum in women and its development in children in *Proceedings of the APPICON 2016* (AIIMS, Patna).
15. Gulia KK (2016). Sleep loss during pregnancy and cognitive deficits in offspring: Novel evidences in the *Proceedings of ISDN2016*: 21st Biennial meeting of the International Society for Developmental Sciences held in Antibes, France.
16. Gulia KK (2015). Sleep loss during pregnancy and cognitive deficits in offspring: Recent evidences in the *Proceedings of the Annual Conference of the Association of Physiologists and Pharmacologists of India* held at AIIMS-Jodhpur, Rajasthan.

ii. Manpower trained on the project

a) Research Scientists or Research Associates: **PhD (one); MPhil (one): JRF (three)**

b) No. of Ph.D/ MPhil. produced :

One (PhD thesis submitted by Ms Aswathi BS), 2018
Thesis Title: "**Sleep profile of rat offspring of sleep deprived mothers**"

MPhil thesis produced: One (Ms Jonhsy Mary); 2017
Thesis titled "**Evaluation of Sleep-wakefulness profile and the behavioral phenotype of male rats during middle age that are born to REM sleep deprived rats during pregnancy**"

c) Other Technical Personnel trained : 3 JRF: Ms Neelima Sivadas, MTech,
Ms Anjuprabha, MTech
Ms Athira, MTech

ii. Patents taken, if any : None

1. Financial Position:

No	**Financial Position/ Budget Head**	**Funds Sanctioned**	**Expenditure**	**% of Total cost**
I	Salaries/ Manpower costs	8,06,400	8,11,453	-
II	Equipment	15,00,000	15,36,441	-
III	Supplies & Materials	4,00,000	2,42,810	
IV	Contingencies	40,000	1,32,623	
V	Travel	70,800	94,414	
VI	Overhead Expenses	3,99,000	3,99,000	-
VII	Others, if any, Interest	59542	-	-
	PI paid	6,894	6,894	-
Total				

14. Procurement/ Usage of Equipment

S No	Name of Equipment	Model & make	Cost (Rs in lakhs)	Date of Installation	Utilisation Rate (%)	Remarks Maintenance/ Breakdown
1	DSI transmitter	T: F50-EEE (DSI)	2,59,246	2015	70%	T Battery over
2	MP150 system & ERP and ECG	Biopac systems	9,37,059	2015	70%	Working
3	Ultrasound playback speaker (Avisoft)	Avisoft Bioacoustics, Germany	3,40,136	2015	70%	Working

b) Plans for utilising the equipment facilities in future

Applied for new projects

Name and Signature with Date

Kamlesh

a. Kamalesh K Gulia

(Principal Investigator)

V. Mohan Kumar

b. V Mohan Kumar

(Co-Investigator)

29.05.2020