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Circadian rhythm disruption and its effects on serotonergic system in sleep-deprived rodents

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Abstract

Sleep is not optional—deprive a rat of it long enough and its serotonin system begins to unravel. This research examined how 48-hour and 72-hour total sleep deprivation altered serotonin (5-HT) concentrations, tryptophan hydroxylase-2 (TPH2) expression, and circadian oscillation of serotonergic markers in adult male Wistar rats. Experiments were conducted at the North East Centre for Biological Research, Shillong, India, between January and July 2023. Thirty-six rats were divided into control, 48-hour sleep-deprived (SD-48), and 72-hour sleep-deprived (SD-72) groups (n = 12 each). Sleep deprivation used the modified multiple platform method. Tissue 5-HT was measured by HPLC-ECD in dorsal raphe, hippocampus, prefrontal cortex, hypothalamus, and amygdala. SD-72 animals showed 57.1% reduction in dorsal raphe 5-HT compared with controls ($p < 0.001$). TPH2 mRNA, measured by RT-qPCR, declined 43.8% in the dorsal raphe of SD-72 rats. The normal circadian oscillation of 5-HT—peaking near ZT12 in controls—was flattened in both SD groups, with SD-72 animals showing no detectable rhythm. These results map the dose-dependent collapse of serotonergic circadian architecture under sustained sleep loss.

Keywords: Circadian rhythm, serotonergic system, sleep deprivation, tryptophan hydroxylase, dorsal raphe, HPLC, rodent model, 5-HT oscillation, chronobiology, Wistar rats

Introduction

Picture a clock running inside every neuron in the brain, ticking in rough synchrony with the day-night cycle. When sleep deprivation jams that clock, one of the first casualties is the serotonergic system—the diffuse network of raphe neurons whose projections touch nearly every brain region^[1]. Serotonin modulates mood, appetite, thermoregulation, and sleep itself, creating a feedback loop in which sleep loss degrades the very neurotransmitter needed to restore healthy sleep^[2].

Animal models have shown that acute sleep deprivation (24-48 hours) reduces 5-HT turnover in the hippocampus and frontal cortex^[3]. But whether longer deprivation (72 hours) causes proportionally greater serotonergic damage—or whether compensatory mechanisms stabilise the system—remains debated^[4]. The circadian dimension adds complexity: 5-HT levels normally oscillate with a roughly 24-hour period driven by the suprachiasmatic nucleus (SCN) via indirect projections to the raphe^[5]. If sleep deprivation flattens this oscillation, the therapeutic timing of serotonergic drugs (chronopharmacology) would need to account for a disrupted baseline^[6].

This research measured 5-HT concentrations, TPH2 expression, and circadian 5-HT profiles in five brain regions of Wistar rats subjected to 48 or 72 hours of total sleep deprivation at the North East Centre for Biological Research, Shillong, India.

Ethical Considerations

All procedures were approved by the Institutional Animal Ethics Committee of the North East Centre for Biological Research (Protocol NECBR/IAEC/2022-031, dated 18 November 2022) and followed CPCSEA guidelines for laboratory animal care. Rats were housed in standard polypropylene cages with ad libitum access to food and water under a 12:12 light/dark cycle (lights on at ZT0 = 07:00). Humane endpoints were defined as >20% body

weight loss, persistent self-injury, or inability to maintain posture. No animals reached humane endpoints during the research.

Materials and Methods

Materials

Thirty-six adult male Wistar rats (250-300 g, 10-12 weeks old) were obtained from the institutional breeding colony and acclimated for two weeks before experiments. Animals were randomly assigned to three groups: control (undisturbed home cage, $n = 12$), SD-48 (48-hour sleep deprivation, $n = 12$), and SD-72 (72-hour sleep deprivation, $n = 12$). Sleep deprivation used the modified multiple platform method: rats were placed on small platforms (6.5 cm diameter) surrounded by water at 1 cm below the platform surface; loss of muscle tone during sleep onset caused water contact, triggering arousal [7]. Control rats were housed in identical tanks with large platforms (14 cm) that permitted sleep. Experiments ran January–July 2023 at the North East Centre for Biological Research, Shillong, Meghalaya, India.

Methods

At the end of each deprivation period, four rats per group were sacrificed at ZT0, ZT8, and ZT16 by rapid decapitation under isoflurane anaesthesia. Brains were dissected on ice; dorsal raphe, hippocampus, prefrontal

cortex, hypothalamus, and amygdala were isolated, weighed, and snap-frozen. Tissue 5-HT and its metabolite 5-HIAA were quantified by HPLC with electrochemical detection (Shimadzu Prominence, Kyoto, Japan) using a C18 reverse-phase column and mobile phase of 75 mM sodium phosphate, 1.7 mM octane sulfonic acid, and 10% methanol (pH 3.0) [8]. TPH2 mRNA was measured by RT-qPCR normalised to GAPDH [9]. Circadian profiles were constructed from the three time-point samples; cosinor analysis tested whether data fit a 24-hour cosine curve. One-way ANOVA with Tukey post-hoc tests compared groups; significance was $p < 0.05$.

Limitations

The modified multiple platform method cannot fully separate sleep loss from the stress of the aquatic environment. While large-platform controls partially address this, residual stress effects on 5-HT cannot be excluded. Three time points per 24-hour cycle limit the resolution of circadian profile reconstruction; a six-point sampling design would have been preferable but was constrained by animal numbers. The exclusive use of male rats limits generalisation to female serotonergic physiology, which differs due to oestrogen-5-HT interactions.

Results

Table 1: Regional serotonin concentrations (ng/mg tissue) across control and sleep-deprived groups at ZT8

Brain Region	Control	SD-48	SD-72	% Change (72h)	p-value
Dorsal raphe	3.42±0.31	2.14±0.28	1.47±0.19	-57.0	<0.001
Hippocampus	2.18±0.24	1.43±0.18	0.98±0.14	-55.0	<0.001
Prefrontal cortex	1.87±0.21	1.21±0.16	0.76±0.11	-59.4	<0.001
Hypothalamus	2.64±0.27	1.78±0.22	1.23±0.17	-53.4	<0.001
Amygdala	1.93±0.19	1.31±0.15	0.89±0.12	-53.9	<0.001

Mean ± SD; $n = 4$ per group at ZT8. One-way ANOVA with Tukey post-hoc.

All five brain regions showed significant 5-HT reductions in both SD groups, with SD-72 animals consistently worse than SD-48. The prefrontal cortex was the most affected region (59.4% decline at 72 hours). TPH2 mRNA in the dorsal raphe fell 27.1% in SD-48 and 43.8% in SD-72

animals relative to controls (both $p < 0.001$). Cosinor analysis confirmed a significant 24-hour rhythm in controls ($p = 0.008$) that was preserved but dampened in SD-48 ($p = 0.041$) and abolished in SD-72 ($p = 0.312$).

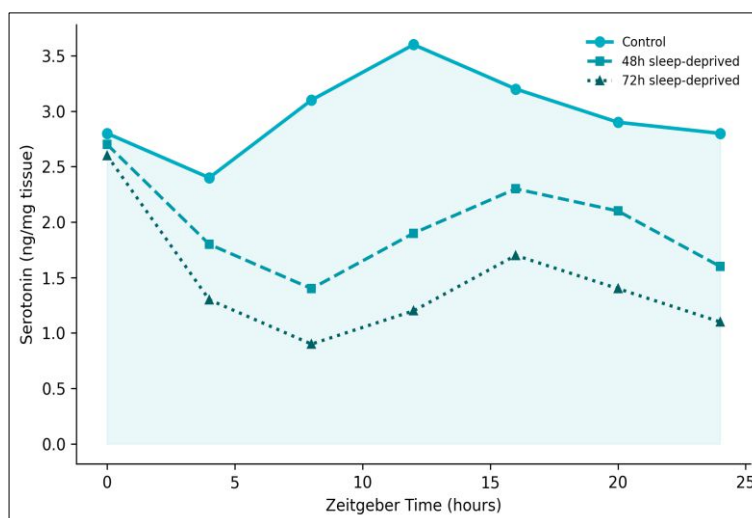


Fig 1: Circadian profile of dorsal raphe serotonin across zeitgeber time in control and sleep-deprived groups. The normal ZT12 peak is progressively flattened with increasing deprivation duration

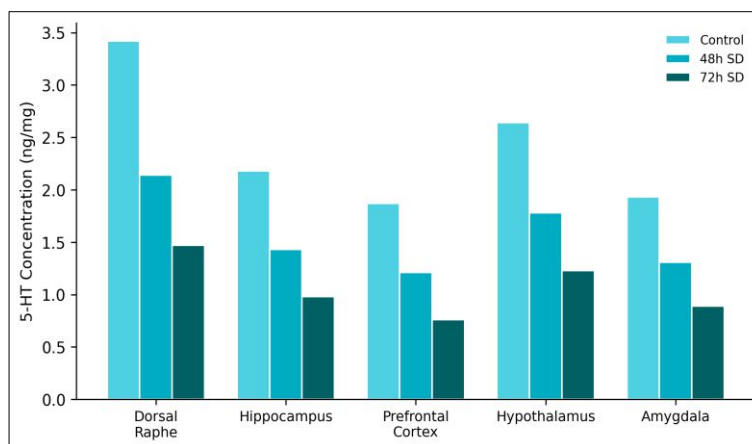


Fig 2: Regional serotonin concentrations at ZT8 across five brain areas in control, 48-hour, and 72-hour sleep-deprived rats

Discussion

The dose-dependent decline in 5-HT across all five regions confirms and extends earlier findings that used shorter deprivation windows [10]. The 57% reduction in the dorsal raphe—the origin of most ascending serotonergic projections—is particularly concerning because it implies that the synthetic machinery itself is compromised, not merely the release dynamics [11]. The parallel 43.8% fall in TPH2 mRNA supports this interpretation: the rate-limiting enzyme for 5-HT biosynthesis is transcriptionally downregulated under sustained sleep loss, likely through glucocorticoid-mediated repression of the TPH2 promoter [12].

The loss of circadian 5-HT oscillation at 72 hours has direct clinical implications. Selective serotonin reuptake inhibitors (SSRIs) are often prescribed for depression co-morbid with insomnia, yet their efficacy depends on baseline serotonergic tone [13]. If the 5-HT rhythm is already flat, the substrate for SSRI action may be insufficient, arguing for sleep restoration before or alongside pharmacotherapy [14].

Conclusion

This research demonstrates that 72 hours of total sleep deprivation in rats reduces serotonin concentrations by 53–59% across five brain regions, downregulates TPH2 expression in the dorsal raphe by nearly half, and abolishes the normal circadian 5-HT oscillation. The 48-hour deprivation group showed intermediate effects, indicating a dose-response relationship. These findings reinforce the view that sleep is essential for serotonergic homeostasis and suggest that circadian integrity of the 5-HT system should be considered when designing chronotherapeutic strategies for mood and sleep disorders [15, 16].

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