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Behavioural Alterations in Wistar rats induced by Mobile Phone Auditory Stimuli in a Controlled Setting.

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ABSTRACT

Researchers, staff of the animal centre, students and trainers frequently use their mobile phones inside the animal centre. Therefore, this study was designed to determine the effect of mobile phone ringtones as an auditory stimulus on the behaviour of Wistar rats. This study was conducted at the Medical Research Institute, Colombo, Sri Lanka. Rats were separated into a controlled area and left for 1 hour before experimentation. The initial behaviour was examined for 10 minutes as the control. Rats were exposed to mobile sample ringing from 200-850Hz for one minute and the ethograms were recorded for 10 minutes. This procedure was followed for female and male rats (gender) and young and adult rats (maturity) under diverse housing conditions. After the intervention, the ethogram showed increased freezing and sleeping, and decreased walking and rearing behaviours. The study indicates that these alterations in rat behaviour upon exposure were independent ($p>0.05$) of gender, maturity, or housing style (single, pair, or group of four). This study concluded that the results of behavioural research using Wistar rats may be affected by mobile phone ringing at the animal centre, which can induce stress or defensive-related behaviour.

Keywords : Behavioral studies, ethogram, mobile phone ringing, Wistar rats

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INTRODUCTION

Animal studies play a pivotal role in preclinical research by facilitating the assessment of drug efficacy, toxicological effects, chemical antigenicity, and other physiological consequences. These animal-based studies ensure the safety and effectiveness of scientific interventions before their application to humans (clinical research), thereby preventing potential health-related suffering (Mukharjee et al., 2022). The widely utilized laboratory animal models in such research include mice, rats, rabbits, guinea pigs, birds, non-human primates and their genetically or biologically modified variants (Robinson et al., 2019). Changes in behavioural patterns in laboratory animals serve as indicators of underlying physiological and psychological consequences. Therefore, intervention-based behavioural assessments are an integral element of animal studies, serving as a tool for studying outcomes by altering hor-

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mone levels and other biological parameters through interventions (Yoruk et al., 2022). The behaviour of laboratory animals could also be observed to identify physical health conditions before their use in research (Dawkins, 2003). And also, behavioural studies of animals are used as a parameter for estimating animal welfare status in animal husbandry (Temple et al., 2011). Laboratory animals are particularly sensitive to their environmental variables, including temperature, humidity, lighting, and, notably, noise. Variations in environmental factors alter animals' overall well-being, including behavioural patterns, potentially impacting research outcomes (Mogil, 2017). Therefore, controlling environmental factors is essential for ensuring the reliability and validity of experimental results (Castelhano-Carlos & Bauman, 2009). Noise, defined as an unwanted or disruptive sound, is a significant environmental stressor in animal facilities. Animal facilities are usually established according to guidelines to ensure minimal interference from environmental factors in experimental observations. Sources of noise within animal research facilities include ventilators, equipment operation, and human activities, such as talking, loud speaking, shouting and equipment handling. Studies have demonstrated that such noises can induce stress responses, phenotypically or physiologically, in laboratory animals, leading to alterations in their behaviour, physiological parameters, and anatomical features (Krohn et al., 2011). There were many studies conducted to assess the effect of noise on alteration of the laboratory animal behaviour and social interactions, and anatomical and physiological parameters of health (Krohn et al., 2011; Corbani et al., 2021; Lauer et al., 2009; Voipio et al., 2006; Naqvi et al., 2012). In many of these studies, it is emphasized that the effect of noise on laboratory animals varies depending on the animal species, exposure duration, noise type, and noise volume or frequency. However, few studies have assessed the effects of noise on rats. None of the studies has evaluated the effects of sudden mobile phone sounds on behavioural alterations in laboratory animals. Thus, this study focused on the influence of mobile phone ringing sounds on the behavioural patterns of rats. Usually, animal centre staff, researchers, and other handlers (e.g., students and trainers) use their mobile phones there. Some researchers use their mobile phones without being in silent mode, even though they are observing behavioural changes as a study outcome. As animals are very sensitive to noise, they could change their behaviour in response to short-term sound exposures as well. Therefore, it is important to study the effects of mobile phone ringtones in behavioural studies. The present study aimed to address this gap by evaluating the behavioural changes in Wistar rats exposed to mobile phone sounds in an animal research facility. By assessing the effects of sudden auditory stimuli, such as ringing tones and vibrations, this research seeks to provide insights into how mobile phone use in animal centres might impact behavioural studies. The findings of this study have the potential to inform best practices for minimizing auditory disturbances in research environments, thereby enhancing the accuracy and reliability of behavioural assessments.

MATERIALS & METHODS

Study design, setting, animals & housing and sample size determination

The ARRIVE 2.0 guidelines (Animal Research: Reporting of In Vivo Experiments) were followed during the testing and reporting of this study. This interventional study was conducted at the Medical Research Institute, Colombo, Sri Lanka. Wistar rats were selected as the choice of animal to observe changes in behaviour due to the stimulation of a mobile phone ringing in the animal husbandry, as Wistar rats exhibit stable and well-known behaviour, facilitating accurate observations and have a low tendency of hearing defects (Casarrubea et al., 2019b; Koch et al., 2021). Due to a lack of data in the literature on the "power equation", the sample size was calculated using the "resource equation" (Festing, 2016), accounting error degrees of freedom (E), total number of animals per group (N), Number of animal groups (G), with the consideration of $10 < E < 20$. The calculated total number of animals for a protocol was 24 (6 Wistar rats per study group across 4 groups), with an acceptable E value $(6 \times 4) - 6 = 18$. Overall, 32 2-month-old and 6-month-old Wistar rats were recruited as per the protocols. As all procedures were designed to ensure no harm or undue stress to the animals, the repeated-measures design reduced the total number of animals used, in compliance with the 3Rs principle in animal research. Therefore, the animals used in one protocol were subjected to a lag period (24 hours) and reused in another protocol, indicating that the lag period was sufficient to return to baseline behaviour. Also, sound exposures were separated by 60-minute intervals within each protocol to minimize habituation and stress to animals (Grieco et al., 2021). The use of ascending order of sound stimuli and sufficient inter-stimulus intervals was implemented to minimize carry-over effects, which may occur due to non-randomization. This reuse strategy is used to reduce animal use while maintaining valid observations in the study.

Procedure

All the study protocols were conducted during the light phase of a 12:12 light-dark cycle.

The selected rats were qualitatively observed for any response to a noise (clapping) applied to their normal auditory sensitivity. The rats that responded to noise were selected for the study.

Preparation of a controlled exposure area

The Principal Investigator was allowed to acclimate the rats to testing to avoid behavioural changes caused by human contact. An acoustically insulated, separate experimental room (2.5m x 2.5m) was arranged to allow the observation of the sounds of mobile phones to rats without other noise-emitting instruments, i.e., laptops, radios, laboratory equipment, or other speakers.

The rats were housed in standard polycarbonate transparent rat cages (56 cm × 35 cm × 20 cm) with autoclaved wood shavings as bedding. Each cage accommodated groups of four, pairs, or single housing, where possible, under a 12:12 light-dark cycle with controlled temperature (24°C), humidity (50-70%), and ventilation (15-20 air circulations per hour). Food and water were provided ad libitum.

Gender differences in behavioural responses to auditory stimuli in rats

A total of 12 male and 12 female rats (6 months old) were kept in groups of 4 each (6 groups). The animals were taken to separate areas where the study was conducted and allowed to habituate to the new environment, minimizing stress and ensuring baseline behavior before exposure to the stimuli. As the control, the initial behavioural patterns of experimental animals were observed for 10 minutes. The mobile phone sound stimuli were played from a speaker positioned 20 cm horizontally and this distance was consistently maintained for all cages to ensure uniform sound exposure across all experimental animals. The animals were exposed for 1 minute to each of the mobile sample ringing sounds, varying in its frequencies as sample ringing sound 1 (SR-S1) (0.20-0.25kHz, low frequency), sound 2 (SR-S2) (0.40-0.45kHz, moderate low frequency), sound 3 (SR-S3) (0.60-0.65kHz, moderate high frequency), and sound 4 (SR-S4) (0.80-0.85kHz, high frequency) with a 60 minutes-intervals between each stimulus (**Figure 1**). Sound intensity (dB SPL) was not directly measured; however, all auditory stimuli were delivered using the same device, fixed volume setting, and constant distance (20 cm), ensuring consistent sound exposure across all experiments. These auditory stimuli consisted of recorded real-world mobile phone polyphonic, melodic ringtones commonly used in Sri Lanka. The sample ringing sounds were presented to Wistar rats in ascending order of frequency (lowest to highest), with a lag between each sound to prevent habituation and auditory overlap. The behavioural changes were observed for 10 minutes immediately after each stimulus, and the ethograms (**Table 1**) were recorded and videoed. These video footages of protocols were blinded to the group assignments to reduce observer bias. The ethogram, including behavioural patterns of accessing activity levels, anxiety-related behaviours and changes in movement or posture, was developed by referring to the literature to record the frequencies of their behaviours (Casarrubea et al., 2019a).

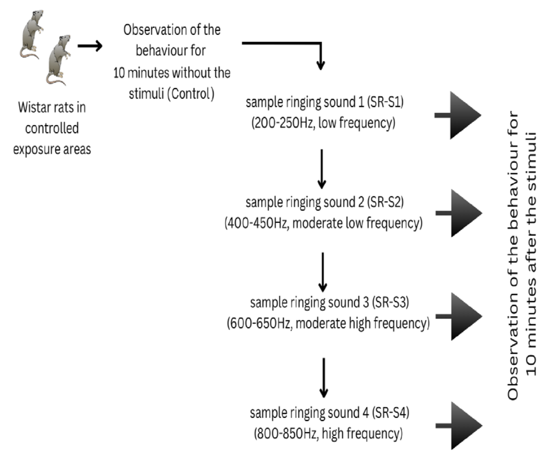


Figure 1: Schematic diagram of the study protocol of introducing mobile phone ringing sounds as an auditory stimulus for Wistar rats. The ethograms were developed in the control and after each stimulation. Adult, young, male, or female Wistar rats in suitable caging were used as per the testing protocol.

Evaluation of behavioural changes in young and adult rats

If a total of 24 animals were needed for the protocol, as calculated, an additional 8 rats (4 young and 4 adults, in equal numbers of males and females) were included to avoid interference from gender or housing differences. Therefore, the 16 young (2 months old) and 16 adults (6 months old) rats were separated into groups of four animals (8 groups), and the procedure described in **Figure 1** was followed.

Evaluation of behavioural changes of rats in different housing patterns

12 male and 12 female rats (6 months old) were kept in single housing, pairs, or groups of 4 as per the testing protocol. All 24 rats were assessed for each housing pattern, with a 1-day settlement period after changing the pattern to facilitate their normal behaviours. The random selec-

tion of animals was followed by pairing and grouping. As the control group for a particular experimental procedure (i.e., single, pair, or group housing), rats in each housing pattern without exposure to sound stimuli were observed. The experimental procedure for the intervention and the observing ethograms described in **Figure 1** were followed for all three housing patterns.

Table 1 Descriptions and abbreviations of the behavioural ethogram of Wistar rats in response to stimuli used in behavioural assessment

Abbreviation of the behaviour	Description of the behaviour
WA	The rat walks around the animal cage
RA	The rat walks around against a stimulus
BA	The rat slightly arched the back of its body
ST	The rat falls off the balance
IS	The rat is immobilized at a place and sniffs
CL	The rat tends to climb in the cage
FD	The rat eats the provided food pellets
DK	The rat drinks water via the water pipe
FS	The rat scrubs its face and body
FPL	The rat licks its forepaws
HPL	The rat licks its hind paws
SL	The rat sleeps
FR	The rat expresses a fixed position for a period

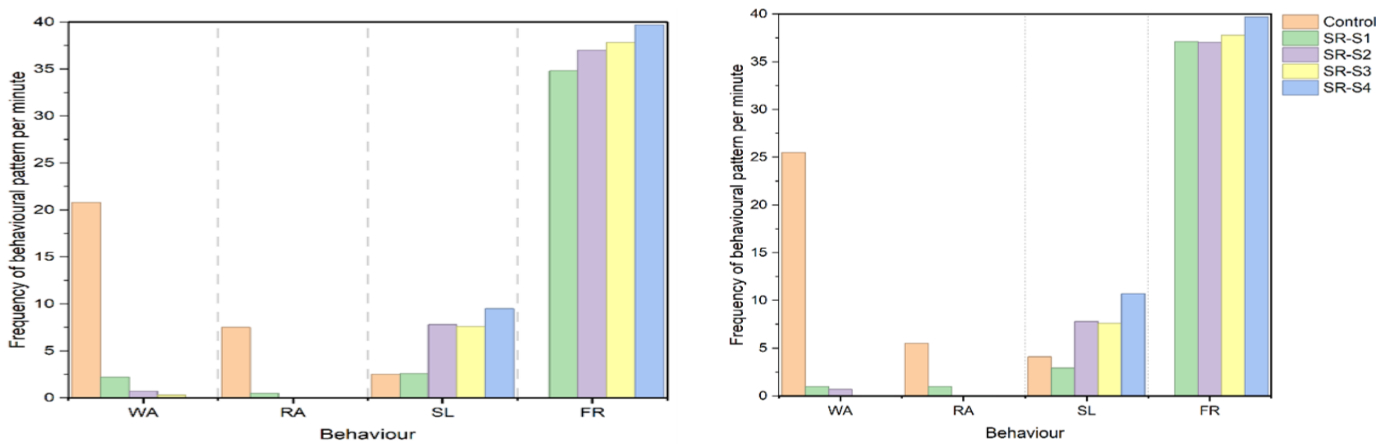
Data analysis

The data were assessed qualitatively and quantitatively using IBM SPSS version 25.0 and OriginPro 2025. The ethograms for each protocol were presented separately in a table as frequencies. The median frequencies of each behaviour between pairs and groups were assessed. The differences between regular behavioural patterns and behaviour after the intervention were statistically analysed using the Kruskal–Wallis H test at a 95% significance level, after confirming non-normality with the Shapiro-Wilk test. The frequencies of behavioural patterns before and after the sound stimulation in between groups were compared using the Mann-Whitney U test at a 95% significance level. Effect sizes (r) were calculated using the formula $r = Z/\sqrt{N}$, following the standard interpretations (small = 0.1, medium = 0.3, large = 0.5). Although formal power calculations were not performed, the sample size was determined using the resource equation method, ensuring an adequate error degree of freedom (E = 18), which is acceptable for exploratory animal studies (Festing, 2016).

RESULTS

Gender differences in behavioural responses to auditory stimuli in rats

Six-month-old, twelve adults male Wistar rats (193.0±8.0g) and twelve adult female Wistar rats (124.0±13.0g) were shown behavioural changes to the different frequencies of mobile phone ringing sounds, as in **Figure 2**. The male and female control groups exhibited a similar behavioural pattern ($p = 0.850$) at the 95% significance level. Before the auditory stimulation, in the male and female control groups, rats commonly exhibited "walking (WA)" (23.2±3.3/min), "rearing (RA)" (6.5±1.4/min) and "immobilization & sniffing (IS)" (5.5±2.8/min) behaviours while the absence of behaviours in ethogram i.e., "freezing (FR)", "back-arching (BA)", "stumbling (ST)" and "face or body scrubbing (FS)" during the observational period of the control groups. However, three of them (BA, ST, and FS) were also not expressed after the sound stimuli. Rats showed relatively lower frequencies for "feeding (FD)" (0.2±0.1/min), "hind paw licking (HPL)" (0.2±0.1/min) and "forepaw licking (FPL)" (0.1±0.0/min) behaviours during the whole observational period (Table 2). There was no significant difference ($p=0.068$) between the behaviour of male and female rats after each sample of ringing sounds (Significance level $p=0.05$, 95% CI). Significant differences in behaviour were observed between the control and experimental groups in both male ($p=0.012$) and female ($p=0.034$) Wistar rats, with a large effect size ($r > 0.5$), indicating substantial behavioural alterations following auditory exposure. The majority of male and female rats commonly expressed FR (37.6±1.6/min), "sleeping (SL)" (7.1±2.9/min) and IS (5.1±2.1/min) behaviours after exposure to all low and high frequencies. The FR and SL were increased dramatically from 36.0±1.6/min in SR-S1 to 39.7±0.0/min in SR-S4 and from 2.75±0.2/min in SR-S1 to 10.1±0.8/min in SR-S4, respectively. Although rats' IS was comparatively increased after applying SR-S1 to 6.9±2.2/min, it was decreased eventually from SR-S2 (6.0±2.8/min) to SR-4 (5.1±2.1/min). On average, WA was observed at 0.6±0.3/min in male and female Wistar rats after sound stimulation. There was a sharp drop of WA (1.6±0.8/min) in SR-S1 compared to the frequencies of the control groups and it was totally absent in SR-S4.



(a) (b)

Figure 2: Prominent behavioral frequency changes in male (a) and female (b) Wistar rats in control (without intervention) and different sound stimulations. The sample ringing sounds (SR-S) are categorized as follows: SR-S1 (0.20-0.25kHz, low frequency), SR-S2 (0.40-0.45kHz, moderate low frequency), SR-S3 (0.60-0.65kHz, moderate high frequency), and SR-S4 (0.80-0.85kHz, high frequency). WA, RA, SL and FR refer to walking, rearing, sleeping and freezing behaviours, respectively.

Table 2: Descriptive statistics (Median±SD) of behavioural frequencies of Wistar rats in the categories under gender variation, maturity of animal and different housing patterns; Abbreviations of behavioural patterns are stated in Table 1.

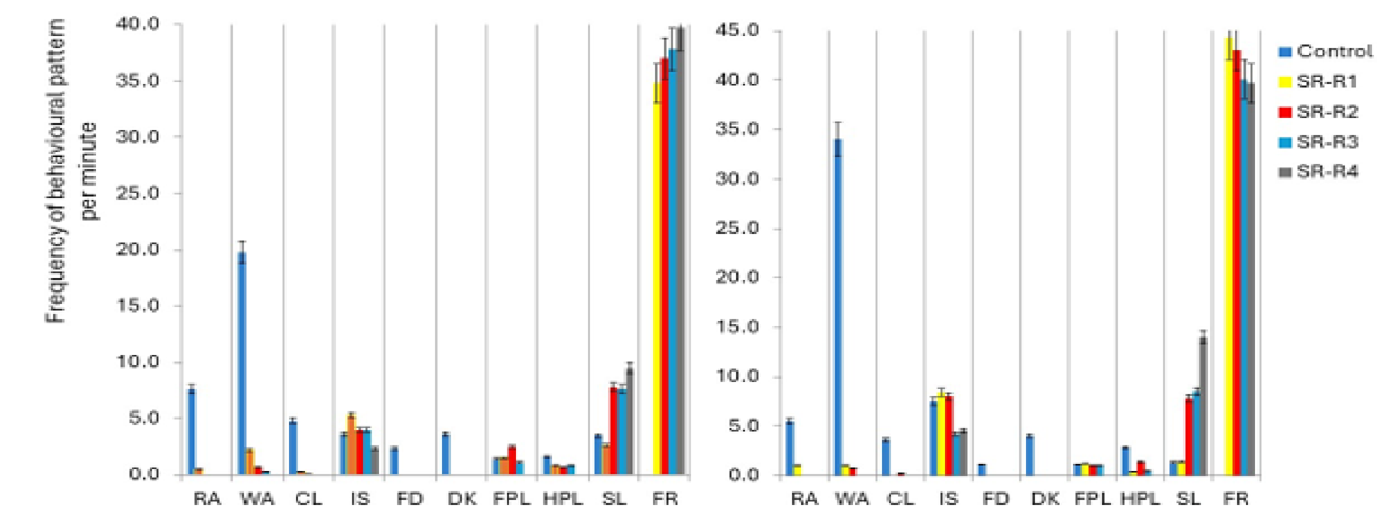
Behaviour	Group	Average frequency of the behaviour (per minute)						
		Gender variation (n=24)		Maturity of animal (n=32)		Housing patterns (n=24)		
		Male	Female	Young	Adult	Single	Pair	Group of four
WA	Control	20.8±0	25.5±0	34.0±0	19.8±0	45.0±0	38.0±0	25.0±0
	Experimental	0.8±0.9	0.4±0.5	0.4	0.8	0.4	0.4	0.4
RA	Control	7.5±0	5.5±0	5.5±0	7.6±0	8.9±0	7.3±0	5.5±0
	Experimental	0.1±0.3	0.3±0.5	0.3	0.1	0.3	0.3	0.3
BA	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Experimental	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ST	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Experimental	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IS	Control	3.6±0	7.5±0	7.5±0	3.6±0	7.5±0	7.5±0	7.5±0
	Experimental	3.9±1.2	6.3±2.2	6.3	3.9	6.3	6.3	6.3
CL	Control	4.8±0	3.6±0	3.6±0	4.8±0	16.2±0	3.6±0	3.6±0
	Experimental	0.1±0.1	0.1±0.1	0.1	0.1	0.1	0.1	0.1
FD	Control	2.2±0	1.1±0	1.1±0	2.3±0	1.1±0	1.1±0	1.1±0
	Experimental	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DK	Control	3.6±0	4.0±0	4.0±0	3.6±0	4.0±0	4.0±0	4.0±0
	Experimental	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FS	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Experimental	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FPL	Control	1.4±0	1.1±0	1.1±0	1.4±0	1.1±0	1.1±0	1.1±0
	Experimental	1.3±1.0	0.8±0.5	0.8	1.3	0.8	0.8	0.8

HPL	Control	1.6±0	2.8±0	2.8±0	1.6±0	2.8±0	2.8±0	2.8±0
	Experimental	0.6±0.4	0.6±0.5	0.6	0.6	0.6	0.6	0.6
SL	Control	2.5±0	4.1±0	1.3±0	3.5±0	1.3±0	1.3±0	1.3±0
	Experimental	6.9±2.9	7.3±3.2	7.9	6.9	7.7	8.8	7.9
FR	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Experimental	37.3±2.0	37.9±1.3	41.8	37.3	44.3	41.8	38.2

Behavioural changes in young and adult Wistar rats to auditory stimuli

Six-month-old, 16 adult rats, including n=8 male rats (199.0±8.0g) and n=8 female rats (146.0±6.0g), and 2-month-old, 16 young rats, including n=8 male rats (171.0±11.0g) and n=8 female rats (99.0±13.0g), were included. As shown in **Figure 3**, both adults and young rats showed higher frequencies of WA (26.9±10.0/min), RA (6.0±1.5/min), and IS (5.5±2.6/min) before the intervention (control group). The behaviours such as BA, ST and FS were not shown across all experimental conditions. The FR behaviour was absent in the control group. The very low frequencies were expressed for FPL (1.3±0.2/min), FD (1.7±0.8/min), HPL (2.2±1.6/min) and SL (2.4±1.6/min) by the control group. After the sound stimulations, WA and CL suddenly decreased to 1.7±0.8/min in SR-S1, and then further decreased in SR-S3 and SR-S4. The behaviours, i.e., SL (7.4±3.9/min) and FR (39.6±3.1/min), were prominent after applying the sound stimulation. Similarly, RA (0.8±0.4/min during SR-S1) declined progressively and became absent after SR-S2. The frequencies of IS experienced a sudden surge in SR-1 (6.9±22/min) and gradually decreased up to SR-S4 (3.4±1.6/min).

Except for FR, SL and IS, all other behaviours were absent in SR-S4. There was no significant difference between adults' and young rats' behavioural pattern alteration between control groups (p=0.685) and after the stimulation (p=0.154). However, there was a statistically significant difference in the behaviour of the control and experimental groups of adults (p=0.018) and young (p=0.001) Wistar rats (r>0.5).



(a) (b)

Figure:3 Behavioural frequency changes in young (a) and adult (b) Wistar rats in control (without intervention) and different sound stimulations. The sample ringing sounds (SR-S) are categorized as follows: SR-S1(0.20-0.25kHz, low frequency), SR-S2 (0.40-0.45kHz, moderate low frequency), SR-S3 (0.60-0.65kHz, moderate high frequency), and SR-S4 (0.80-0.85kHz, high frequency). Abbreviations of behavioural patterns are stated in Table 1.

Behavioural changes of Wistar rats in different housing patterns to auditory stimuli

Six-month-old, twelve adult male Wistar rats (198.0±12.0g) and twelve adult female Wistar rats (128.0±11.0g) were kept in different housing (group, pair, and single) at different time intervals. Rats showed behavioural changes at different frequencies to mobile phone ringing sounds, as shown in Figure 4. The majority of adult rats initially expressed FR (41.4±4.5/min) behaviour, then SL (8.1±5.1/

min) behaviour after exposure to low or high frequencies across all three housing patterns. There were no significant differences in behavioural patterns between group and pair housing of adult rats (p=0.068) and between the group and single housing of adult rats (p=0.285) after the auditory stimulation.

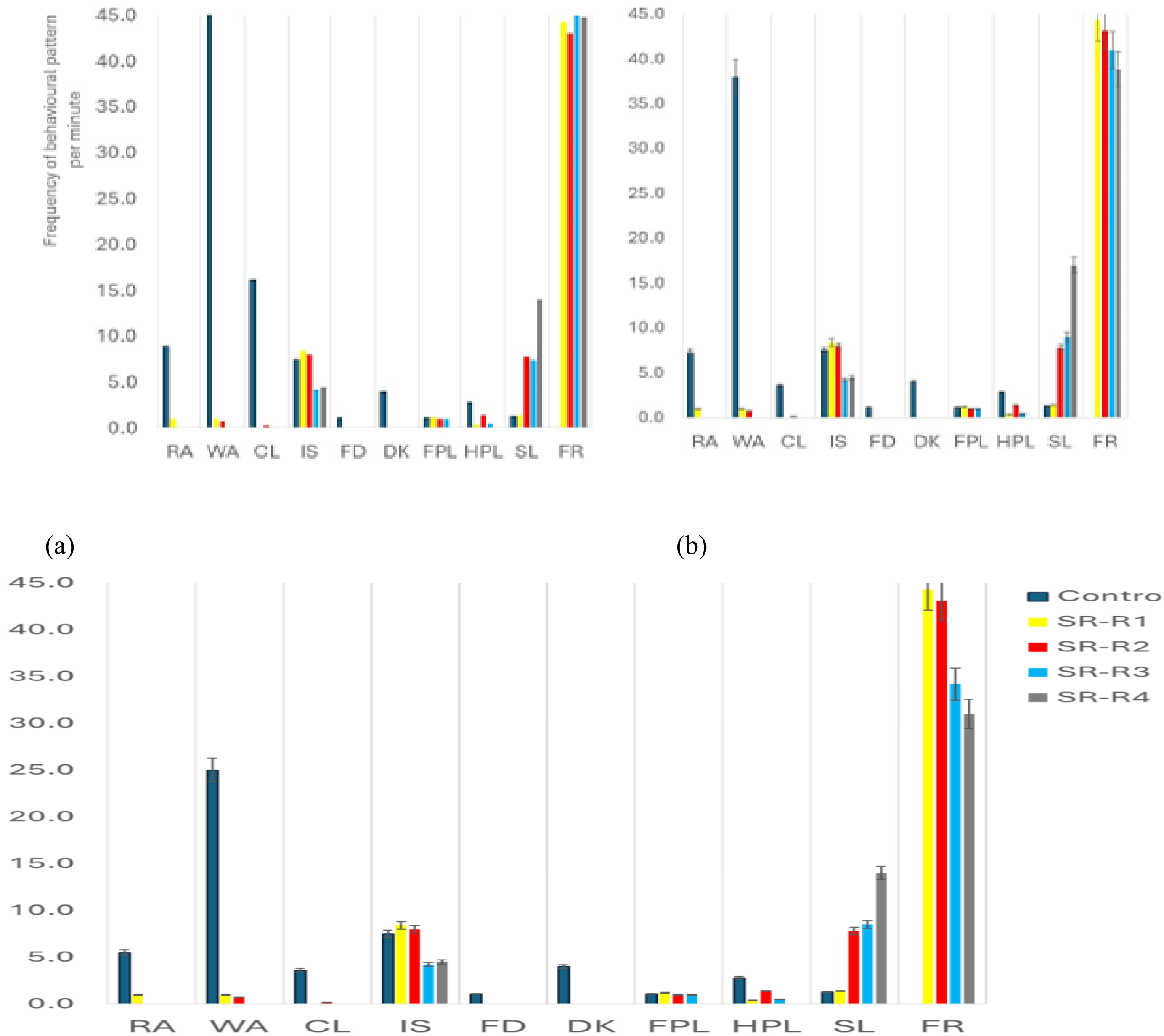


Figure 4: Behavioural frequency changes with single (a), pair (b) and group of four (c) housing of Wistar rats in control (without intervention) and different sound stimulations. The sample ringing sounds (SR-S) are categorized as follows: SR-S1 (0.20-0.25kHz, low frequency), SR-S2 (0.40-0.45kHz, moderate low frequency), SR-S3 (0.60-0.65kHz, moderate high frequency), and SR-S4 (0.80-0.85kHz, high frequency). The intensity of the color gradient in each area indicates the relative frequency of each behavior, with warmer colors indicating higher frequencies. Abbreviations of behavioral patterns are stated in Table 1.

DISCUSSION

Laboratory animals are often exposed to various noises during daily care, which may significantly affect their welfare, as they are highly sensitive to sounds and other environmental changes. They can also change their behaviour in response to short-term sound exposures, e.g., mobile phone ringing. Mobile phones are being used all the time because of modern amenities. Thus, the animal centre staff and researchers, and other handlers tend to use their mobile phones in the animal centre. Some researchers use their mobile phones without being in silent mode, even though they are observing behavioural changes as a study outcome. Previous studies have shown the importance of evaluating the effects in rats upon their exposure to noise due to their sensitivity and the use in studies. A study conducted to assess the effect of sub-chronic exposure to noise on changing the locomotive process and induction of behavioural patterns of depression in rats has proved that the sub-chronic exposure to noise could induce behaviour of anxiety and depression, and later psychological disorders in long-term exposure (Voipio et al., 2006). A similar study assessed locomotor activity and anxiety-like behaviour in adult rats by exposing them to 100dB noise for 1 hour for 10 consecutive days, concluding that this exposure induced changes in mental state and ultrastructural changes in the auditory hypothalamus (Zhvania et al., 2020). Another study showed that rats tended to mutagenic formation upon exposure to low-frequency noise (Vasilyeva et al., 2017). The current study was conducted to analyse any alteration in rats' behaviour in response to mobile phone ringtones as auditory stimuli at Medical Research Institute, Colombo, Sri Lanka. The effects of maturity (adult or young), sexuality (male or female), and housing (group, pair, or single) on behavioural responses to mobile phone ringing were assessed in this study. The Wistar rats showed their regular behaviour, including RA, WA, FD, DK, and licking, at different frequencies before the intervention. After mobile phone ringing-sound stimulation, many rats across study categories exhibited FR as a passive defensive response. As reported in other similar studies using various sound stimuli (Alexandrov et al., 2023; Totty et al., 2021), the regular behaviour they had expressed was completely replaced by fear-like responses during the experimental procedure. The association between auditory stimulation and reported FR frequencies may be influenced by the intensity of the ringing sound, as the experimental group reported higher FR frequencies at higher volumes. Therefore, the frequency of FR behaviour was higher at higher intensities, i.e., SR-S3 and SR-S4. Nevertheless, previous studies have found that females are more stress-sensitive than males due to hormonal influences (Berry & Zucker, 2011). The gender (male or female) of the Wistar rats did not show a statistically significant difference in defensive behavioural changes under the tested conditions in our study. Young and adult Wistar rats were used to assess age-related behavioural alterations induced by mobile phone ringtones. Although young rats showed greater tendencies toward exploratory behaviors, i.e., WA and RA, in previous studies (Menegassi et al., 2017), the control groups in the current study did not exhibit statistically significant differences in behavioral patterns. After the exposure, these behavioural patterns were significantly reduced, and enhanced FR and SL behaviours were noted. This controversial pattern may suggest an acute stress-like or defensive behavioural response in the experimental group, as reported in adults and young rats in response to auditory stimuli. Wistar rats housed individually tend to exhibit a slightly higher frequency of FR behaviour than those in pair or group housing when exposed to auditory stimuli, suggesting that social buffering is probably due to a sense of protection (Casarrubea et al., 2024). As a result of the current study, this stress mitigation due to socialization was explained in other studies as being driven by various environmental stimuli (Eraslan et al., 2023; Perkins et al., 2019). However, the statistically insignificant differences across housing conditions (single, pair, and group) in responses to mobile phone ringing in the current study may be due to prior social interactions. Therefore, the mobile phone ringing during behaviour testing at animal centres may alter the findings of behaviour-related studies conducted using Wistar rats. So, minimizing the use of mobile phones at the animal centre will be an added advantage for generating accurate results in animal-based studies.

LIMITATIONS

The reuse of animals across protocols may introduce potential habituation or sensitization effects. Although intervals were incorporated to minimize this, future studies should consider independent cohorts or counterbalanced designs. This study was the absence of direct measurement of sound intensity (dB SPL), which may influence behavioural responses independently of frequency. The absence of physiological stress markers (e.g., corticosterone and heart rate) limits the ability to definitively confirm stress responses. Future studies incorporating physiological stress markers such as serum corticosterone levels, heart rate monitoring, and automated behavioural tracking systems (e.g., EthoVision) would strengthen the interpretation of stress-related responses.

CONCLUSION

According to the study, Wistar rats altered their regular behaviour in response to mobile phone ringing, regardless of gender, maturity, or housing pattern. The ringing sounds significantly suppressed movement-related behaviours, likely indicating an increase in stress-like or defensive behavioural responses. Therefore, the mobile phone ringing at the animal centre during experiments could alter the findings of behaviour-related studies conducted with Wistar rats. Further studies are recommended to determine any neuroanatomical or cognitive effects on rats from exposure to mobile phone ringing.

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ETHICS APPROVAL

The ethics approval was obtained from the Ethics Review Committee of the Medical Research Institute, Colombo (06-2022/17.11.2022).

AUTHORS CONTRIBUTION

Pulwansha T. Amandi Thilakarathna: Proposal writing; obtaining ethics approval; conducting the study; data collection; formal analysis of data; writing the draft manuscript. **Mangala Gunatilake**: Conceptualization; methodology; supervision; validation; manuscript reviewing and editing. **Mayuri G. Thammitiyagoda**: Conceptualization; methodology; resources; supervision; validation. **Ramani Karunakaran**: Conceptualization; methodology; resources; supervision; validation.

CONFLICT OF INTEREST

No conflicts of interest are declared by any author.

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