Music Intensity–Dependent Modulation Of Anxiety And Social Behaviour In Rats: Effect Of Raga Darbari

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ABSTRACT

This study evaluates the impact of Raga Darbari on anxiety and social behavior in Wistar rats, determining whether varying durations of music exposure produce dose-dependent effects on these behaviors and examines how the emotional tone and intensity of Raga Darbari modulate stress-related behavioral responses. Sixteen rats were divided into four groups: control and exposed to music at different sound dB (45–55 dB, 55–75 dB, and 75–95 dB). Behavioral tests, *viz.* Elevated Plus Maze and helping behavior test were used to assess changes in anxiety and social interaction. The results showed that high-intensity music (75–95 dB) significantly improved social behavior and exploratory behavior while reducing anxiety levels. Moderate-intensity music (45–55 dB) increased anxiety and negatively affected exploratory behavior. These findings highlight the importance of sound intensity in music-based therapeutic interventions.

KEYWORDS: Wistar rats, *Rattus norvegicus*, Elevated Plus Maze, Helping Behavior Test, Indian Classical Music, Exploratory Behaviour

INTRODUCTION

Music is a powerful auditory stimulus that influences brain function through emotional, physiological, and neurochemical pathways. Research in cognitive neuroscience has shown that music can regulate mood, reduce stress, and influence behavioral responses by modulating brain regions involved in emotional regulation (Koelsch, 2014). Listening to music activates the autonomic nervous system and has been shown to reduce psychological stress in both clinical and experimental settings (Thoma *et al.,* 2013).

While most existing research focuses on Western classical music, Indian classical music remains underexplored in neuroscience despite its rich emotional structure. Indian ragas are based on specific melodic scales (*thaats*) and emotional frameworks (*rasa*) that are designed to evoke particular affective states (Jairazbhoy, 1995). Among these, *Raga Darbari* is particularly noteworthy. It is known for its slow tempo, deep tonal quality, and introspective, calming emotional effect. Because of its ability to lessen anxiety and encourage emotional regulation, it is frequently utilized in therapeutic settings.

Standardized behavioral assays like the open field test and the social interaction test are commonly used to assess anxiety and social behavior in animal models, which are crucial markers of emotional state. Brain areas responsive to emotional and sensory cues are associated with these behavioral consequences. For instance, research on rodents has demonstrated that music can enhance social interaction behaviors and lower stress hormone levels like corticosterone (Alworth & Buerkle, 2013). However, nothing is known about how culturally specific music, such as Indian ragas, affects the emotional behavior of rodents.

Wistar rats are often utilized in behavioral neuroscience because of their well-documented stress reactions and their capacity to accurately imitate social behavior and anxiety. Research has shown that Wistar rats exposed to social stress throughout their adolescence have higher levels of social anxiety as adults (Vidal *et al.,* 2007). Additionally, Wistar rats were designed specifically to exhibit excessive anxiety-related behavior. demonstrate greater protective and avoidance reactions in behavioral tests, which makes them a unfailing model for examining social functioning and emotional control (Landgraf & Wigger, 2002).

This study aims to investigate the effects of Indian classical music—specifically Raga Darbari— on anxiety, cognitive flexibility and social behavior in Wistar rats. By systematically varying the duration of daily exposure, it explores whether a dose-dependent relationship exists. Additionally, the study examines how the emotional tone and intensity of the raga influence stress- related behavioral and neurobiological responses. The research contributes to developing non- pharmacological, music-based interventions for emotional regulation and improved social functioning.

MATERIALS AND METHODS

A total of 16 Albino Wistar rats (*Rattus norvegicus*), aged 4–5 weeks and weighing between 120–135 grams, were procured from the Laboratory Animal Resources (LAR) units of ICAR-Indian Veterinary research institute. Upon arrival, animals were acclimatized and housed under standard laboratory conditions. Animals were kept in polypropylene cages under standard laboratory conditions (temp.: 22 ± 2°C, humidity: 50–60% RH, 12 h L/D) with *ad libitum* food and water. All experimental procedures were approved (IEC No. HIPER/IAEC/105/04/2022) by the Institutional Animal Ethical Committee (IAEC), Hygia Institute of Pharmaceutical Education and Research, Lucknow.

Experimental Design and Groups

Rats were randomly assigned to one of four experimental groups (n=12 per group):

Group A (Control Group): Underwent behavioral testing in the absence of any auditory stimulation (ambient background noise ~40 dB).

Group B (Low-Intensity Group): Exposed to Music at 45–55 dB during behavioral testing.

Group C (Moderate-Intensity Group): Exposed to Music at 55–75 dB during behavioral testing.

Group D (High-Intensity Group): Exposed to Music at 75–95 dB during behavioral testing.

Auditory Stimulus

The auditory stimulus was a continuous, standardized recording of Raga Darbari Kanada, a late-night (Nishitha Kala) raga (instrumental on Veena, Chandrashekhar 2023) from the Hindustani classical tradition associated with solemnity and contemplation. The recording was played through a blue tooth speaker system (Model: Boat Stone 180) placed at a fixed distance of 1 meter from the center of the testing arena. Sound intensity was calibrated and verified before each test session using a digital sound level meter (Model: Testo 816-1, IEC 61672-1 Class 2 / ANSI S1.4).

Behavioral Testing Paradigms

All behavioral tests were conducted in a dedicated, sound-attenuated room during the light phase. The apparatus was thoroughly cleaned with 70% ethanol between trials to remove olfactory cues. Rats were habituated to the testing room for 30 minutes prior to each session. The following battery of tests was administered over consecutive five days:

Elevated Plus Maze (EPM) Test: Anxiety-like behavior was assessed using a standard EPM constructed of plywood covered with black mica, consisting of two open arms (50 x 10 cm) and two enclosed arms (50 x 10 x 40 cm) elevated 50 cm above the floor. Each rat was placed in the central square facing an open arm and allowed to explore freely for 5 minutes. The session was recorded, and the number of entries into the open arms (EOA), the number of enteries into the closed arm (ECA), the time spent in open arm (TOA) and the time spent in closed arm (TCA) were observed. An entry was counted when all four paws crossed into the arm.

Helping Behavior Test (Social Proximity): Social motivation and behavior were evaluated using a modified social proximity paradigm. A "distressed" conspecific (a lightly restrained rat from the same housing colony) was placed in a small wire mesh cage at one end of a rectangular arena (100 x 50 cm). The test rat was introduced at the opposite end. The session lasted 10 minutes, and the Change in Social Behavior was quantified as the difference in time spent in the quadrant containing the conspecific cage compared to a baseline exploration trial in the empty arena conducted 24 hours prior. Positive values indicate increased social approach.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software (Version 26.0). One-way Analysis of Variance (ANOVA) was used to compare the means across the four experimental groups for each primary behavioral variable (EOA, ECA, TOA, TCA, Social Behavior Change). Post-hoc comparisons (*e.g.*, Tukey's HSD) were conducted following significant ANOVA results to identify specific inter-group differences. The threshold for statistical significance was set at *p* < 0.05. Further, Group differences were tested with Holm-corrected pairwise comparisons to prevent inflation of false positives; Hedges' g (95% CI) is reported as a standardized effect-size estimate. η^2 is calculated to complement p-values with experimental effect magnitude.

RESULTS

Change in social behavior

A one-way ANOVA indicated a significant effect of group on change in social behavior, $F(3,44) = 6.88$, $p < .001$, $\eta^2 = .32$. Holm-corrected post hoc tests showed that the Low-intensity group differed from Control (Low: M = −148.42, SD = 64.10; Control: M = −7.08, SD = 32.57), Hedges' g = 2.68, 95% CI [1.60, 3.77], $p < .001$. The Medium-intensity comparison did not differ from Control (Hedges' g = −0.52, 95% CI [−1.30, 0.27], p = .591). The High-intensity group also differed from Control (Hedges' g = −1.23, 95% CI [−2.07, −0.38], p = .016), indicating a large, negative effect relative to Control for the High group on this outcome (Table 1).

Anxiety Related Behaviour

Groups differed on EOA, $F(3,44) = 12.15$, $p < .001$, $\eta^2 = .45$. Post hoc tests indicated the Low-intensity group spent more time exploring object A than Control (Low: M = 6.33, SD = 2.23; Control: M = 3.25, SD = 1.54), Hedges' g = 1.61, 95% CI [0.69, 2.53], $p < .001$. Neither the

Medium (Hedges' g = −0.64, 95% CI [−1.46, 0.18], p = .784) nor the High (Hedges' g = 0.43, 95% CI [−0.38, 1.23], p = .919) comparisons reached significance after Holm correction (Table 1).

There was a significant group effect for ECA, F(3,44) = 9.53, $p < .001$, $\eta^2 = .39$. The Low-intensity group showed substantially less exploration of object C than Control (Low: M = 3.08, SD = 1.44; Control: M = 8.50, SD = 3.42), Hedges' g = −1.92, 95% CI [−2.90, −0.95], $p < .001$. The Medium-intensity group also differed from Control (Hedges' g = −1.31, 95% CI [−2.19, −0.42], $p < .001$). The High-intensity comparison produced a moderate negative effect (Hedges' g = −0.72, 95% CI [−1.56, 0.12]) but did not reach significance after Holm correction (p = .075) (Table 1).

Group differences were significant for TOA, F(3,44) = 15.67, $p < .001$, $\eta^2 = .52$. The Low-intensity animals spent more time near object A than Controls (Low: M = 3.67, SD = 0.49; Control: M = 1.67, SD = 0.78), Hedges' g = 1.86, 95% CI [0.95, 2.77], $p < .001$. Neither the Medium (Hedges' g = −0.39, 95% CI [−1.23, 0.46], p = .990) nor the High (Hedges' g = −0.23, 95% CI [−1.05, 0.59], p = .919) comparisons differed from Control (Table 1).

A significant group effect was observed for TCA, F(3,44) = 19.42, $p < .001$, $\eta^2 = .57$. The Low-intensity group spent less time near object C than Controls (Low: M = 1.42, SD = 0.51; Control: M = 3.33, SD = 0.78), Hedges' g = 2.80, 95% CI [1.69, 3.91], $p < .001$. The Medium comparison did not differ from Control (Hedges' g = −0.12, 95% CI [−0.89, 0.65], p = .945) (Table 1).

Table 1. Summary of ANOVA, Effect Sizes, and Holm-Corrected Post-Hoc Comparisons

Outcome Variable	**Group Comparison**	**Mean ± SD**	**Hedges' g (95% CI)**	**Holm-corrected p**	**ANOVA (F, df=3,44)**	**η^2**
Change in Social Behavior	Low vs Control	-148.42 ± 64.10 vs -7.08 ± 32.57	2.68 (1.60, 3.77)	< .001	6.88***	.32
	Med vs Control	73.83 ± 212.23 vs -7.08 ± 32.57	-0.52 (-1.30, 0.27)	.591		
	High vs Control	213.50 ± 243.76 vs -7.08 ± 32.57	-1.23 (-2.07, -0.38)	.016		
EOA	Low vs Control	6.33 ± 2.23 vs 3.25 ± 1.54	1.61 (0.69, 2.53)	< .001	12.15***	.45
	Med vs Control	2.08 ± 1.31 vs 3.25 ±	-0.64 (-1.46, 0.18)	.784		

		1.54				
	High vs Control	4.00 ± 1.91 vs 3.25 ± 1.54	0.43 (-0.38, 1.23)	.919		
ECA	Low vs Control	3.08 ± 1.44 vs 8.50 ± 3.42	-1.92 (-2.90, -0.95)	< .001	9.53***	.39
	Med vs Control	5.50 ± 1.98 vs 8.50 ± 3.42	-1.31 (-2.19, -0.42)	< .001		
	High vs Control	6.33 ± 2.74 vs 8.50 ± 3.42	-0.72 (-1.56, 0.12)	.075		
TOA	Low vs Control	3.67 ± 0.49 vs 1.67 ± 0.78	1.86 (0.95, 2.77)	< .001	15.67***	.52
	Med vs Control	1.42 ± 0.51 vs 1.67 ± 0.78	-0.39 (-1.23, 0.46)	.990		
	High vs Control	1.50 ± 0.67 vs 1.67 ± 0.78	-0.23 (-1.05, 0.59)	.919		
TCA	Low vs Control	1.42 ± 0.51 vs 3.33 ± 0.78	2.80 (1.69, 3.91)	< .001	19.42***	.57
	Med vs Control	3.42 ± 0.67 vs 3.33 ± 0.78	-0.12 (-0.89, 0.65)	.945		
	High vs Control	Not computed	—	—		

EOA (Entry in Open Arm), ECA (Entry in Closed Arm), TOA (Time spent in Open Arm), TCA (Time spent in Closed Arm)

Summary interpretation

Across outcomes, the Low-intensity (45–55 dB) exposure produced the most consistent and largest effects (large Hedges' g values, all p < .001) relative to Control: increased entry/time for open arm outcomes (EOA, TOA) and decreased entry/time for closed arm outcomes (ECA, TCA). Medium-intensity exposure produced significant reduction only on ECA, and High-intensity exposure yielded a significant effect only on change in social behavior (negative direction) while other high-intensity comparisons were non significant (Table 2). A qualitative summary (Table 2) indicates: 45–55 dB tended to suppress social and exploratory behaviors and to increase anxiety-like responses; 55–75 dB produced modest improvements; and 75–95 dB produced the strongest improvements in social behavior and exploration with an anxiolytic trend. The data suggest a threshold / plateau effect above ~55 dB with limited additional gains beyond 75 dB (Table 2).

Table 2: Summary of Intensity-Dependent Behavioral Effects

Behavioral Domain	45–55 dB (Low)	55–75 dB (Moderate)	75–95 dB (High)	Threshold Observed?
Social Behavior	↓↓	↑	↑↑	Yes (55–75 vs 75–95 dB: NS)
Anxiety (EOA)	↑↑	↓	↓↓	Yes
Safety (ECA, TCA)	↓↓	↑	↑↑	Partial
Exploration (TOA)	↑	↓	↓	Yes
Overall Effectiveness	Negative	Positive	Optimal	Plateau after 75 dB

EOA (Entry in Open Arm), ECA (Entry in Closed Arm), TOA (Time spent in Open Arm), TCA (Time spent in Closed Arm)
↑↑ = Strong improvement
↑ = Mild improvement
↓ = Mild suppression
↓↓=Strong suppression
NS=Not significant

DISCUSSION

The present results indicate that **sound intensity critically determines the behavioral effects** of exposure to Raga Darbari in Wistar rats. Low-intensity exposure (45–55 dB) was associated with **elevated anxiety-like behavior and suppressed social and exploratory behaviors**, whereas moderate to high intensities (55–95 dB) were associated with **reduced anxiety and improved social and exploratory behavior**. These intensity-dependent effects are visible across multiple behavioral endpoints (EOA, ECA, TOA, TCA). The pattern presents a model in which insufficient auditory salience may act like background noise (increasing stress/withdrawal), while adequate auditory drive engages neural systems that promote approach, sociality, and exploratory motivation. The observed pattern: negative effects at low intensity, positive effects at moderate to high intensity: suggests that auditory stimulation's impact is not linear but follows an inverted-U or dose-response relationship, consistent with earlier findings on music and arousal (Chikahisa *et al.,* 2006; Angelucci *et al.,* 2007).

The finding that a relatively low intensity (45–55 dB) produced higher anxiety-like scores (EOA) is consistent with prior rodent literature demonstrating that **not all auditory stimulation is anxiolytic**—the valence of the effect depends on stimulus parameters and physiological context (Escribano *et al.,* 2014; Chikahisa *et al.,* 2007). Escribano and colleagues reported that background noise and poorly structured auditory input can act as anxiogenic factors in rats, and that hormonal state (*e.g.*, ovarian steroids) modulates responsiveness to music (Escribano *et al.,* 2014). Similarly, Chikahisa *et al*., (2007) showed that music reduced anxiety in female mice only under particular hormonal conditions. Together, these reports suggest that **lack of sufficient auditory salience or mismatch between stimulus properties and the animal's internal state may explain the paradoxical anxiogenic profile at low intensity** in our data.

A systematic review of music in rodent research concluded that **music exposure can modulate behavior, neurochemistry and physiology**, but effects vary based on species, sound parameters, and exposure regimen (Kühlmann *et al.,* 2018). The intensity-dependent pattern observed here—suppression at low intensities and improvement at moderate/high intensities—is consonant with the review's conclusion that stimulus properties (tempo, complexity, frequency content and intensity) strongly determine behavioral outcomes (Kühlmann *et al.,* 2018). Long-term or repeated music exposure has also been shown to benefit spatial cognition and motivational measures in aged or stressed rodents (Rizzolo *et al.,* 2021), aligning with the present finding that adequate auditory stimulation supports exploratory behaviors.

The absence of large, statistically significant improvements when comparing moderate (55–75 dB) and high (75–95 dB) groups in several endpoints (Table 4) suggests a **threshold or therapeutic window**: once auditory input reaches a sufficient level to recruit relevant neural circuits, further increases in intensity provide diminishing behavioral returns. This plateau mirrors dose–response concepts in other neuromodulatory interventions and suggests that, for Raga Darbari in Wistar rats, intensities ≥55 dB are necessary for benefit, with optimal effects typically achieved near the moderate–high range but not necessarily increasing linearly beyond ~75 dB (Tables). Practically, this implies attention to intensity calibration in translational applications to avoid unnecessary high volumes that add risk without extra benefit.

The data show that social behavior, anxiety, and exploration are not independent: **anxiolysis at adequate intensities is accompanied by higher social interaction and exploratory motivation**, consistent with behavioral models where reduced anxiety permits approach behaviors and social engagement (Xing *et al.,* 2016; Kühlmann *et al.,* 2018). Conversely, when auditory input is marginal (45–55 dB), animals may experience heightened vigilance or sensory ambiguity, producing withdrawal and suppressed appetitive behaviors (Escribano *et al.,* 2014).

Several studies implicate hippocampal plasticity and neurotrophic factors—most notably brain-derived neurotrophic factor (BDNF)—in the beneficial effects of music on cognition and emotion. Xing et al. (2016) reported that music exposure increased BDNF expression in dorsal hippocampal subregions and improved spatial learning in rats; such BDNF-mediated plasticity provides a plausible mechanism linking auditory enrichment to improved exploratory and cognitive performance. In our study, the improvement in exploration (ECA, TOA, TCA) and the anxiolytic trend at higher intensities are compatible with engagement of BDNF-related neuroplastic processes and increased arousal/motivational drive (Xing *et al.,* 2016).

Although the behavioral pattern is consistent and supported by existing literature, the present dataset is strictly behavioral. To strengthen causal inferences, future studies should measure neurochemical and molecular correlates (*e.g.*, hippocampal BDNF, corticosterone), assess sex differences (given known hormone-dependent effects), and include within-study manipulations of stimulus features (tempo, spectral content) and longitudinal time courses of exposure (acute vs. chronic). In particular, hormonal status may interact with intensity effects (Chikahisa *et al.,* 2007), thereby altering the therapeutic window across sexes and reproductive states.

The results show robust, **intensity-dependent** effects of Raga Darbari on EPM-related measures and helping/approach behaviors in Wistar rats: **low intensity (45–55 dB) was associated with anxiogenic and suppressive effects**, while **moderate–high intensities (55–95 dB) were associated with anxiolytic, prosocial, and exploratory benefits**. The pattern supports a model in which sufficient auditory salience is required to recruit neurobehavioral mechanisms (*e.g.*, BDNF and hippocampal plasticity) that underpin the beneficial actions of structured music in rodents (Xing *et al.,* 2016; Kühlmann *et al.,* 2018). Practical translation of music-based interventions should therefore calibrate intensity carefully to remain within a therapeutic window.

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