Annexure-IX

PROJECT COMPLETION REPORT

Title of the project:

The effects of maternal sleep deprivation on cognition in the offspring in an animal model

DST File No: SR/CSI/110/2011

SCTIMST Project File No: 8063

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(No of copies: TEN)

1. Title of the project: **The effects of maternal sleep deprivation on cognition in the offspring in an animal model**

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

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4. Date of commencement: **01.04.2012**

5. Planned date of completion: **01.04.2015**

6. Actual date of completion: **30.09.2015**

7. Objectives as stated in the project proposal:

1.To evaluate the effects of maternal sleep deprivation on learning and memory in the offspring using radial arm maze.

2.To quantify the offspring's emotional changes through ultrasonic vocalizations, elevated plus maze test, open field test and light-dark transition test during different stages of development.

3.To investigate the effect of maternal sleep deprivation on neuronal interaction in the offspring by measuring theta activity coherence from hippocampus, amygdala and prefrontal cortex.

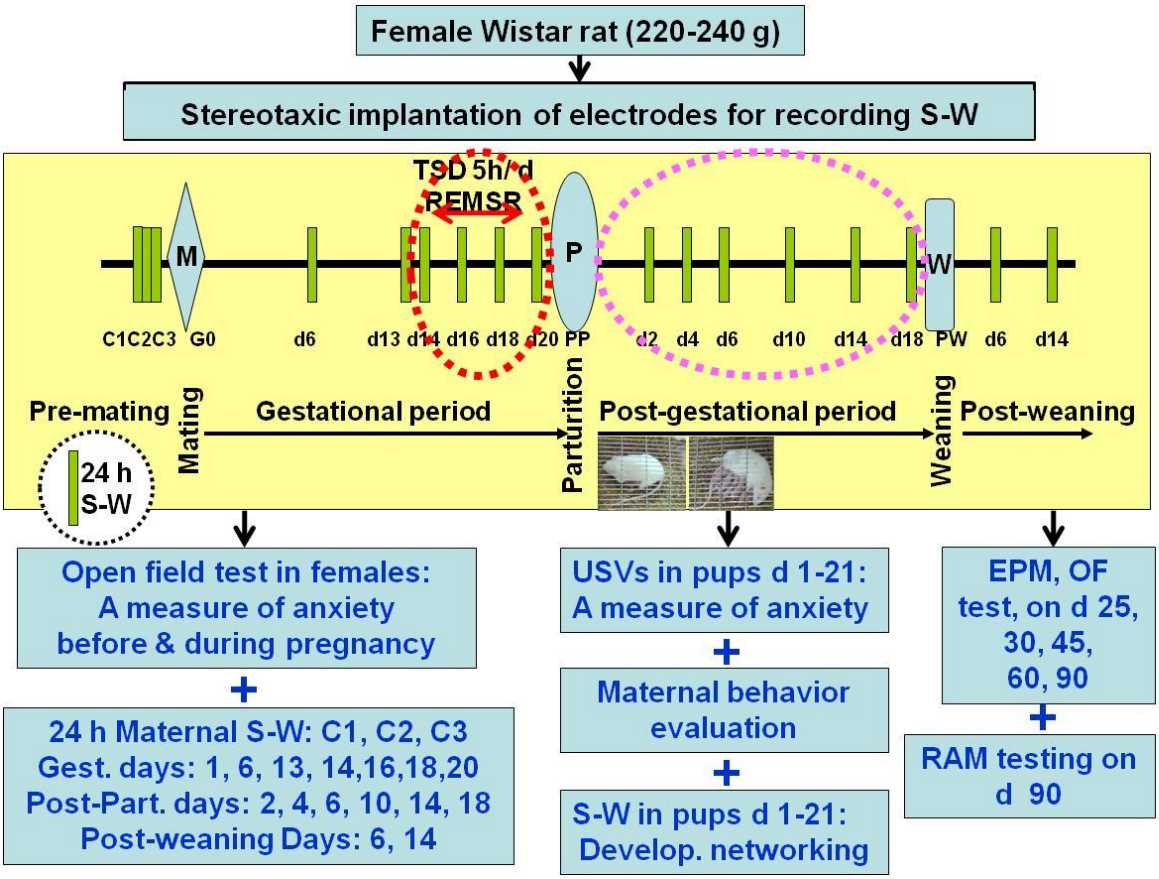
8. Deviation made from original objectives if any, while implementing the project and reasons thereof: None

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. The effects of maternal total sleep deprivation of 5 hr (TSDX5h) and rapid eye movement (REM) sleep restriction (REMSR) during 3rd trimester of pregnancy (gestation day 14 to 19) were studied on the offspring's emotional changes through ultrasonic vocalizations (USVs), elevated plus maze (EPM) test and open field (OF) test during different stages of postnatal development as per experimental protocol (**Figure 1**).

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).

Figure 1 Experimental protocol for recording sleep-wakefulness (S-W) in dams, pups and the behavior tests in offspring

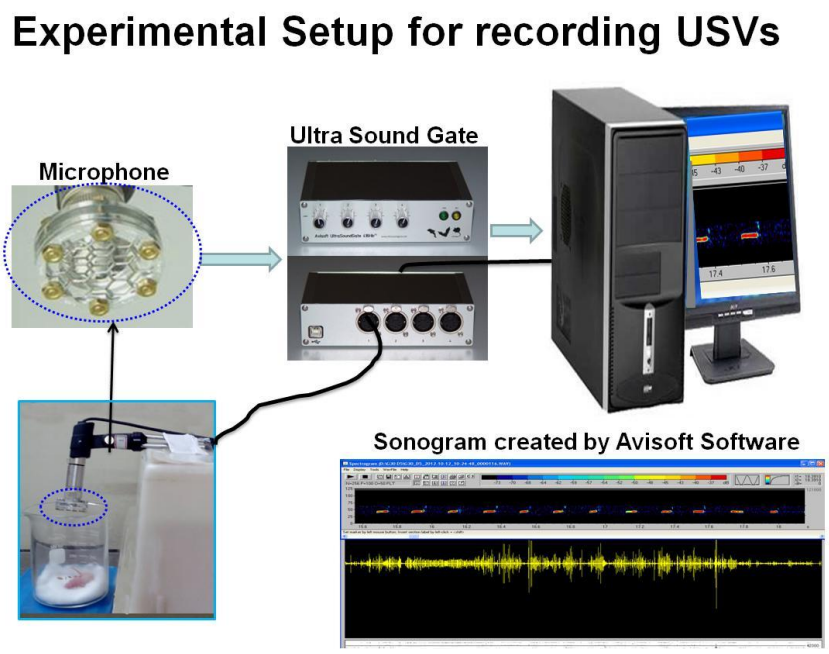


The USVs of the pups were studied from postnatal days 1-21 (**Figure 2** is showing the set-up), and their anxiety were tested during peri-adolescence in EPM and OFT.

For monitoring the sleep during pregnancy, and for electrophysiological based sleep deprivation protocol, adult female rats were implanted with electrodes for recording sleep-wakefulness (S-W), prior to mating. Under anesthesia (ketamine (50mg/kg) and xylazine (5mg/kg)), electroencephalogram (EEG) and electromyogram (EMG) electrodes were implanted stereotaxically. After post surgical recovery, their S-W was recorded for 24 hrs, on three days, for control values. The female rats were put for mating with male rats of same strain and age. Following mating, pregnancy confirmed female rats were kept in separate cages and their body weights were monitored throughout the period of gestation.

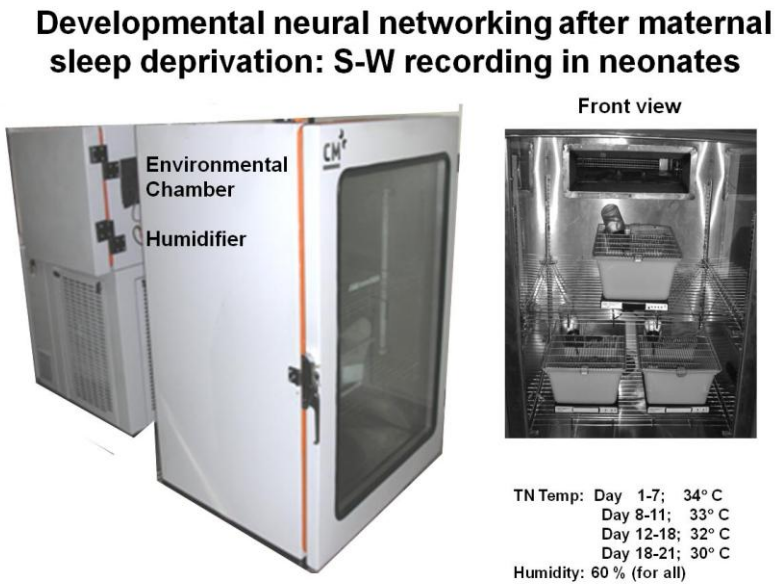
Animals were housed in a room having lights on from 6:00 to 18:00 hours (12:12 light: dark cycle), controlled temperature (26 ± 1ºC) with *ad libitum* supply of food and water.

Figure 2 USV recording setup for rats



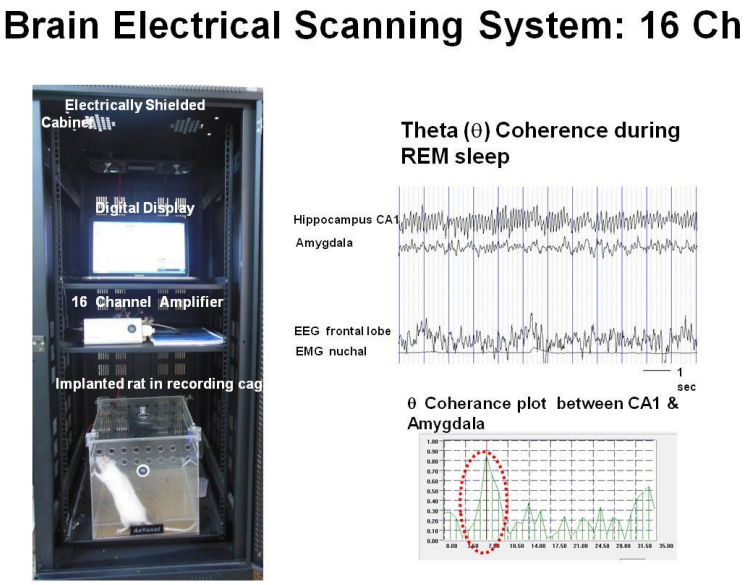
The post gestational recordings of S-W (24 hr) were taken on once in each trimester (day 2, 7, 14, 20). The TSDX5h was achieved through gentle handling of female from 9 am to 2 pm from post-gestational day 14 to 19. Effectiveness of gentle handling on sleep deprivation was monitored (and ensured) on the basis of EEG and EMG for any occurrence of sleep (Recording using MP 150 system from Biopac system). For REMSR, platform over water was used and sleep deprivation was performed from 11 am to 9 am the next day. On completion of the sleep deprivation protocol of 6 days, dams were monitored till the delivery of pups. Sleep of mother undergoing acute total sleep deprivation and their pups were studied simultaneously. The S-W in the pups born to sleep deprived and control rats (n=6 in each group) were taken on the post-natal days (pnds) 1, 5, 10, 15 and 20 (Experimental set up for neonatal sleep recording, **Figure 3**). The maternal sleep was recorded on weekly basis post-partum and post weaning.

Figure 3



To study the effect of maternal sleep deprivation on neuronal interaction in the offspring by theta activity coherence from hippocampus, amygdala and prefrontal cortex was analyzed. For this, the deep brain electrodes were stereotaxically implanted in hippocampus, amygdala and prefrontal cortex these areas of brain in addition to electrodes for recording S-W in the adult males. The field potentials from deep brain areas and S-W were recorded using multiplexer BESS recording system from Axxonet Systems (**Figure 4**).

Figure 4 Experimental set up for the deep brain recordings and sleep



The data collected from all these experimental setups during different development days as per the protocol was analyzed and presented in the next section (10).

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

- To measure the anxiety state in the neonates (rats), we have standardized the USV recordings techniques using isolation paradigm.
- The technique of 24-hr sleep-wakefulness recording during pregnancy, post-delivery, and post-weaning is standardized. This is an ideal animal model for pregnancy related sleep research.
- We have also established the technique of recording sleep-wakefulness in neonates, infants and adolescents in rats. This provides an opportunity to study the development of neural network, which is crucial for the study of cognitive functions.
- To study the changes in neural networks during sleep, we have standardized the procedures of simultaneous recording of sleep-wakefulness and field potential from various areas of brain, using 16 channel BESS system.

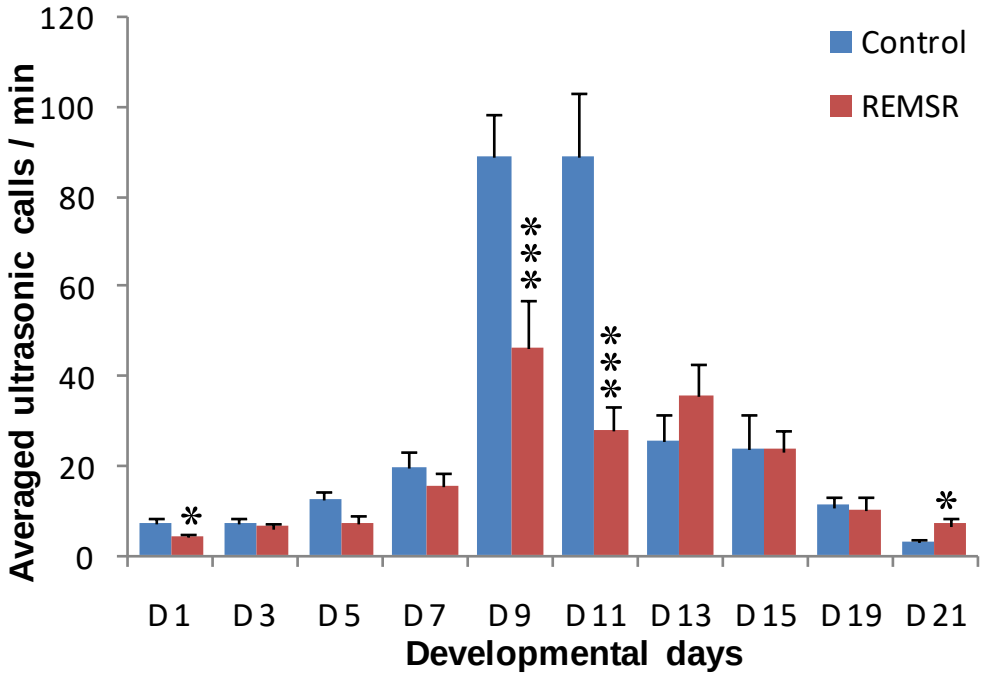
Effects of sleep deprivation, REMSR (A) and TSDX5h (B), during 3rd term of pregnancy on USVs (pups) and emotional behavior during adolescence (C)

A. <u>Effects of REMSR</u> on:

Rate of Ultrasonic vocalization

A total of 17991 ultrasonic calls were obtained from pups on various days. The total numbers of USVs per min were averaged for each developmental day studied in 19 male pups, in each group, obtained from 10 litters. The intra and inter group multiple comparisons were made for all days for pups in control and REMSR group. The total numbers of calls in the pups of the control group remained low during initial days (Day 1-5). These were increased on 7th day and reached to highly significant values on peak days 9 and 11 (Figure 5). Thereafter, the total number of calls was reduced, reaching to a very low value on day 21.

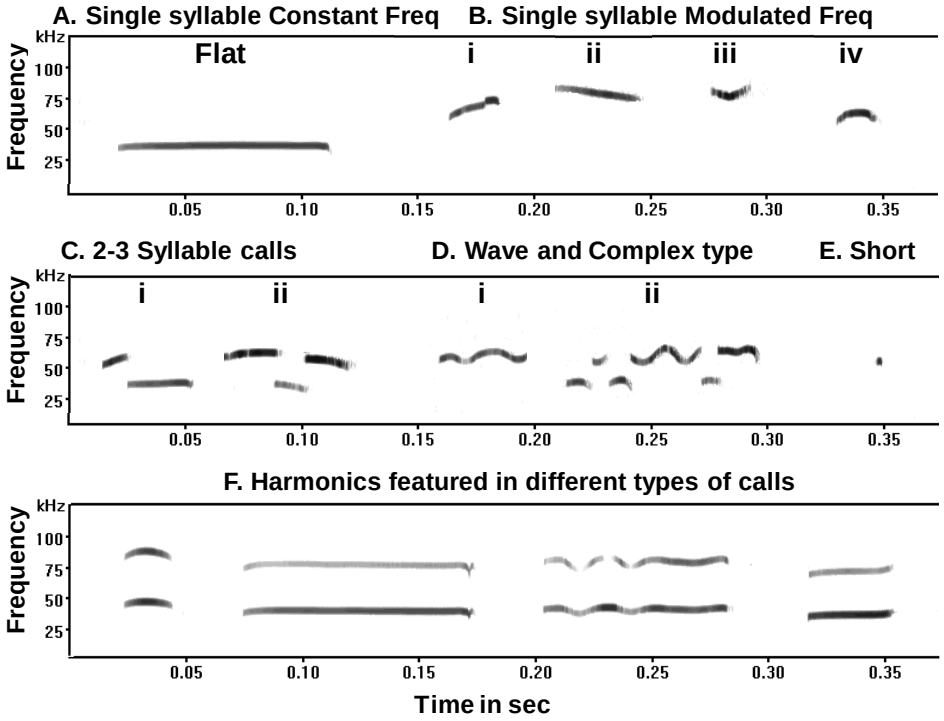
Figure 5: Average no of USVs/ min in pups after maternal separation



Vocalizations are presented as averaged calls/ min ± SEM (Standard error of mean) in the Y axis and different developmental days on X axis. 'D' refers to days, Control values are represented in blue bars while the REMSR group values are shown in brown bars. The comparison of values between Control v/s REMSR group is depicted in '*'. The significance values are given as '*' for $p < 0.05$, '***' for $p < 0.001$.

The USVs of the pups born to REMSR mothers were nearly half or lower than the control values on peak vocalization days of 9 and 11. A comparison of the total USVs in the pups born to REMSR mothers with the pups of the control group showed a reduction in calling rate on most of the initial days, though statistical significance was observed only on days 1, 9 and 11 (Figure 5). Thereafter, the numbers of calls of pups born to REMSR mothers were not only comparable; it was even higher than pups of the control group on day 21.

Figure 6: Sonograph of different call types produced by pups in the isolation paradigm



The frequency is shown in kHz on the Y axis and time scale is expressed in sec on the X axis. The CF and MF refers to constant and modulated frequency respectively. In single syllable MF category, numeral i, ii, iii and iv denotes Upward, Downward, U-shaped and Inverted U-shaped calls.

Types of ultrasonic vocalizations

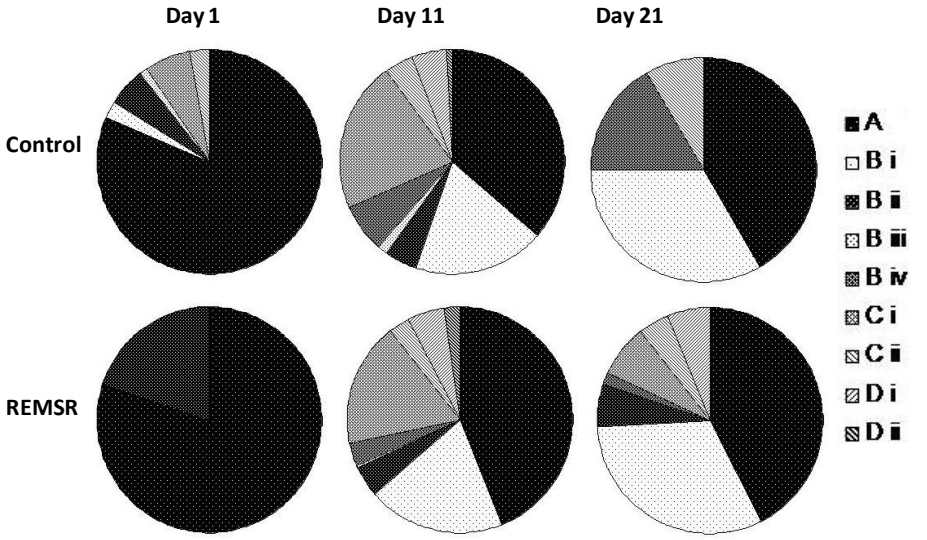
Individual calls were labelled on the basis of shape and sonographic features. USVs were grouped into five categories on the basis of number of syllables, frequency modulation and duration (Figure 6). The calls having constant frequency (CF) with a variation of ± 5 kHz from start to end were designated as Flat calls (Figure 6A). The single syllable calls having simple frequency modulation were grouped into Single syllable frequency modulated (MF) category (Figure 6B) which consisted of upward sweep (Figure 6A-i), downward sweep (Figure 6A-ii), U-shaped (Figure 6A-iii), and inverted U shaped (Figure 6A-iv) calls. In 3rd category (Figure 6C), all two or three syllable calls were clustered. All the calls displaying complex frequency modulation were grouped together as Wave and Complex type (Figure 6D). Calls with durations of less than 5 ms were taken as short calls (Figure 6E). The calls in the form of harmonics or additional overtones over the fundamental call were also common, which were referred to as harmonics (Figure 6F). The percentage of calls in the

form of harmonics was calculated separately from the total calls/ day, irrespective of the types.

Call types on different days in control and REMSR group pups

The distribution of call types on postnatal days 1, 11 and 21 are shown in Figure 7. On day 1, call types were minimal (flat and downwards) in the pups of REMSR mothers. From day 9 (the peak calling day) onwards all the call types were found in both the groups; however, there were significant variations among them.

Figure 7: Distribution pattern of isolation calls during different developmental days in the control and REMSR group

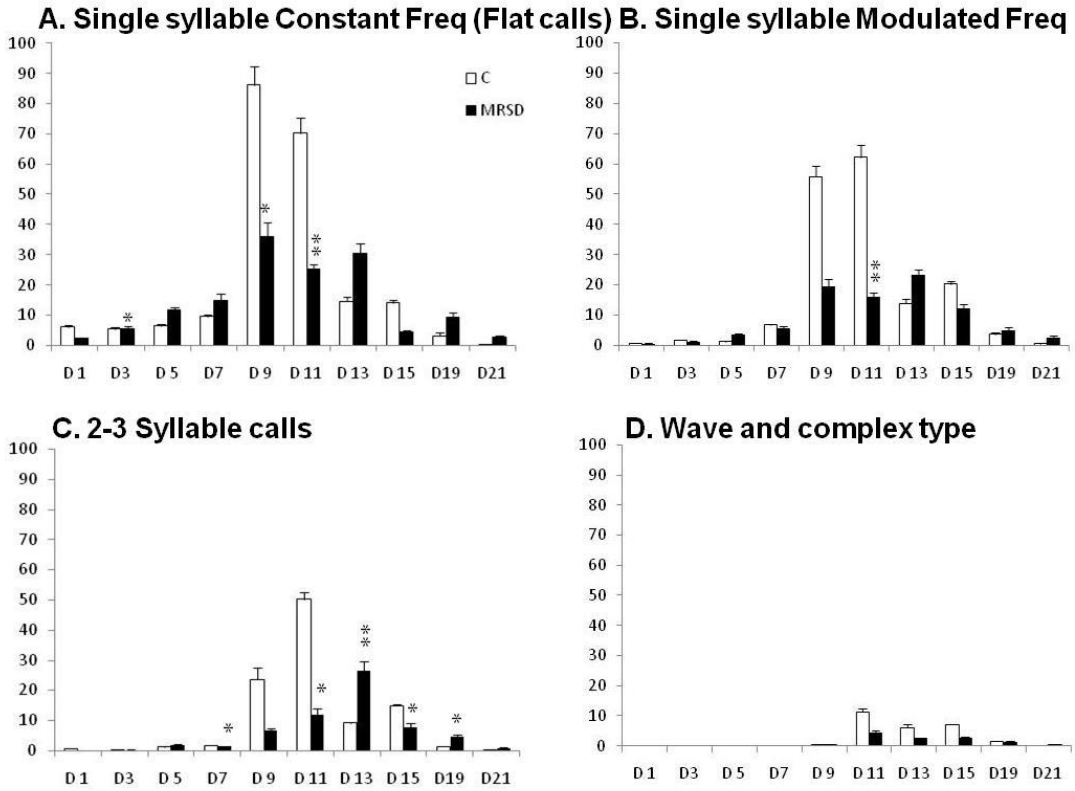


The percent distribution of various calls grouped in four categories obtained on isolation at postnatal day 1, 11 and 21 is shown in pie diagram for pups in the control and REMSR group. A denotes category consisting of flat calls; B having upwards (i), downwards (ii), U shaped (iii), and inverted-U (iv); C is sum of 2 and 3 syllable and D contains wave and complex calls.

In the control group pups during initial week of postnatal days, the predominant calls were the flat calls, ranging from 80% on day 1 to 60 % on day 7. They decreased during the later developmental days when the other call types in higher frequency modulation and complexity increased in number (Figure 8). A comparison of the call types in different categories between the control and REMSR groups was performed. Of all the categories of calls, flat calls consisting of constant frequency were more frequent in comparison to remaining call types in both the groups (Figure 8). Pups in REMSR group made

significantly lower number of flat calls on days 3, 9 and 11 in comparison to the control group, even though calls in this group were also lower (not statistically significant) on days 1 and 15 (Figure 8A). On day 5, the vocalization in flat, MF single syllable and 2-3 syllable calls were higher in number.

Figure 8: Distribution pattern of isolation calls grouped in four categories in the control and REMSR group



Distribution pattern of isolation calls grouped in four categories during different developmental days in the control and REMSR group. Developmental days are shown in horizontal axis and averaged numbers of calls are shown in Y axis.

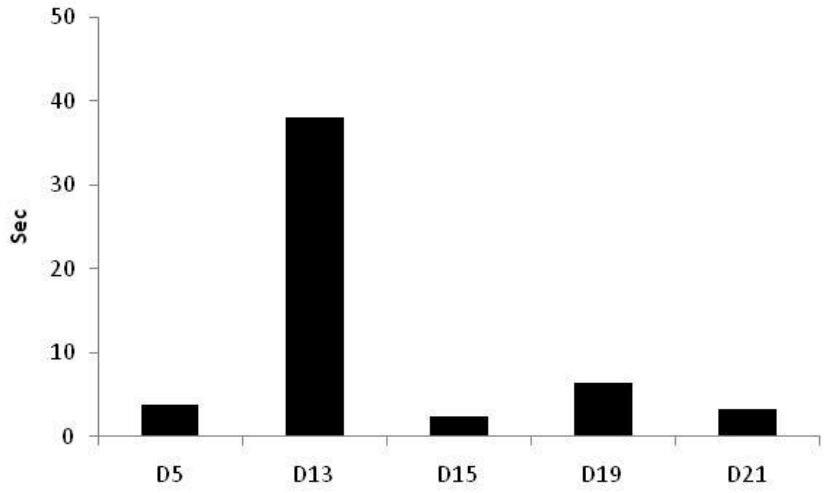
There was a significant decrease in single syllable MF calls on day 11 in REMSR group (Figure 8B). However, a comparison of calls within this group showed that the downward calls were higher on day 5 in the pups of REMSR mothers, whereas upward calls were higher on days 9 and 11. Inverted-U calls remained higher on day 11 in control group pups (not displayed in Figure 8).

A shift in the peak calling days of 2-3 syllable USVs of REMSR group towards the end of the second week resulted in significant differences between the two groups on days 7, 11,

13, 15 and 19 (Figure 8C). The 2 syllable calls remained higher on day 7, and 3 syllable calls were higher on day 13 in the pups of the REMSR mothers. Wave and complex calls could be observed only after postnatal day 7 and their numbers were low in both the groups of pups during the observed days (Figure 8D). No significant variation was observed in dot calls between the control and the REMSR groups, except on day 21.

To compare total time spent in vocalization on various days, the difference in total time was calculated between the two groups. The excess time spent in ultrasonic vocalization, on the days 5, 13, 15, 19 and 21, by the pups of REMSR mothers is plotted in Figure 9.

Figure 9: Plot of excess time spent in ultrasonic vocalization by the pups in sleep challenged mothers



Plot of excess time spent in ultrasonic vocalization by the pups in sleep challenged mothers (difference taken from total time spent in vocalization by control pups) during testing days in the isolation paradigm.

Fundamental frequency

The carrier/ fundamental frequency in the flat calls in the control group pups remained around 40 kHz in majority of cases on day 5 (80 %), days 9-11 (80-90 %) and increased to higher frequencies on later days. Flat calls of higher frequencies were also observed on day 5 (45-46 %) in the pups of REMSR mothers. The remaining MF calls were centred at higher frequency of 55-65 kHz in both the groups. The frequency overtones/ harmonics in ultrasonic vocalizations were also common in both the groups.

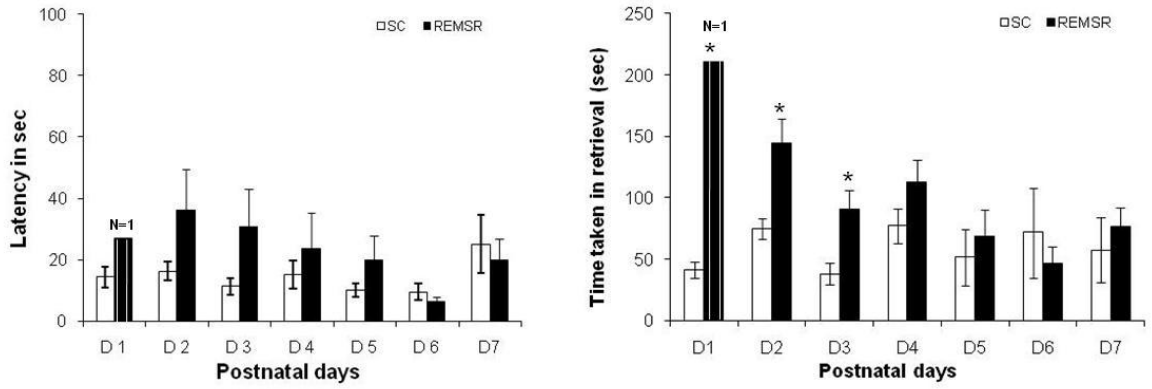
USV distribution over first and second min

The numbers of calls made during the initial first minute was higher till day 15 in control groups of pups. Similar trends were observed in pups from REMSR group except on initial (days 1-3) and later days (19-21) when a higher number of calls were observed during second minute.

Retrieval test

In comparison to control group mothers which showed normal retrieval behaviour, only one animal of REMSR group showed retrieval behaviour on day one. In addition, time taken in retrieving all the pups of the REMSR group was significantly higher in comparison to the pups of the control group on initial three days (Figure 10). Thereafter, a gradual increase in the retrieval response was observed. There was an increase in latency to retrieve the first pup in the REMSR group, though the trend did not reach the level required for statistical significance.

Figure 10: Latency of retrieval of first pup and time taken for retrieving all the pups on different postnatal days in control and REMSR group rats.



Latency to retrieval of first pup is shown in A. The post-natal days are shown on horizontal axis and time in sec is shown on the vertical axis. The open bars depict Control and closed bars depict REMSR group data. The hatched closed bar depict retrieval by few mothers (such number of cases are written above the bar).

Discussion of results of REMSR and USVs:

The temporal profile of isolation induced distress calls produced by control group pups on different postnatal days is comparable to earlier reports on some of the postnatal days.

However, the present detailed study on different postnatal days showed that the USVs followed a distinct developmental pattern. In addition, the present study showed that a profound change in USVs in the pups born to the REM sleep-compromised mothers. These pups showed less number of USVs during initial days, with a significantly reduced calling rate during second week of peak vocalization. Their peak vocalization was shifted to the end of 2nd week. Strikingly lowered USVs in these pups of REMSR mothers during the second week (days 9 and 11), can be due to their reduced sensitivity to isolation stress or to a reduction in their ability to call. A poor display of retrieval response by these REMSR mothers on initial crucial days (days 1-3) and a consequent drop in body temperatures of pups when lying scattered would definitely be contributing factors that modified their vocalization response on the following days. The diminished response in experimental pups on isolation strongly indicates a potential link between the expression of USVs and the maternal sleep.

It is claimed that the distress induced vocalizations emitted by neonates are the acoustic signals for their mother to carry out an appropriate retrieval response. Reduced vocalization in pups would result in reduced care giving response by mothers. A weaker calling response during initial post-natal days can be attributed to comparatively reduced sensitivity to hypothermia. Subsequent days when pups call more, probably for retrieval by their mothers, coincide with the period of development of thermoregulation. Finally, during the end of 3rd week, ultrasonic vocalizations nearly cease with the growth of fur in pups. However, the extreme reduction in calling rates of REMSR group pups on peak vocalization days suggests that they had different levels of sensitivity and responsiveness to the maternal isolation which could be contributed by prenatal stress as a result of maternal sleep restriction during pregnancy. This is supported by finding wherein the prenatal stress is indicated to suppress USVs in pups. This might probably also reflect an early onset of a distorted/ malformed social bonding with their mothers.

The second peculiar observation in this study was an altered vocalization pattern in the pups born to REM sleep-compromised mothers. Though these pups produced a lower number of ultrasonic vocalizations on peak calling days (days 9-11), unlike the control pups, their calling rate did not show a decrease on day 13. This extended vocalization response, and inability of these pups to reduce ultrasonic calls prior to weaning (day 21) possibly reflects a subtle developmental delay produced by REMSR.

An evaluation of qualitative aspects of the USVs provided further details on their distribution across development. Since the isolated dot calls did not show change between two groups during various developmental days, it can be assumed that these might not have a specific role in the current paradigm. Predominance of flat calls during initial days of post-natal development indicates that pups are capable of making only simpler constant frequency calls during initial days. The ability to make the frequency modulated calls gradually increased during later days of growth. However, it can be seen that the pups from REMSR group made more efforts in making larger number of calls in both constant and high pitched single/ multiple syllable frequency modulated calls on day 5. In addition, these pups of REM sleep-compromised mothers made longer duration calls on day 5 which however was lacking during the further days of development. This indicated that initially these pups attempted to vocalize for a longer time supplemented with high pitched sounds. But they discontinued their effort in absence of an optimal care response from their mother. This may be responsible for their lower calling rate on day 5. The trend for an increased count in the 2-3 syllable calls which display higher frequency modulation is suggestive of increased signs of anxiety in pups born to the REM sleep-deprived mothers. The reduced numbers of complex and wave types of calls in pups whose mothers were REM sleep- deprived also indicate a reduction in their ability to produce these frequency modulated calls. The increased time in vocalization on day 13 onwards, in pups of REM sleep deprived- mothers, is due to the persistent, but delayed, increase in calling rate. The control group pups generally showed reduction in calling rate from day 11 onwards.

Increase in duration and calling rate of flat calls, and existence of the higher frequency calls in REMSR pups on day 5, probably suggests that these pups tried high pitch modality during early development. The reduction in rate of downward calls in these pups indicates that the flat calls may be easier to manoeuvre. The lower number of flat, downward, inverted-U and 2 syllable calls in the pups of REM sleep-compromised mothers on day 11 indicate reduction in both the constant and frequency modulated calls.

Increased total time of low intensity USVs on day 5 in pups of REMSR mothers is probably suggestive of an attempt to attain mother’s attention in spite of making less

number of calls. The reduction in calling rates, and vocalization time in these pups on day 9-11 might be suggestive of their reduced sensitivity to isolation stress.

An altered crying pattern with abnormal cries is related to brain damage, in human babies. It is thus possible that the ultrasonic vocalization in rat pups, altered in the absence of an optimal environment during early postnatal period, might get reflected in emotional deficits during later life. The exact message hidden in the individual call types are not yet deciphered but it would be interesting to decode these acoustic signals to understand importance of the vocalizations. These conclusions are supported by the reports that the USVs of the pups shape the mother's responsiveness towards the pups. In contrast, the USVs emitted by adults display more distinct acoustic features than the pups and are largely grouped into two broad categories. The long 22-kHZ USVs are emitted in aversive conditions such as on exposure to painful stimulus, after defeat in aggressive encounter, after ejaculation during mating whereas the short 50-kHZ calls are emitted during copulatory act, play with mates and positive motivational states.

Present findings reiterate that isolation calls in pups vary in carrier frequency and display change in the sonographic structure during development. Quantitative and qualitative alterations in USVs of pups may be affecting the message to their mother for retrieval process. The mechanism and expression of anxiety might vary across various phases of ontogeny (neonatal, adolescent, adult and old age) which could be defined by specific experiences in age dependent manner for emotional learning. However, during early development, distress giving variables may be limited and common to all pups. Since evaluation and assessment of newborn's behaviour is comparatively difficult, it is emphasized that the USVs measurement in neonates can be taken as a possible tool to study various indices of emotional development.

The disturbance in REM sleep is associated with depression in mothers [2]. The results of current study support recent human studies associating maternal stress with adverse neural developmental in child. It is difficult to assert from the present study that the alterations in USVs were due to prenatal factors alone and it did not have a contribution from postnatal factors. Though the brain development during postnatal period is more important in determining cognitive function, the impaired neural development during foetal life may increase the susceptibility to emotional development .

The results of the present study suggest the importance of maternal REM sleep in modulation of USVs of the neonates born to them. The pups born to REM sleep-compromised mothers not only showed qualitative changes in calling rates but also displayed an altered vocalization pattern during development. Reduction in vocalization response to isolation on peak calling days, variations in the types and characteristics of call types, and alteration in temporal profile, together highlight the importance of maternal sleep in shaping the emotional behaviour in neonates.

It is reasonable to assume that the ultrasonic vocalizations can be utilized as a reliable early marker for an affective state in the rat pups and this model could be used to understand the mother-child bonding for an optimal cognitive development during post-partum life. These findings may have an important clinical implication in human neonates, as delayed vocalization may be associated with REM sleep deprivation in mothers during late pregnancy. It would be interesting to see whether reduced USVs on the peak calling days has any relationship with depression traits. Further studies are required to decode USVs to understand plasticity in the pups' vocalization and the maternal responses in shaping their behaviour during post-natal period. This is the first report showing a link between maternal REM sleep deprivation and the vocalization in neonates and infants. REMSR during pregnancy give rise to depression like symptoms in offspring.

Further details are mentioned in the published work: Gulia KK, Patel N, Radhakrishnan A, Kumar VM (2014). Reduction in ultrasonic vocalizations in pups born to rapid eye movement sleep restricted mothers in rat model. **PLoS One.** 2014 Jan 13;9(1):e84948.

B. <u>Effects of TSDX5h during 3rd term of pregnancy on USVs and behavior</u>:

Ultrasonic vocalizations (USVs) in rodent pups are analogous to cries in human babies. There is significant reduction in USVs in pups after experimental deprivation of rapid eye movement sleep during pregnancy. Though all components of sleep are reduced in any sleep restricting situation, including those happening during pregnancy, the effects of total sleep deprivation on the USVs of newborns, and their emotional development are not documented.

USVs of the pups born to the pregnant Wistar rats undergoing sleep deprivation for 5h during third trimester were tested on postnatal days (pnds) 1,5,9,11,15 and 21 in isolation paradigm followed by evaluation of anxiety-related behaviours during pnds 25-28, using elevated plus maze (EPM). The USVs, which are inaudible to human ears, were recorded using microphones, and analysed using SASPro software. In comparison to the control pups, neonates of sleep-deprived dams made higher vocalizations with lesser amplitude during pnds 1-9. The weanlings of sleep-deprived dams made increased entries into the open arms and showed higher mobility in the EPM, when tested during pnds 25-28. The results suggest that the maternal sleep deprivation during later pregnancy affects emotional plasticity in the pups. Enhanced distress calling during early development on the one hand and reduction in risk assessment in weanlings on the other indicate a link between the two altered behaviours. Thus, the enhanced USVs, similar to human baby's cries during critical window of early ontogeny, may provide a vital early signal indicative of altered emotional development.

HIGHLIGHTS of the study:

- Acute total sleep deprivation during pregnancy increases crying during early postnatal days.
- Total sleep deprivation (acute) during pregnancy increases risk taking behaviour in pre-adolescents.
- Maternal sleep deprivation affects emotional development of babies.
- Ontogenetic profiles of USVs, similar to human baby's cries during critical window are the first signals to modulate mother-child bonding.

Further details are discussed in the published work: Gulia KK, Patel N, Kumar VM (2015). Increased ultrasonic vocalizations and risk-taking in rat pups of sleep-deprived dams. **Physiol Behav** 139: 59-66.

C. <u>Effects of TSDX5h during 3rd term of pregnancy on anxiety profile of offpsring</u>:

Sleep deprivation in women resulting from their modern lifestyle, especially during pregnancy, is a serious concern as it can affect the health of the newborn. Anxiety disorders and cognitive deficits in the offspring are also on the rise. However,

experimental studies on the effects of sleep loss during pregnancy, on emotional development and cognitive function of the newborn, are scanty in literature. In the current study, female rats were sleep-deprived for 5 h by gentle handling, during the 6 days of the third trimester (days 14-19 of pregnancy). The effects of this sleep deprivation on anxiety-related behaviors of pups during their peri-adolescence age were studied using elevated plus maze (EPM). In addition to body weights of dams and offspring, the maternal behavior was also monitored. The weanlings of sleep-deprived dams showed heightened risk-taking behavior as they made increased explorations into the open arms of EPM. They also showed higher mobility in comparison to the control group. Though the body weights of sleep-deprived dams were comparable to those of the control group, their newborns had lower birth weight. Nevertheless, these pups gained weight and reached the control group values during the initial post-natal week. But after weaning, their rate of growth was lower than that of the control group. This is the first report providing evidences for the role of sleep during late pregnancy in shaping the neuropsychological development in offspring.

HIGHLIGHTS of the study:

- First report on hyperactive offspring if mothers had sleep loss during pregnancy.
- First demonstration of risk-taking behavior in adolescents on prenatal sleep loss.
- Babies born to sleep-deprived mothers are low in body weight.
- First evidence on poor growth rate during peri-adolescence in these offspring.
- Highlights importance of maternal sleep in shaping anxiety behavior in children.

Further details are discussed in the published work: Radhakrishnan A, Aswathy BS, Kumar VM, Gulia KK (2015). *Sleep deprivation during late pregnancy produces hyperactivity and increased risk-taking behaviour in offspring.* **Brain Research**. 1596: 88-98.

D. <u>The S-W profile of rat during normal pregnancy</u>

To develop an animal model for studies on peri-partum sleep disorders, sleep patterns in female Wistar rats during pregnancy, post-partum and after weaning, were assessed and associated adaptive changes in their anxiety were examined. Adult nulliparous female rats, maintained in standard laboratory conditions with *ad libitum* food and water, were

surgically implanted with electroencephalogram and electromyogram electrodes under anaesthesia for objective assessment of sleep-wakefulness (S-W). After post-surgical recovery, three control recordings of S-W were taken for 24 h before the animals were kept for mating. After confirmation of pregnancy, S-W recordings were acquired during different days of pregnancy, post-partum lactation/nursing days, and also after weaning. Their anxiety levels were tested in the elevated plus maze. During pregnancy, sleep increased primarily due to increase in light non-REM sleep during dark period. There was an increase in non-REM sleep delta power after parturition, though the sleep was fragmented, especially during daytime. 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.

HIGHLIGHTS of this study:

- First report on sleep-wakefulness during pregnancy-postpartum continuum
- Sleep fragmentation during last trimester of pregnancy and post-partum
- Non-REM sleep delta power was increased during late pregnancy and after parturition
- Post-partum sleep and anxiety were reduced in contrast to ante-partum
- An animal model for drug trials and study of sleep disorders during pregnancy

Further details are discussed in the published work: 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.

E. Evidences for changes in theta coherence and P waves with memory deficits after sleep deprivation in rat model

The theta waves and theta coherence have been shown to facilitate the neuronal communication between the hippocampus, amygdala and the prefrontal cortex for the memory-retrieval and synaptic remodelling. However, the effects of either total sleep deprivation or REM sleep deprivation, on theta coherence between these regions have not

been studied. In rats, P waves were also shown to be associated with learning and memory. In this longitudinal study, changes in the P waves, and theta coherence between the hippocampus, amygdala and the prefrontal cortex, were studied before and after exposing the animals to different regimens of sleep deprivation. Changes in reference and working memory were also simultaneously evaluated. Concomitant with a rebound increase in non-REM sleep after total sleep deprivation, and REM sleep after REM sleep deprivation, there was a decrease in the coherence of theta waves between all the studied areas, especially between hippocampus and amygdala. Though there was an increase in the number of P waves after sleep deprivation, the ratio of P wave cluster to singlet was reduced. There was a decrease in correct memory performance after sleep deprivation. 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 various neural networks. The data for this work is prepared for manuscript submission soon.

The importance of maternal sleep on foetal neural development is evident from the results obtained so far. The findings have provided important information for defining the optimal sleep for a better mother-child health and appropriate development of cognition in pups. The study has thrown open the possibility of having an early electrophysiological marker for assessing later derangements in neuronal development. Role of different components of sleep on various components of cognition were elucidated:

- The study provided the first internationally published report showing a link between maternal REM sleep during late pregnancy and isolation-induced acoustic signals (ultrasonic vocalizations) in rat pups.
- Acute total sleep deprivation during pregnancy increases crying during early postnatal days.
- Total sleep deprivation (acute) during pregnancy increases risk taking behaviour in pre-adolescents.
- Maternal sleep deprivation affects emotional development of babies.

- Ontogenetic profiles of USVs, similar to human baby's cries during critical window are the first signals to modulate mother-child bonding

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

The study provided landmark information on role of sleep during pregnancy in shaping brain for effective cognitive abilities during prenatal to postnatal life. This study provided first scientific evidence on the early developmental risks to the offspring by maternal sleep deprivation. The babies born the sleep deprived mothers (during third term of pregnancy) had clear emergence of hyperactivity and increased risk taking behaviour during adolescence. The depression like symptoms in babies born to the REM sleep deprived mother was another crucial finding of this study. The altered vocalization patterns during neonatal and early infancy could be an early acoustic signal maker for altered cognitive behaviour.

The finding of this study will address sleep associated alteration in neuronal milieu early in life, for a cognitive development later in life. The identification of differences in the effects of acute and chronic sleep deprivation is of practical importance. Moreover, studying the magnitude of changes in cognitive development of the offspring, by sleep deprivation during the three trimesters of pregnancy, will not only be of clinical significance but will also help in policy making. The scope of this study is vast.

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 (2012). Dynamism in activity of the neural networks in brain is the basis of sleep-wakefulness oscillations. **Front Neurol.** 3:38. doi: 10.3389/fneur.2012.00038. eCollection 2012.
2. Gulia KK, Patel N, Radhakrishnan A, Kumar VM (2014). Reduction in ultrasonic vocalizations in pups born to rapid eye movement sleep restricted mothers in rat model. **PLoS One.** 2014 Jan 13;9(1):e84948. doi: 10.1371/journal.pone.0084948. eCollection 2014 Jan 13. (Impact factor 3.5).
3. Gulia KK, Patel N, Kumar VM (2015). Increased ultrasonic vocalizations and risk-taking in rat pups of sleep-deprived dams. **Physiol Behav** 139: 59-66 (Impact factor: 3.03).
4. Radhakrishnan A, Aswathy BS, Kumar VM, Gulia KK (2015). Sleep deprivation during late pregnancy produces hyperactivity and increased risk-taking behaviour in offspring. **Brain Research**. 1596: 88-98 (Impact factor 2.83).
5. 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 3.0).

Book chapter in Handbook of Ultrasonic vocalizations

6. Invited Chapter in Book: **Ultrasonic vocalizations and behavior of rat pups born from sleep-deprived dams** authored by Kamalesh K Gulia and Velayudhan Mohan Kumar. Chapter 11.4, In Handbook of Ultrasonic Vocalization Edited by Stefan M Brudzynski, Handbook of Behavioral Neuroscience series by Elsevier (Accepted)

Reports/ abstracts in Journals/ Proceedings

7. Gulia KK (2014). Sleep restriction during pregnancy and neural plasticity in infants. **Sleep Biological Rhythms** 12; 4: 237. (Impact factor 0.59)
8. Radhakrishnan A, Gulia KK**,** Kumar VM (2014). Altered neuronal communication between the amygdale-hippocampal region and the pontine region on acute sleep deprivation. **Sleep Biological Rhythms** 12; 4: 262. Sleep Biological Rhythms 12; 4: 260. (Impact factor 0.59)

9. Aswathy BS, Gulia KK, Kumar VM (2014). Characterization of the sleep profile in offspring born to dams undergoing sleep deprivation during late pregnancy. **Sleep Biological Rhythms** 12; 4: 265. (Impact factor 0.59)

10. Aswathy BS, Sharaf SS, Kumar VM, Gulia KK (2014). Sleep alterations and anxiety-related changes during pregnancy and postpartum in an animal model. **Sleep Biological Rhythms** 12; 4: 265.

11. Gulia KK. Can sleep loss during pregnancy affect plasticity in neural networks in offspring during early development? in proceedings of International Conference on Integrative and Comparative Physiology & 1st Annual meeting of the Society for Evolutionary and Integrative Biology, **SEIB-2013** held at University of Kerala, 18-20 December 2013.

12. Patel N, Radhakrishnan A, Gulia KK, Kumar VM. Reduced ultrasonic vocalizarions in pups born to REM sleep deprived mothers in rat model: An early marker for depression!" in International Conference on Integrative and Comparative Physiology & SEIB-2013 held at University of Kerala, 18-20 December 2013. **Niraj Patel received 1st poster award for this work.**

13. Aswathy BS, Nair PP, Gulia KK, Kumar VM. Dynamics in sleep-wake parameters during the early ontogenetic development in rat neonates, **ICNS-2013** at Bhubaneshwar, Odisha

14. Gulia KK. Changing sleep dynamics during pregnancy and neural plasticity in offspring in proceedings of International symposium on Emerging Trends and challenges in Neuroscience & 31th Conference of Indian Academy of Neurosciences (**IAN**) held at Allahabad, from 25-27 October 2013.

15. Gulia KK. Maternal sleep loss and plasticity in neural networks in offspring during early development, in proceedings of International Conference in Neurosciences (**ICNS-2013**) held at Raveshaw University, Cuttack, Odisha from 9-11 November 2013.

16. Gulia KK. Rapid Eye Movement (REM) sleep in National Sleep Medicine Course 2013 (**NSMC 2013**), Chennai from 14-15 December 2013

17. Gulia KK, Kumar VM (2012). Relationship between maternal sleep restriction and anxiety in offspring. **Annals of Neurosciences**, Vol 19 Suppl, p 10

18. Radhakrishnan A, Gulia KK, Kumar VM. Effects of acute sleep deprivation on amygdalar-hippocampal theta coherence and pontine wave density in rats in proceedings of International Conference on Integrative and Comparative Physiology & 1st Annual meeting of the Society for Evolutionary and Integrative Biology, **SEIB-2013** held at University of Kerala, Trivandrum from 18-20 December 2013.

The result published in PLoS One was also reported by **e-Telegraph** as following.

http://epaper.telegraphindia.com/paper/4-0-26@01@2014-1001.html

ii. Manpower trained on the project

a) Research Scientists or Research Associates: **JRF (Six)**

b) No. of Ph.D. produced : **One (Continuing)**

c) Other Technical Personnel trained : **2**

ii. Patents taken, if any : None

19. Financial Position:

No	**Financial Position/ Budget Head**	**Funds Sanctioned**	**Expenditure**	**% of Total cost**
I	Salaries/ Manpower costs	11,84 ,600	10,71,382	
II	Equipment	20,00,000	20,37,804	
III	Supplies & Materials	2,50,000	1,76,190	
IV	Contingencies	1,00,000	1,25,085	
V	Travel	1,00,000	68,552	
VI	Overhead Expenses	3,00,000	3,00,000	
VII	Others, if any	-	-	
Total	100%	39,34,600	37,79,013	96.04%

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 Receiver & transmitter	Receiver-RPC1 (DSI) T: F50-EEE (DSI)	2,32,000	2013	90%	Working T Battery low
2	Avisoft USV rat	Avisoft, Avisoft-SAS Pro	6,19,381	2013	70%	Working
3	ANYMAZE	Anymaze system	5,67,381	2013	90%	Working
4	Fabricated mazes	LD;EPM;RAM: OF	57,500	2013	60%	Working
5	Axxonet system	16 channel BESS	5,61,225	2913	80%	Working

b) Plans for utilising the equipment facilities in future

Already being used in the current project sanctioned under DST-CRSI -102

Name and Signature with Date

a. Kamalesh K Gulia

(Principal Investigator)

b. V Mohan Kumar

(Co-Investigator)